The Cooperative Research Centre for
Water Quality and Treatment

Characterisation of Natural Organic Matter of Drinking Water Reservoir-Catchment Systems

Research Report 12

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PROJECT 2.1 CHARACTERISATION OF NATURAL ORGANIC MATTER: REPORT 2

CHARACTERISATION OF NATURAL ORGANIC MATTER OF DRINKING WATER RESERVOIR-CATCHMENT SYSTEMS.

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FOREWORD

Characterisation of Natural organic Matter

Project Leader: 1997-April 2000 : Dr Kaye Spark
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Research Node: Australian Water Quality Centre

CRC for Water Quality and Treatment
Project 2.1.1 Characterisation of Natural Organic Matter.

Note:
The work reported here is that of Dr Kaye Spark, during the time she was employed by the South Australian Water Corporation. After acceptance of a position as senior research scientist with CSIRO, in April 2000, completion of the project and its reporting became the responsibility of the project officer.
The analyses of data and interpretation of the work reported here, except that of Chapter 6, was done by Dr John van Leeuwen. The report is based on the available data to April 2000, with some additions advised by Dr Spark. All attempts were made to interpret them in the context of the original project aims and objectives (as detailed in Report 1). Chapter 6 is an extract of a draft paper prepared by Dr Spark during the time of the preparation of this report. Her co-operation in this and in other ways to facilitate the preparation of this report is gratefully acknowledged.
It should be noted that experimental work and data gathering after April 2000 may have been different, albeit slightly, had staff changes not occurred during the project. No assertion is made that the interpretations detailed in this report, other than Chapter 6, are the same or similar as those that would have been made had staffing changes not occurred.

EXECUTIVE SUMMARY

This report is the second that describes the work performed for CRCWQT Project 2.1 "Characterisation of Natural Organic Matter". The first deals with NOM in relation to water treatment processes. The second deals with the characterisation of natural organic matter from various catchment sources and in reservoirs, evaluation and application of the infrared (DRIFT) and UV-vis spectroscopy for characterisation of NOM and adsorption of NOM by minerals.

The results include those of the application of DRIFT spectroscopy to characterise NOM isolated from field sources, concurrently with results of experiments performed to evaluate the suitability of the technique for NOM in various matrices. This was done because DRIFT is readily able to be performed and is inexpensive compared with other techniques, but knowing that there was a risk of application under sub-optimum conditions for some sample types. Application of the technique on soil derived samples is well reported in the literature and was similarly applied in this study, while its suitability for characterising NOM isolated from natural waters, without desalting, was not well known. From the work reported here, it was recognised that salts in reservoir waters and soils can markedly impact on DRIFT spectra and subsequent efforts to remove salts from natural waters using dialysis is described in Chapter 5.

Chapter 6 gives the results of a separate study that examined the adsorption of NOM from catchment and reservoir sources onto soil minerals such as goethite, kaolinite and silica.

Surveys aimed to determine variations in the character of NOM in reservoir catchment systems were conducted in Victoria and South Australia.

In Victoria nine reservoirs were sampled in February 1999 and analysed by DRIFT, and UV-vis spectroscopy, and for total organic and inorganic compositions. From these, three reservoir-catchments (Moorabool, Lake Wartook and West Gellibrand) were selected and further studied in April and July 1999. DRIFT spectra of samples collected from the same reservoirs in February and April showed consistency in relative absorbances at ~ 1633, ~1428, 1140 and 1080 WN/cm, but were different between the three reservoirs. The results indicate that DRIFT spectra of reservoir samples may be of value as a finger-print, though the causes of absorbances may be due to both organic and inorganic components.

DRIFT spectra of extracts from soils and litter layers from Victorian reservoir-catchments indicate differences in the functionalities of organics, particularly at about 1610 and 1400 WN cm^{-1}. Lower absorbance at ~ 1610 WN/cm, possibly due to low aromaticity, was found for samples from a grass site at Moorabool.

UV/vis data obtained for raw surface waters and extracts of litter layers and soils included parameters reported in the literature to be of value for characterising NOM, ie E4/E6 ratio, and SUVA. Also, two other parameters were determined to have potential significance in characterising NOM, and were (1) the ratio of 254nm/456nm (UV/COL) and (2) 254nm x 456nm x 1000/DOC (UVCOL/DOC).

These ratios were selected on the basis of inclusion of absorbance in the visible region, which at 456 nm indicates the level of colour of the naural water or extract. The reason for this colour may be from humic material, polyaromatics and from dissolved iron compounds. Several trends were found in these ratios dependant on sources. The UV/COL ratio values were in the order of reservoir~litter layer > soils. The UVCOL/DOC ratio values had an opposite trend of soils>reservoir~litter layer.

Two reservoir catchments (Mt Bold and Myponga) were studied in South Australia from which three study sites were selected, a pine forest, grass and native trees. These sites were examined on three occasions in autumn and winter. Differences were found in the organic loads in soils from the three sites with highest loads associated with the pine forest. Trends in the UV/COL ratio values for litter layers and soils were similar to those found for samples collected in Victoria. There also appeared to be a trend between the ratio value and the position on a catchment slope, with higher values occurring at the top of slopes. This ratio is postulated to be a potential indicator of the degree of degradation of NOM, with high values indicative of lower degradation. SUVA values were found to be higher at some lower positions of catchment slopes, perhaps a result of higher humification of organics at these locations.

DRIFT spectra of soil and litter layer extracts contained few well resolved peaks that could be assigned to organic functionality. However, differences in the relative peak heights of these appeared to be related to some differences in vegetation sources ie from grass versus treed sites. The influence of salts on DRIFT spectra of extracts from soils appeared to be high for some samples, demonstrating the need for de-salting samples when characterisation of NOM is paramount. Dialysis of samples prior to DRIFT analysis appears to reduce interference caused by salts on the spectra, though the time required for dialysis is about one full day. Extensive ultrafiltration can also be used to remove salts, as described in Report 1, though both methods result in a loss of organics that could be important to the drinking water industry.

Recommendations from the work of this study include,

1. the application of DRIFT to be conditional on the type of information that can be derived from spectra,
2. the use of dialysis or ultrafiltration for desalting of natural water samples if DRIFT is to be used to characterise NOM,
3. further testing of the UV/COL and UVCOL/DOC ratios for their apparent source specificity, and worth in characterising NOM
4.

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CHAPTER 1

INTRODUCTION

Organic matter in soils (SOM) is very important in the global carbon cycle with estimates of 14-15 x 10^{17} g of C (Eswaran *et al.*, 1993; Sclesinger, 1984; Sclesinger 1995; Hayes 1997) in total. This has been estimated to be 2.5-3 times the terrestrial biomass C (Hayes, 1997). The mean residence time of organic carbon in soils has been estimated to be 32 years, accounting for the short turnover time of readily bio-transformed organic substances and the long residence time of more stable organic materials (Hayes, 1997). The highly transformed amorphous dark coloured materials are classified as humic substances (HS) while the identifiable classes of organic macromolecules, such as carbohydrates, peptides and nucleic acids are classified as non-humic. Humic substances can constitute 70-80% of SOM and are resistant to microbial degradation through self association to amorphous macromolecules, to soil minerals and entrapment in soil aggregates (Hayes, 1997). These are too large to pass across cell membranes, and their chemical structural variability makes them have a low compatibility with the specific structural requirements of extracellular enzymes and their purpose designed activities.

OM has significant impacts in soils as it forms and stabilises soil aggregates. HS are important cation exchanges in the release of nutrients when SOM is mineralised (Hayes,1997). OM from terrestrial sources is transported to waterways by surface and sub-surface flows in various chemical states dependant on the source type, the degree of degradation and the transport processes. Although in itself not a health hazard, OM in drinking water, whether from allochthonous or autochthonous sources, does support microbial growth in waters treated for drinking purposes. Conventional water treatment, which incorporates inorganic coagulants such as aluminium and ferric-based compounds, tends to remove the large molecular weight hydrophobic fraction but little of the uncharged, hydrophilic compounds. The latter are representative of the non-humic fraction, though the degree to which they have undergone microbial degradation would be varied. These coagulant recalcitrant organics persist in waters after conventional treatment and pose little impact on aesthetic water quality due to their lack of colour imparting properties. However, as they can be a substrate for microbial growth in a water distribution system, waters are often disinfected using chemicals. When chlorine-based disinfectants are used OM in the water acts as a precursor for the formation of chlorinated disinfection by-products. For these reasons the presence of organic matter is important to reservoir-catchment management authorities. The sources and sinks of organic matter in these systems, and knowledge of their degradation and transport processes might assist in minimising the impact of OM on the water treatment industry and consumers of the treated drinking water. Practical considerations at a catchment level include the suitability of vegetation cover types for minimising erosion and transport of organics into waterways.

1.1 Characterisation techniques:

1.1.1 IR spectroscopy: A description and applications of Diffuse reflectance Fourier transform infrared spectroscopy has been given in section 2.1.1 of Report 1. For ease of reading, this section is also given in this report.

DRIFT is an infrared spectroscopic technique applied for the characterisation of organic and inorganic compounds that gives information on the types and relative amounts of various molecular functional groups. This technique relies on the interaction of infrared radiation with samples, measuring the frequencies at which absorbances occur and their intensities.

Infrared spectroscopy deals with transitions between vibrational energy levels in molecules. Each vibrational motion of a molecule occurs at a certain frequency, which is characteristic of the molecule and of the particular vibration. Vibrational motions include symmetric and asymmetric stretching and bending (scissoring, rocking, wagging and twisting). Only certain vibrational energies are allowed to the molecule and the molecule may be made to go from one energy level by absorption of a quantum of electromagnetic radiation. In undergoing such a transition the molecule gains vibrational energy, manifested by an increase in amplitude of the vibration. The frequency of the light required to cause the transition for a particular vibration is equal to the frequency of that vibration. Consequently vibrational frequencies may be measured by measuring the frequencies of light that are absorbed by the molecule. The wavelengths of light which cause most of these vibrational transitions lie in the infrared region of the electromagnetic spectrum. Function groups have vibrational frequencies characteristic of that functional group within the range of the infrared spectrum between 4000 cm^{-1} and 625 cm^{-1}. Extensive data now exists and is detailed in the literature of the absorption frequency bands of functional groups. Examples of these are given by Parker and Frost (1996), Williams and Fleming, (1997), and Francioso *et al*. (1998). Commercial software libraries are also available to assist in interpretation of DRIFT spectra.

DRIFT spectroscopy has been used to address a variety of questions relating to the structure of natural organic matter (NOM) including characterisation of peat fulvic acids (Francioso *et al*. 1998), differentiation of coniferous wood species (Nault and Manville, 1997), the influence of soil texture on the organic composition in soils (Capriel *et al.* 1995), sorption of NOM to soils and mineral matrices (Kaiser *et al*. 1997), heating affects on the structure of humic sodium salts (Woelki and Salzer, 1995) and soft rot in potatoes caused by *Erwinia carotovora* (Stewart *et al*. 1994),

The application, advantages and disadvantages of DRIFT spectroscopy has been described by Page (2000), Spark (1998), Capriel *et al*. (1997) and Frost and Parker (1997).

DRIFT spectroscopy provides some important advantages over other techniques used to characterise compounds, namely only a small sample size is needed and the technique is relatively quick and inexpensive to perform. However, in the application of DRIFT spectroscopy, functional groups from organic and inorganic compounds are detected, with the potential of one confounding the other, where information on one of them is sought. Hence, if salts are present in a sample to be analysed for its organic content, an unambiguous interpretation of the spectra is unlikely. Therefore, knowledge of the relative compositions of the inorganic/organic matrices would influence the confidence of interpretation that can be made of the IR spectra. The impact of clay on DRIFT spectra was shown by Downes (2000) in the course of this study. According to Hayes (1997) IR spectra of humic substances show broad bands characteristic of functional groups in a variety of environments and provides only

limited information about their composition. Humic substances, although significant in relation to water treatment processes, constitutes a fraction of the total NOM in water and soil. Despite humic substances being the larger proportion of the total NOM in these matrices in most cases, it is the non-humic fraction that is of particular interest to the water industry. This is so because of the recalcitrance of this fraction to removal by the most common reagents used for water treatment, inorganic coagulants. We therefore report the application of DRIFT spectroscopy in an attempt to better characterise NOM.

1.1.2 Ultra violet – visible spectroscopy (UV-vis). Hayes (1997) also described this method as having limited applications in the humic substance sciences. The peaks of UV-vis spectra are described as being broad which provide little information that is interpretable. Ratios used include the E4/E6 (absorbance at 465/665 nm) values for comparing humic samples (Chin *et al*. 1994) from different sources. Distinct differences can be found but the meaning of these is not clear. Simpson *et al*. (1997) provided E4/E6 data for various humic fractions (humic acids, HA and fulvic acids, FA). These authors found that the ratio values for the HS isolated from podzol soils were, in general, lower for the HAs and for the FAs . They interpreted this on the basis that HAs are of a larger molecular weight. The highest ratio values were obtained for fractions isolated at pH 7. This was postulated to be due to lower associations of molecules, greater aromaticity or unsaturation.

Another interpretation of the ratio is that its magnitude is related to the degree of condensation of the aromatic humic components (Simpson *et al*. 1997). Early studies associated low E4/E6 with increased condensation of aromatic C and a greater degree of humification (Gressel *et al.* 1995) with humic acids having ratios of between 3 to 5, while fulvic acids of 5.5 to 8 (Stevensen, 1982). Gressel *et al*. (1995) determined E4/E6 ratio values for extracts from pine and understory extracts. They also found that these ratios were impacted by addition of a buffer solution and pH. They recommended that they be measured at the original pH with no alteration beyond any dilution necessary to meet a standard ionic concentration and compliance with the Beer-Lambert law. These authors found that the ratio values for pine extract samples were in the range of 7.8 to 8.5, while understory extracts were in the range of 6.3-7.7. They concluded from their work that the ratios are probably indicative of molecular size or degree of complexation but a decrease in ratio is not necessarily indicative of DOM humification.

According to Korshin *et al.* (1997) UV spectra of NOM can be represented by three bands, each a gaussian function of energy. These bands can be ascribed to three types of electronic transitions typical of aromatic compounds and are referred to as local excitation (LE), benzoid (Bz) and electron-transfer (ET). The LE occurs at 180 nm, the Bz at 203 nm and the ET is centred at 253 nm. The ET band is a distinctive feature of the electronic spectra of aromatic compounds and is affected by the presence on the aromatic ring by polar functional groups such as hydroxyl, carbonyl, carboxyl, and ester groups. Non-polar aliphatic groups do not increase its molar extinction coefficient (Korshin *et al.* 1997). According to these authors, the ratio of absorbances of ET/Bz will be low for NOM in which the aromatic rings are substituted predominantly with aliphatic groups and increase for NOM in which the aromatic groups are highly substituted with hydroxyl, carbonyl, ester and carboxyl groups. Chin *et al*. (1994), measured the absorbances of aquatic humic substances at

280 nm. From about 270 nm to 280 nm π-π* electron transistions occur for a number of aromatic substances such as phenolic substances, aniline derivatives, benzoic acids, polyenes and polycyclic aromatic carbons. They also used the E4/E6 ratio in their studies. An advantage of using 280 nm, according to Chin *et al*. (1994) is that nitrate, ubiquitous in natural waters, does not absorb at this wavelength. Korshin *et al*. (1997) state that nitrate does not significantly absorb light at greater than ~230 nm, but between 200 nm and 230 nm low levels of nitrate can absorb enough light to alter the spectrum of natural waters significantly. Krasner *et al*. (1996) used UV absorbance at 285nm as, according to these authors, it is a highly specific indicator of benzene carboxylic acids and phenol. They also used fluorescence measurements using an excitation wavelength at 345 nm and an emission wavelength of 415 nm.

The measurement of UV absorbance at 254 nm is often used by the drinking water industry (eg. Chow *et al.* 1999, Korshin *et al*. 1997, Owen *et al*. 1995; Edzwald, 1993; Edzwald *et al*. 1987) as a rapid, inexpensive indirect measurement of dissolved organic carbon. Conjugated, unsaturated double bonds, as of aromatic compounds absorb UV light. The removal of organics can be monitored by removal of UV absorbing compounds and maximum or near maximum is achieved when further treatment causes little or no further removal of these compounds. The humified, coloured organics generally have relatively high UV absorbing properties compared with non-humic substances and it is the former which is more readily removed by conventional treatment processes (Krasner and Amy, 1995).

A further derivation of this UV measurement is to standardise it to the total concentration of dissolved (less than 0.45 μm) organic carbon (Amy *et al*. 1992, Chow *et al.* 1999) as follows:

SUVA= (254nm x 100) / DOC mg/L (Chandrakanth *et al*. 1998; Edwards, 1997, Edzwald, 1993). This ratio has been interpreted as a measure of the relative aromaticity of NOM (Chandrakanth *et al*. 1998).

The brown colour of water attributable to NOM, particularly humic substances, tannins and lignin derived compounds absorb in the visible region of the light spectrum. The colour of natural surface waters as determined relevant to the drinking water industry is measured at a range of wavelengths of about 400-460 nm; including 408 nm (Fu *et al*. 1994); 436 nm (Bernhardt and Schell, 1993); 456 nm (Bennet and Drikas, 1993).

In this study, UV-vis spectra were measured from ~190 to 700 nm for a range of isolates of organic matter from waters and from soil and litter layer leachates. These were then further investigated for other ratios that might be related to their sources such as type of soil, vegetation, catchment topography, climate etc. As humic substances are the fraction of organics that are more readily removed by conventional water treatment processes using inorganic coagulants (Krasner and Amy, 1995) and have higher relative UV absorbance and colour, it was considered that these features might provide other ratios that are useful in water treatment studies.

1.1.3 High performance size exclusion chromatography (HPSEC): In the work reported here HPSEC was not extensively used, ie only on NOM samples collected from Victoria in April 1999. The technique adopted in this study is as described by

Chow *et al*. (1999). Following separation of organics, based on molecular size, they are detected using UV absorbance at 260 nm. Although this analytical system is useful in relation to the study of efficiencies of water treatment it does not allow for detection of non-UV absorbing organics. These organics may be of small or large molecular weight and may be precursors to disinfection by-products and bacterial growth in a distribution system.

1.2 Organic matter in soils and natural waters.

Organic matter present in soils and waters have been characterised in terms of their humic and non-humic fractions, as described above. Humic substances from a range of sources have been extensively studied in terms of humic acid, fulvic acid, humin and non-humic acid components (examples include Hatcher and Clifford, 1994; Saiz-Jimenez, 1992; Bruchet *et al.* 1990 and Gonzalez-Vila *et al*. 1995). In soils, organic matter is seen as important in relation to the health or condition of the soil for various land management practices, such as pasture, cropping and maintaining a natural environment. Organic loads are related to the fertility of the soil, its structure, capacity for supporting microbial and invertebrate activities and agricultural value. Terrestrial sources of organics in water are mostly significant for streams, rivers, lakes and reservoirs. Organic loads in catchments may vary according to vegetation cover type resulting in different concentrations of organic matter in throughflow water in soils (Naidu *et al.* 1993). In that study it was found that the concentrations of DOC in waters from pine forest was about twice that of pasture and native vegetation.

Findlay *et al*. 1997 studied streams draining catchments vegetated with pasture, native forest and pine catchment. They found that there were differences in the activities of extracellular enzymes (cellobiohydrolase, N-acetylglucosaminidase and dihydroxylphenylalanine) in relation to the terrestrial vegetation type.

Bandick and Dick (1999) determined the activities of soil enzymes as an indicator of soil quality. Soil enzymes, primarily of microbial origin catalyse reactions that are part of the nutrient cycle. These authors found differences in enzyme activities in relation to agricultural land management practices, which in turn impacts the organic type and loads. Hence vegetation type and density and land management practice impacts on the organic loads in soils. In soils, variation would exist in the degree to which organics have undergone bio-degradation and consequently their resultant character. Differences in the character of organics and in their concentrations in surface and subsurface flows to waterways would be affected by climatic and topography conditions in the catchment.

1.3 Objectives of project work:

For the work reported here, the objectives were as follows:

1. To improve and/or validate methods for the characterisation of NOM in soils and waters,
2. to study the loadings of organic matter in soils of drinking water reservoir catchment systems, in relation to various vegetation types, topography, geographical differences and season.
3. to study the diversity of the character of NOM in drinking water reservoir catchment systems, in relation to various vegetation types, topography, geographical differences and season.

The work performed to address the first objective is described, in part, in Report 1, where the application of the techniques, thermochemolysis and pyrolysis-gas chromatography/mass spectrometry for characterisation of NOM are detailed.

In the work report here, the technique of Diffuse reflectance Fourier-transform IR (DRIFT) was extensively applied in attempts for characterisation NOM. Work was performed with the aim of improving and validating this technique for characterisation of NOM isolates from natural waters, soils and litter layers. Also, the results of surveys of organic matter in soils and waterways in South Australia and in Victoria are given. These surveys were conducted for several reasons, including

1. Isolation of organic matter that could be used in the evaluation of characterisation techniques.
2. Determination of the variability in the character of organics in soils and water in relation to differences in vegetation, topography and climate.
3. To gain a better understanding of organic loadings and potential leaching capacities from different catchments varying in soil type, vegetation cover etc.
4. Sample collection made in conjunction with other studies; these being those of the project PhD candidate and the Honours degree study.

The PhD study included an investigation into the effect of alum treatment to remove turbidity, colour and organics from a range of natural and synthetic waters. The aim was to better understand the relationships between the various sources of NOM and the efficiency of the common treatment coagulant, aluminium sulphate, to achieve drinking water quality.

The honours study investigated microbial enzyme activities and microbial respiration rates in catchment soils and associated organics were characterised by chemical structural techniques such as high performance size exclusion chromatography with UV detection and pyrolysis-gas chromatography/mass spectrometry. The reader is referred to the two theses (Page 2000; Anstis, 1999), to be read in conjunction with this report.

CHAPTER 2

MATERIALS AND METHODS

Analytical procedures frequently used for this part of the study are described in this chapter. Those infrequently used and relating to specific project objectives are described in the respective, subsequent chapters.
Also in this chapter, are the procedures used for sampling waters, soils and soil litter layers for the surveys of NOM in southern Australia. The analyses applied to these samples are detailed.

2.1 Analyses of natural waters and aqueous extracts of soils, soil litter layers and terrestrial vegetation.

2.1.1 Analytical techniques

Dissolved Organic Carbon Analysis: DOC concentrations were determined using a total organic carbon analyser (Model 820, Sievers Instruments Inc., USA). UV absorbance at 254 nm is often used as a surrogate for DOC (Edzwald, 1993). The absorbance at 254 nm was measured using a UV/VIS spectrophotometer (Model 918, GBC Scientific Equipment Ltd., Australia) with a 1 cm quartz cell. The specific UV absorbance (SUVA), UV/DOC x 100, can be used to determine the character of the organics.

UV-vis Scans: Scans were performed using a UV/VIS 918 spectrometer (GBC, Australia) with a 1 cm square quartz cell. Scans were typically determined from 189 to 700 nm.

Colour: Colour was determined spectrophotometrically by comparing the absorbance of a sample at 456 nm with a platinum/cobalt standard. Samples were first filtered through a 0.45 µm membrane filter and measured using a UV/VIS 918 spectrophotometer (GBC, Australia) with a 5 cm cell. The instrument was calibrated using a deionised (Milli-Q®) water blank and a 50 Hazel Units (HU) platinum/cobalt standard; allowing a direct reading of colour in HU.

Turbidity: A Hach ratio turbidimeter (model 18900) was used to measure turbidities of water samples in units as nephelometric turbidity units (NTU).

Metals: Metal concentrations of the samples were determined using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The samples were prefiltered through a 0.45 µm membrane filter and acidified to ~1% with concentrated nitric acid (Aristar) prior to the analysis.

pH: The pH of the samples were measured using a portable WTW pH meter with a combined pH electrode. Calibration was carried out with pH 6.86 and pH 9.18 buffers.

Alkalinity: Alkalinity was determined by an autotitrator (Radiometer Autotitrator comprising TTT85 Titrator, ABU80 Autoburette, SAC80 Automatic sample changer and

PRS12 printer) with 0.02M hydrochloric acid titrated to pH 4.5. Alkalinity was determined as mg/L $CaCO_3$.

Bicarbonate ion concentration: This was determined using the same method as for alkalinity as $CaCO_3$. The multiple factor applied for bicarbonate in relation to carbonate is 2.03.

Conductivity: A cell consisting of two platinum plates is immersed in the sample and the conductivity measured directly using a conductivity meter, expressed in microsiemens/cm (µs/cm). Conductivity is temperature dependent and is corrected to a standard reference temperature of 25°C. The conductivity reading is dependent on the dimensions of the measuring cell and therefore, the instrument is calibrated using solutions of known conductivity.

Total dissolved solids (TDS): TDS is determined by measurement of electrical conductivity (EC) using a platinum cell and auto-ranging conductivity meter. TDS is calculated from the conductivity at 25°C using an empirical factor. The factor used varies with the water type, and takes into account the expected changes in the relative concentration of ionic species at different conductivities. This factor is detailed in the AWQC Test Methods as of July 2001.

Total Kjeldhal Nitrogen (TKN): Total Kjeldhal nitrogen (TKN) (organic nitrogen) was determined using automated flow colourimetry. The TKN present in a sample is converted to ammonium sulfate by digestion with sulphuric acid and potassium sulphate. The ammonia formed reacts with dichloroisocyanate to form monochloroamine which then undergoes oxidative coupling with salicylate to form an indophenol dye, the concentration of which can be determined by measuring the optical density at 630 nm.

HPSEC analysis: The samples were first filtered through a 0.45 µm membrane filter. Size exclusion chromatography (SEC) was performed using a Waters 501 pump, 717 autosampler, 484 tunable UV detector, an Inter Action column oven set at 30 degrees centigrade and a Showa Denko, Shodex KW-802.5 packed column (Shoko.Co., Ltd.). The carrier solvent was a 0.1 M phosphate buffer solution (pH 6.80) adjusted to an ionic strength of 0.1 M with sodium chloride. The flow rate was 1mL/min. Calibration was performed using polystyrene sulfonate (PSS) standards (Polysciences Inc. MA) of molecular weights 35,000, 18,000, 8,000 and 4,600 daltons. Detection was based on UV absorbance (260 nm/cm). The apparent molecular weight was expressed as number average molecular weight and calculated using the equation stated below:

2.1.2 Analyses performed on dried soil samples**:**

Analyses of dried soil samples for Al, Fe, Na, Ca, Mg and TOC were performed at the Analytical Chemical Unit of CSIRO Land and Water, Adelaide Laboratory, Waite Rd Urrbrae, South Australia.

2.1.3 Analyses performed on freeze-dried samples:

Diffuse reflectance Fourier transform infrared spectroscopy (DRIFT). Drift analysis was carried out on freeze dried samples after adjustment to pH 7, using a Nicolet Magna 750 Spectrometer with a KBr filter and a MCP/B detector. The freeze dried samples were diluted to 5% using potassium bromide (Merck Spectroscopic grade).

Sample preparation: Samples were mixed with KBr at about 1% (w/w). Approximately 0.005 g of finely ground sample was mixed with about 0.5 g KBr. The mixture was shaken to evenly distribute the sample in the KBr and this was placed into a sample receptacle of the Nicolet instrument. A flat spatula blade was used to make a flat surface, level with the edges of the receptacle. The sample was then placed into the instrument and left for 5-10 minutes for nitrogen to remove any residual water and carbon dioxide.

Instrumentation settings:
Number of scans: 64; resolution: 4; apodisation: Happ-Ganzel, final format: absorbance; data spacing: 1.928 cm^{-1}.

Optical bench settings: Max: 10.00 Min: -10.00, location: (1027 or 1028), gain: 4; detector: MCT/B; beamsplitter: KBr; aperture: 32; velocity: 1.8988; spectral range: 4000-400.

DRIFT spectra of bio-polymer standards, pure compounds in various NOM isolates and reference NOM isolates are given in Figures 2.3 to 2.39. Details describing these spectra are given in the section, 'Evaluation of DRIFT analysis and interpretation of spectra'.

Assignments of absorbance bands to functionality from literature references are shown in Appendix 2.1

Semi-quantification of functional groups was determined as detailed by Gressel *et al.* (1995), where peak height ratios were calculated using the OH peak at ~3340 to 3380 WM/cm, at 1610 WN/cm (aromatic and COO^-) and 1070 WN/cm (polysaccharides). In the present study only the broad peak at ~3360 WN/cm was used. Gressel *et al*. (1995) found the peak at 1610 WN/cm to be highly influenced by COO^- and to drastically change with pH, while work in this study showed that the peak at ~ 1070 WN/cm can be highly influenced by sulphate (see Report 1).

2.2 Surveys of the variation of the character of natural organic matter in Victoria and South Australia

2.2.1 Victorian surveys:

A total of three sampling trips were made to Victoria for collection of surface water samples, vegetation samples, soil litter layers and soil samples. Samples were collected in an endeavour to study and evaluate variation in the characters of DOM from its sources to that in the recipient streams and reservoirs.

2.2.1.1 Isolation of natural organic matter from water samples**:**

Raw and treated surface water samples were collected from Victoria in February 1999, (Allen Dam, Lake Wartook Reservoir, Moora Moora Reservoir, Moorabool Reservoir, Mt Cole Reservoir, Mt. Langi Ghiran Reservoir, Pankalak Reservoir, Pankalak treated water (WTP), a stream from the Wartook Reservoir, West Gellibrand Reservoir).

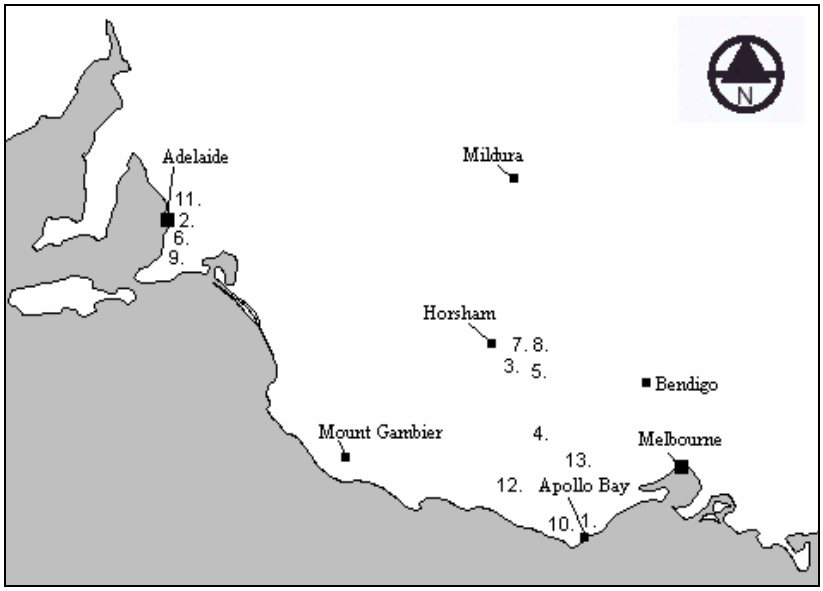


Figure 2. 1 Locations of reservoir-catchment study sites in south-eastern Australia: 1. Allen Reservoir; 2. Hope Valley Reservoir; 3. Lake Wartook; 4. Moorabool Reservoir; 5. Moora Moora Reservoir; 6. Mount Bold Reservoir; 7. Mount Cole Reservoir; 8. Mount Langhi Ghiran Reservoir; 9. Myponga Reservoir; 10. Pankalak Reservoir; 11.Warren Reservoir; 12. West Gellibrand Reservoir; 13. Wilson Reservoir. (Source: Page 2000, PhD thesis).

In April 1999, raw surface waters were collected from Lake Wartook, Moorabool and West Gellibrand reservoirs. Water samples were not collected in July 1999, (the third survey trip to Victoria). On this occasion only soil, soil litter and vegetation samples were collected. Water samples were collected into 1.25 L PET bottles, 10 or 20 L plastic containers and were transported to the AWQC, Bolivar, SA by commercial courier service or at the end of the sampling trip. Samples were stored at 4^{o}C on arrival at the laboratory. Water samples were used in jar test experiments to meet objectives of the PhD study by Page (2000), and also analysed for a range of organic and inorganic water quality parameters. The latter was to assess the diversity of waters in Victoria to enable comparisons with South Australian waters. Additionally

this allowed an evaluation of techniques, particularly the characterisation of NOM by UV-vis and DRIFT spectrometry. Sub-samples were adjusted to pH 7 using 0.1 M NaOH or HCl prior to freeze-drying in preparation for DRIFT analysis.

2.2.1.2 Isolation of natural organic matter from soil litter layers**.**

Soil litter layers were collected from Moorabool, sites 1 to 5 and West Gellibrand, sites 1 to 3 in April 1999 and from Moorabool, sites 1 to 5, Wartook, sites 1 to 3 and from West Gellibrand sites 3 to 5, in July 1999 (see Figure 2.2).

At the Moorabool sites, vegetation types varied from exotic to native flora. Predominant vegetation at the various sites were as follows: Site 1: Douglas Fir, Site 2: Californian redwood, Site 3: *Pinus radiata* Site 4: Natives, mostly eucalypts and Site 5: grass.

At Lake Wartook, vegetation at all three sites had mixed native trees and grasses. At West Gellibrand, the vegetation was as follows: sites 1 and 5: *Pinus radiata* and sites 2, 3 and 4: eucalypts.

Further descriptions of these locations, including soil analysis have been reported by Page (2000).

Litter samples were sieved with 1 mm sieves to remove soil; larger pieces were cut to approximately 1 cm and then stored in air tight bags at < 4^{o}C.

Procedure used to extract soluble organic compounds from soil litter samples:

Samples of litter (0.5 kg) were gently mixed with 2L reverse osmosis (RO) water and then left to stand overnight at <4^{o}C. The supernatant was decanted, centrifuged at ~9,000 rpm and filtered through a 0.45 µm GFC filter.

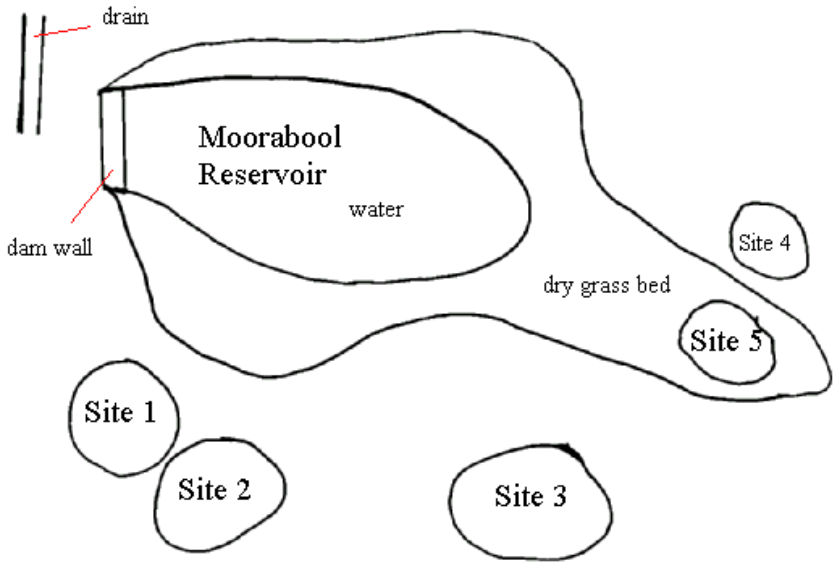


Figure 2. 2 Schematic diagram of Moorabool Reservoir and locations of sampling sites (not to scale).

2.2.1.3 Isolation of natural organic matter from soil samples**.**

No soil samples were collected in February 1999. Soil samples were collected from Moorabool sites 1 to 5, Wartook sites 1 to 3 and West Gellibrand, sites 1 to 3, in April 1999. In July 1999, samples were collected from Moorabool sites 1 to 5 (x 4 samples per site), Wartook sites 1 to 3 (x 4 samples per site) and West Gellibrand sites 3 to 5 (x 4 samples per site).
Samples were sieved through a 2 mm mesh sieve and stored in air-tight bags at ~4^{o} C. Sub-samples (~ 200 g) were air dried at ambient temperature, ground with a mortar and pestle and stored in plastic jars at room temperature. Analyses performed on the dried samples for the samples detailed in this report and for the Honour's thesis were: totals for Al, Fe, Na, Ca, Mg, total organic carbon and XRD.

For extraction of soluble organic material the following procedure was used. Soil sample (1 kg) was gently mixed with RO water (~1000 ml), and left to stand overnight. The supernatant was decanted, centrifuged and filtered through GFC and 0.45 μm filters. Analyses of the resultant samples included pH, DOC, TKN, TDS, Ca, Mg, Na, Al, Fe alkalinity, conductivity. Solutions were adjusted to pH 7 as previously described and UV-vis scans performed. Samples were then freeze dried for DRIFT, and potentially ^{13}C NMR and Py-GC/MS analyses.

Analyses performed on samples included the following: pH, high performance size exclusion chromatography (HPSEC), dissolved organic carbon (DOC), total Kjeldhal nitrogen (TKN), total dissolved solids (TDS), Ca, Mg, Na, Al, Fe, alkalinity and conductivity. UV-vis scans were performed between 200 and 700 nm. Samples were adjusted to pH 7 using 0.1 M NaOH or 0.1M HCl for DRIFT analysis. Samples were freeze-dried for analyses such as DRIFT, ^{13}C NMR, Py-GC/MS, though not all of these were performed on all samples.

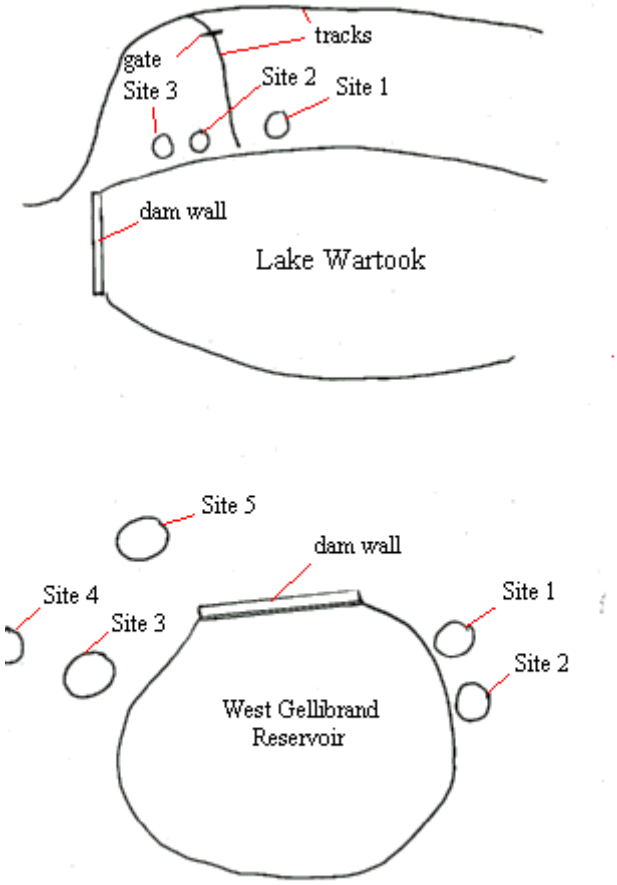


Figure 2.3 Schematic diagrams of sampling sites at Lake Wartook and West Gellibrand Reservoir (not to scale).

2.3 South Australian surveys:

The survey of the variation in the characters of NOM was also studied in South Australia to coincide with the studies by Anstis (1999) and Page (2000), supported through CRC Project 2.1.1. Objectives of the Anstis (1999) study included comparison of the variation in the character of NOM from contrasting vegetation sites, ie. *Pinus radiata* forest and grasses (Myponga) and native vegetation (Mt Bold). Characterisation of NOM was attempted on the basis of microbial activities as a reflection of organic types, chemical structural evaluations using pyrolysis-gas chromatography/mass spectrometry and the assimilability of organics based on bacterial regrowth potential. In the study by Page (2000) the treatability of organic isolates from Myponga and Mt Bold catchments were also studied.

2.3.1 Isolation of natural organic matter from waters, soil litter layers and soil samples.

2.3.1.1 Sample collection in October 1998:

Soil samples were collected from the Mt Bold catchment (adjacent to the Soil and Land Management study sites CRCWQ&T 2.1.2, located at Scott Creek) and from the

Myponga catchment (in proximity to the S&L M study sites CRCWQ&T 2.1.2) in October 1998.

At Mt Bold, soil samples were collected at various heights along a grassed slope, near or away from trees and along the same heights of the slope. Samples were collected from the bottom or lower sections of the study area, at the middle and at the top of the slope. Soil samples were also collected near to an adjacent dirt road, and near a pond. The soils of this area have a texture contrast between the A and B horizons. A horizons have a silty clay loam texture, generally less than 20 cm in thickness. The B-horizons are of medium to heavy clays with an open structure.

At Myponga, soil samples were collected along a gentle slope at the top, middle and lower sections. The soils have a strong texture contrast between the A and B horizons, with the A-horizon being loamy sand to sandy loam and the B-horizon a heavy clay.

Samples (~ 1 kg) were collected using a shovel. Sub-samples were gently mixed with RO water (1:1) and left refrigerated overnight. The extracts were decanted, centrifuged for ~ 30 minutes at 9000 rpm and then filtered through 0.45 um. Analyses performed on these samples were pH, UV-vis scan (189 to 700 nm) and DRIFT, after adjustment to pH 7.

2.3.1.2 Sample collection in March 1999.

In March 1999, soil samples were again collected from both the Mt Bold and Myponga catchments, except that the sites selected were the same as those selected for the Anstis (1999) study. Both locations, in contrast to the October 1998 collections, were adjacent to their respective reservoirs.

The Myponga sites studied in March 1999 had soils that differed to those studied in October. Two locations were selected, one along a steep slope heavily forested with *Pinus radiata* and the second, along a gentle slope covered with pasture grasses. The pine site was distinctive in having a thick layer of rotting vegetation above the soil (~ 10 cm thick), with the A-horizon having loam to clay texture. The grass site had a similar texture but had a very low load of litter layer. The soil compositions differed to those previously samples and are detailed by Anstis (1999). The Myponga pine site had a mean percentage sand content of 55%, the grass site 47% and the Mt Bold site 75% (a reversal of the trend in sand content at the various locations compared with the October 1998 sites). The March (and July) sampling sites had clay contents of 14 to 18 % except for the Mt Bold top and middle sites, which had clay contents of 5 and 7 %, respectively.

At both locations, three blocks (7mx7m) were randomly selected along slopes, at the top, middle and near to the waters edge. Using an auger, eight samples were collected from each block at the three study sites, Mt Bold (1. native vegetation) and at Myponga (2. pine, *Pinus radiata* and 3. grass areas). The samples were combined to give one sample from each block, resulting in three samples per slope height and therefore 9 samples in total from Mt Bold and 18 from Myponga.

Samples were sieved through 2mm and sub-samples gently mixed with RO water (1:1 vol/vol). These were left to stand overnight at ~ 4^{o}C; were then decanted and centrifuged as previously described, and filtered through a GFC and 0.45 μm filters. Analyses performed on the leachates were DOC, TKN, C/N, UV-vis scan, pH, conductivity, bicarbonate, total dissolved solids, Al, Ca, Fe, Na, Mg and moisture content. Leachates were adjusted to pH 7 and DRIFT analysis performed on the freeze-dried materials. Dried soil samples were analysed for total Al, Ca, Fe, Na, Mg.

2.3.1.3 Sample collection in July 1999**.**
Coinciding with the study of Anstis (1999) soil litter layers and soil samples were collected in July 1999. At Mt Bold, 18 soil samples were collected from 3 blocks (7m x 7 m) at each slope height (top, middle and bottom). Thirty six samples were collected from Myponga (18 from the pine forest site and 18 from the grass site). Two samples (each of four cores) were collected from each block and analysed either separately or combined.

At the time of the sample collection, heavy rains had fallen making the soil saturated, and precluding the sieving of soils (without initially drying the soil). DOM was extracted from the soil samples as previously described. Analyses performed on litter layer and soil extracts included DOC, TKN, C/N, UV-vis scan, pH, conductivity, bicarbonate, TDS, Na and moisture content. Leachates were adjusted to pH 7 and DRIFT analysis performed on the freeze-dried materials.

2.4 Evaluation of DRIFT analysis and interpretation of spectra.

DRIFT analysis was applied extensively for analyses of samples collected from vegetation, litter layers, soils and natural waters. Key reasons for its application were the short time to perform the analysis, the low cost and the potential for determination of the relative presence of functionalities of organics comprising the samples. The potential advantages over other techniques such a Py-GC/MS, thermochemolysis and ^{13}C-NMR include the short time for analysis and the low cost.

A disadvantage of DRIFT analysis is that the absorbance spectral bands are a result of the total of the inorganic and organic compound functionalities and differentiation between the two may be speculative. Where a sample is determined to be predominantly composed of organics, assignment of functionalities can be made with some confidence.

A series of commercially available bio-polymers were analysed by DRIFT in order to compare absorbance bands with the known chemical structures of these organics.
Bio-polymers analysed by DRIFT were as follows:

1. Hydrolytic lignin (Aldrich), (8072-93-3)
2. Cellulose (Aldrich), (9004-34-6)
3. Chitosan (Aldrich), (9012-76-4)
4. Pectin (Aldrich), (9000-69-5)
5. Polygalacturonic acid (Sigma)
6. IHSS Suwanee River Natural Organic Matter, 1N101.
7. IHSS Suwanee River Fulvic Acid, 1R101F.

Their DRIFT spectra are given in figures 2.3 to 2.8, respectively. Assignments of these bands to specific functionalities are based on published literature and data supplied by the Nicolet Instrument Corporation.

Lignin is a heterogenous macromolecule, comprising of a range of aromatic monomers including courmaryl, guaiacyl and syringyl moieties. The relative abundances of these differ between lignin types such as those from grasses, softwoods and hardwoods.

The Aldrich lignin showed large absorbance bands at about 3400 (OH stretching), 2950 (very strong, CH_3 and CH_2), 2850 (alkyl CH stretching), 1700 (C=O), 1620 (carboxylate), 1505 (higher lignin and aromatic skeletal structure), 1495, 1250 (aryl ethers, phenols), and 1050 WN/cm (C-C stretching of aliphatic and aromatic CH groups).

Aldrich cellulose (∃ 1-4 linked D-glucopyranose) spectrum had absorbance bands at about 3350, 2980 (very strong, CH_3), 1620 (carboxylate), 1410, 1300-1380 (CH deformation, C-O stretching of carbohydrates, OH bending) and 1050-1100 WN/cm (carbohydrates).

Aldrich chitosan [poly(D-glucosamine)] spectrum had absorbance bands at 3450, 2920 (CH_2), 2850 (alkyl CH), 1680 (amide), 1600 (C=C), 1400 (alkyl CH), 1390 (amide) and 1100-1150 (carbohydrates,C-O stretching on alkyl ethers and alcohols and polysaccharides).

Aldrich pectin (polymer containing arabinose, galactose and galacturonic acid) spectrum had absorbance bands at 3500, 2950 (CH_2), 1750 (carboxylate), 1600 (C=O stretching of ketones, 1420 (C-O stretching of carbohydrate), 1220 (C-O ethers and esters) and 1100 WN/cm (carbohydrates).

Sigma polygalacturonic acid spectrum: bands at 3450, 2950, 1600 (C=O stretching of ketones), 1400 (CH deformation), 1350 (CH of alcohols and COO^-), 1050-1150 WN/cm (carbohydrates, polysaccharides).
IHSS reference NOM (1N101) had broad, lower resolved bands than the bio-polymers investigated, indicative of the heterogeneity of NOM, with respect to the likely diversity of functionality. Bands were evident at about 3400, 2960, 2920, 1720 (C=O of acids), esters, aldehydes and ketones), 1600 (C=O of ketones and amides), 1200 (backbone vibrations of carbonic acids of phenolic or aliphatic alcohols or of aryl ethers) and 1070 WN/cm (carbohydrates).

The IHSS fulvic acid reference 1R101F was very similar to the NOM sample except that the NOM sample also had a clear band at ~ 1070 WN/cm. This agrees with the understanding of the components of NOM and fulvic acids in that NOM also contains a neutral fraction that includes carbohydrates. Although fulvic acids may have carbohydrates enmeshed into the macromolecule, generally the fulvic acid fraction, as isolated would be expected to have a lower relative amount of carbohydrates present. This is also based on carbohydrates being more assimilable by soil and aquatic micro-organisms in general, than aromatic structures that are thought to be prominent in humic substances.

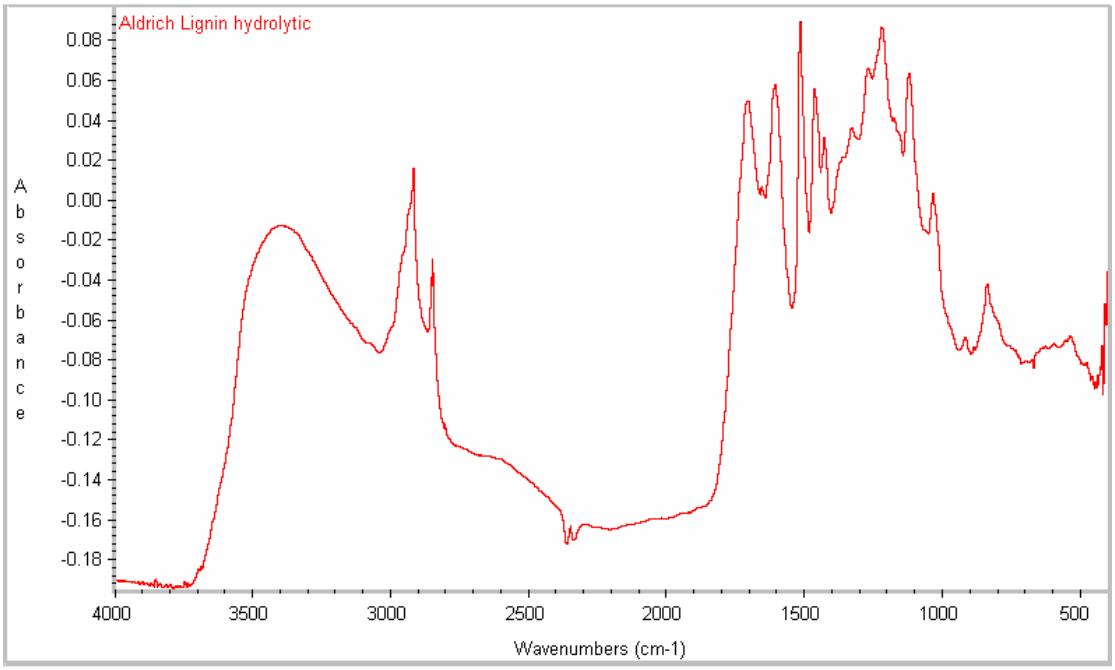


Figure 2.4 DRIFT spectrum of hydrolytic lignin (Aldrich).

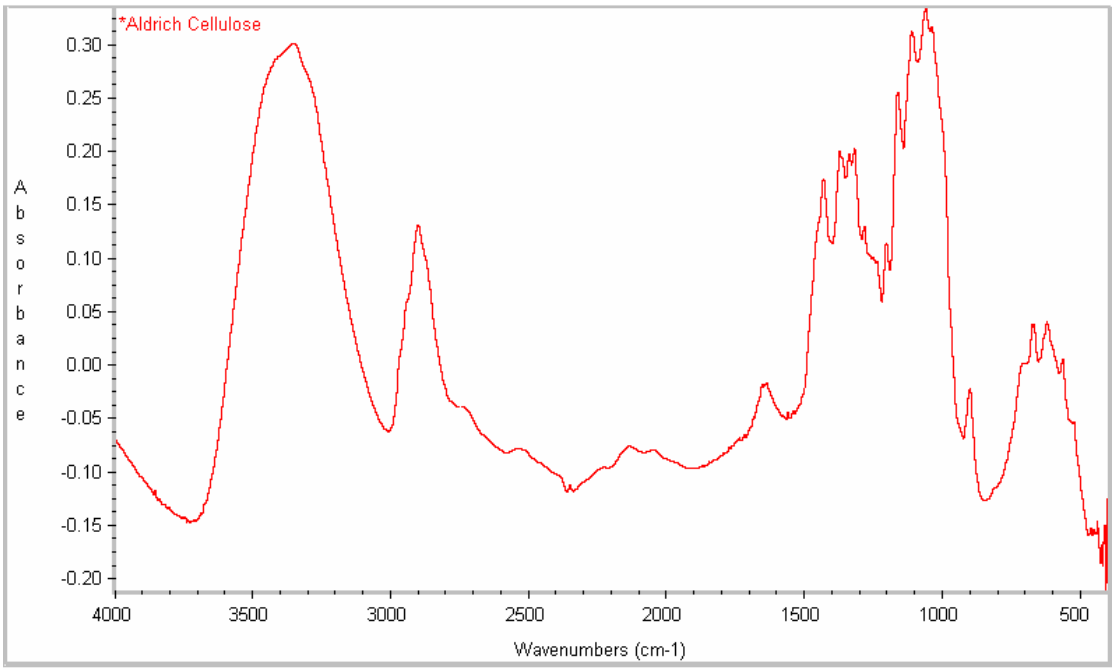


Figure 2.5 DRIFT spectrum of cellulose (Aldrich).

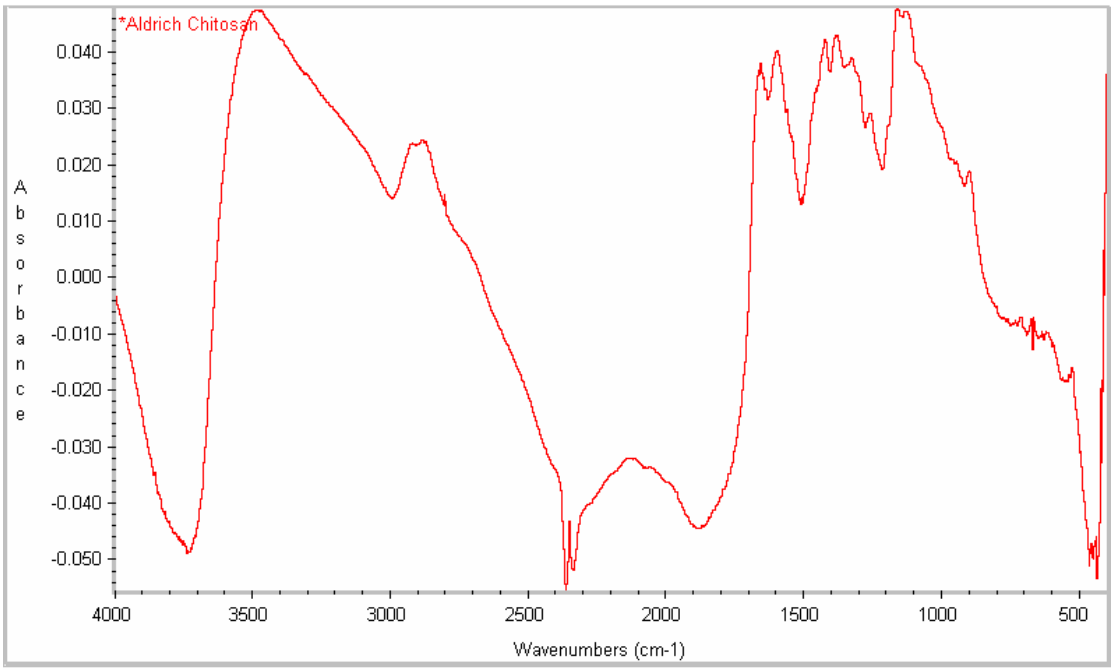


Figure 2.6 DRIFT spectrum of chitosan (Aldrich).

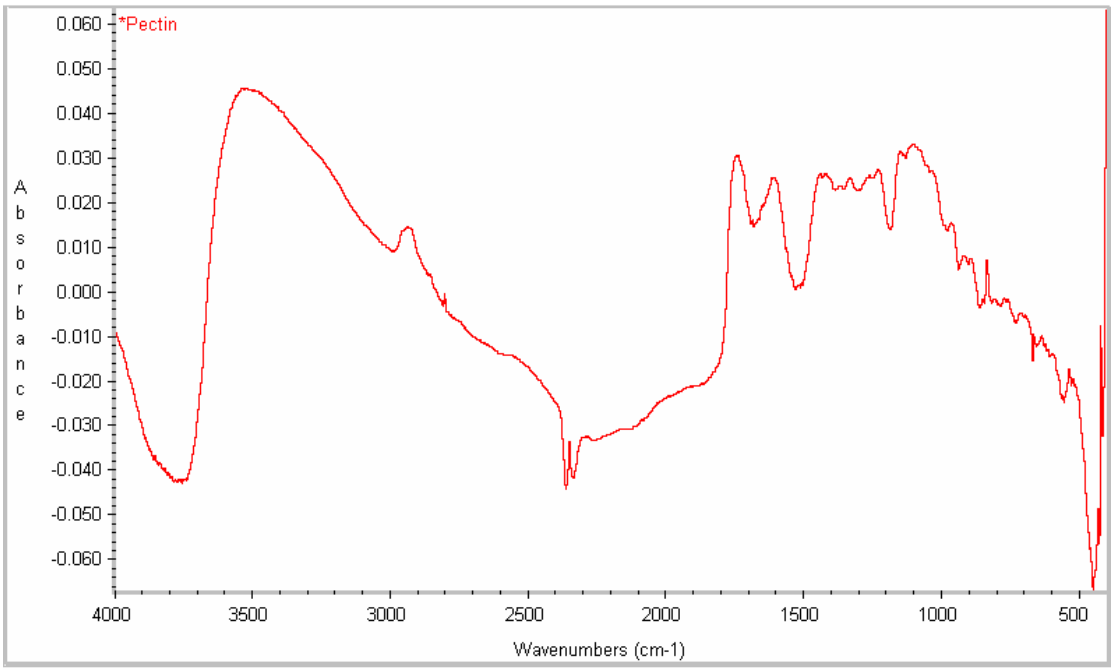


Figure 2.7 DRIFT spectrum of pectin.

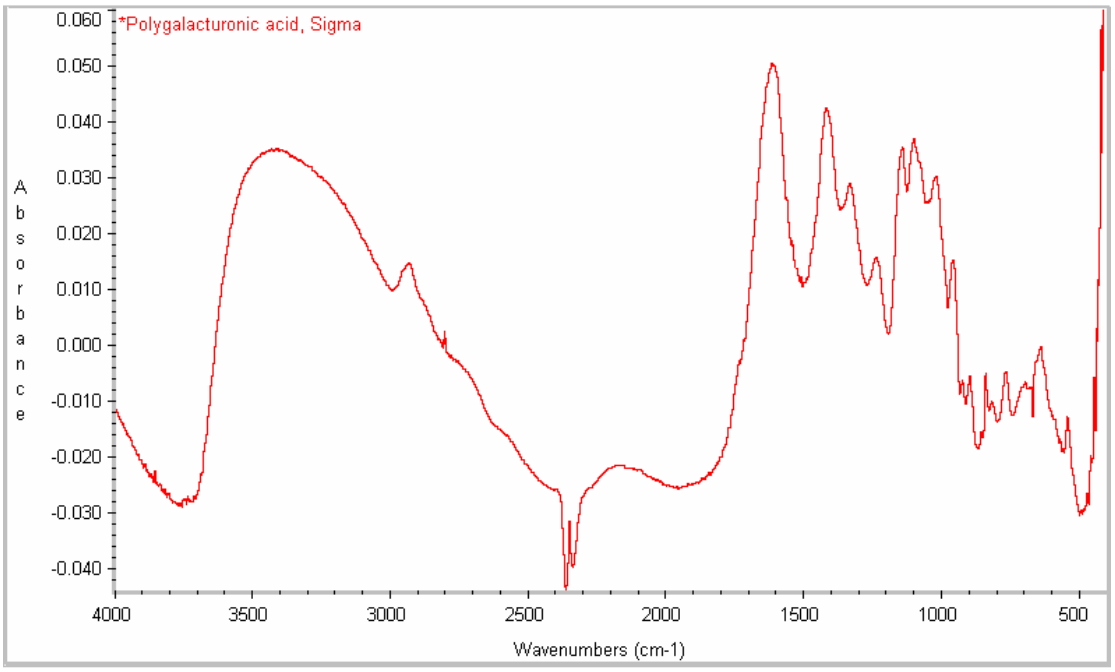


Figure 2.8 DRIFT spectrum of polygalacturonic (Sigma).

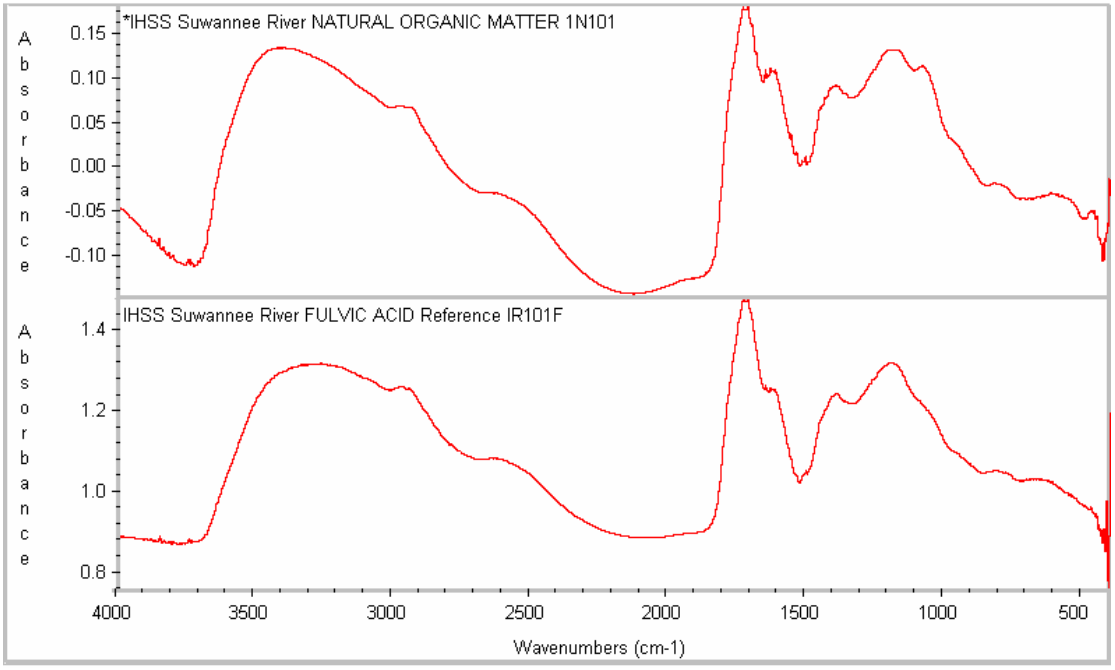


Figure 2. 9 DRIFT spectra of IHSS reference NOM (1N101) and fulvic acid (IR101F).

2.4.1 DRIFT spectra of various standard compounds – Series 1.

The feasibility of DRIFT for detection of specific functional groups was investigated where pure organic compounds were mixed with other organic matrices. Two experiments were performed, one where standard organic compounds were mixed with a sample of humic substances and two, mixed with natural organic matter isolated using magnetic ion exchange resin (MIEX™).

Experiment 1. A series of standard compounds (Table 2.1, Nos.1 to 6) were diluted in high purity water (20 mg to 200 mL, 100 mg/L)

A sample of coal derived humic substances (Coal HS) was diluted in high purity water, (160 mg to 800 mL, 200 mg/L).

Table 2. 1 List of standard compounds analysed by DRIFT spectroscopy.

No.	Compound/matrix	formula
1	Sodium benzoate	C_6H_5COONa
2	Sodium acetate	CH_3COONa
3	Sodium gluconate	$COONa\text{-}(CHOH)_4\text{-}CH_2OH$
4	Acetamide	CH_3CONH_2
5	Acetanilide	$C6H_5\text{-}NH\text{-}COCH_3$
6	Acetaminophenol	$HO\text{-}C_6H_4\text{-}NH\text{-}COCH_3$
9	Loy Yang (Vic.)HA (Coal HS)	

Using the above standards, the following solutions were prepared:

Solution 1-0 100 mL of coal HS

Solutions 1-1 to 1-6 were prepared by mixing 100 mL of coal HS with 20 ml of the various standard solutions.

These solutions 1-0 to 1-6 were divided into two equal volumes, A and B.

Volume A samples were adjusted to pH 7 and filtered through 0.45 μm. Five mL of each of these was diluted to 100 mL in high purity water and analysed for DOC and TKN concentrations. The remaining solution was freeze-dried and analysed using DRIFT.

Volume B samples were filtered through 0.45 μm and 5 mL of each solution diluted to 100 mL with high purity water. These samples were analysed for DOC and TKN. The remaining solutions were adjusted to pH 7, freeze-dried and analysed using DRIFT.

These preparations were designed to determine if there were any differences associated with pH correction to 7 and the results of analyses for DOC and TKN concentrations.

The results of DOC and TKN analyses on these samples are shown in Table 2.2. Both DOC and TKN values were similar for the A and B samples, indicating little or no difference between the sample preparation procedures for these parameters. Although

C/N ratios differed for some pairs, this is likely to be due to the low concentrations of the TKN in these samples and with minor variation of TKN between the A, B pairs.

Table 2. 2 DOC and TKN concentrations of Series 1 samples.

Sample solutions (1:20)	DOC (mg/L)	TKN (mg/L)	C/N ratio
1-0a	4.05	0.07	58
1-0b	3.75	0.10	38
1-1a	3.84	0.06	64
1-1b	3.99	0.10	40
1-2a	3.40	0.07	49
1-2b	3.25	0.08	41
1-3a	3.33	0.05	67
1-3b	3.33	0.05	67
1-4a	3.40	0.25	14
1-4b	3.46	0.27	13
1-5a	3.94	0.17	23
1-5b	3.87	0.15	26
1-6a	3.93	0.16	25
1-6b	3.86	0.13	30

DRIFT spectra of Coal HS (A and B, Figure 2.9) were similar with few distinct absorbance bands. These were at about 3380 (OH stretching), 2950 (CH_3 and CH_2), 1600 (benzene ring breathing, C=C stretching and C-C olefinic bands; carbonyl stretching) and 1400 (COO^-, CH deformation) WN/cm.

Spectra of these humic substances with sodium benzoate added resulted in no obvious spectral change except for a shoulder at ~ 1590 WN/cm and a shoulder at ~1700 WN/cm (carbonyl group stretching) appeared more evident (Figure 2.10).

The addition of sodium acetate resulted in a loss of the shoulder at ~ 1590 WN/cm but the shoulder at ~1700 WN/cm remained. Although these are slight changes in overall features of the spectra, these changes do relate to the structural chemistry of the compounds added to the HS.

Spectra of Coal HS with sodium gluconate added are shown in Figure 2.12. A more distinct peak shoulder occurs at ~ 1080 WN/cm, which can be assigned to carbohydrates. With the addition of acetamide (Figure 2.13), a peak is now evident at ~ 1630WN/cm, which is the absorbance wavelength for primary amines and amides.

The secondary amide of sodium acetaminophenol showed no peak at ~1630 WN/cm, but a very small spike at~1500, which is assigned to aromatic skeletal vibration (Figure 2.14).

The spectrum of acetoaminophenol (Figure 2.15) had shoulder peaks at ~1500 WN/cm and at 1250 WN/cm. These may be assigned to aromatic skeletal vibrations and to phenols, respectively.

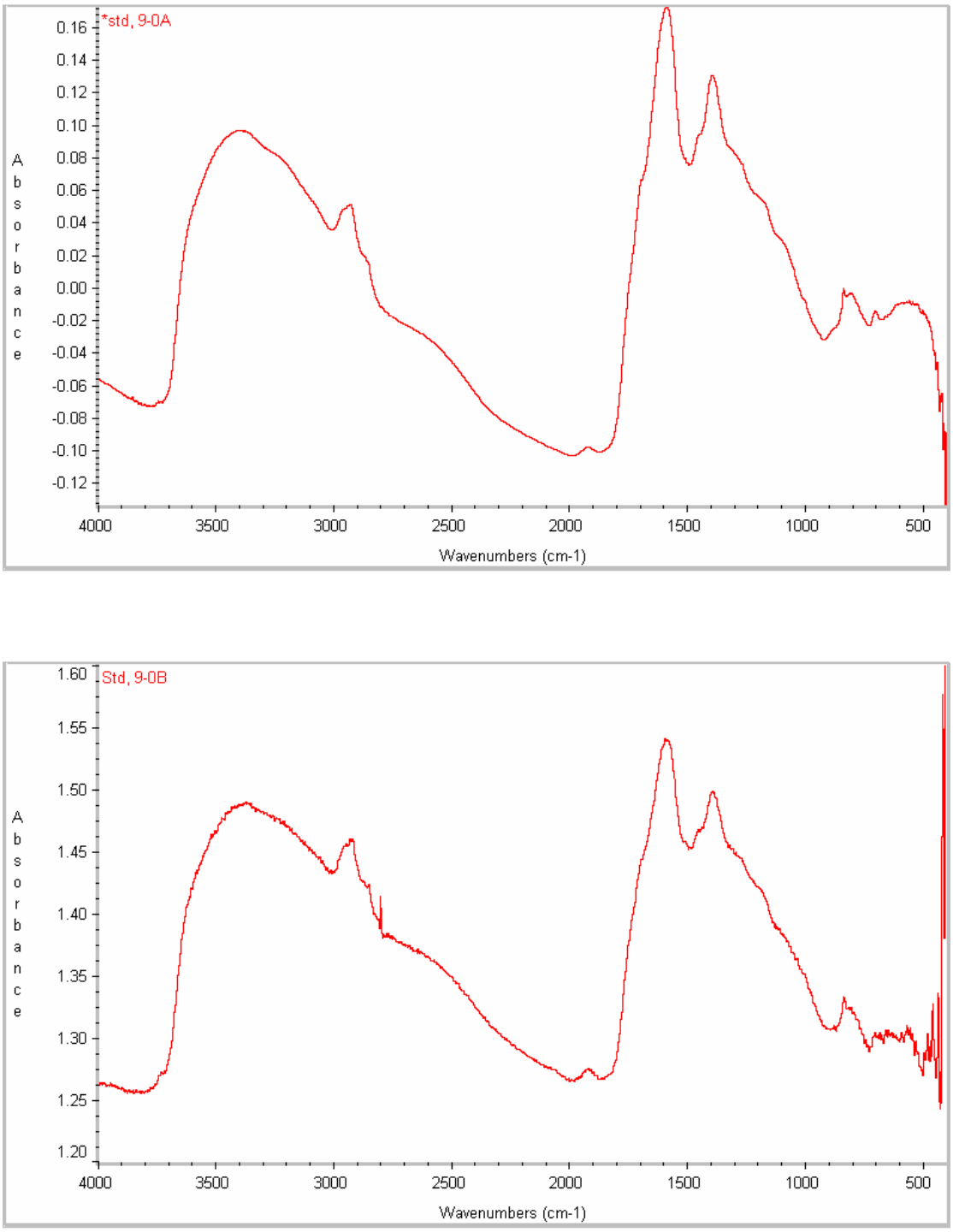


Figure 2.10 DRIFT spectra of Series 1 samples, Coal HS-A (above) and Coal HS-B (below).

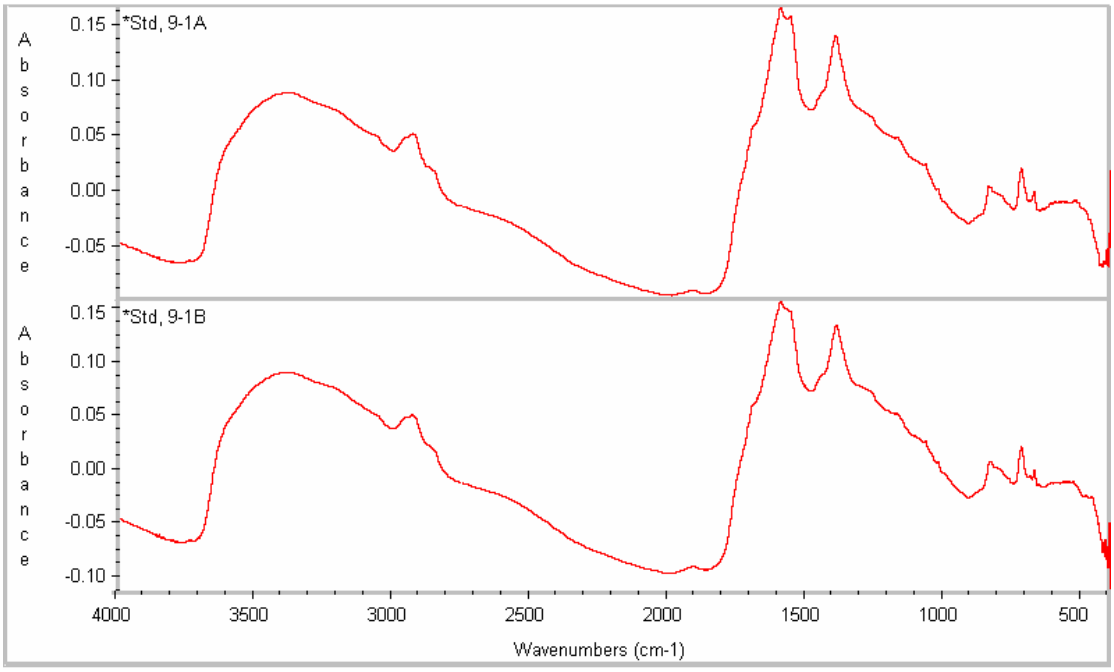


Figure 2.11 DRIFT spectra of Series 1 samples, Coal HS with sodium benzoate, A (above) and B (below).

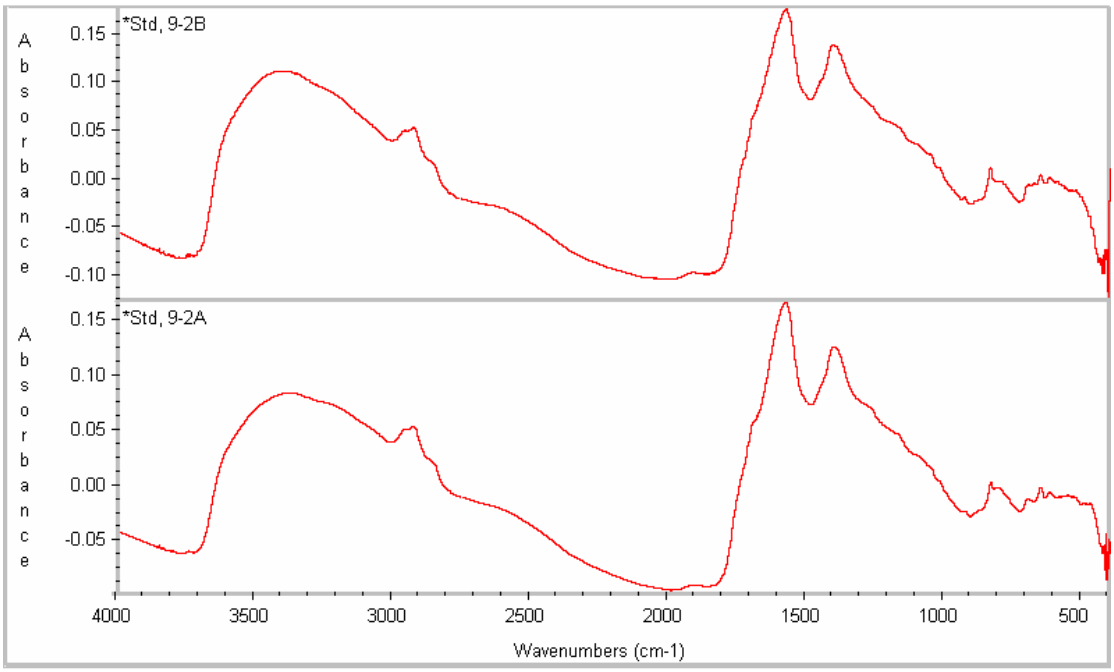


Figure 2.12 DRIFT spectra of Series 1 samples, Coal HS with sodium acetate, A (below) and B (above).

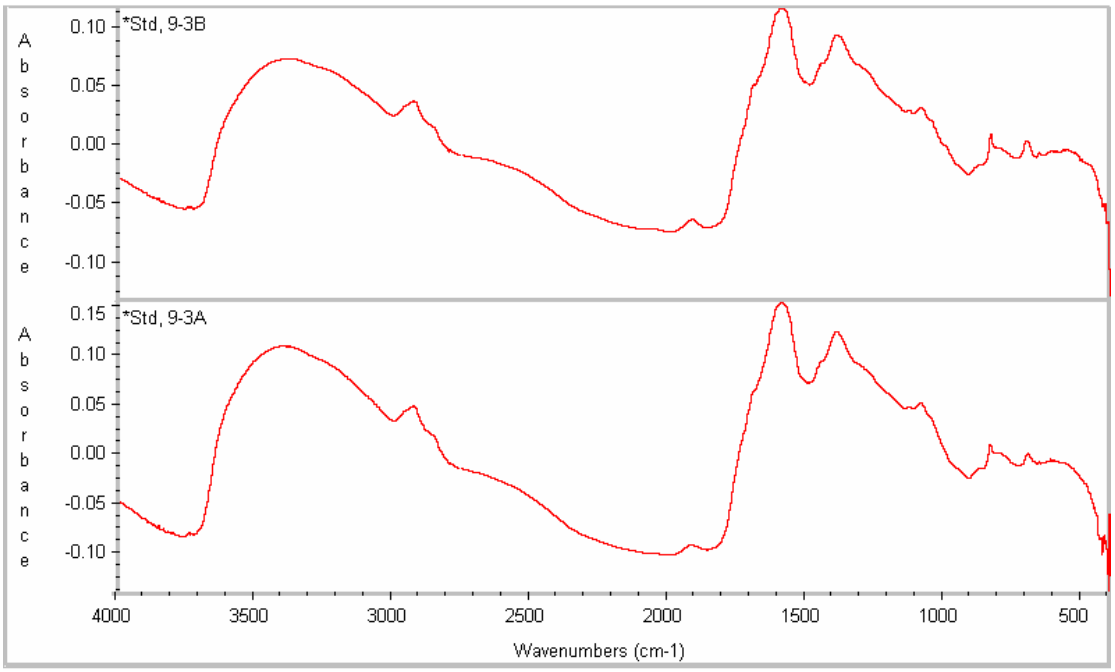


Figure 2.13 DRIFT spectra of Series 1 samples, Coal HS with sodium gluconate, A (below) and B (above).

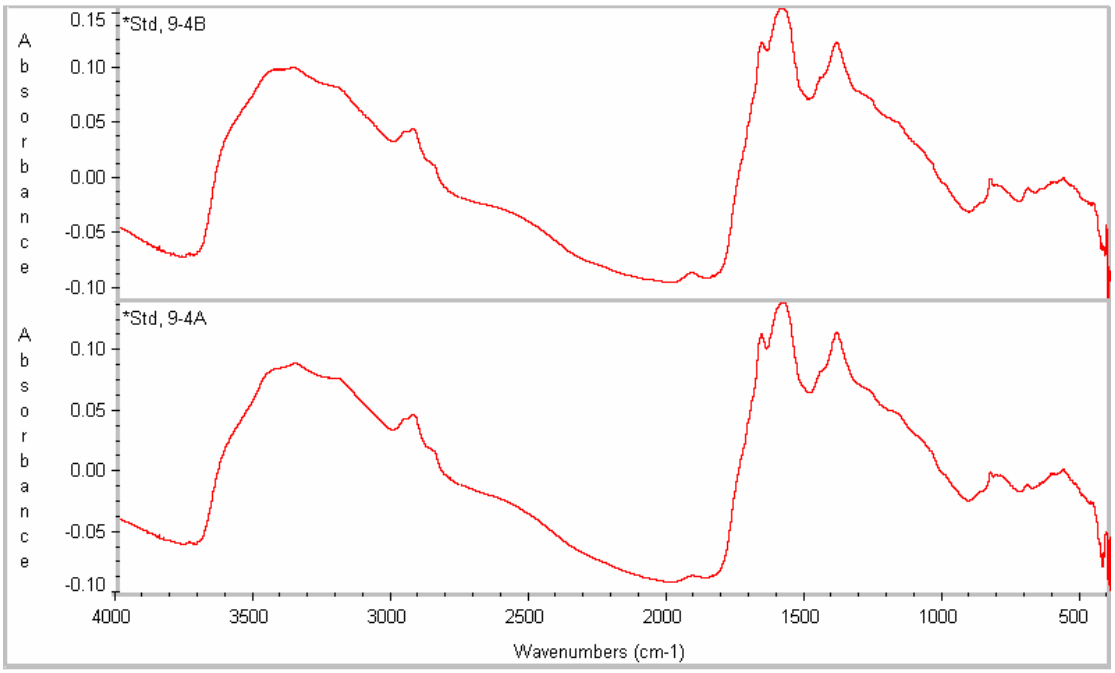


Figure 2.14 DRIFT spectra of Series 1 samples, Coal HS with sodium acetamide, A (below) and B (above).

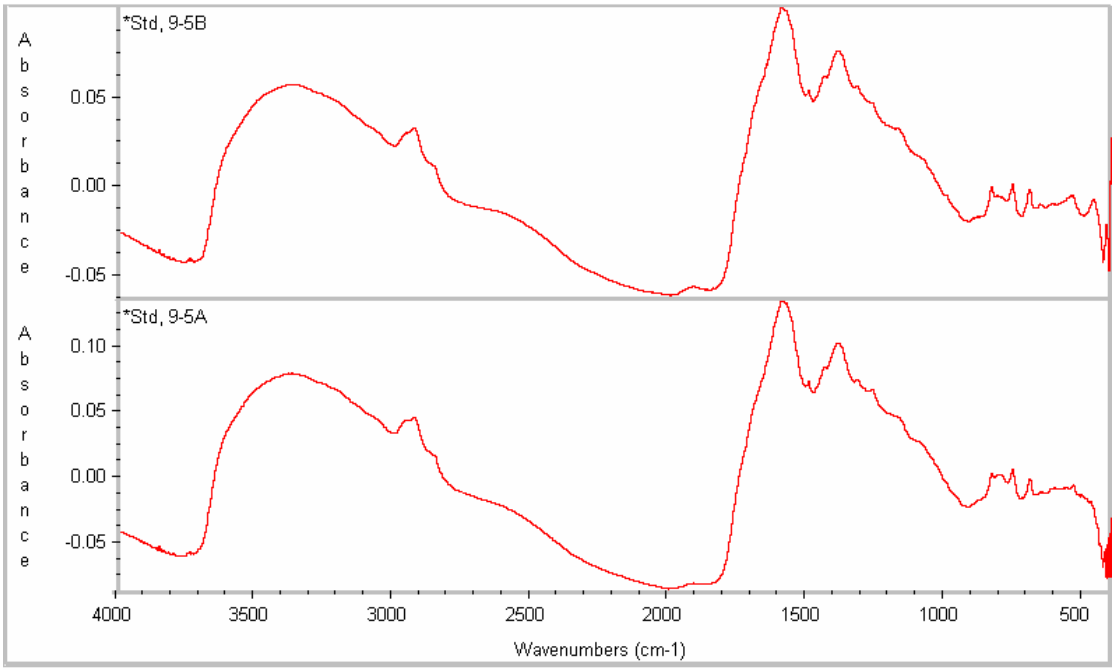


Figure 2.15 DRIFT spectra of Series 1 samples, Coal HS with sodium acetanilide, A (below) and B (above).

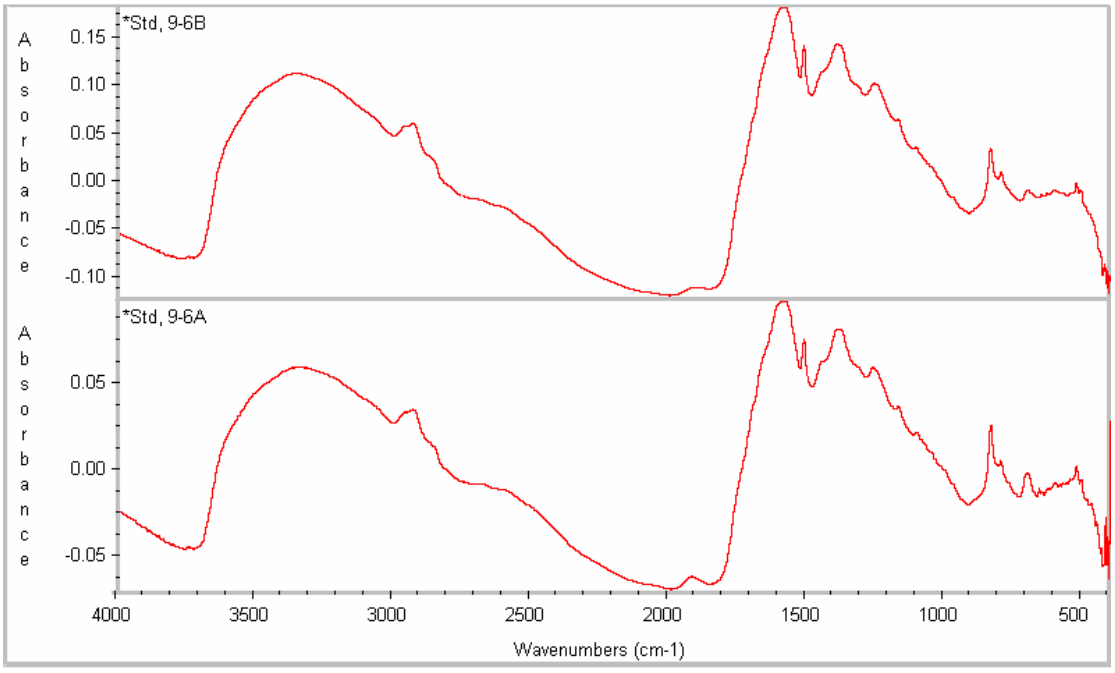


Figure 2.16 DRIFT spectra of Series 1 samples, Coal HS with sodium acetaminophenol, A (below) and B (above).

2.4.2 DRIFT spectra of various standard compounds in a MIEX extract (Hope Valley Reservoir) lignin and cellulose – Series 2**.**

The feasibility of DRIFT for detection of specific functional groups was further investigated where the same organic compounds as series 1 were used, as well as cellulose and lignin. These were mixed with NOM isolated using magnetic ion exchange resin (MIEX™).

Experiment 2 Compounds (Table 2.3, Nos.1 to 6) were diluted in high purity water (20 mg to 200 mL, 100 mg/L).

A MIEX extract from Hope Valley Reservoir (freeze-dried sample) was used to prepare a solution (1L) at 200 mg/L. The MIEX fraction was >500MW, based on ultrafiltration fractionation. A 1:1000 dilution of this fraction in high purity water had a DOC concentration of 7.7 mg/L and TKN of 0.5 mg/L, (C/N 15.4)

Table 2. 3 Standard compounds, bio-polymers and a MIEX extract used in DRIFT evaluation**.**

No.	Compound/matrix	Formula
1	Sodium benzoate	C_6H_5COONa
2	Sodium acetate	CH_3COONa
3	Sodium gluconate	$COONa\text{-}(CHOH)_4\text{-}CH_2OH$
4	Acetamide	CH_3CONH_2
5	Acetanilide	$C_6H_5\text{-}NH\text{-}COCH_3$
6	Acetaminophenol	$HO\text{-}C_6H_4\text{-}NH\text{-}COCH_3$
7	Cellulose	$(C_6H_{10}O_5)_n$
8	Lignin, hydrolytic	
10	MIEX fraction	

From the above standards, bio-polymers and extract, the following solutions were prepared:

2-0 200 mL of MIEX fraction.
2-1 100 mL of MIEX fraction, 100 mL of standard 1.
2-2 100 mL of MIEX fraction, 100 mL of standard 2.
2-3 100 mL of MIEX fraction, 100 mL of standard 3.
2-4 100 mL of MIEX fraction, 100 mL of standard 4.
2-5 100 mL of MIEX fraction, 100 mL of standard 5.
2-6 100 mL of MIEX fraction, 100 mL of standard 6.
2-7* 100 mL of MIEX fraction stir with 10 mg standard 7.
2-8* 100 mL of MIEX fraction stir with 20 mg standard 8.
2-7* 100 mL of MIEX fraction stir with 10 mg standard 7 (adjusted to pH 7, freeze-dried).
2-8* 100 mL of MIEX fraction stir with 10 mg standard 8 (adjusted to pH 7, freeze dried).

*Because cellulose and lignin are practically insoluble in water, the solid bio-polymers were added to standard solutions and sonicated for 10 minutes.

The above solutions were adjusted to pH 7 and filtered through 0.45 μm. Ten millilitres of these samples were diluted 1 in 10 with high purity water and analysed for DOC and UV-vis scans performed. The remaining solutions were freeze-dried and analysed using DRIFT. DOC concentrations are given in Table 2.4 and UV-vis spectral data (and in relation to DOC concentrations) are given in Table 2.5.

Table 2.4 Results of DOC analysis performed on Series 2 samples.

Solutions (1:10)	DOC [mg/L]
2-0	8.3
2-1	7.3
2-2	5.2
2-3	6.0
2-4	6.0
2-5	7.3
2-6	7.5
2-7	7.9
2-8	9.1

Table 2. 5: UV-vis spectral data and ratios to DOC concentrations for Series 2 samples.

	Series 2 standard samples								
Parameter	0	1	2	3	4	5	6	7	8
SUVA	4.5	2.9	3.6	3.1	3.1	3.9	5.8	4.6	4.6
(254nm x 456nm x 1000)/DOC	1.02	0.34	0.44	0.40	0.36	0.44	0.61	1.05	1.17
254nm/456nm	16.2	18.3	15.3	14.6	16.0	24.9	40.9	15.9	16.4
E4/E6	8.5	7.9	7.6	5.3	10.7	7.3	11.0	10.1	5.9

DRIFT spectra of the various samples are given in figures 2.16 to 2.19.
Similar absorbance bands were found in spectra of series 2 samples as those of series 1. Sodium benzoate addition resulted in a extra small resolved peak ~ 1500, which can be attributed to aromatics. Sodium acetate did not appear to alter the spectrum of NOM alone. Additions of sodium gluconate, acetamide and acetanilide (Figure 2.17) lead to extra peaks/absorbance bands being detected at about 1020-1100, 1650 and 1500 WN/cm, respectively. These can be assigned to carbohydrates, amides and aromatics, which concurs with the standards added. Addition of acetaminophenol to MIEX (Figure 2.18) resulted in small shoulder peaks at ~1650, 1500, 1400, 1350 and a more distinct peak at ~1200 WN/cm. These can be assigned to amide, aromatic, CH deformation, sec. amide and ester functionalities. All except the last correlating with the addition of the standard. The addition of cellulose did not seem to impact greatly on the spectrum of the NOM alone (Figure 2.18). Addition of lignin (Figure 2.19) resulted in very small peaks at ~1500 (aromatics) and 1250 WN/cm (phenols).

Generally, despite the high relative addition of standards to the MIEX extracted NOM (1:2 W/W) changes in spectral absorbance bands were mostly small or very small.

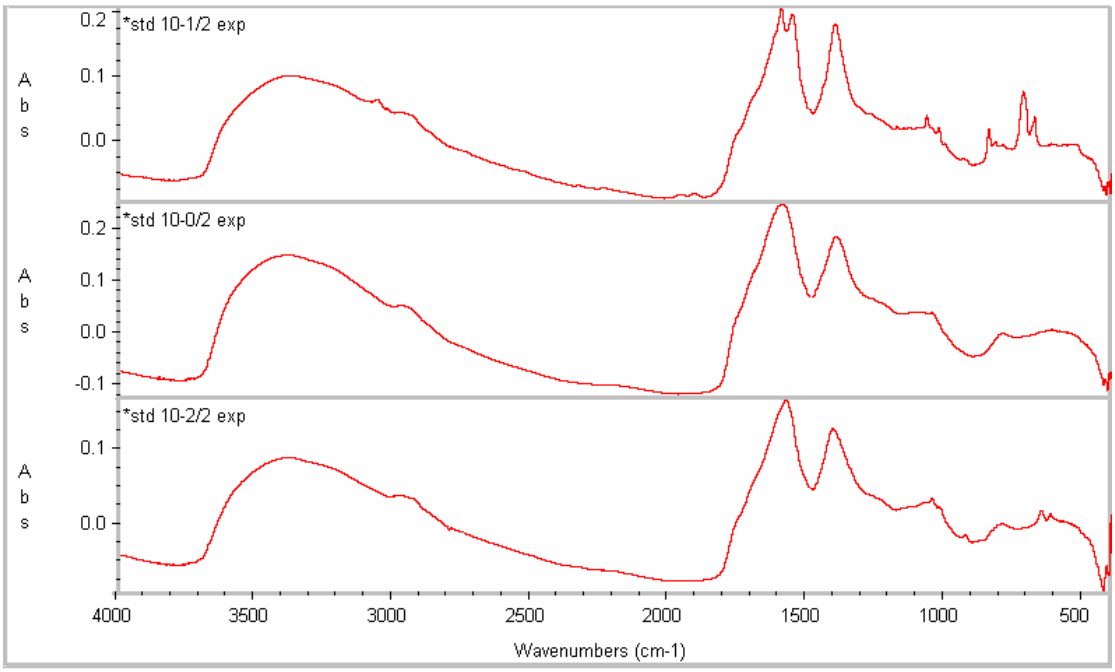


Figure 2. 17 DRIFT spectra of Series 2 samples, numbers 0 to 2.

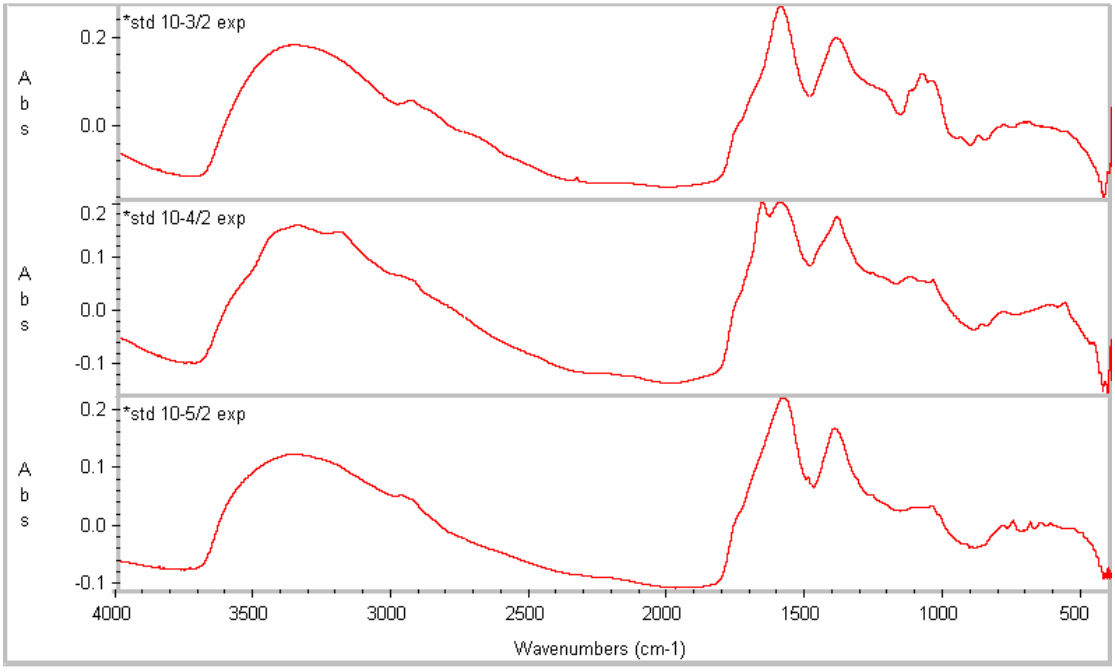


Figure 2.18 DRIFT spectra of Series 2 samples, numbers 3 to 5.

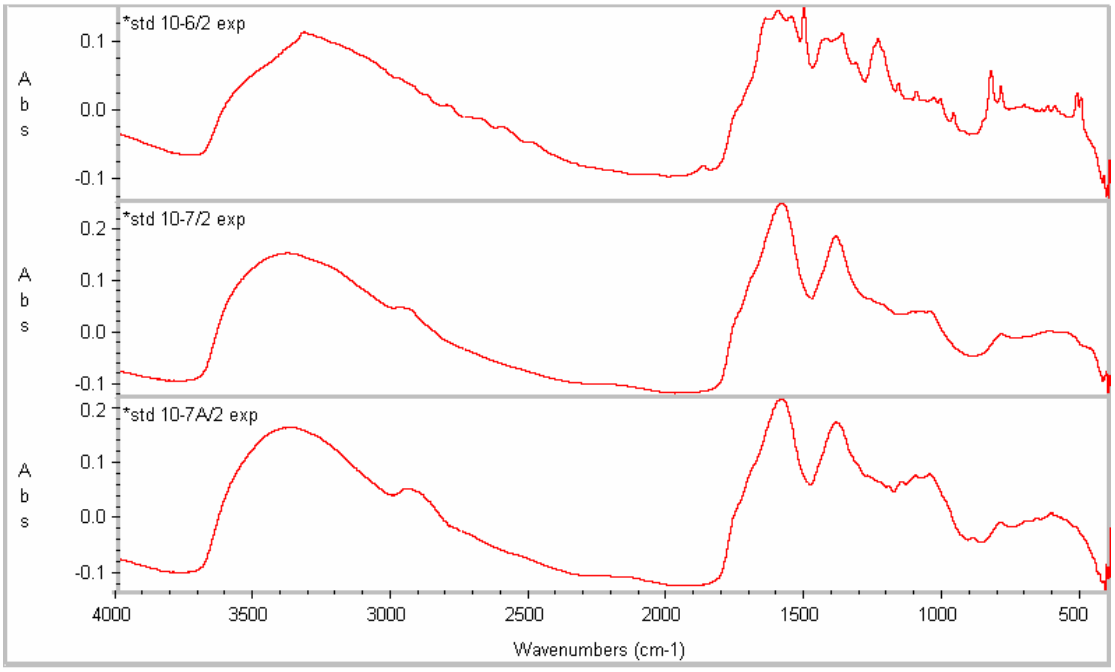


Figure 2. 19 DRIFT spectra of Series 2 samples, numbers 6, 7 and 7A.

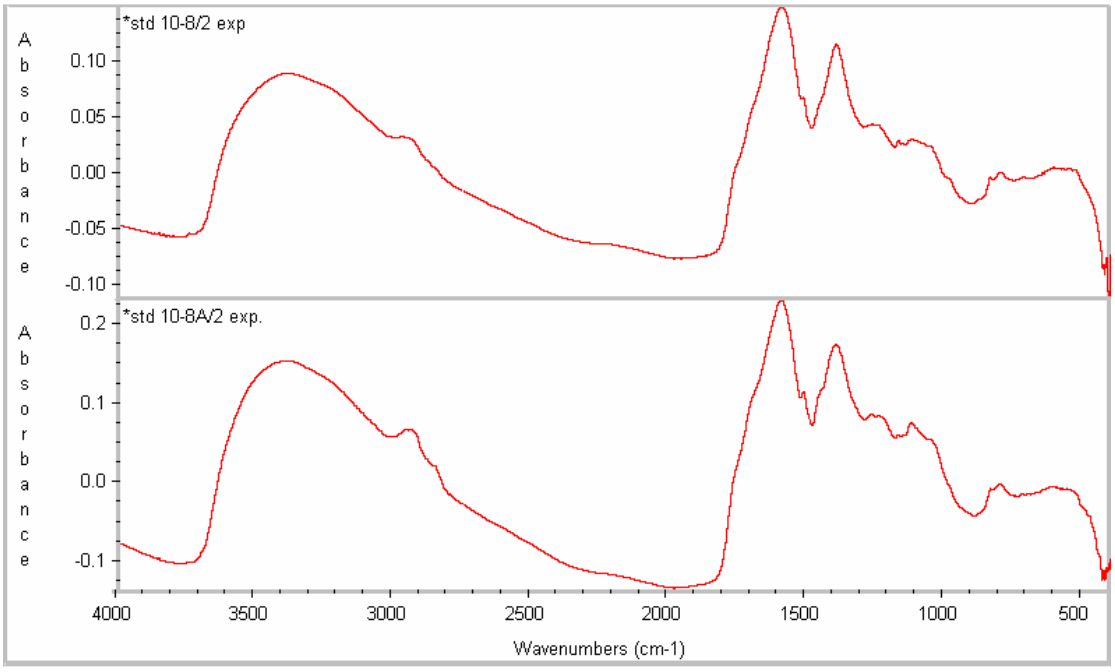


Figure 2.20 DRIFT spectra of Series 2 samples, numbers 8 to 8A.

A DRIFT spectrum of para-nitrophenol is shown in Figure 2.20. Absorbance peaks between 1000 and 2000 WM/cm were narrow and sharp, centred at about 1650 (C=C), 1550 (-NO_2), 1450 (C=C), 1300, 1170 (phenol),1100 WN/cm (C-O alcohol bond). Possible functional group assignments are those in brackets.

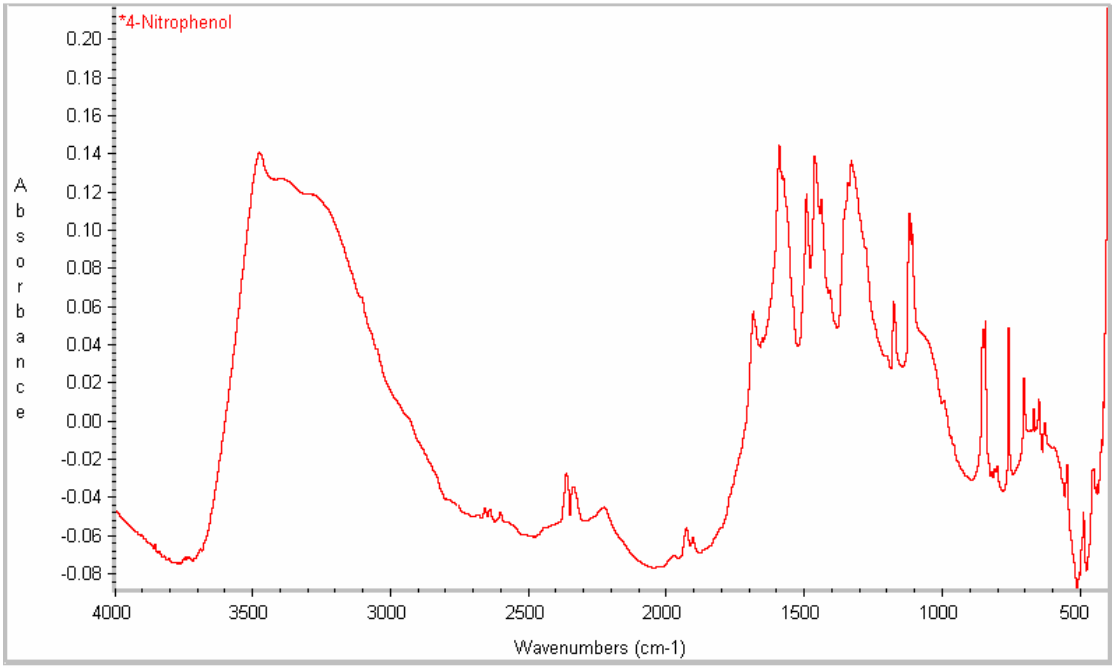


Figure 2.21 DRIFT spectrum if 4-nitrophenol.

DRIFT spectra of aluminium-based coagulants are given in Figures 2.21 to 2.23. Aluminium sulphate, as $Al_2(SO_4)_3.18H_2O$, (alum) showed a strong peak at ~1150 WN/cm which can be attributed to sulphate ion. This is the spectral region where carbohydrates also absorb, causing confounding of analysis for these organics in water treated with this coagulant. The spectrum of aluminium chloride had a range of prominent absorbance bands which would also make the use of this coagulant of limited value, in relation to the characterisation of organics in drinking water.

Spectra of alum with sodium bicarbonate, sodium bicarbonate, calcium carbonate, potassium bicarbonate, sodium bicarbonate and sodium chloride are shown in figures 2.24 to 2.28. The potential of inorganic compounds contributing to absorbance bands in the DRIFT spectra is evident from these, though there is marked variability in the level of absorbances between these compounds.

The impacts of pH (3, 7 and 10) on DRIFT spectra are shown in figures 2.29 to 2.31 (soil litter and soil extracts) and figures 2.34 to 2.36 (raw water samples). Major impacts are evident from the relative heights of peaks in these spectra, showing the need for consistency in the pH of samples prior to freeze-drying for DRIFT analysis. For all DRIFT analysis of field samples a pH of 7 was selected.

Spectra of a field collected, reference set of organic samples are shown in figures 2.32, 2.33 (extracts from the O-horizon from Mt Bold), 2.37 (South Australian reservoirs), 2.38 (pond sediment) and 2.39 (vegetation from the Moorabool Reservoir,

Victoria). These samples are described in Report 1, Chapters 2. Most of these spectra had few well resolved peaks, at about 1600, 1400 and 1100-1000 WN/cm, varying in relative absorbance peak heights. None of these samples were desalted, and except for the vegetation leachates, it is likely that inorganic compounds would have impacted on the spectra. The sharpness of peaks at ~ 3400 and at 1600 WN/cm for raw reservoir water samples and pond sediment samples indicates impacts from inorganics (organic heterogeneous organics exhibit broad, rounded peaks). Peaks with these features are evident from the spectra of extracts from the vegetation collected from Moorabool, Victoria (Figure 2.39). UV-vis data of samples from the Mt Bold catchment and from reservoirs in South Australia are given in Table 2.6. Ratios of 254 nm:456 nm were highest in the pond sediment and in the reservoirs. This may be interpreted as reservoir DOM having a higher abundance of conjugated double bonds, (eg of aromatics) in relation to colour imparting organics, than in the catchment soils, at that time (1997). This might be explained by the presence of higher levels of free proteins (consisting of UV absorbing benzene or other unsaturated groups, such phenylalanine, tryptophan, tyrosine and histidine) and/or small molecular weight aromatics being present in reservoir water. Proteins impart little, if any colour and hence would increase the ratio of UV absorbance to coloured organics ie. increase the ratio value in the reservoir waters.

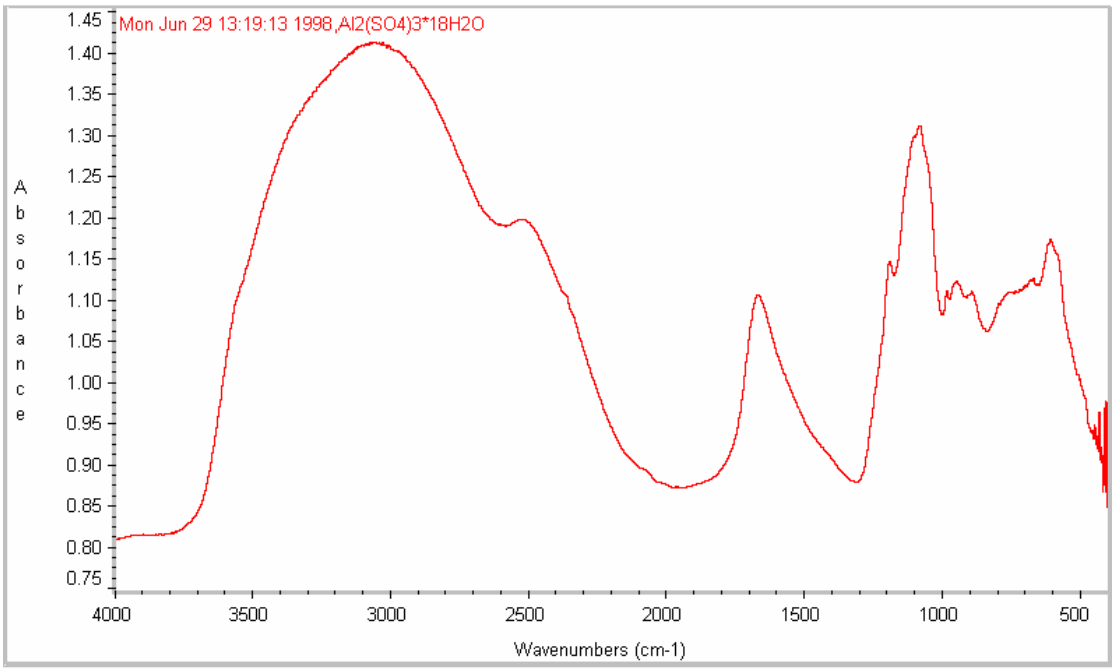


Figure 2.22 DRIFT spectrum of aluminium sulphate.

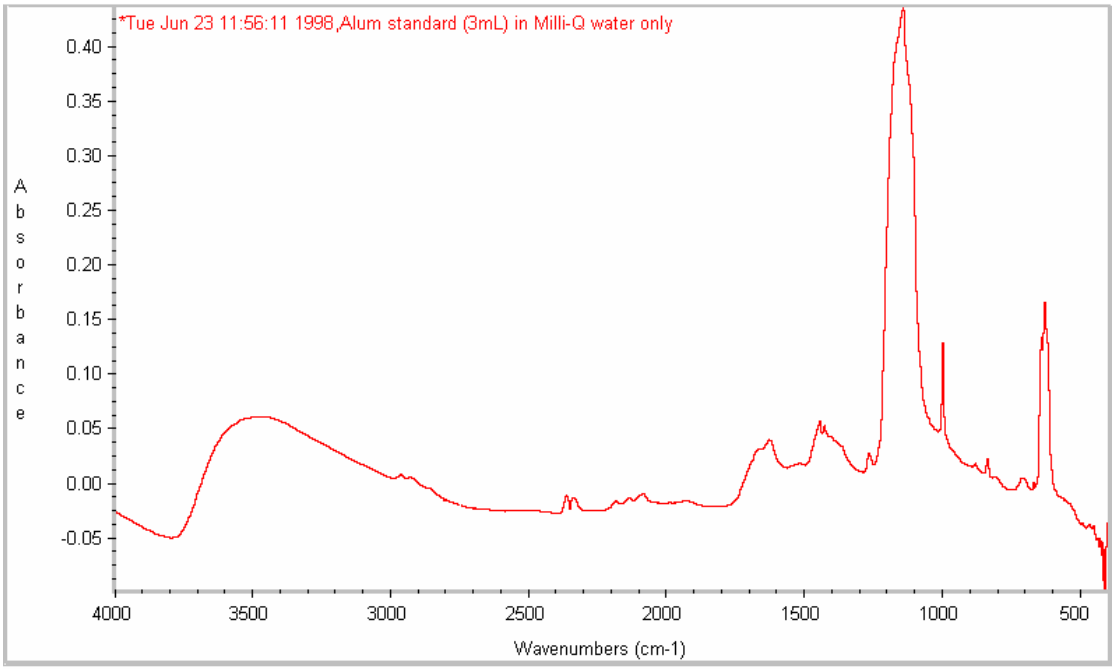


Figure 2. 23 DRIFT spectrum of a solution alum sulphate diluted in high purity Milli-Q Plus water.

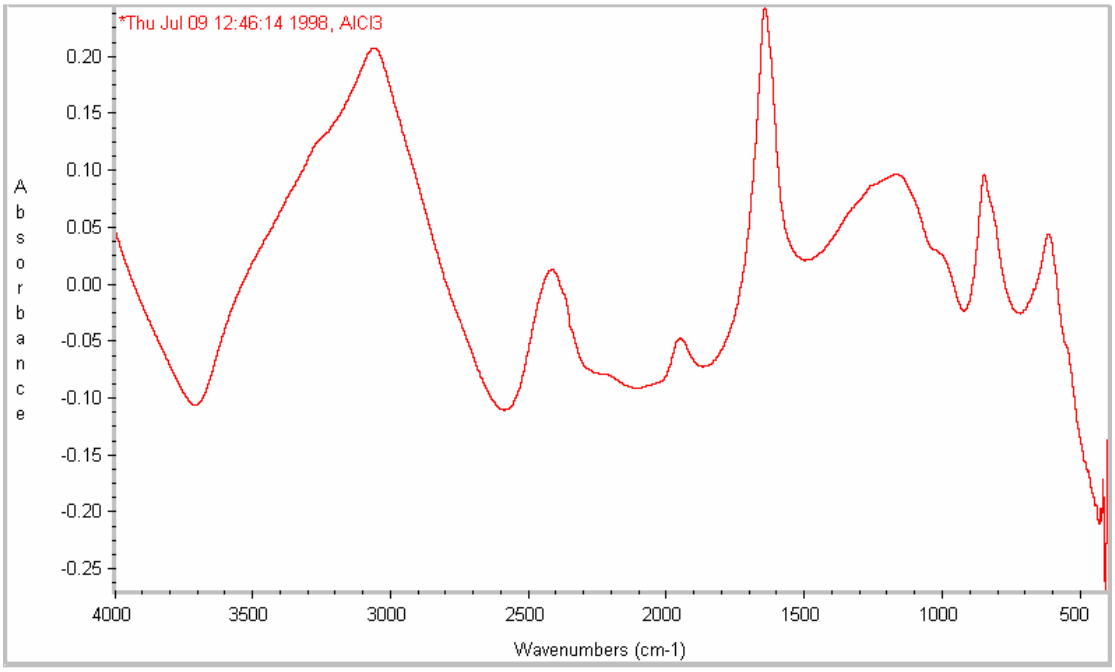


Figure 2.24 DRIFT spectrum of aluminium chloride.

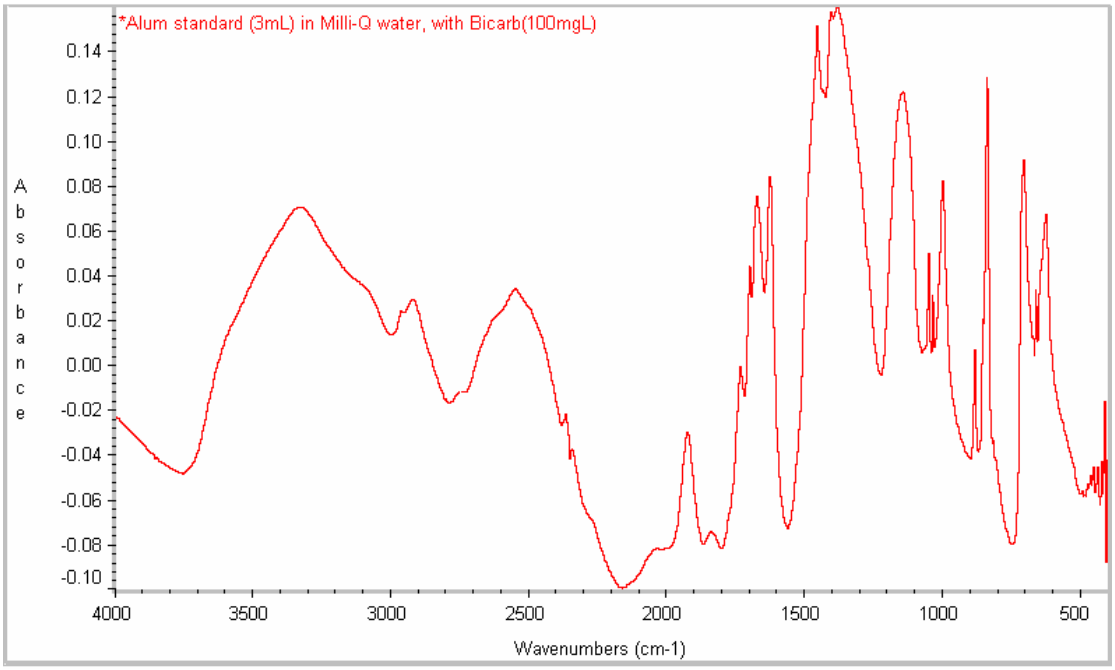


Figure 2.25 DRIFT spectrum of a aluminium sulphate (3mL, 20,000 ppm) in Milli-Q Plus water and sodium bicarbonate (100 mg).

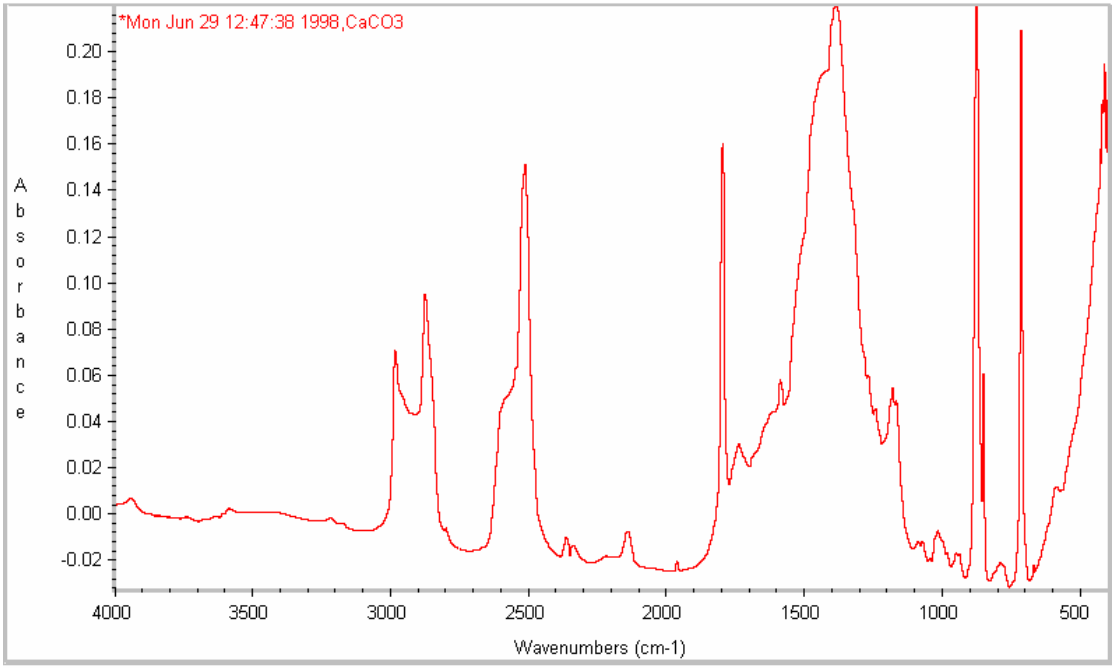


Figure 2.26 DRIFT spectrum of calcium carbonate.

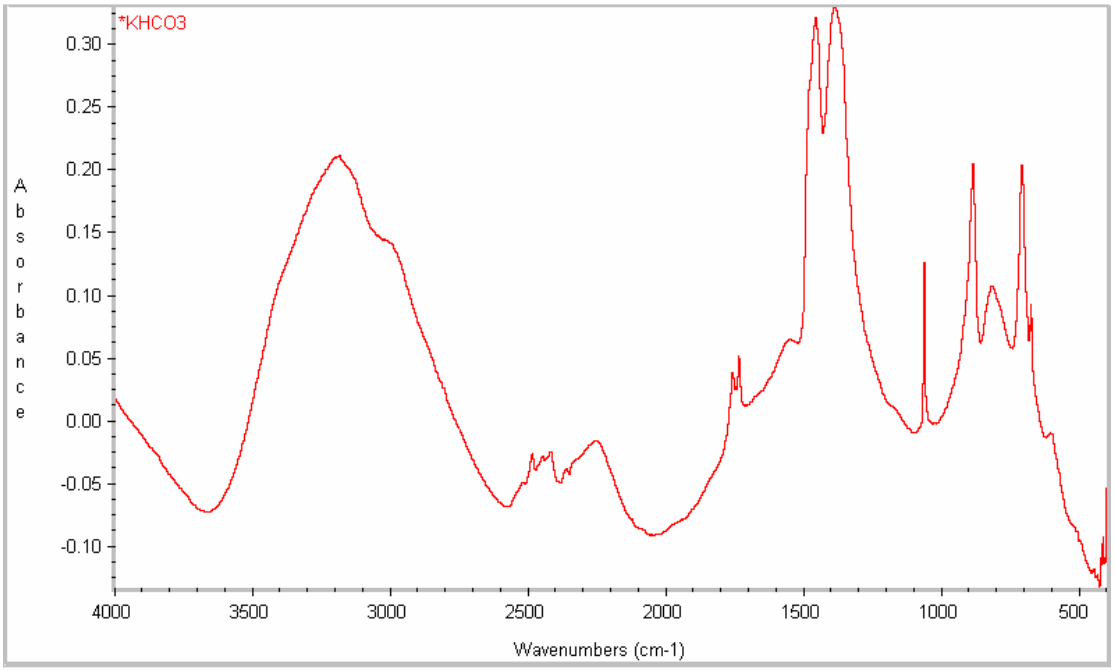


Figure 2.27 DRIFT spectrum of potassium bicarbonate.

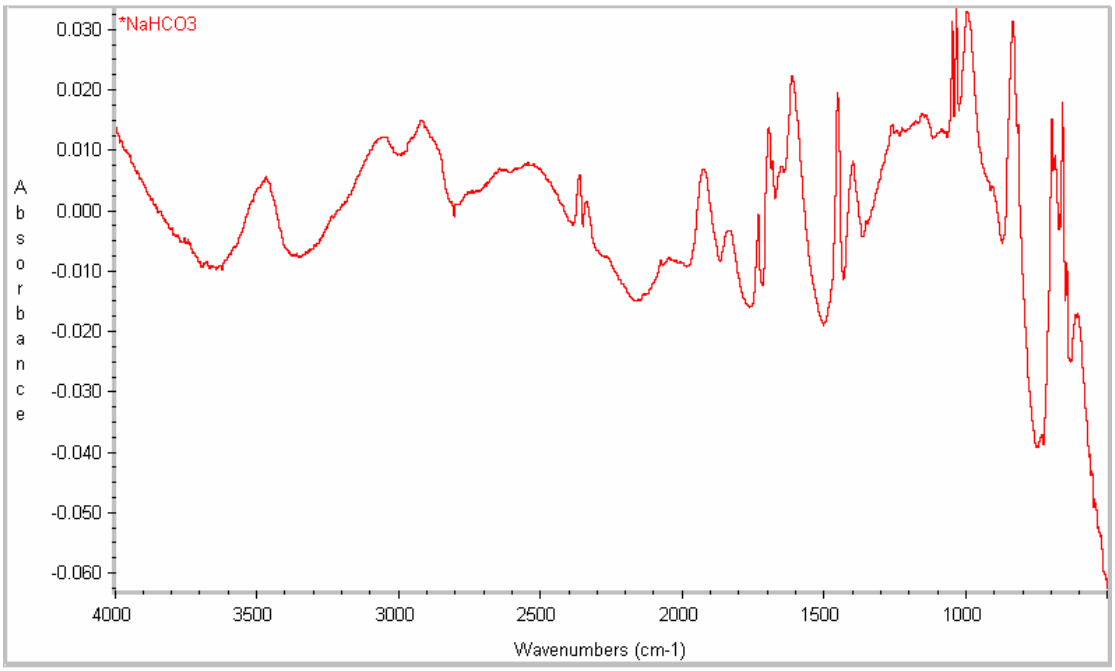


Figure 2.28 DRIFT spectrum of sodium bicarbonate.

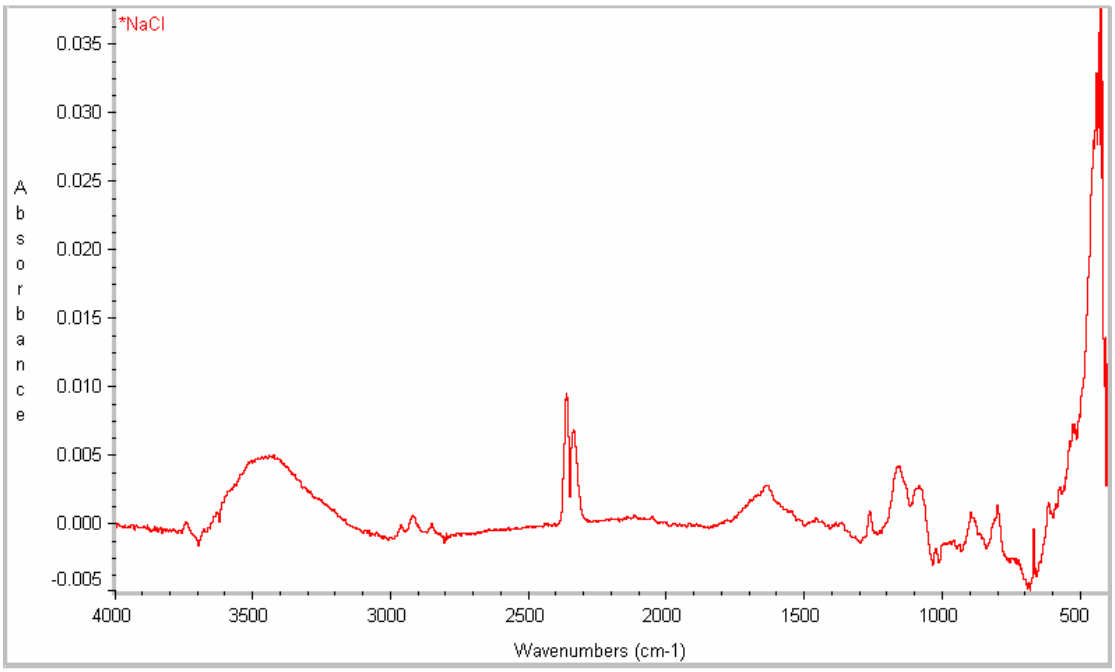


Figure 2.29 DRIFT spectrum of sodium chloride.

DRIFT spectra of reference samples:

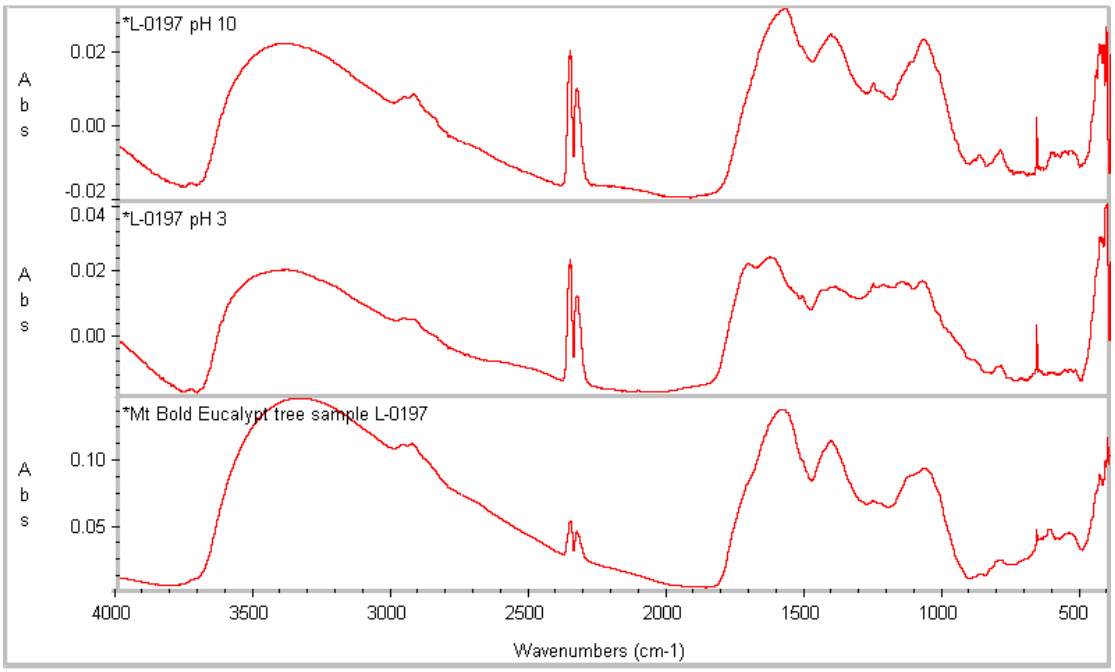


Figure 2.30 DRIFT spectra of a leaf litter extract, under eucalypt tree, Mt Bold, at pH 3, 7 and 10.

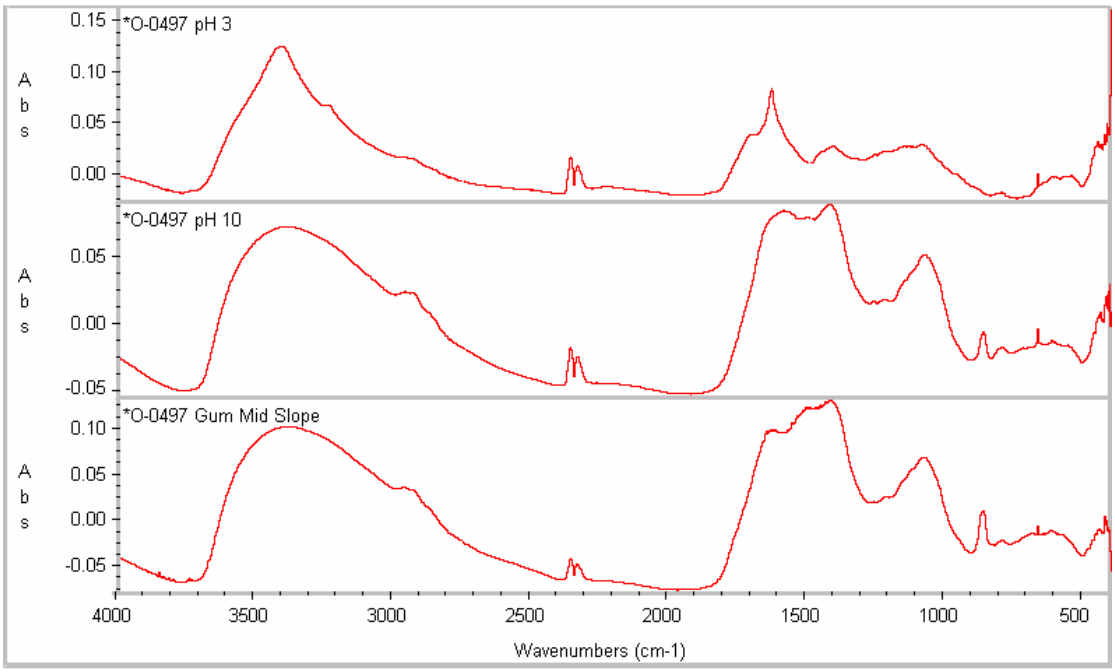


Figure 2.31 DRIFT spectra of a soil extract (#0497) from under eucalypt tree, Mt Bold, at pH 3, 7 and 10.

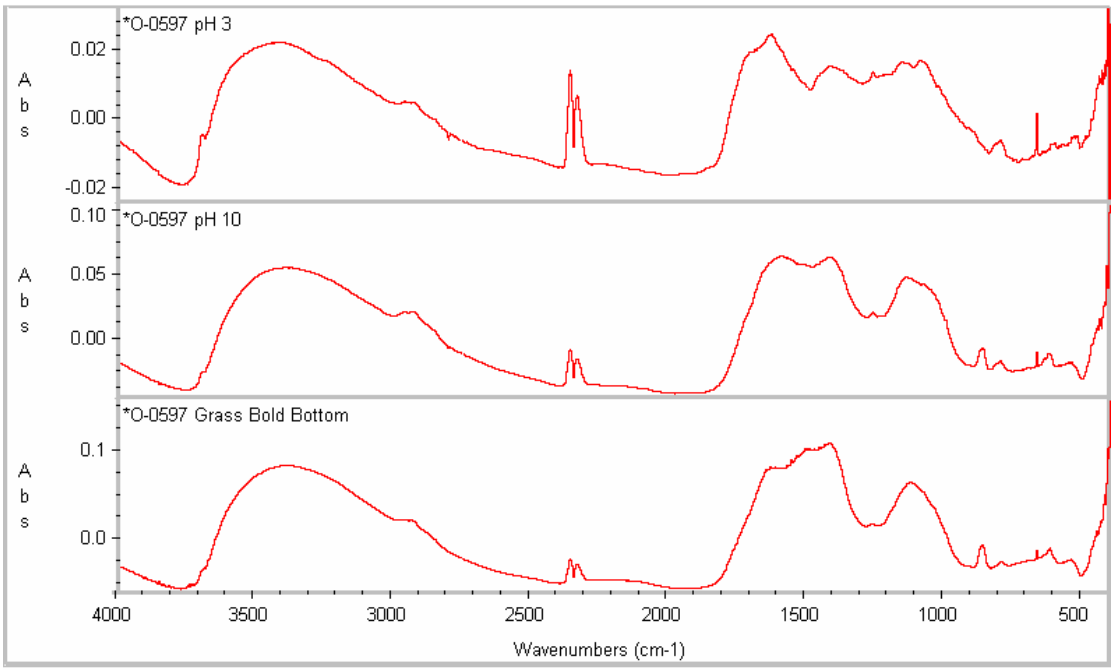


Figure 2.32 DRIFT spectra of a soil extract (#0597) from Mt Bold, bottom of slope, grass site, at pH 3, 7 and 10.

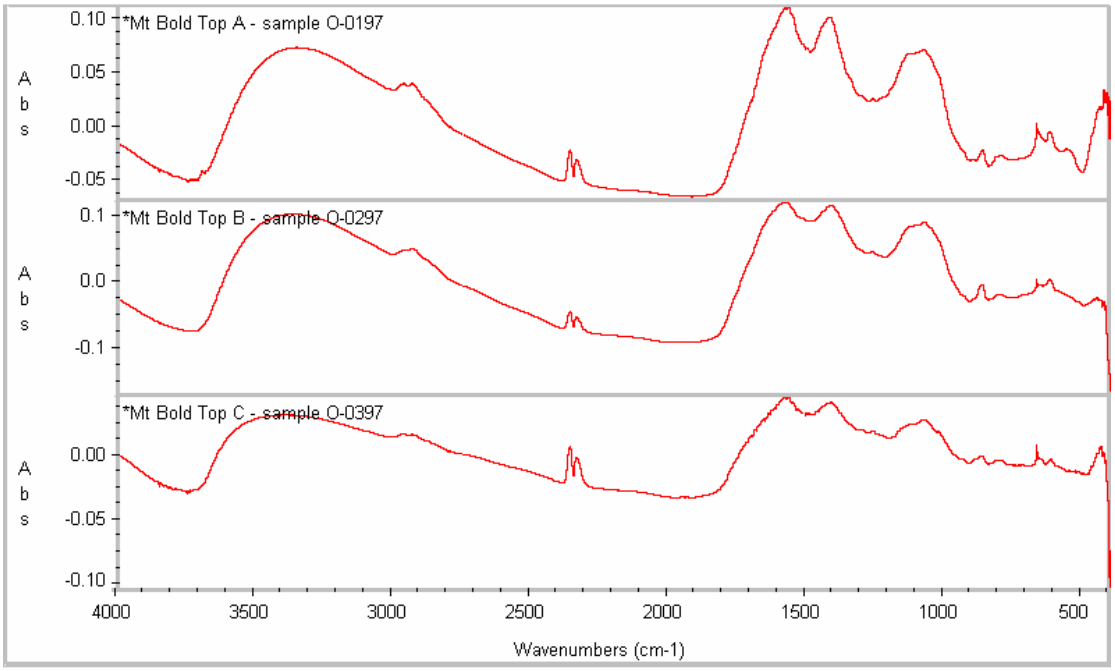


Figure 2.33 DRIFT spectra of extracts from soil O-horizons from Mt Bold, top of a slope, at pH 7.

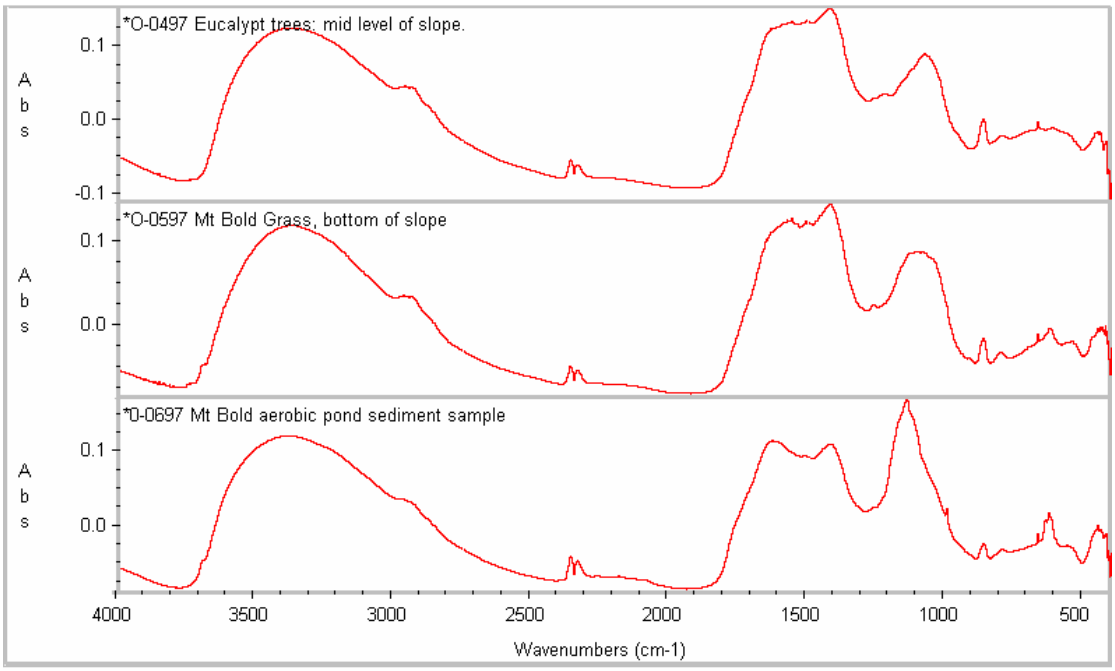


Figure 2.34 DRIFT spectra of samples collected from Mt Bold (Scott Creek).

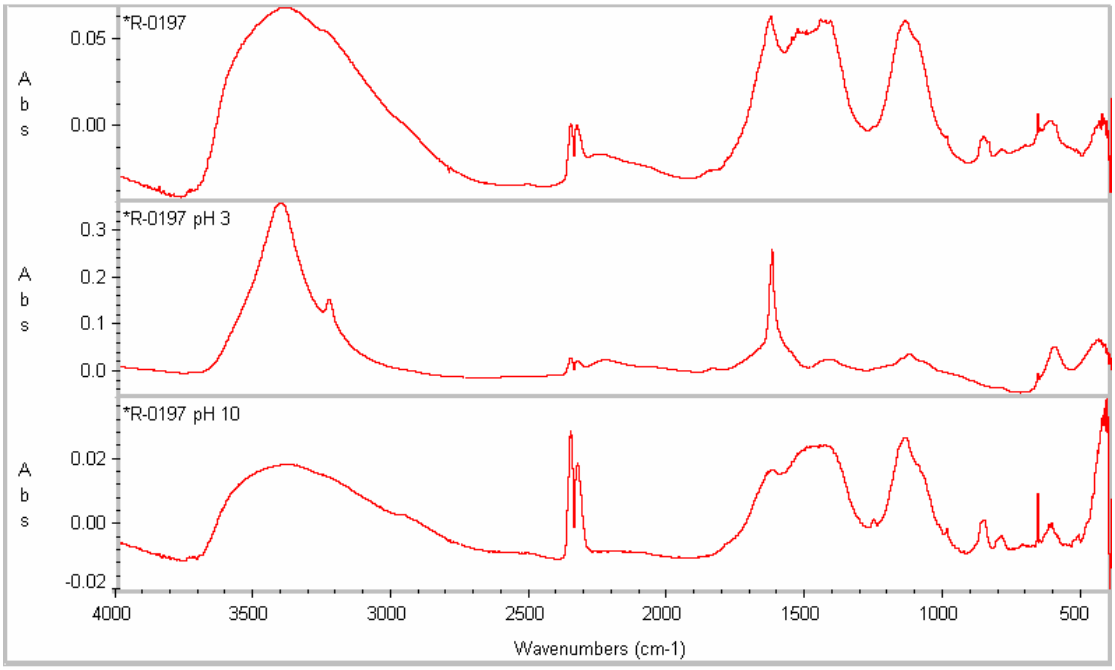


Figure 2.35 DRIFT spectra of a raw water sample from Myponga Reservoir at pH 3, 7 and 10.

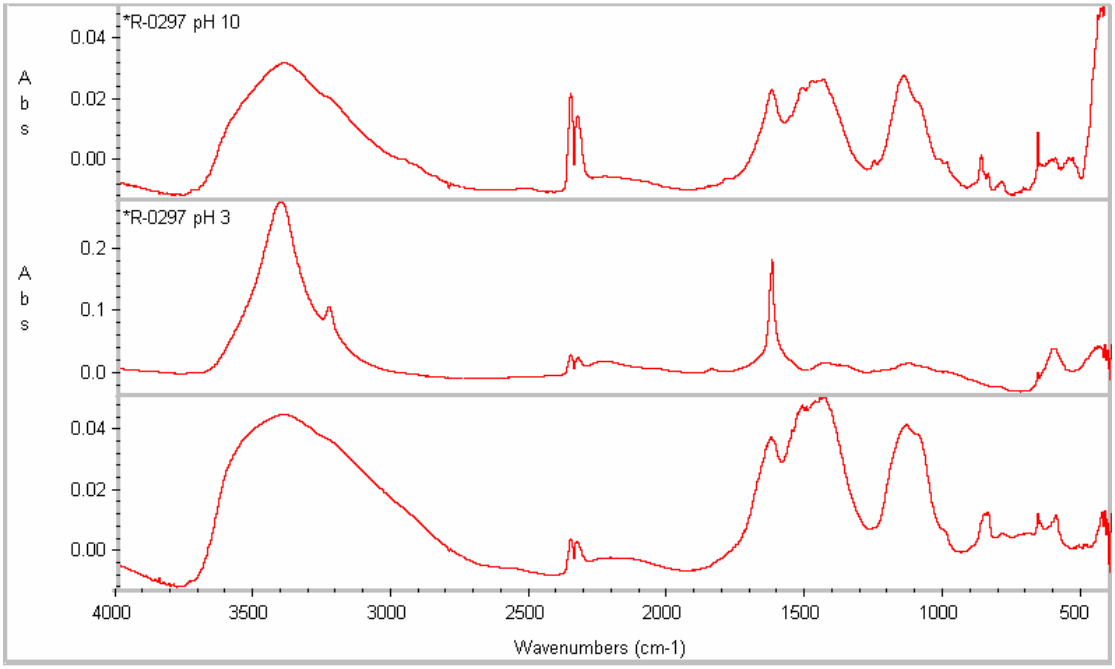


Figure 2. 36 DRIFT spectra of a raw water sample from Hope Valley Reservoir at pH 3, 7 and 10.

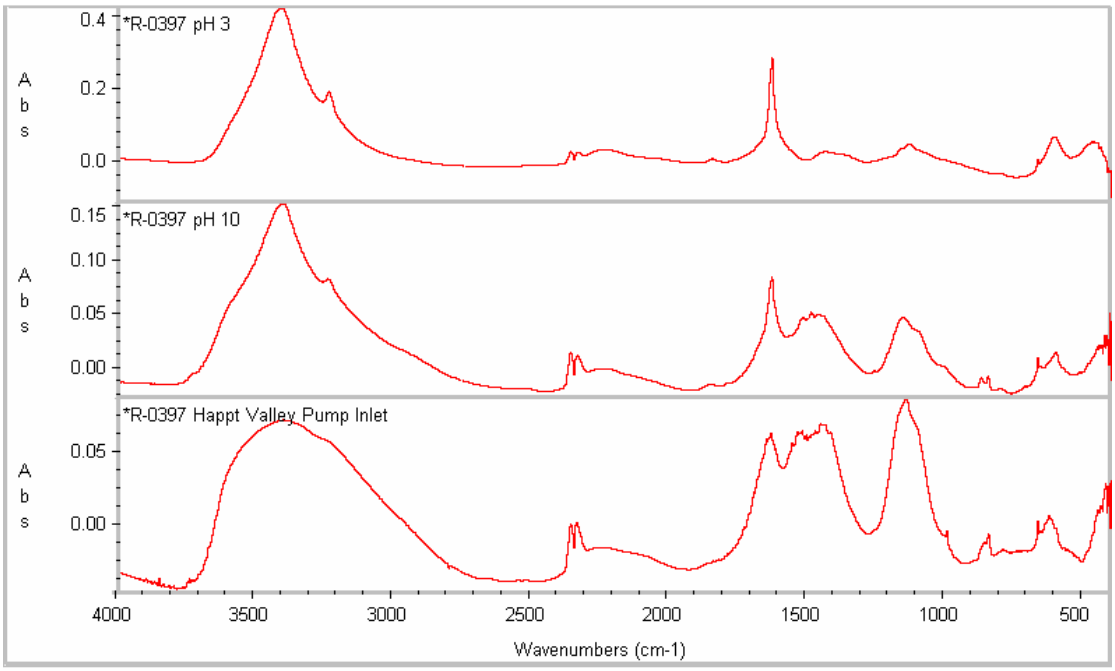


Figure 2.37 DRIFT spectra of a raw water sample from Happy Valley Reservoir at pH 3, 7 and 10.

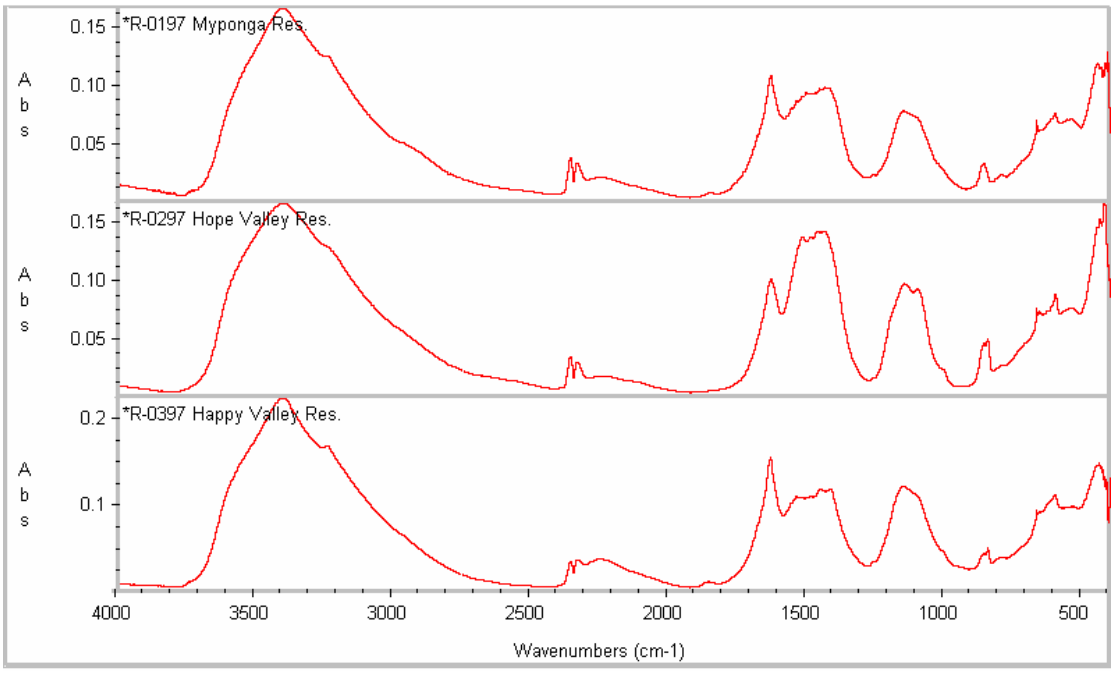


Figure 2.38 Comparisons of DRIFT spectra of raw waters from Myponga, Hope Valley and Happy Valley reservoirs, at pH 7.

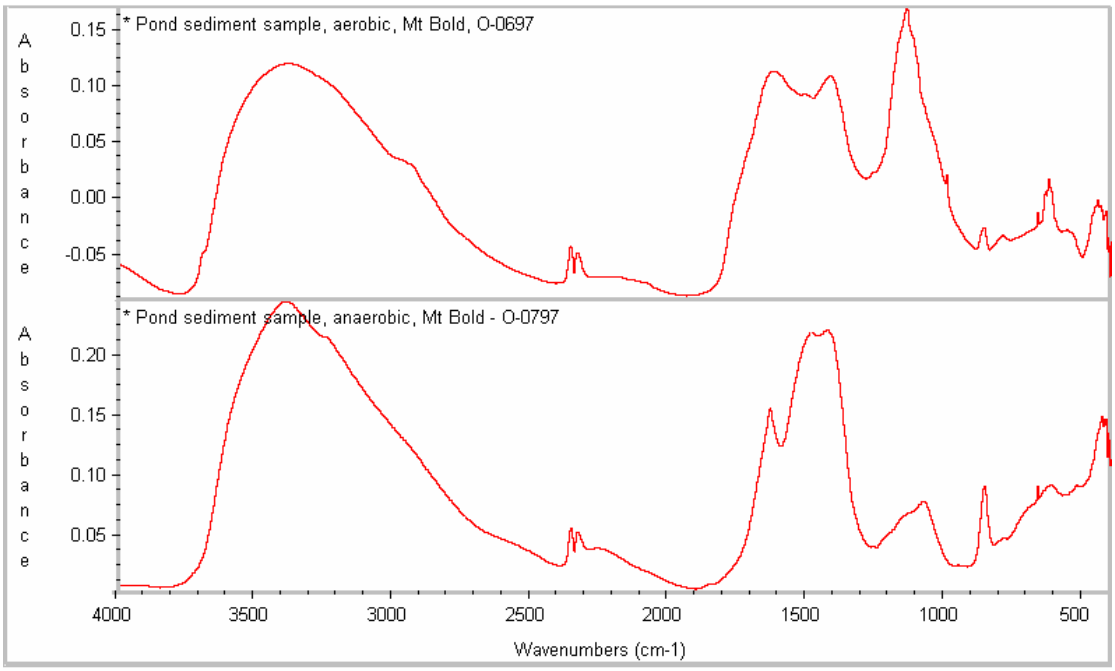


Figure 2.39 DRIFT spectra of extracts from pond sediment, collected from Mt Bold.

Table 2.6 UV-vis spectral data of reference NOM samples collected from Mt Bold in 1997.

UV-vis parameter	Sample site L-0197	O-0197	O-0297	O-0397	O-0497	O-0597
254nm/456nm	13.1	10.1	12.8	7.6	14.8	13.5
E4/E6	6.4	3.1	4.0	2.5	5.0	4.1

parameter	O-0697	O-0797	R0197	R0297	R-0397
254nm/456nm	20.6	25.9	22.7	27.7	25.1
E4/E6	11.6	15.4	7.8	6.4	5.3

Sample list.
O-0197 Mt. Bold top 1, O-horizon soil
O-0297 Mt. Bold top 2, O-horizon soil
O-0397 Mt. Bold top 3, O-horizon soil
O-0497 Mt. Bold midslope, O-horizon soil, under eucalypt tree
O-0697 Mt Bold sediment of pond above water level
O-0797 Mt. Bold O-horizon soil, probable anaerobic conditions
L-0197 Mt. Bold, eucalypt leaf litter
R-0397 Happy Valley Reservoir raw water at pump inlet
R-0297 Hope Valley Reservoir raw water
R-0197 Myponga Reservoir raw water

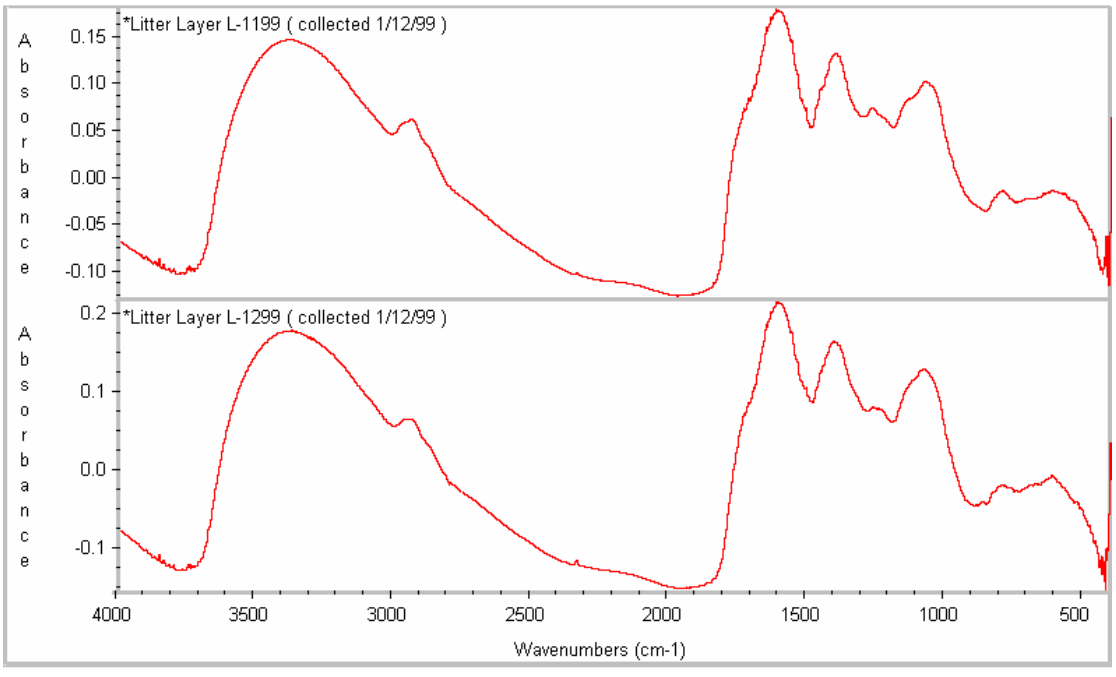


Figure 2.40 DRIFT spectra of leachates of vegetation from the Moorabool catchment (Victoria, April 1999).

2.4 Conclusions:

DRIFT spectra of freeze-dried materials from soils and natural waters contain few well resolved absorbance peaks, and these may be influenced by inorganics. Where specific compounds or bio-polymers are analysed by this method, evidence of specific chemical functionality pertaining to the standards can be found in the spectra. DRIFT spectra of NOM from soils and waters may be better viewed as a fingerprint of a matrix that comprises inorganic and organic matter. Changes in spectra may therefore be better used to reflect or detect changes in overall water quality, than to attempt specific assignment of absorbance bands to functionality. The pH of samples prior to freeze-drying was found to have a marked impact on the DRIFT spectra, demonstrating the need for pH adjustment to a consistent value in order for comparisons between spectra to be allowed.

Appendix 2. 1 List of absorbance peak assignments of DRIFT spectra based on published literature.

Peak or Range WN/cm	Assignment	References
730	C-H stretch of carbonyl conjugated cis alkenes	Stewart *et al. 1*994.
750, 770	CH aromatic	Cagniant *et al.* 1994. Gressel et al. 1995
812	CH aromatic	Cagniant *et al.* 1994.
820	Aromatic CH deformations	Gressel et al. 1995.
841; 856	CH of aromatic rings *(p*-substitutni and C-C in aliphatic moieties	Francioso *et al.* Cagniant *et al.* 1994. 1998.
995	C-H stretch of carbonyl conjugated trans alkenes	Stewart *et al.* 1994.
1040-1075	C-O stretching in polysaccharides and mineral impurities	Gressel et al. 1995.
1050	C-C skeletal vibration of aliphatic and aromatic CH groups	Francioso *et al.* 1998.
1050-1030	Phospholipids, due to P-O stretch	Stewart *et al. 1*994.
1000-1100	Carbohydrates	Kaiser *et al.* 1997.
1138	C-O stretching on alkyl ethers and tert alcohols	Francioso *et al.* 1998.
1180-1090	Polysaccharide absorbances	Stewart *et al.* 1997.
1160, 1115, 1058	C-O stretching in carbohydrates	Pandey and Theagarajan, 1997.
1150-980	C-O stretching in polysaccharide	Stewart *et al. 1*994.
1215	C-O stretching and OH deformation in COOH	Gressel et al. 1995.
1235-1270	COOH deformations, phenolic OH stretching	Gressel et al. 1995
1240	clay absorbed propanate C=0	Parker and Frost, 1996.
1250-1100	Backbone vibrations of carbonic acids of phenolic	Woelki *et al.* 1997.
	or aliphatic alcohols or of aryl ethers	Woelki *et al.* 1997.
1250-1220	C-O ethers and esters	Cagniant *et al.* 1994.
1260	aromatic ethers and phenols (C-O stretch)	Francioso *et al.* 1998; Cai and Smart, 1994.
1270	aromatic ethers and phenols	Francioso *et al.* 1998.

Appendix 2.1 (cont.) List of absorbance peak assignments of DRIFT spectra based on published literature.

Peak or Range WN/cm	Assignment	References
1265, 1237	C-O stretching in lignin	Pandey and Theagarajan, 1997; Stewart *et al* 1994.
1265-1270	phenolic groups	Kaiser *et al.* 1997.
1300-1440	C-H deformation and OH bending	Stewart *et al.* 1997.
1312	CN stretching	Parker and Frost, 1996.
1370	CH of alcohols and COO^-	Francioso *et al.* 1998.
1373, 1371	CH deformation	Parker and Frost, 1996.
1373	C-O stretching in carbohydrates	Pandey and Theagarajan, 1997.
1375-1420	COO- stretching, C-O stretching of phenolic OH	Gressel et al. 1995
1383	C-H alkyl deformation	Parker and Frost, 1996.
1384	COO^-, amide II	Woelki *et al.* 1997.
1397	CH deformation	Parker and Frost, 1996.
1427	CH_2 and COO^- symmetric motion	Francioso *et al.* 1998.
1425	C-O stretching in carbohydrates	Pandey and Theagarajan, 1997.
1430-1450	Aliphatic CH2 and CH3 deformation and bending	Gressel et al. 1995
1446	C=C band	Cai and Smart, 1994.
1450	CH motion of aliphatic groups	Francioso *et al.* 1998; Parker and Frost 1996.
1458	CH deformation	Parker and Frost, 1996.
1460	C-O stretching in carbohydrates	Pandey and Theagarajan, 1997.
1460	C-H deformation absorption	Liauw *et al.* 1995.
1472	C-H alkyl deformation	Parker and Frost, 1996.
1474	NH stretch of trimethylammonium ion	Parker and Frost, 1996.

Appendix 2.1 (cont.) List of absorbance peak assignments of DRIFT spectra based on published literature.

Peak or Range WN/cm	Assignment	References
1506	higher lignin content, aromatic skeletal	Pandey and Theagarajan, 1997.
1515	Aromatic C=C and secondary amides (NH deformation of polypeptides	Gressel et al. 1995
1543	clay absorbed propanate C=0	Parker and Frost, 1996.
1550	secondary amide N-H bend	Stewart *et al.* 1994.
1553	asymmetric C=O for absorbed propanoic acid	Parker and Frost, 1996.
1570	carboxylate carbonyl stretching	Liauw *et al.* 1995.
1590-1610	C=C stretching of aromatics, COO- stretching, primary amines	Gressel et al. 1995.
1596	benzene ring breathing; C=C stretching and C-C olefinic bands	Pandey and Theagarajan, 1997; Stewart *et al.* 1994.
1597	carbonyl stretching	Francioso *et al.* 1998.
1600	C=O stretching of ketones, chinones or amides; C=C	Woelki *et al.* 1997.
1600	C=C	Cagniant *et al.* 1994.
1625-1610	carboxylate band	Kaiser *et al.* 1997, Francioso *et al.* 1998.
1644	C=C of olefin	Francioso *et al.* 1998.
1650	proteins	Stewart *et al.* 1994.
1650-1750	carbonyl band	Cai and Smart, 1994.
1660	secondary amide or protein carbonyl	Stewart *et al.* 1994.
1660	conjugated C=O stretching vibration	Pandey and Theagarajan, 1997.
1666	C=0 of amide 1	Francioso *et al.* 1998.
1693	COOH	Francioso *et al.* 1998.
1700; 1710; 1697	C=O stretching	Frost and Parker, 1997; Heitz *et al.* 1995; Parker and Frost, 1996.
1700-1720	C=O stretching of COOH	Gressel et al. 1995.
1709	esters	Stewart *et al.* 1994.

Appendix 2.1 (cont.) List of absorbance peak assignments of DRIFT spectra based on published literature.

Peak or Range WN/cm	Assignment	References
1710	carboxylic acid carbonyl stretching	Liauw *et al.* 1995; Parker and Frost, 1996
1720-1650	carbonyl group stretching	Heitz *et al*. 1995.
1720	C=O of carbon acids, esters, aldehydes and ketones	Woelki *et al.* 1997; Parker and Frost, 1996.
1740	C-O stretching in carbohydrates	Pandey and Theagarajan, 1997.
1740	higher xylan content, C=O band	Pandey and Theagarajan, 1997.
2300-2000	nitriles	Woelki and Salzer, 1995.
2539	propanoic acid-dolomite	Parker and Frost, 1996.
2755, 2752	aldehyde CH stretch	Parker and Frost, 1996.
2783	alkyl CH stretch	Parker and Frost, 1996.
2832	alkyl CH bands	Parker and Frost, 1996.
2871	alkyl CH stretch	Frost and Parker, 1997; Parker and Frost, 1996.
2900	alkyl C-H stretching	Frost and Parker, 1997.
2928	CH stretching vibrations	Parker and Frost, 1996.
2928	asymmetric CH_2 stretching	Capriel *et al.* 1995.
2935	Aliphatic CH_2 and CH_3	Gressel et al. 1995
2956	symmetric CH_2 stretching	Capriel *et al.* 1995; Parker and Frost, 1996; Francioso *et al.* 1998.
2957	alkyl CH stretch	Parker and Frost, 1996.
2971	CH stretching vibrations	Parker and Frost, 1996.
2980	CH3	Francioso *et al.* 1998; Parker and Frost, 1996.
3000-2800	aliphatic C-H	Capriel *et al.* 1995*.
3100-3000	aromatic and olefinic C-H stretching	Capriel *et al*. 1995.
3240, 3220	NH stretching	Parker and Frost, 1996.

Peak or Range WN/cm	Assignment		References
3340-3380	O-H stretching		Gressel et al. 1995.
3360	O-H stretching		Pandey and Theagarajan, 1997.
3535	OH band		Cai and Smart, 1994.
3630	O-H stretching		Frost and Parker, 1997.
3800-3600	mineral OH bands	sharp, weak band	Cai and Smart, 1994.

Other tabulated assignments, see Spark, 1998.

CHAPTER 3

SURVEYS OF THE VARIATION IN THE CHARACTER OF NOM FROM SOIL LITTER LAYERS, CATCHMENT SOIL AND RESERVOIR WATERS.

3.1 Introduction:

In this chapter is described the results of surveys performed to assess the diversity of natural organic matter in raw water sources, in soils and in soil litter layers in Victoria.

Data presented below are of three surveys conducted in February, April and July of 1999. The aim in obtaining this data was to compare the character of organic matter from a range of sources and locations. Knowledge of the variation in the character of the NOM from various sources could then be applied in further studies on the character of NOM in relation to its treatability with alum, THMFP and BRP. Although the data presented here describes the characterisation of NOM only, the same data also relates to the study by Page (2000). This study examined the treatability of organics isolated from the same or similar sources.

The types of samples collected were varied over the collection period. In the first collection, only reservoir waters were obtained afterwhich soil litter samples and soil samples were also collected. In February 1999, collections were made to study variations in organic loads and in the NOM characters of Victorian reservoirs. Subsequent to this, three reservoir were selected on the basis of ease of access and diversity of raw water NOM, in Victoria. These, together with data of South Australian reservoir-catchment systems, would give information on the diversity of organics in waters, litters layers and soils in south-eastern and southern Australia.

3.2 Methods

The descriptions of sampling locations, extraction procedures and analytical methods applied are reported in Chapter 2 of this report.

Initially, nine reservoirs were selected to study variations in water quality and in the concentrations and characters of NOM. From the results of these, three representative reservoir-catchment systems were selected (Moorabool, Lake Wartook and West Gellibrand) for further study in April and July. A range of sites were selected at each location, varying on the basis of the immediate local vegetation type and replication. Five sites were sampled at Moorabool (site 1: Douglas Fir; site 2: California redwood; site 3: *Pinus radiata;* site 4*: E. ovata; E. radiata; A. melanoyxlon* (blackwood); messmate; silverwattle and black wattle; site 5: grass; three sites from Lake Wartook, all predominantly eucalypts; and five sites from W. Gellibrand (sites 1and 5: *Pinus radiata* and sites 2-4 eucalypts).

3.3 Results and Discussion:

3.3.1 Reservoir samples collected in February 1999.

Organic and inorganic chemistry data of reservoirs sampled in February 1999 are shown in Table 3.1. Most of these raw waters were low in buffering capacity (low alkalinities) and varied markedly in DOC concentrations (5.8 to 17.8 mg/L). Lower raw water pH values are associated with lower alkalinity values. TKN values varied from 0.26 to 0.77 mg/L and were not related to the DOC concentration alone. Hence the C/N ratios also varied markedly, indicating variation in autochthonous input to the DOC.

Table 3. 1 Water quality data of reservoirs surveyed in Victoria in February 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N	Conductivity [µs/cm]	Bicarbonate (mg/L)
Allen Dam	6.98	5.8	0.26	22.3	145	29
Lake Wartook Res.	6.45	9.0	0.44	20.5	76	6
Moora Moora	6.69	16.3	0.77	21.2	138	10
Moorabool Res.	7.42	9.9	0.65	15.2	165	42
Mt Cole Res.	6.58	6.8	0.27	25.2	60	10
Mt.Langi Ghiran Res.	6.83	17.8	0.65	27.4	204	15
Pankalak Res.	7.21	15.4	0.56	27.5	266	23
Pankalak treated water (WTP)	7.32	7.0	0.22	31.8	368	50
Stream from Wartook Res.	6.42	8.4	0.41	20.5	74	6
West Gellibrand Res.	6.96	4.8	0.28	17.1	102	14

UV-vis scans of waters collected in February 1999 are shown in Figure 3.1. The morphologies of the curves are similar and featureless. The intensity of absorbances at 254nm are related to the DOC concentrations, and compared with absorbances above ~ 500 nm, are relatively high. Analyses of UV-vis data in relation to DOC concentrations (SUVA, #1 ratio) are shown in Table 3.2. Again, marked variation was found to occur in this parameter indicating differences in the relative abundances of conjugated double bonds amongst the NOM isolates.

UV-vis spectrometry alone has limited applications for characterisation of humic substances. Use is made of the E4/E6 ratio (absorbance ratio of 465/665 nm), for comparing humic samples from different sources and environments but there are no definite interpretations of the meanings of these (Hayes, 1997). The magnitude of the E4/E6 ratios have been considered to be related to the degree of condensation of the aromatic humic components Simpson *et al*. (1997). Others have considered that aromaticity influences the ratio only as a secondary factor and are primarily governed by the molecular sizes of the HS, with small ratios indicative of large molecular sizes (Simpson *et al*., 1997). E4/E6 ratios varied from ~ 3.3 to 9.8 for raw waters, while the one treated water (from the Pankalak WTP) was very low ie ~1.8. This latter result is in conflict with the premise that smaller ratios are indicative of larger HS, as it is generally held that larger HS are removed with alum treatment. It may be that with the bulk of HS removed by optimum alum treatment, the remaining organics bear little or no relationship to the E4/E6 ratio (#2). Accepting that the ratio does

relate to the size of the HS, the results indicate variation of HS sizes between the reservoir samples. Other ratios were determined for comparison, ie #3, 254nm / 456nm and #4, (254 nm x 456 nm x 1000) / DOC^2 [or designated (254 nm * 456 nm *1000)/DOC^2]. Although these are not conventional they were determined on the following assumptions: for #3, high colour per absorbance at 254nm (low ratio) reflects humified and/or aromatic compounds from lignin/tannin sources, (in contrast to compounds such as proteins or those with conjugated double bonds that impart little colour) and for #4, higher values are indicative also of higher aromatic input to DOC. Low ratios of #3 and high of #4 may indicate higher terrestrial source inputs. Interestingly, the treated water from Pankalak had the highest #3 ratio which is in agreement with the concept that alum treatment removes more of the large molecular weight, coloured hydrophobic material, that may be assumed to be terrestrially derived. This water, as might be expected, had the lowest #4 ratio.

Of the raw waters, Allen Dam, Mt Langi Ghiran and Pankalak had the highest #4 ratio values which contrasted markedly with Wartook Reservoir water and the Moorabool Reservoir water. The ratio of the water from the stream from Lake Wartook was very similar to that of the sample from the lake itself, as might also be expected.

DRIFT analysis was performed on the reservoir samples (Table 3.3), in the context of the potential high interferences from inorganic salts. Functionality assignments were based on literature data as detailed in Chapter 2 Appendix 2.1.

Spectra of samples were standardised to percentage absorbances with baselines at ~ 3800 and 1930 WN/cm and 100% at ~3380 WN/cm in Region 1. Absorbances at ~ 3300 are predominantly attributed to OH stretching of carboxylic and alcoholic groups (Francioso *et al*. 1998); bonded hydrogen (Painter *et al*. 1981). In this region absorbances from NOM isolates generally result in a broad single peak, sometimes with higher resolved shoulder peaks at ~2970 WN/cm and ~2930 WN/cm attributed to $–CH_3$ and $–CH_2$ functional groups, respectively. Spectra are predominantly compared on the basis of differences in Region 2 (1000 to 2000 WN/cm).

DRIFT spectra of freeze-dried material (FDM) of reservoir waters are shown in Table 3.3. Major peaks occurred at about 1620, 1500, 1420, 1140, 1100, 1080, 860 and 800. Peaks from 1610 to 1633 WN/cm (*carboxylate band*), varied from ~74% (Moorabool) to ~95% (L. Wartook); only Allen Dam and Moorabool Reservoir had a band at ~1500 (*aromatic skeletal*); 1415-1442 WN/cm (CH_2, COO^-, C-O stretching of carbohydrates) varied from 63% (W. Gellibrand) to 97% (Allen Reservoir); 1133-1144 WN/cm (C-O stretching of alkyl ethers and tertiary alcohols) from 77 % (Pankalak) to 105% (Mt Langi Ghiran); 1078-1111 WN/cm (carbohydrates) from 76% (Moora Moora Res.) to 112% (Lake Cole). Peaks at ~860 and ~800 WN/cm (CH of aromatic rings) were found in spectra of most samples at ~ 25 to 35%. Distinguishing peaks varied between these samples by about 20 to 35%. No relation was evident between DRIFT and UV-vis data.

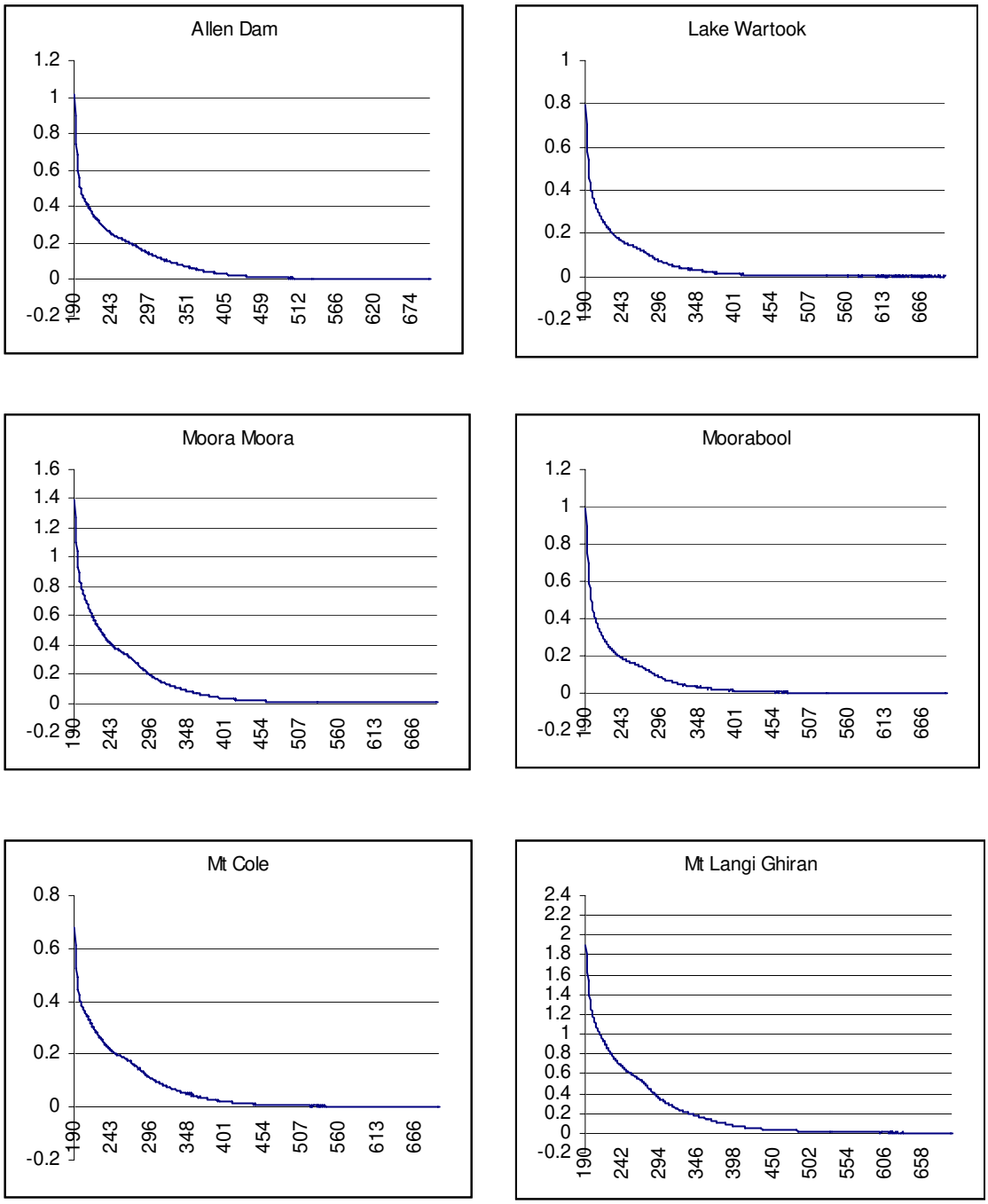


Figure 3. 1: UV-vis data (absorbance, y-axis versus wavelength, x-axis) of surface waters collected from Victorian reservoirs in February 1999.

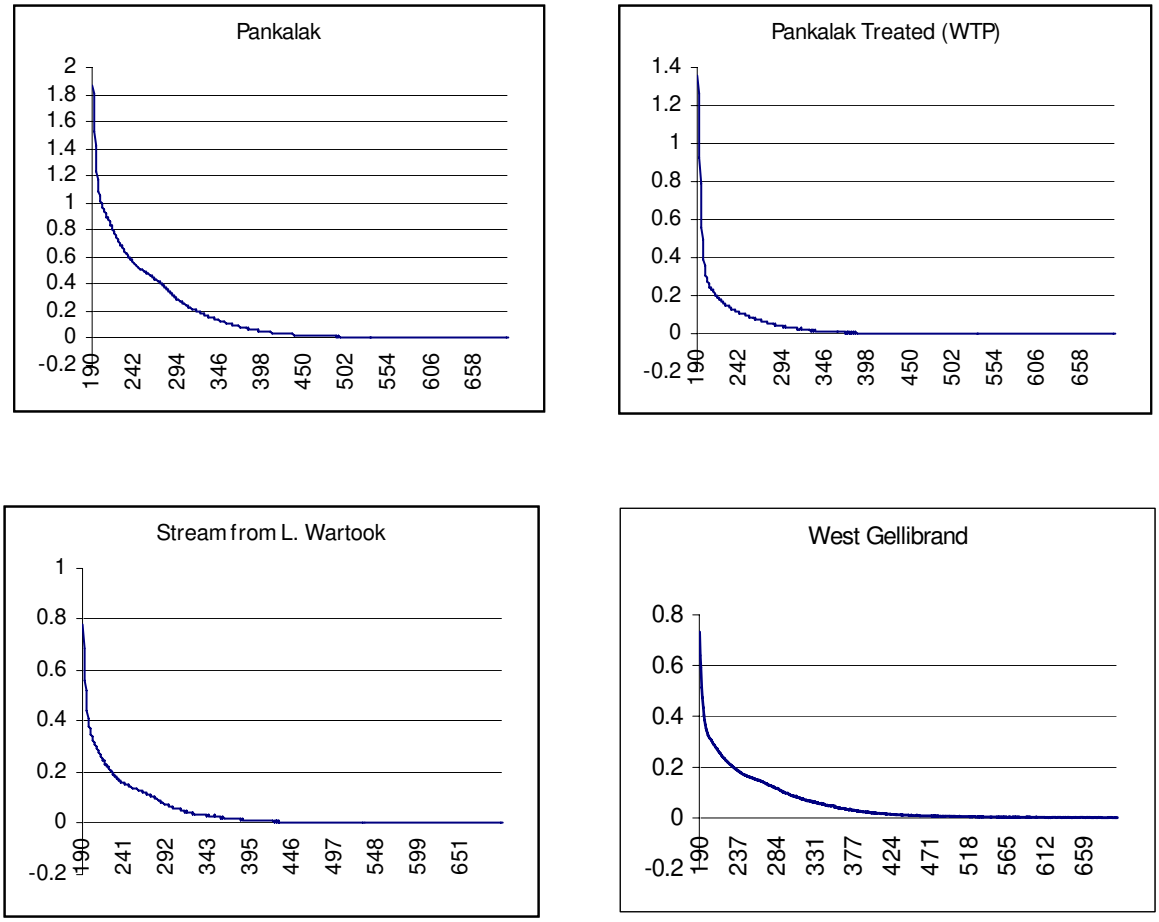


Figure 3.1 (contin.) : UV-vis data (absorbance, y-axis versus wavelength, x-axis) of surface waters collected from Victorian reservoirs, a stream and a water treatment plant in February 1999.

Table 3. 2: UV-vis and DOC (mg/L) data of Victorian surface waters collected in February 1999.

Water source	DOC mg/L	SUVA	E4/E6	254nm/ 456nm	(254*456*1000)/ DOC^2
Allen Dam	5.8	3.92	5.96	15.8	0.098
Lake Wartook	9.0	1.59	3.37	23.8	0.011
Moora Moora	16.3	2.19	9.84	25.9	0.018
Moorabool	9.9	1.66	4.37	29.3	0.009
Mt. Cole	6.8	2.83	5.81	19.3	0.042
Mt.Langi Ghiran	17.8	3.35	7.04	18.7	0.060
Pankalak	15.4	3.18	7.53	22.4	0.045
Pankalak Treated	7.0	1.26	1.78	46.2	0.003
Stream from Wartook Res.	8.4	1.60	3.34	24.3	0.011
West Gellibrand	4.8	3.26	4.48	17.1	0.062

Table 3. 3 Relative percentages of absorbance band heights of DRIFT spectra of materials from Victorian reservoirs, February 1999.

Allen Dam		L. Wartook		Moora Moora		Moorabool		Mt Cole	
WaveNo.cm-1	Percentage	WaveNo.cm-1	Percentage	WaveNo.cm-1	Percentage	WaveNo.cm-1	Percentage	WaveNo.cm-1	Percentage
3788	0	3777	0	3761	0	3799	0	3766	0
3381	100	3375	100	3376	100	3381	100	3370	100
2963	48.2	2968	53.8	2974	52.5	2968	52.6	2957	52.2
2262	8.2	2930	53.8	2936	52.5	2930	51.2	2924	53.1
		2219	7.6	2240	8.6	2229	71.6	2262	5.9
1937	0.0	1936	0.0	1920	0.0	1936	0.0	1947	0.0
		1714	56.9						
1627	74.7	1627	87.8	1633	91.4	1632	73.5	1616	88.4
1502	92.6					1507	91.2		
1431	96.5	1415	76.0	1420	77.8	1442	94.9	1415	71.6
		1257	57.3	1247	54.9				
1133	98.8	1143	81.3	1144	74.7	1143	101.9	1138	101.6
1084	105.4	1078	81.6	1073	76.5	1078	89.8	1078	111.6
861	27.2					856	37.2		
		796	35.1	790	31.5			796	26.9

Table 3.3 (cont.): Relative percentages of absorbance band heights of DRIFT spectra of materials from Victorian reservoirs, February 1999.

Mt Langi Ghiran		Pankalak		Stream from L. Wartook		West Gellibrand	
WaveNo.cm-1	Percentage	WaveNo.cm-1	Percentage	WaveNo.cm-1	Percentage	WaveNo.cm-1	Percentage
3799	0	3766	0	3766	0	3804	0
3370	100	3386	100	3376	100	3365	100
2973	57.9	3239	86.2	2968	52.0	2968	55.3
2930	54.4	2968	43.1	2936	50.3	2240	14.0
2257	75.4	2267	13.8	2246	7.6		
1920	0.0	1936	0.0	1920	0.0	1942	0.0
1610	88.6	1638	83.7	1616	94.7	1627	77.4
1420	72.8	1431	75.0	1426	88.3	1437	63.8
1133	105.3	1143	77.2	1144	91.2	1138	100
1111	106.1	1105	73.2	1100	81.9	1089	98.1
915	38.6	856	22.1	850	29.8		
796	21.9	785	22.5	796	32.7	801	31.1

3.3.2 NOM isolates obtained from samples collected in April 1999.

Water quality data of reservoir waters collected in April 1999 (Moorabool, Lake Wartook and West Gellibrand reservoirs) are shown in Table 3.4.
DOC concentrations represent general variations in waters of southern Australia. The pH values correlate to the bicarbonate/alkalinity levels of the waters.

DOC and TKN concentrations, and consequently the C/N ratios were very similar to those determined for these waters in February, indicating little change over the summer/autumn period. Further, the bicarbonate concentrations were virtually unchanged over this period, indicating consistency in the alkalinity levels of these waters.

Table 3. 4: Water quality data of reservoirs surveyed in Victoria in April 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N ratio	Cond [µs/cm]	Bicarb. (mg/L)	TDS (by EC) mg/L
Moorabool Res(8/4/99)	7.90	10.1	0.58	17.4	168	47	92
Lake Wartook Res(8/4/99)	6.87	8.9	0.42	21.2	100	6	55
West Gellibrand Res(9/4/99)	7.02	5.0	0.32	15.6	110	14	60

Organic and inorganic data of leachates of soil litter layers from the Moorabool (sites 1-5) and West Gellibrand (sites 1-3) catchments are given in Table 3.5. Of marked contrast to reservoir waters, the C/N ratios of the soil litter leachates were low (~ 2.6 to 6.1). This result can be explained by higher relative microbial activities in soils than in raw surface waters, leading to the formation of higher relative concentrations of nitrogenous compounds. Variation between sites within the same location were similar for both Moorabool and West Gellibrand.

Organic and inorganic data of soil leachates are shown in Table 3.6. DOC concentrations were generally between raw reservoir water and litter layer extract concentrations. These varied markedly between sites and locations with the lowest being 5.9 mg/L and the highest, 80.7 mg/L, indictaing large differences in organic loads to soils. C/N ratios were variable but higher than for the litter layer extracts and comparable to the reservoir waters. The cations measured, particularly sodium, varied markedly between sites and locations. Organic and inorganic chemistry data were also obtained on whole dried soil samples (Table 3.7), which further demonstrated large variations between sites and locations. These variations however, are not correlated to the data obtained of soil leachates.

UV-vis scans of the samples collected in April 1999 are shown in Figures 3.2 to 3.5 and summarised data is given in Table 3.8. Curve morphologies UV-vis scans of reservoir samples were again similar to each other and featureless, as those of the February collections. Scans of litter and soil extracts did show some variations particularly at about 286 nm where a peak shoulder appeared on the curve (Moorabool litter layers, sites 4 and 5; West Gellibrand litter layers, sites 2 and 3) and similarly for some soil samples. Also, the UV-vis scan curves of soil samples varied in relation to the steepness in absorbance declines to ~ 236 nm, most notably Moorabool site 2 (Figure 3.3) and site 5 (Figure 3.4) and W. Gellibrand site 3 (Figure 3.5). The basis of these differences is not known.

Summarised UV-vis data showed several trends as follows:

1. SUVA values for the reservoir samples collected in April were virtually the same as those collected in February, indicating little summer/autumn (UV radiation) impact on the character of the NOM in these waters.

2. The values of the parameter 254 nm/456 nm was variable for the three reservoirs sampled in April, though were generally higher than the litter layer and soil extracts. The higher ratio values result from lower coloured water in relation to the content of UV absorbing compounds. Soils tended to have the smallest ratios and this may also be due to colours of extracts being contributed to by iron.

3. The most distinguishing data between the various sample types was of the (254 nm x 456 nm x 1000)/DOC^2 parameter. Consistency in values, in terms of comparative magnitudes, was found for the three reservoirs sampled in February and in April 1999. This indicates that this parameter (254 nm x 456 nm x 1000)/DOC^2 is of potential value in discriminating different sample types.

4. The values of the (254 nm x 456 nm x 1000)/DOC^2 parameter showed further trends in relation to the sample type, with the highest ratios from soil, followed by reservoirs and then litter layer extracts. By use of this parameter, the three sample types could, in most cases, be readily distinguished. However, considerable variation in this ratio was found between sites of the same location eg. West Gellibrand soil sites 1-3.

Table 3. 5: Organic and inorganic data of extracts of soil litter layers collected in April 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N ratio	Cond. [µs/cm]	Bicarb. (mg/L)	TDS (by EC) mg/L
Moorabool site 1	5.96	126.7	36.0	3.52	142	49	78
Moorabool site 2	5.95	310.0	121.0	2.56	304	88	170
Moorabool site 3	5.75	119.0	28.0	4.25	133	21	73
Moorabool site 4	4.76	222.0	36.2	6.13	115	4	63
Moorabool site 5	6.03	128.0	43.4	2.95	262	49	140
West Gellibrand site 1	5.14	234.3	42.2	5.55	134	27	73
West Gellibrand site 2	5.18	181.7	54.0	3.36	109	20	60
West Gellibrand site 3	5.32	422.3	154.0	2.74	261	63	140

Table 3. 6: Organic and inorganic data of extracts of soil extracts collected in April 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N ratio	Cond. [µs/cm]	Bicarb. (mg/L)	TDS (by EC) mg/L	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Al (mg/L)	Fe (mg/L)	Alkalinity $CaCO_3$ (mg/L)
Moorabool site 1	6.84	18.0	1.04	17.3	34	10	19	3.0	1.6	8	1.2	0.568	8
Moorabool site 2	6.39	16.2	1.58	10.2	69	8	38	8.6	3.2	21.4	11.5	5.82	6
Moorabool site 3	5.09	19.7	0.78	25.3	154	6	84	5.2	5.6	56.8	3.2	1.38	4
Moorabool site 4	5.08	40.5	1.62	25.0	60	6	33	1.8	2.4	19.2	5.0	2.32	4
Moorabool site 5	6.22	5.9	0.30	19.6	53	6	29	2.0	2.4	9.8	2.0	0.89	4
Wartook site 1	4.36	80.7	2.88	28.0	56	0	31	1.4	2.4	9.2	4.3	2.52	0
Wartook site2	6.07	10.8	0.52	20.7	8	8	4	0.4	<0.6	<1.0	2.7	1.53	6
Wartook site3	5.46	29.3	1.74	16.8	27	6	15	0.8	1.2	4.80	5.2	2.62	4
West Gellibrand site1	4.86	35.5	1.44	24.7	62	<10	34	1.8	3.4	15	1.7	2.78	8
West Gellibrand site 2	4.49	22.7	0.72	31.5	73	0	40	1.4	2.6	14.2	1.0	1.77	0
West Gellibrand site3	5.86	11.9	1.30	9.2	54	6	30	4.2	1.4	7.4	4.8	5.32	4

Table 3. 7: Organic and inorganic analyses data of dried soil samples collected in April 1999.

Sample Description	Total Al*	Total Fe*	Total Na*	Total Ca*	Total Mg*	TOC %
Moorabool site 1	24000	97700	<417	2440	1420	4.7
Moorabool site 2	20100	48300	<444	5330	1190	5.6
Moorabool site 3	16000	11800	<478	543	870	4.4
Moorabool site 4	18200	13900	<428	440	852	7.0
Moorabool site 5	16900	15600	<479	1240	1180	3.8
Wartook site 1	1340	1120	<463	301	445	3.0
Wartook site 2	2000	1720	<436	90.5	371	2.4
Wartook site 3	2740	2080	5290	244	329	2.1
West Gellibrand site 1	25400	30400	1940	350	4170	5.4
West Gellibrand site 2	22600	34200	1160	397	2530	3.4
West Gellibrand site 3	21500	28700	1890	1360	2410	5.7

* results mg/kg dry

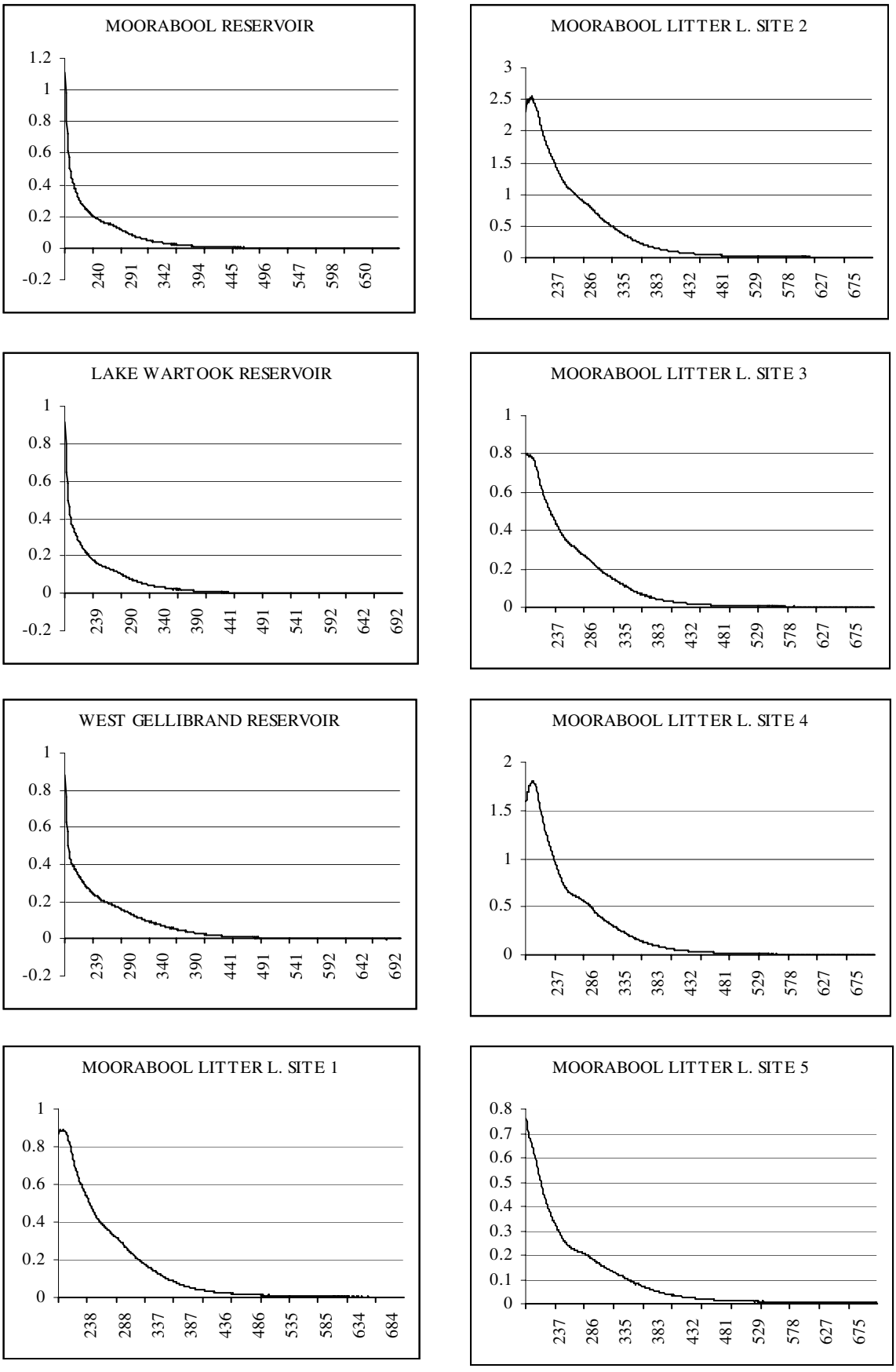


Figure 3. 2: UV-vis data (absorbance, y-axis versus wavelength, x-axis) of reservoir waters and soil litter extracts of samples collected from Victorian catchments in April 1999.

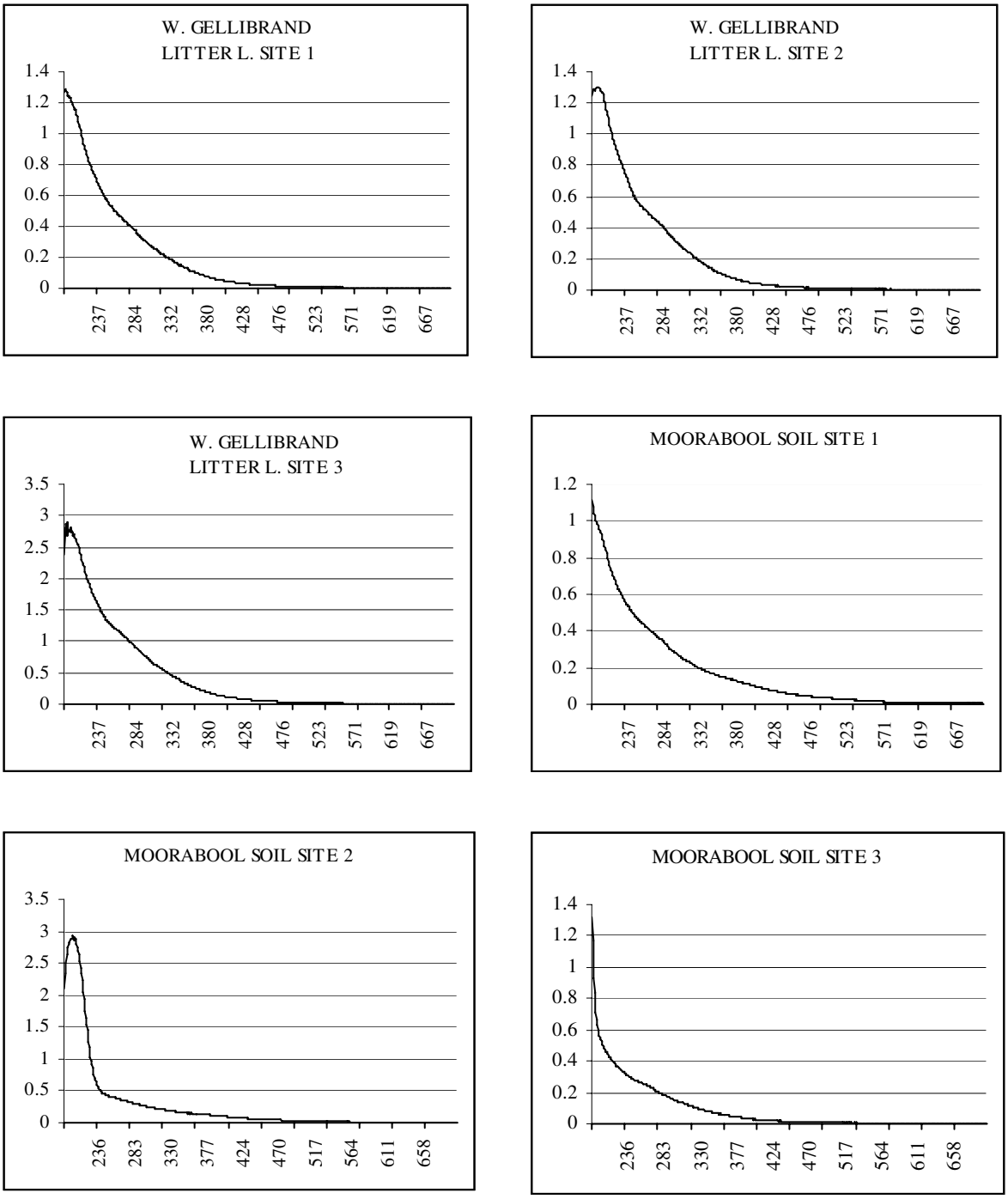
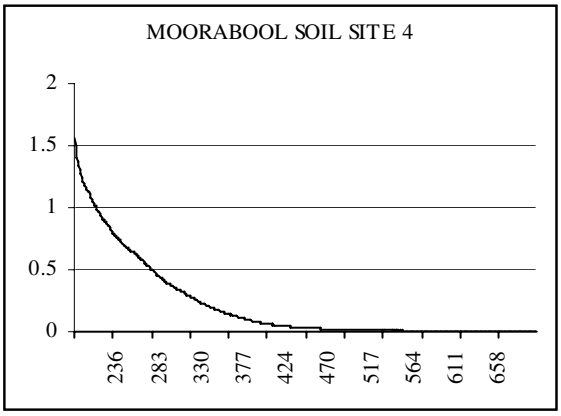
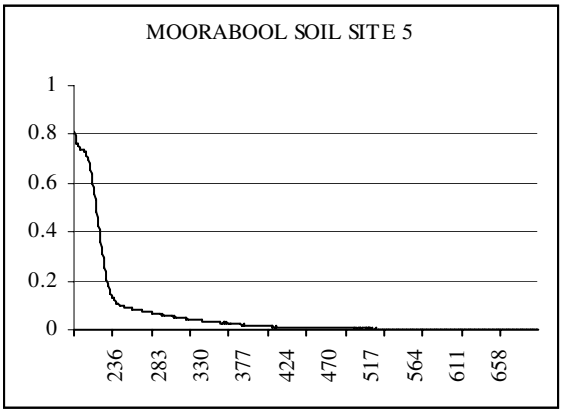
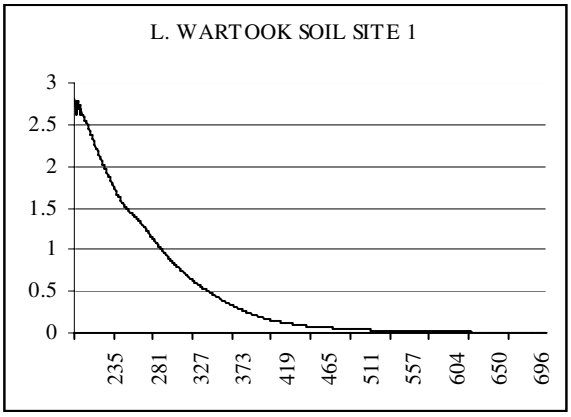
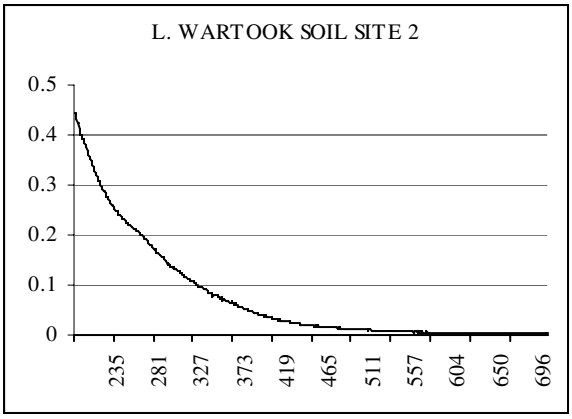


Figure 3. 3: UV-vis data (absorbance, y-axis versus wavelength, x-axis) of soil litter and soil extracts of samples collected from Victorian catchments in April 1999.









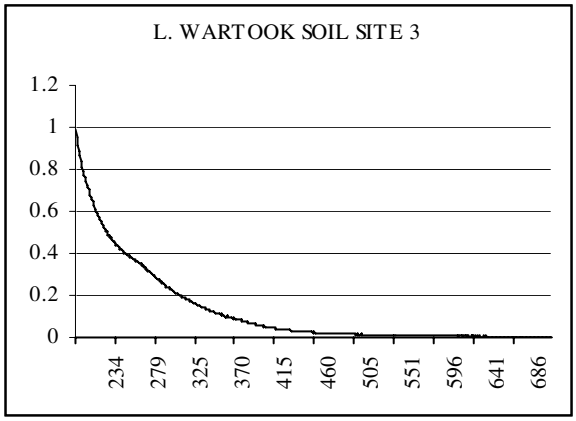


Figure 3. 4: UV-vis data (absorbance, y-axis versus wavelength, x-axis) of soil extracts of samples collected from Victorian catchments in April 1999.

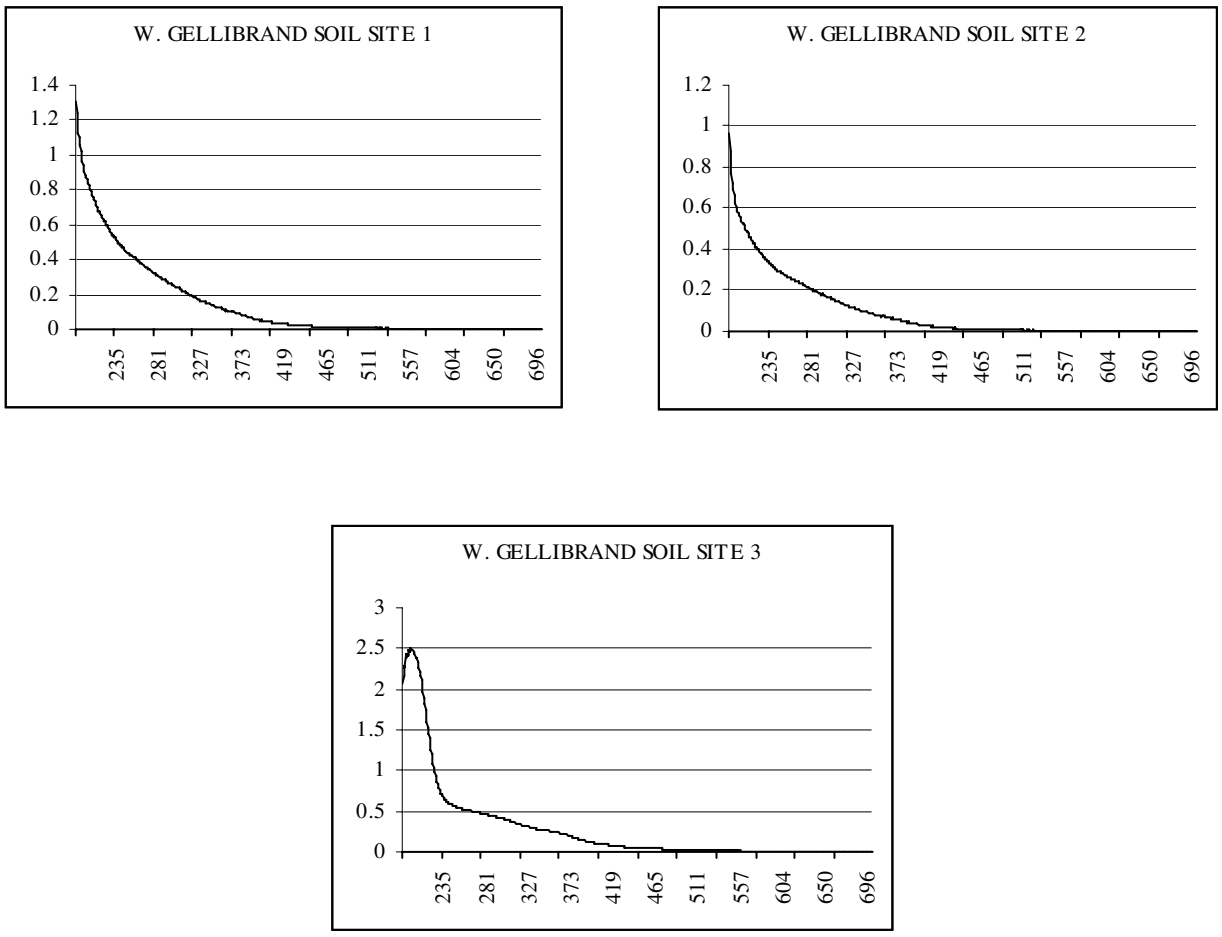


Figure 3. 5: UV-vis data of soil extracts of samples collected from Victorian catchments in April 1999.

Table 3. 8: UV-vis data of samples from reservoirs, soil litter layers and soils collected in April 1999.

Source	E4/E6	SUVA	254nm /456nm	254nm*456nm *1000/DOC^2	Colour/DOC (HU/mg/L)
Moorabool Reservoir	n.a.	1.55	41.2	0.00584	0.30
Lake Wartook Reservoir	n.a.	1.51	41.2	0.00551	0.30
West Gellibrand Reservoir	n.a.	3.86	19.2	0.07760	1.50
Mean	n.a.	2.31	33.9	0.02965	0.70
SD	n.a.	1.34	12.7	0.04153	0.69
Moorabool Litter L. site 1, 1:10	5.08	0.31	21.9	0.00043	0.10
Moorabool Litter L. site 2, 1:10	5.08	0.35	22.8	0.00053	0.11
Moorabool Litter L. site 3, 1:10	5.81	0.28	26.5	0.00029	0.08
Moorabool Litter L. site 4, 1:10	9.33	0.29	23.8	0.00035	0.09
Moorabool Litter L. site 5, 1:10	3.45	0.18	13.1	0.00025	0.10
Mean	5.75	0.28	21.6	0.00037	0.10
SD	2.18	0.06	5.1	0.00011	0.01
W.Gellibr.Litter L. site 1, 1:10	9.02	0.22	25.9	0.00019	0.06
W.Gellibr.Litter L. site 2, 1:10	5.60	0.30	27.4	0.00032	0.08
W.Gellibr.Litter L. site 3, 1:10	8.74	0.29	24.5	0.00034	0.08
Mean	7.79	0.27	25.9	0.00028	0.08
SD	1.90	0.04	1.5	0.00008	0.01
Moorabool soil, site 1, 1:2	8.21	2.48	8.4	0.07289	2.11
Moorabool soil, site 2, 1:2	9.24	2.43	8.4	0.07000	2.07
Moorabool soil, site 3, 1:2	4.70	1.31	21.4	0.00802	0.45
Moorabool soil, site 4, 1:2	8.00	1.58	25.2	0.00985	0.45
Moorabool soil, site 5, 1:2	3.45	1.45	12.9	0.01627	0.86
Mean	6.72	1.85	15.3	0.03541	1.19
SD	2.50	0.56	7.7	0.03306	0.84
L. Wartook soil, site 1, 1:2	8.04	1.73	20.2	0.01486	0.61
L. Wartook soil, site 2, 1:2	4.65	1.97	13.0	0.02991	1.11
L. Wartook soil, site 3, 1:2	4.95	1.24	17.7	0.00874	0.51
Mean	5.88	1.65	16.9	0.01783	0.75
SD	1.87	0.37	3.7	0.01089	0.32
W.Gellibrand soil, site 1, 1:2	15.41	1.15	25.2	0.00524	0.33
W.Gellibrand soil, site 2, 1:2	8.56	1.17	27.0	0.00507	0.32
W.Gellibrand soil, site 3, 1:2	7.16	4.44	12.8	0.15462	2.50
Mean	10.37	2.25	21.7	0.05498	1.05
SD	4.42	1.89	7.8	0.08630	1.25

Table 3. 9 Summary data of DRIFT spectra of reservoir samples collected in April 1999.

Moorabool		Lake Wartook		West Gellibrand	
WaveNo. cm-1	Percentage	WaveNo. cm-1	Percentage	WaveNo. cm-1	Percentage
3777	0	3767	0	3782	0
3392	100	3386	100	3376	100
2979	48	2968	45	2974	53
2930	47	2936	46	2924	50
2230	9	2213	9	2278	9
1926	0	1948	0	1937	0
1633	73	1638	86	1627	76
1513	84				
1426	90	1426	72	1431	82
		1252	45		
1149	102	1133	74	1144	110
1073	88	1078	70	1090	113
				948	35
856	30	856	17	802	26

Summary data of DRIFT spectra of raw reservoir waters, litter layer and soil extract samples are shown in Table 3.9, tables 3.10-3.11 and tables 3.12 – 3.14, respectively. Consistency in spectral features for the three reservoirs sampled in April and in February was found, again indicating minor changes in the waters over these sampling times. Absorbance shoulders at ~2975 WN/cm (C-H stretching of -CH_3) and ~ 2930 WN/cm (C-H stretching of –CH_2) were found, ranging from 52 to 55% in February and from 45 to 53% in April. The peaks at ~ 1633 WN/cm (carboxylate band) also showed high consistency, with February / April percentage values as follows: Moorabool, 74/73; Lake Wartook, 88/86 and West Gellibrand, 77/76. The peaks at ~1513 WN/cm (aromatic skeletal structure) was only detected in the Moorabool samples, 84/91 as was the ~1252 WN/cm (C-O) for L. Wartook only (57/45). Peaks at ~1428 WN/cm (CH_2, COO^- symmetric motions: C-O stretching) were found in all reservoir samples, ie Moorabool 95/90, Lake Wartook, 76/72 and W. Gellibrand (64/82). The peaks at ~ 1140 WN/cm (C-O stretching of alkyl ethers) were also consistent (Moorabool, 102/102; Lake Wartook, 81/74 and W. Gellibrand (100/110) as were the peaks at ~1080 WN/cm (carbohydrates, Moorabool, 90/88; Lake Wartook 82/70; W. Gellibrand 98/113). This data, although having a high potential of being impacted by salt interference, is remarkably consistent in peak trends over the sampling period for each reservoir. This, if true (but requiring further work to substantial this observation), could provide a ready method for finger-printing individual water sources and tracing their overall changes, though the specific reasons for these changes would probably need to be investigated separately.

Table 3. 10 Summary data of DRIFT spectra of soil litter layer samples collected from the Moorabool catchment in April 1999

Moorabool s1 Douglas fir		Moorabool s2 Cali. redwood		Moorabool s3 *P. radiata*		Moorabool s4 Native trees		Moorabool s5 grass	
WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage
3761	0	3772	0	3777	0	3755	0	3767	0
3370	100	3381	100	3381	100	3386	100	3376	100
2963	59	2974	67	2974	59	2974	61	2974	61
2935	62	2925	67	2936	65	2930	63	2936	66
						2702	29	2713	29
2202	5	2186	7						
				2159	1	2142	4	2121	4
1926	0	1947	0	1947	0	1958	0	1937	0
		1714	76	1720	68	1719	74	1714	64
1605	119	1595	119	1611	101	1600	113	1611	96
1399	99	1410	106	1404	88	1405	100	1415	93
1236	73								
1263	77	1269	83	1269	76	1258	83	1247	75
1133	80			1139	84	1128	100	1138	95
1073	95	1062	93	1068	95	1062	98	1073	99
802	41	785	52	796	41	802	45	802	40

Table 3. 11 Summary data of DRIFT spectra of soil litter layer samples collected from West Gellibrand catchment in April 1999.

West Gellibrand s1 *P. radiata*		West Gellibrand s2 Eucalypts		West Gellibrand s3 Eucalypts	
WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage
3767	0	3771	0	3799	0
3397	100	3391	100	3370	100
2974	66	2968	64	2963	71
2930	69	2924	66	2930	72
2713	30	2729	33	2170	8
2170	5	2153	6		
1958	0	1925	0	1910	0
1714	75	1724	75	1720	69
1616	107	1605	111	1610	98
1405	94	1399	97	1410	85
1247	78	1263	82	1253	69
1133	86	1138	85		
1073	97	1068	95	1073	86
785	43	802	45	791	45

A comparison of the major absorbance peaks of the DRIFT spectra of litter layer and soil extracts and reservoir water is shown in Table 3.15. Declines in relative absorbances (from litter layer to reservoir water) was found for peaks ~2970 WN/cm (C-H stretching of CH_3 groups) and ~2932 WN/cm (C-H stretching of CH_2). The reduction in responses of these peaks may be explained by demethylation/oxidation reactions of organics as they are leached from their sources, degraded in the soil and then in the receiving waters by micro-organisms. Peaks at ~1717 WN/cm (carbonyl stretching of esters, carboxylic acid, aldehyde and/or ketones) and ~1261 WN/cm (phenols and/or aromatic esters) were found in the litter layer samples but not in the soils or water. Again, their absence in soils and reservoirs infers their degradation in the matrices. The highest absorbance band at ~ 1050 WN/cm occurred in spectra of the litter layer samples, which indicates higher levels of carbohydrates.

This band is probably due to these samples having undergone less exposure to degradation processes. Similar trends were found for the samples from West Gellibrand, except for the peak at ~1070 WN/cm, indicating a capacity to characterise these sample types (Table 3.16). Absorbance peaks in spectra of Wartook samples differed to those of W. Gellibrand and Moorabool, as follows (litter layer data not available): peaks at ~1720 and 1250 WN/cm were detected in soil and reservoir samples, respectively (Table 3.17). Similar trends were found for Wartook sample peaks at ~ 2960, ~2930 and ~ 1600-1630 WN/cm as for the other locations. Interestingly, the percentages of Wartook soil peaks at ~ 2960, ~2930 WN/cm (64 and 65, respectively) are about the same as those of the litter layers of the other two locations. At these percentages, a peak at ~ 1720 is then detected, at ~ 73%

Table 3. 12 Summary data of DRIFT spectra of soil samples collected from the Moorabool catchment in April 1999.

Moorabool s1 Douglas fir		Moorabool s2 Cal. redwood		Moorabool s3 *P. radiata*		Moorabool s4 Native trees		Moorabool s5 grasses	
WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage
3843	0	3853	0	3777	0	3777	0	3815	0
3370	100	3386	100	3386	100	3381	100	3528	82
2957	60	2963	50	3245	86	2968	59	3403	100
2936	61	2930	49	2941	39	2935	59	3240	77
				2257	16	2165	5	2963	35
								2936	33
								2241	7
2007	0	1947	0	1942	0	1969	0	1948	0
1632	90	1643	76	1627	76	1616	103	1638	64
1415	66	1431	84	1420	47	1410	79	1377	62
		1350	86						
1106	110	1100	79	1117	71	1095	92	1128	77
1040	111	1035	88	1041	67	1041	99	1052	62
915	55	916	51						

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Table 3. 13 Summary data of DRIFT spectra of soil samples collected from the Wartook catchment in April 1999.

Wartook s1 eucalypts		Wartook s2 eucalypts		Wartook s3 eucalypts	
WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage
3783	0	3794	0	3766	0
3381	100	3430	100	3381	100
2941	62	2968	64	2968	66
		2930	65	2930	65
1948	0	1996	0	2029	0
1714	79			1725	68
1605	117	1627	98	1616	104
1399	93	1410	72	1405	85
1106	96	1106	102	1106	121
1041	117	1035	128	1041	146

Table 3. 14 Summary data of DRIFT spectra of soil samples collected from the West Gellibrand catchment in April 1999.

West Gellibrand s1 *P. radiata*		West Gellibrand s2 eucalyps		West Gellibrand s3 eucalypts	
WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage	WaveNo. cm^{-1}	Percentage
3772	0	3767	0	3782	0
3365	100	3365	100	3392	100
2963	53	2974	46	3234	92
2936	53	2930	46	2963	57
2159	3	2230	2		
				2034	0
1953	0	1948	0		
1725	53				
1632	85	1638	85	1627	74
1420	65	1426	63	1421	90
1149	105	1155	71	1366	95
1062	92	1062	86	1100	85
		802	22	1035	95
				910	56
				823	28

Table 3. 15 Comparison of major peaks of DRIFT spectra of samples collected from Moorabool in April 1999.

Litter Layer			Soil Layer			Reservoir water	
Mean WN/cm	Mean %	SD%	Mean WN/cm	Mean %	SD%	WaveNo. cm-1	%
3768	0	0	3813	0	0	3777	0
3381	100	0	3385	100	0	3392	100
2974	61	3.3	2963	51	11.6	2979	48
2932	65	2.1	2936	48	12.2	2930	47
1717	71	5.5	(1725)	(53)			
1604	110	10.6	1631	82	15.0	1633	73
						1513	84
1409	97	6.9	1419	69	16.5	1426	90
1261	79	3.9					
1135	90	9.3	1109	86	15.5	1149	102
1066	96	2.4	1042	85	20.8	1073	88

Parenthesis: detected in single sample.

Table 3. 16 Comparison of major peaks of DRIFT spectra of samples collected from West Gellibrand in April 1999.

Litter layer			Soil			Reservoir water	
Mean WN/cm	Mean %	SD%	Mean WN/cm	Mean %	SD%	WaveNo. cm-1	%
2968	67	3.6	2967	52	5.6	2974	53
2928	69	3.0	2933	50		2924	50
2721	32						
1719	73	3.5					
1610	105	6.7	1632	81	6.4	1627	76
1405	92	6.2	1422	73	15.0	1431	82
1254	76	6.7					
1136	86		1135	87	17.1	1144	110
1071	93	5.9	1053	91	4.6	1090	113
						948	35
793	44	1.2	813	25		802	26

Table 3. 17 Comparison of major peaks of DRIFT spectra of samples collected from Lake Wartook in April 1999.

Soil			Reservoir water	
Mean WN/cm	Mean %	SD%	WaveNo. cm-1	%
2959	64	2.0	2968	45
2930	65		2936	46
1720	74			
1616	106	9.7	1638	86
1405	83	10.6	1426	72
			1252	45
1106	106	13.1	1133	74
1039	130	14.6	1078	70
			856	17

3.3.3 HPSEC data of samples collected in April 1999.

HPSEC chromatograms of individual and mixed polysulphonate standards are shown in Figures 3.6 and 3.7. Chromatograms of a reference NOM [International Humic Substance Society (IHSS) Suwanee River] and Blue Dextran are shown in Figure 3.8. These can be used for comparison with those of NOM isolates from soil samples. Chromatograms of samples taken from Wartook, Moroabool and West Gellibrand catchments are shown in Figure 3.9. UV absorbing compounds of the isolates had three distinct peaks, at about 300-400 AMW, 2000 AMW and at 5 to 6 x 10^4. The relative peak heights at 5 to 6 x 10^4 were clearly different between the isolates, especially Wartook in relation to Moorabool and West Gellibrand.

Chromatograms of various sampling sites at each location are shown in Figures 3.10 (Moorabool), 3.11 (Wartook) and 3.12 (West Gellibrand). Again, common features were found in relation to the number and positioning (AMW) of major peaks in chromatograms. Variation within location in the relative peak heights esp. the highest AMW peak to the others, indicates the ratios are not a feature unique to any one site. However, they do indicate considerable variation in the relative compositions of compounds that constitute the peaks. This is particularly notable in the case of West Gellibrand where the relative peak heights at ~ 6 x 10^4 differed markedly. Shoulder peaks appear to be present in the chromatograms of West Gellibrand (site 2, at ~ 200AMW and ~1000 AMW) and Wartook (site 2, at ~200 AMW).

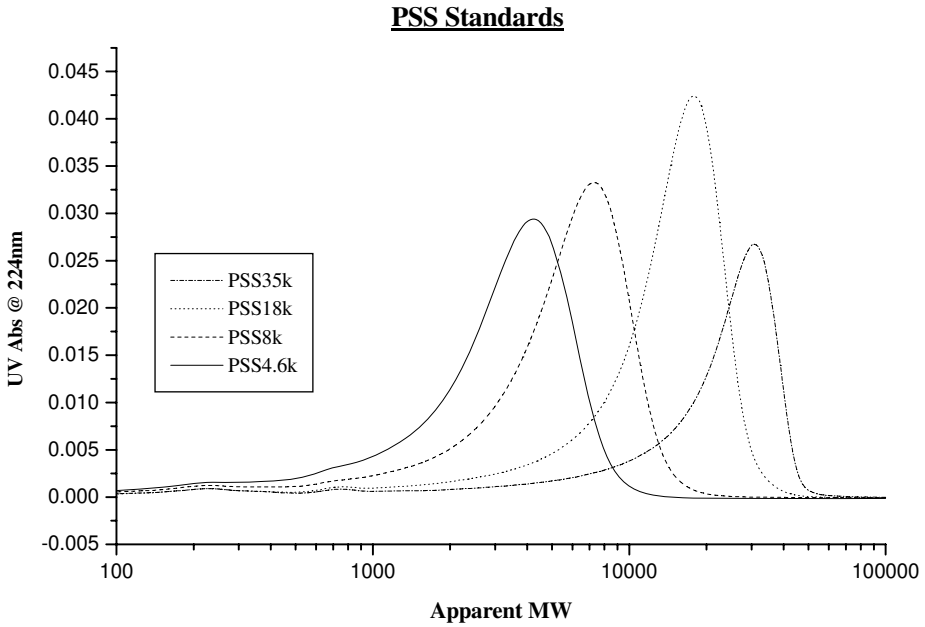


Figure 3. 6 HPSEC chromatograms of polysulphonate standards, for analyses of NOM samples collected in April 1999.

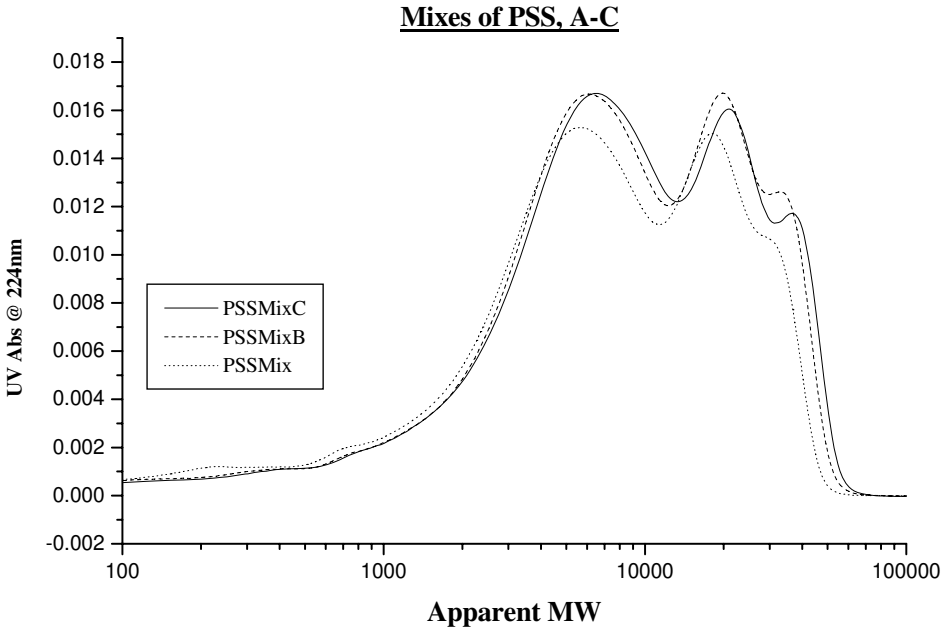


Figure 3. 7 HPSEC chromatograms of polysulphonate mixed standards, for analyses of NOM samples collected in April 1999.

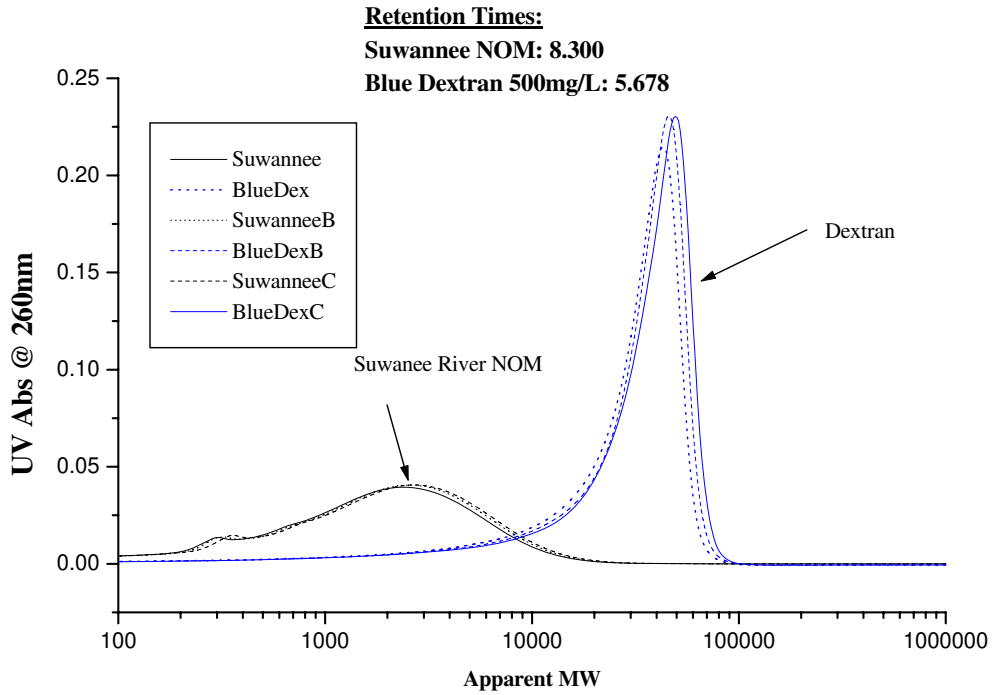


Figure 3. 8 HPSEC chromatograms of dextran (in triplicate) and the IHSS reference NOM, Suwanee River, for comparison to NOM samples collected in April 1999.

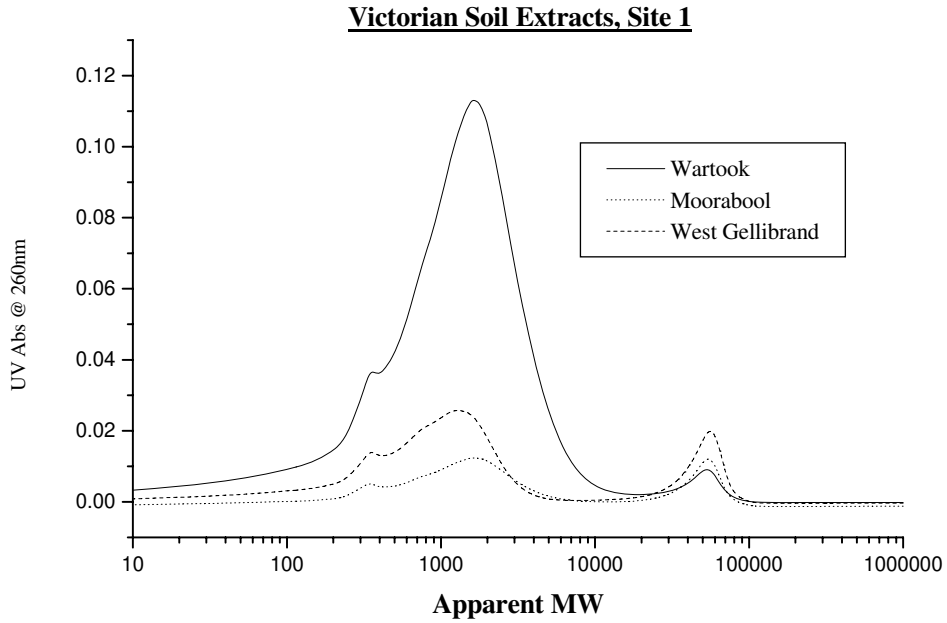


Figure 3. 9 HPSEC chromatograms of extracts of soil samples from each site 1 from Moorabool, Wartook and West Gellibrand catchments, collected in April 1999.

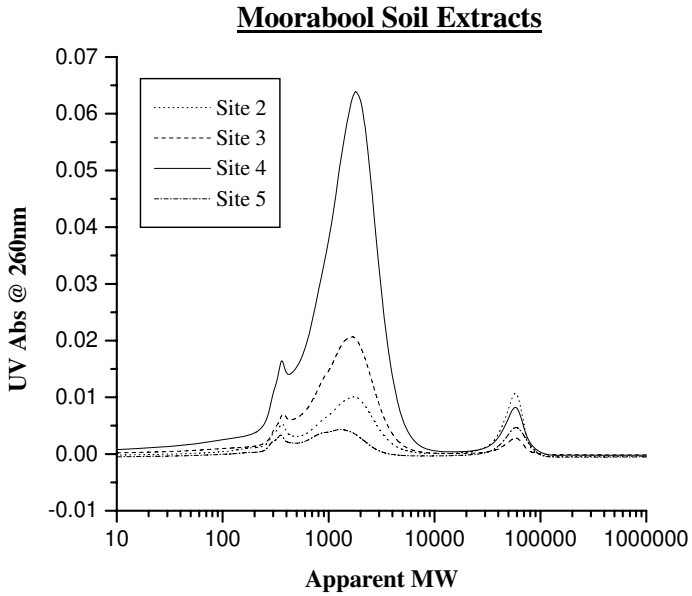


Figure 3. 10 HPSEC chromatograms of soil extracts from Moorabool samples collected from sites 2 to 5 in April 1999.

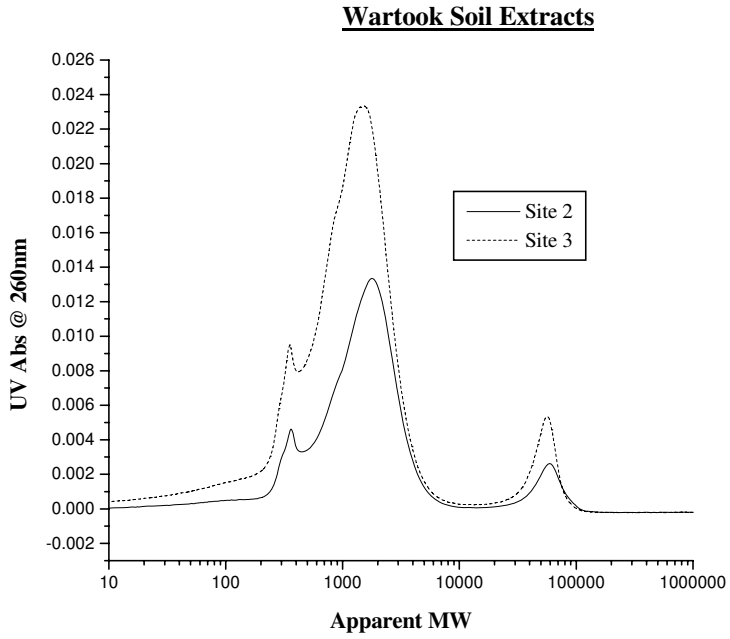


Figure 3. 11 HPSEC chromatograms of soil extracts from Wartook samples collected from sites 2 and 3 in April 1999.

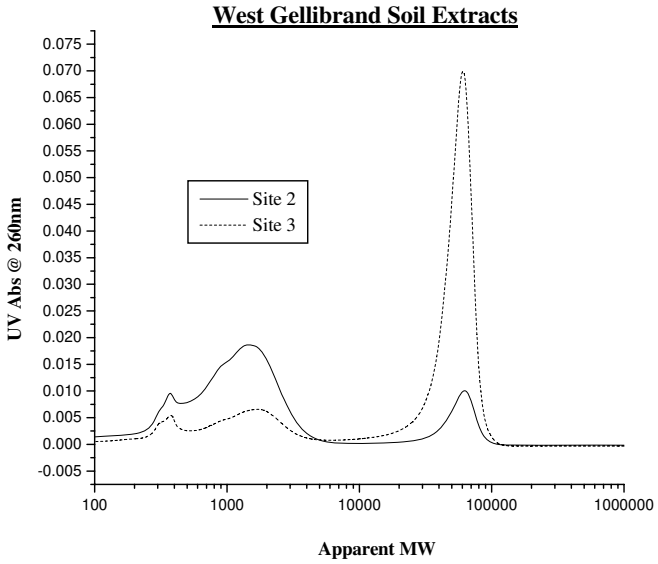


Figure 3. 12 HPSEC chromatograms of soil extracts from West Gellibrand samples collected from sites 2 and 3, in April 1999.

3.3.4 NOM isolates obtained from samples collected in July 1999.

Data from organic and inorganic analyses performed on aqueous extracts of soil litter layers collected from the Moorabool, Lake Wartook and West Gellibrand catchments in July 1999 are shown in Table 3.18.

DOC concentrations were all very high in comparison with concentrations detected in reservoir waters and demonstrated the potential of these sources for organics in soils and reservoirs. High variability in DOC concentrations were also found between the samples from different sites at the same location, indicating that DOC leaching with rainfall or surface flow would be highly variable over relatively small distances. The pH values were also variable but lowest for samples with low bicarbonate concentrations.

C/N ratios varied between and within locations, pointing towards variability in the composition of organics, levels of microbial activities and microbial biomass.

Replicate data from organic and inorganic analyses performed on aqueous extracts of soil samples collected from the Moorabool, Lake Wartook and West Gellibrand catchments in July 1999 are shown in Tables 3.19, 3.20 and 3.21, respectively. DOC concentrations in soil extracts were much less than those of the litter layers, but higher than concentrations detected in the reservoirs on previous sample collections. High levels of variability were found between site replicates, between sites and locations. Hence, analyses of single or few grab samples of soils or litter samples would likely result in unrepresentative

information. C/N ratios were similarly highly variable, reflecting different levels of biodegradation and microbial biomass in the soils, even between very close locations. The pH levels between sites of the same sampling location varied from acidic to neutral soils. A plot of C/N ratios with pH levels are shown in Figure 3.13 (without Moorabool site 1, (5,6)). A trend can be seen in lower C/N ratios corresponding to higher pH values, indicating higher microbial activities in soils with higher pH levels. Removal of data of Moorabool site 1, (5,6) was for the purpose of displaying the apparent trend of the remaining data. The value was considerably higher than any other C/N ratio indicating an error in one of the analyses had occurred.

Table 3. 18 Organic and inorganic data of extracts of soil litter layers collected in July 1999.

Sample Description	pH	DOC mg/L)	TKN (mg/L)	C/N ratio	Conduct. [us/cm]	Bicarb. (mg/L)	TDS (by EC) mg/L	Na mg/L	Alkalinity $CaCO_3$ mg/L
Moorabool site 1	6.47	152	3.5	43.4	130	30	70	1.3	20
Moorabool site 2	7.25	210	10.0	21.0	270	60	150	<0.5	50
Moorabool site 3	5.60	245	7.1	34.5	270	20	150	1.1	20
Moorabool site 4	5.58	428	12.8	33.4	250	40	140	<0.5	30
Moorabool site 5	7.19	127	4.1	31.0	140	30	80	<0.5	20
Wartook site 1	4.70	334	5.8	57.6	310	<10	170	<0.5	10
Wartook site 2	4.70	280	6.8	41.2	150	<10	80	<0.5	10
Wartook site 3	4.76	230	4.2	54.8	130	<10	70	<0.5	10
West Gellibrand site 3	6.31	246	5.2	47.3	170	30	100	<0.5	20
West Gellibrand site 4	5.75	189	2.7	70.0	190	20	100	<0.5	20
West Gellibrand site 5	6.92	121	5.0	24.2	370	40	200	<0.5	30

Table 3. 19 Organic and inorganic data of soil extracts from samples collected from the Moorabool catchment in July 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N ratio	Cond [us/cm]	Bicarb. (mg/L)	TDS (by EC) mg/L	Na (mg/L)	Alkalinity $CaCO_3$ mg/L
Moorabool site 1- (1,2)	7.04	17.2	2.0	8.5	120	12	66	10.4	10
Moorabool site 1- (3,4)	7.08	17.3	1.7	10.0	126	14	70	10.4	12
Moorabool site 1- (5,6)	6.69	66.8	0.7	92.8	314	16	172	19.2	14
Moorabool site 1- (7,8)	7.00	16.1	1.7	9.3	148	14	82	10.2	12
Moorabool site 2- (1,2)	6.85	13.7	1.9	7.1	268	14	146	9.6	12
Moorabool site 2- (3,4)	6.86	16.8	1.6	10.8	84	12	46	5.0	10
Moorabool site 2-(5,6)	7.17	16.6	2.1	7.7	200	22	110	8.6	18
Moorabool site 2- (7,8)	7.23	27.0	2.9	9.4	110	24	60	5.8	20
Moorabool site 3- (1,2)	5.24	41.4	1.6	25.5	104	6	58	14.8	4
Moorabool site 3- (3,4)	5.93	18.0	1.2	15.2	74	6	40	9.4	4
Moorabool site 3-(5,6)	5.60	37.6	1.7	21.9	110	8	60	15.2	6
Moorabool site 3-(7,8)	5.57	30.2	1.6	19.4	86	6	48	12.0	4
Moorabool site 4- (1,2)	5.07	88.5	4.3	20.7	120	10	66	10.0	8
Moorabool site 4- (3,4)	4.82	62.7	3.6	17.5	210	6	116	21.8	4
Moorabool site 4- (5,6)	4.73	58.6	5.9	9.9	260	6	142	26.0	4
Moorabool site 4- (7,8)	4.78	66.8	5.3	12.6	284	6	156	31.4	4
Moorabool site 5- (1,2)	6.10	24.2	1.3	18.1	42		22	<1	
Moorabool site 5- (3,4)	5.78	21.2	0.9	24.7	52		28	<1	
Moorabool site 5- (5,6)	6.01	14.4	1.1	13.1	62	10	34	<1	8
Moorabool site 5- (7,8)	5.87	11.2	0.7	15.1	62	8	34	<1	6

Table 3. 20 Organic and inorganic data of soil extracts from samples collected from the Wartook catchment in July 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N ratio	Cond [us/cm]	Bicarb. (mg/L)	TDS (by EC) mg/L	Na (mg/L)	Alkalinity $CaCO_3$ mg/L
Wartook site 1- (1,2)	4.80	40.1	2.9	13.6	78	8.0	42	4.4	6.0
Wartook site 1- (3,4)	5.05	41.5	3.1	13.5	78	6.0	42	2.6	
Wartook site 1- (5,6)	5.14	18.0	1.3	13.9	62	6.0	34	<1	4.0
Wartook site 1- (7,8)	5.03	32.5	1.9	17.3	58	6.0	32	<1	4.0
Wartook site 2- (1,2)	6.19	14.6	1.1	13.7	28	8.0	14	<1	6.0
Wartook site 2- (3,4)	6.67	16.6	3.2	5.2	38	16.0	22	<1	14.0
Wartook site 2- (5,6)	6.15	18.4	1.4	12.8	30	12.0	16	<1	10.0
Wartook site 2- (7,8)	6.12	23.3	1.6	14.4	42	10.0	22	<1	8.0
Wartook site 3- (1,2)	5.45	13.5	0.6	22.5	32	6.0	18	<1	4.0
Wartook site 3- (3,4)	5.60	18.4	0.8	23.1	36	8.0	20	<1	6.0
Wartook site 3- (5,6)	5.98	19.6	2.0	9.8	36	8.0	20	<1	6.0
Wartook site 3- (7,8)	5.53	37.9	2.1	18.0	54	8.0	30	2.8	6.0

Table 3. 21 Organic and inorganic data of soil extracts from samples collected from the West Gellibrand catchment in July 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N ratio	Cond [us/cm]	Bicarb. (mg/L)	TDS (by EC) mg/L	Na (mg/L)	Alkalinity $CaCO_3$ mg/L
West Gellibrand site 3- (1,2)	5.27	33.0			200	6.0	110.0	7.4	4.0
West Gellibrand site 3- (3,4)	5.63	21.2	2.9	7.2	212	6.0	116.0	8.4	4.0
West Gellibrand site 3- (5,6)	5.38	17.4	2.0	8.6	132	14.0	72.0	8.0	12.0
West Gellibrand site 3- (7,8)	5.43	36.4	2.5	14.6	114	8.0	62.0	4.2	6.0
West Gellibrand site 4- (1,2)	5.18	26.4	2.2	12.0	92	6.0	50.0	10.8	
West Gellibrand site 4- (3,4)	4.99	33.8	2.5	13.6	92	4.0	50.0	6.6	4.0
West Gellibrand site4- (5,6)	5.05	28.0	2.7	10.4	100	4.0	54.0	6.6	4.0
West Gellibrand site 4- (7,8)	4.97	27.8	2.8	9.9	82	2.0	44.0	6.8	2.0
West Gellibrand site 5- (1,2)	5.60	11.0	1.0	11.0	74	6.0	40.0	5.8	4.0
West Gellibrand site 5- (3,4)	5.83	7.6	0.6	13.6	36	6.0	20.0	2.6	4.0
West Gellibrand site 5- (5,6)	5.58	9.2	0.6	15.3	52	8.0	28.0	4.2	6.0
West Gellibrand site 5- (7,8)	5.95	10.2	0.7	14.6	50	8.0	28.0	4.0	6.0

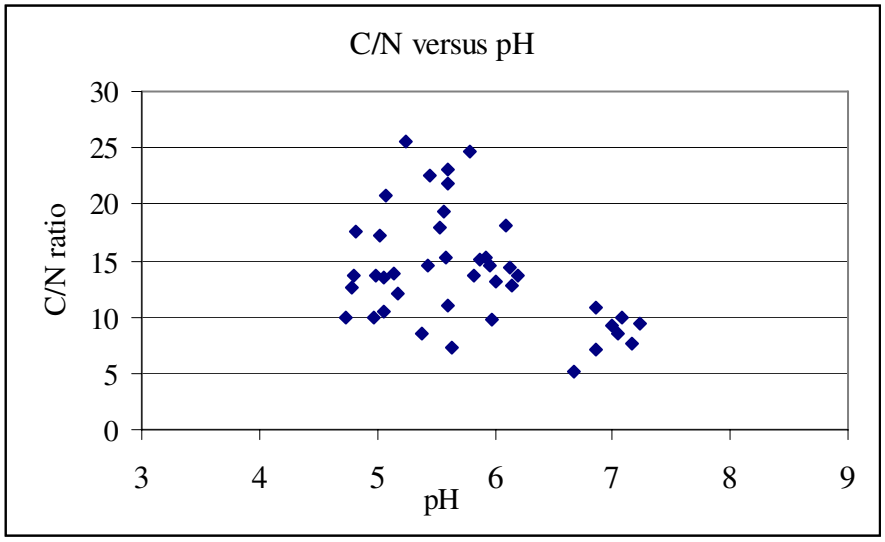


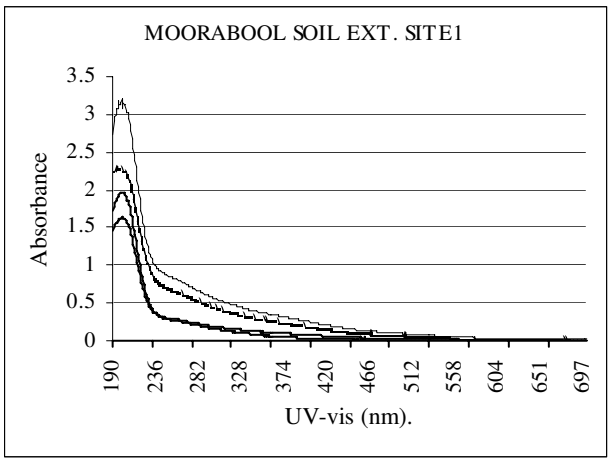
Figure 3. 13 Scatter plot of C/N ratios versus pH values of aqueous extracts from soil samples collected in July 1999.

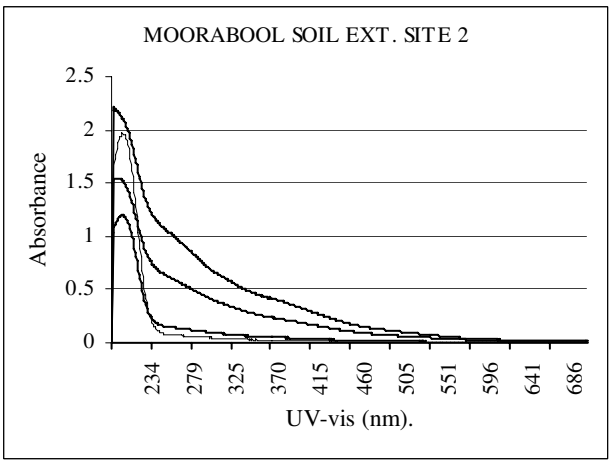
Graphs of UV-vis scans of soils extracts are shown in figures 3.14 (Moorabool), 3.15 (Wartook) and 3.16 (West Gellibrand). Scans of extracts of litter layers are shown in Figure 3.17. Differences can be seen on the curves within and between sites of Moorabool. Curves of samples from site 3 show steady reductions in absorbances while those of site 1 and 2 show rapid declines from ~ 190 nm to ~ 236 nm. These contrast with site 5 where there is a peak shoulder at about 260 nm. Curves of samples from Wartook (Figure 3.15) were similar between replicates from the same site and between sites. Differences in curves of samples from West Gellibrand were found in the same manner as for Moorabool. Replicates from site 1 showed rapid declines in absorbances between ~190 and 233 nm, contrasting with curves of samples from sites 4 and 5. This data is in agreement with data of C/N ratios in the context that both indicate variation in composition of organics between replicates, sampling sites and locations. The curves of litter layers were mostly similar between sites and locations. Summarised data of UV-vis scans are shown in tables 3.22 (soil litter layers), 3.23 (Moorabool soil extracts), 3.24 (Wartook soil extracts) and 3.25 (West Gellibrand soil extracts). Data of litter layer extracts were similar to each other, as might be expected from the similarities in UV-vis curve shapes of these samples. These however, contrasted with data of the soil samples in several ways,

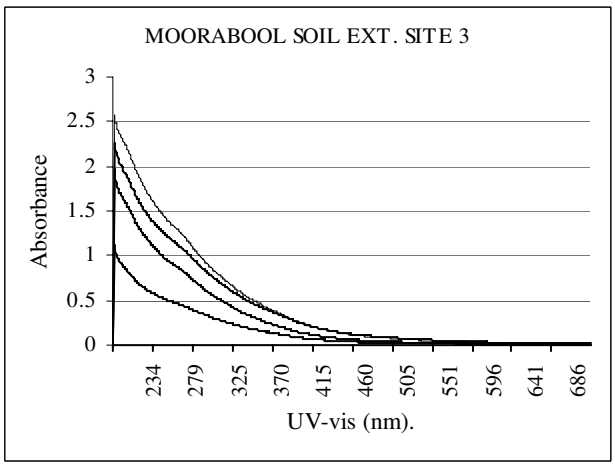
1. the colour/DOC of the litter layer extracts were lower than for soils,
2. E4/E6 data of the litter layers were, in most cases, higher than soils,
3. Ratios of 254nm/456nm were mostly much higher than for soils,
4. Ratios of (254nm x 456nm x 1000/DOC^2) were significantly lower for litter layers than for soils.

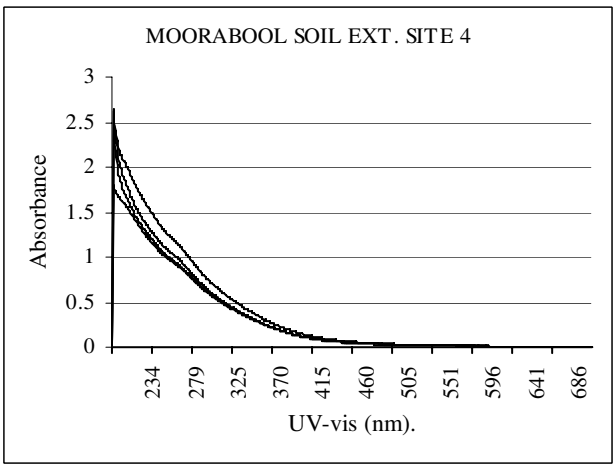
Interestingly, the data of Wartook site 3 and West Gellibrand site 4 (1,2) were similar to the data of the litter layers as were the general shapes of their UV-vis scans. Further work in this area could be performed to determine if the data of the UV-vis ratios can be related to other data such as THMFP or assimilable organic carbon contents.

For the soil samples, differences in UV-vis ratios were found between replicates and between sites, as can be seen from data of Moorabool where the ratios of (254nm x 456 nm x 1000/DOC^2) varied from 0.034 – 2.21. These ratios varied between 0.029 – 0.260 for Wartook samples and between 0.042 and 1.5 for West Gellibrand.









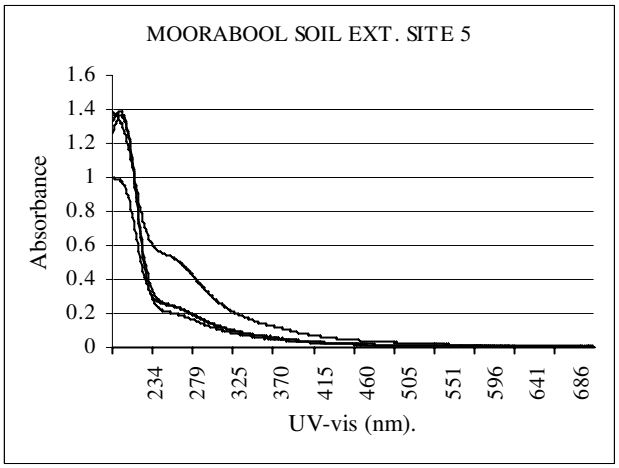


Figure 3. 14 UV-vis data (absorbance, y-axis versus wavelength, x-axis) of soil extracts of samples collected from the Moorabool catchment in July 1999.

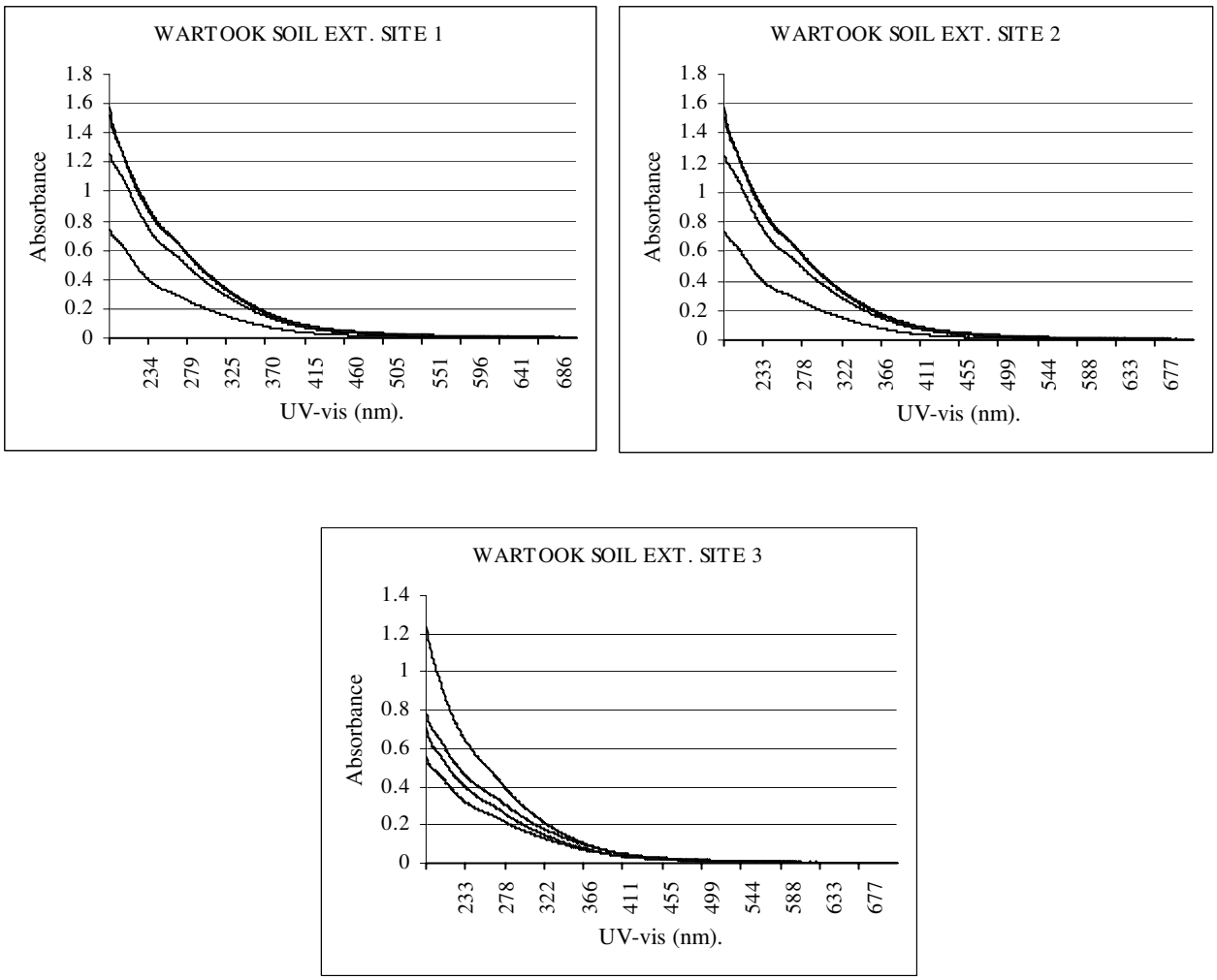


Figure 3. 15 UV-vis data (absorbance, y-axis versus wavelength, x-axis) of soil extracts of samples collected from the Wartook catchment in July 1999.

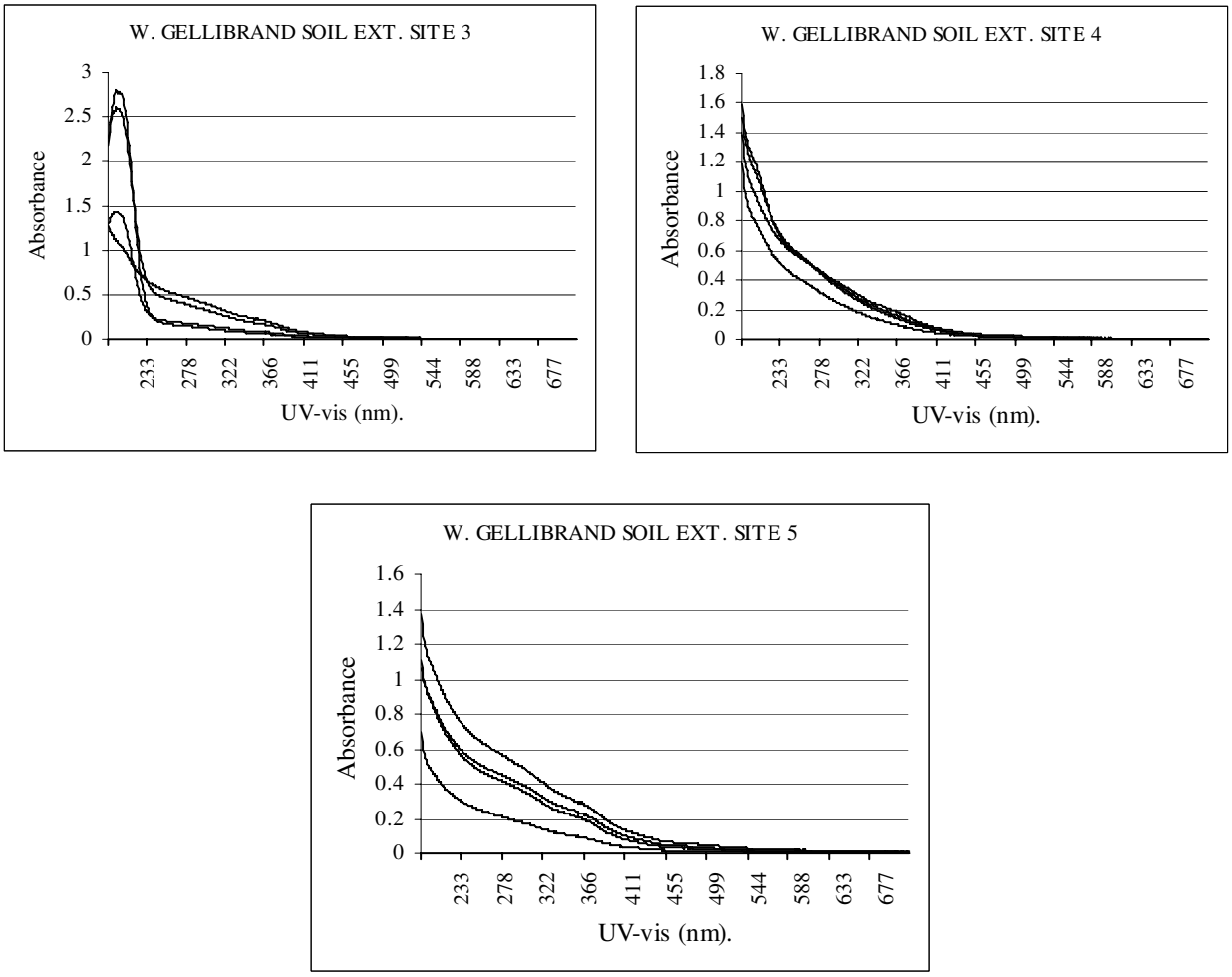
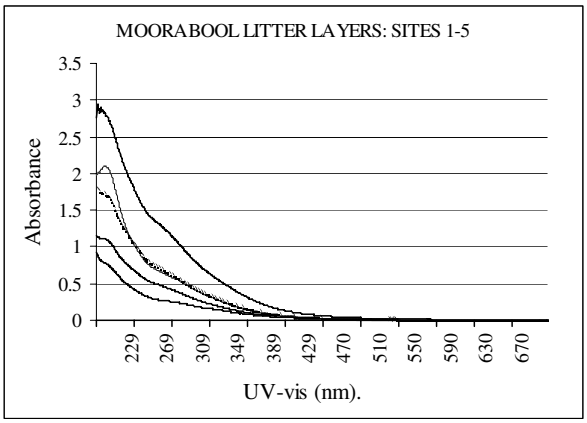
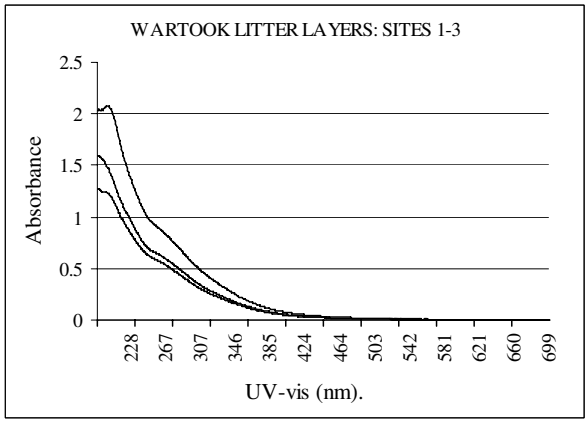


Figure 3. 16 UV-vis data (absorbance, y-axis versus wavelength, x-axis) of soil extracts of samples collected from the West Gellibrand catchment in July 1999.





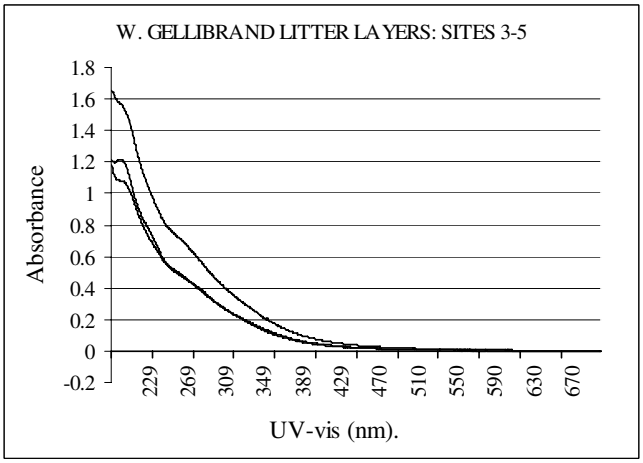


Figure 3. 17 UV-vis data of soil litter layer extracts of samples collected in July 1999.

Table 3. 22 UV-vis data of extracts of soil litter layers collected in July 1999.

Location/ parameter			Sites				
Moorabool	S1	S2	S3	S4	S5	Mean	SD
SUVA	3.35	3.39	3.06	3.21	2.29	3.06	0.448
Colour/DOC	0.749	0.865	0.726	0.840	0.944	0.825	0.089
E4/E6	11.0	10.1	8.6	13.9	5.2	9.7	3.20
254nm/456nm	32.8	28.4	30.5	27.4	17.8	27.4	5.75
(254nm*456nm*1000)/DOC^2	0.00034	0.00040	0.00031	0.00038	0.00030	0.0003	4.52E-05

Wartook	S1	S2	S3	Mean	SD
SUVA	2.77	2.35	2.59	2.57	0.210
Colour/DOC	0.645	0.545	0.572	0.587	0.052
E4/E6	10.0	9.5	12.1	10.5	1.37
254nm/456nm	31.0	31.4	33.0	31.8	1.05
(254nm*456nm*1000)/DOC^2	0.00025	0.00018	0.00020	0.0002	3.70E-05

W. Gellibrand	S1	S2	S3	Mean	SD
SUVA	3.07	2.77	4.25	3.36	0.784
Colour/DOC	0.883	0.738	1.008	0.876	0.135
E4/E6	12.7	13.5	8.6	11.6	2.60
254nm/456nm	25.1	27.3	30.8	27.7	2.89
(254nm*456nm*1000)/DOC^2	0.00037	0.00028	0.00059	0.0004	0.000157

Table 3. 23 UV-vis data of extracts of soil samples collected from the Moorabool catchment in July 1999.

Moorabool site 1	1,2	3,4	5,6	7,8	Mean	SD
SUVA	9.7	7.7	3.3	7.2	7.0	2.7
Colour/DOC	11.5	8.4	1.1	7.2	7.1	4.4
E4/E6	6.2	6.3	7.3	7.4	6.8	0.6
254nm/456nm	6.0	6.5	21.1	7.1	10.2	7.3
(254nm*456nm*1000)/DOC^2	1.5698	0.9075	0.0509	0.7329	0.8153	0.6242

Moorabool site 2	1,2	3,4	5,6	7,8	Mean	SD
SUVA	4.3	12.1	7.0	8.9	8.1	3.3
Colour/DOC	2.9	13.1	6.5	10.0	8.1	4.4
E4/E6	3.8	7.7	7.3	7.1	6.5	1.8
254nm/456nm	10.6	6.6	7.6	6.4	7.8	1.9
(254nm*456nm*1000)/DOC^2	0.1752	2.2143	0.6386	1.2481	1.0690	0.8809

Moorabool site 3	1,2	3,4	5,6	7,8	Mean	SD
SUVA	6.4	5.3	4.8	7.7	6.0	1.3
Colour/DOC	3.5	2.5	2.0	5.0	3.2	1.3
E4/E6	5.8	6.0	6.4	4.7	5.7	0.7
254nm/456nm	13.1	15.2	17.4	10.9	14.2	2.8
(254nm*456nm*1000)/DOC^2	0.3140	0.1833	0.1303	0.5397	0.2918	0.1824

Moorabool site 4	1,2	3,4	5,6	7,8	Mean	SD
SUVA	4.3	3.8	3.3	3.1	3.6	0.5
Colour/DOC	1.3	1.1	1.0	0.8	1.1	0.2
E4/E6	7.1	7.6	6.9	8.1	7.4	0.5
254nm/456nm	22.9	24.3	22.9	27.3	24.3	2.1
(254nm*456nm*1000)/DOC^2	0.0791	0.0606	0.0478	0.0344	0.0555	0.0190

Moorabool site 5	1,2	3,4	5,6	7,8	Mean	SD
SUVA	4.4	2.3	3.4	3.6	3.4	0.9
Colour/DOC	2.3	1.3	1.8	2.2	1.9	0.4
E4/E6	5.3	4.2	4.8	4.0	4.6	0.6
254nm/456nm	13.5	12.4	13.5	12.0	12.9	0.8
(254nm*456nm*1000)/DOC^2	0.1405	0.0425	0.0833	0.1071	0.0934	0.0412

Table 3. 24 UV-vis data of extracts of soil samples collected from the Wartook catchment in July 1999.

Wartook site 1	1,2	3,4	5,6	7,8	Mean	SD
SUVA	3.6	3.4	3.5	3.7	3.6	0.1
Colour/DOC	1.4	1.6	1.5	1.5	1.5	0.1
E4/E6	7.0	6.0	7.0	7.4	6.8	0.6
254nm/456nm	18.8	15.4	17.4	17.8	17.3	1.4
(254nm*456nm*1000)/DOC^2	0.0673	0.0747	0.0722	0.0769	0.0728	0.0041
Wartook site 2	**1,2**	**3,4**	**5,6**	**7,8**	**Mean**	**SD**
SUVA	5.8	5.0	5.2	4.2	5.1	0.7
Colour/DOC	3.2	2.6	2.9	1.7	2.6	0.6
E4/E6	5.2	6.6	5.4	7.6	6.2	1.1
254nm/456nm	13.2	14.1	12.9	17.6	14.4	2.2
(254nm*456nm*1000)/DOC^2	0.2603	0.1807	0.2065	0.0987	0.1866	0.06729
Wartook site 3	**1,2**	**3,4**	**5,6**	**7,8**	**Mean**	**SD**
SUVA	3.9	3.5	3.8	2.7	3.5	0.6
Colour/DOC	1.8	1.3	1.7	0.8	1.4	0.5
E4/E6	4.9	5.8	6.4	7.6	6.2	1.2
254nm/456nm	15.5	20.0	16.6	25.2	19.3	4.4
(254nm*456nm*1000)/DOC^2	0.0963	0.0598	0.0867	0.0290	0.0680	0.0302

Table 3. 25 UV-vis data of extracts of soil samples collected from the West Gellibrand catchment in July 1999.

W. Gellibrand site 3	1,2	3,4	5,6	7,8	Mean	SD
SUVA	5.5	3.3	4.7	5.9	4.9	1.2
Colour/DOC	2.1	1.3	2.3	2.4	2.0	0.5
E4/E6	10.4	6.4	7.8	15.3	10.0	3.9
254nm/456nm	19.0	17.6	14.4	17.5	17.1	1.9
(254nm*456nm*1000)/DOC^2	0.1606	0.0613	0.1552	0.2005	0.1444	0.0590

W. Gellibrand site 4	1,2	3,4	5,6	7,8	Mean	SD
SUVA	3.1	3.4	4.0	4.0	3.6	0.4
Colour/DOC	0.9	1.2	1.4	1.6	1.3	0.3
E4/E6	9.3	11.5	9.7	9.2	9.9	1.0
254nm/456nm	23.7	20.8	20.7	18.0	20.8	2.3
(254nm*456nm*1000)/DOC^2	0.0417	0.0562	0.0786	0.0863	0.0657	0.0205

W. Gellibrand site 5	1,2	3,4	5,6	7,8	Mean	SD
SUVA	11.6	13.3	5.4	9.2	9.9	3.4
Colour/DOC	7.9	8.1	2.3	4.3	5.6	2.9
E4/E6	5.4	6.3	8.4	9.6	7.4	1.9
254nm/456nm	10.4	11.7	17.2	15.4	13.7	3.2
(254nm*456nm*1000)/DOC^2	1.2909	1.5000	0.1630	0.5490	0.8757	0.6263

Summed data of DRIFT spectra of soil litter layers of each of the three catchments are shown in Table 3.26. Relative percentage heights of peaks at ~2970 and ~2930 WN/cm were very similar at 58 to 64. Samples from these locations had an absorbance peak at ~ 1720 WN/cm (carbonyl stretching of acids, esters, aldehydes and ketones) ranging from 70 to 83 %, though were not detected at all sites. Mean peaks heights at ~1610 WN/cm (carboxylate band; C=C) were also similar between locations, ranging from (110 to 120). Variations in peak heights of ~1610 wm/cm between sites and within locations were higher eg. for Moorabool (98 to 121), indicating sampling site specificity in relation to DRIFT data. The peak height for the grass site was lower than for the other vegetation types. The lower absorbance at ~16010 WN/cm was also a general feature for samples collected previously and also for soil organics. The higher absorbances of the treed sites may be due to greater relative proportions of aromatics resulting in C=C stretching. Greater differences in mean values for the peak at ~1400 WN/cm (CH deformation) was found, ranging from 94 to 115, though site variation within location was even higher, ie 98 to 145 for West Gellibrand. The grass site from Moorabool also tended to have a low absorbance at ~1400 WN/cm. Absorbance peak heights of samples from the Lake Wartook catchment taken from different sites generally showed the greatest similarity. These sites also have very similar vegetation.

Data of DRIFT spectra of soil extracts from Moorabool (sites 1 to 3), Moorabool (sites 4 and 5), Wartook and West Gellibrand are shown in Tables 3.27, 3.28, 3.29 and 3.30, respectively. Summed data of these spectra are shown in Table 3.31. The high variations

in many peak heights between replicates of sites, between sites with similar vegetation and between locations indicate the presence of other impacting factors on spectra which preclude differentiation or identification of samples based on these categories alone. Inorganic compounds extracted from the soils are also likely to impact on the DRIFT spectra. Differences in spectra of the various treed sites was not evident.

Nonetheless, the feature of relatively low absorbance bands at ~ 1600 and ~1400 WN/cm was found for the Moorabool grass site, indicating that this might be a distinguishing feature from treed sites, both in litter layers and in soils.

Table 3. 26 DRIFT spectral data of litter layer extracts of samples collected in July 1999.

			Sites			
	Douglas fir	California redwood	*P. radiata*	Native trees	grasses	
Moorabool	S1	S2	S3	S4	S5	Mean
3767-3777	0	0	0	0	0	0
3381-3408	100	100	100	100	100	100
2968-2979	62		60	63	59	61
2930-2947	63	65	64	63	63	64
2127-2138			6	5	5	5
1904-1958	0	0	0	0	0	0
1719-1720			70	82		76
1600-1616	119	121	101	117	98	111
1394-1421	104	108	86	99	90	97
1241-1269	77	79	72	77	72	75
1219-1231	74	75				75
1133-1144	82	88	79	81	109	88
1068-1078	87	98	90	89		91
796-802	45			44		45

	eucalypts	eucalypts	eucalypts	
Wartook	S1	S2	S3	Mean
3761-3777	0	0	0	0
3370-3403	100	100	100	100
2963-2974	58	58	59	58
2925-2936	64	62	63	63
2148-2165	4	4	4	4
1926-1964	0	0	0	0
1720-1725	77	72	77	75
1611-1616	108	112	109	110
1394-1399	93	95	94	94
1252-1263	78	77	81	79
1128-1144	87	86	84	86
1062-1073	99	106	99	101
796-807	33	34	36	34

	Native tree	Native tree	*P. radiata*	
W. Gellibrand	S3	S4	S5	Mean
3772-3777	0	0	0	0
3381-3397	100	100	100	100
2963-2974	64	62	63	63
2936	64	62	65	64
2154-2192	6	5	6	6
1931-1948	0	0	0	0
1714-1725	83	80	77	80
1600-1611	123	116	121	120
1399-1410	145	98	102	115
1258-1269	76	74	82	77
1225			77	77
1122-1139	82	81	87	83
1073-1078	88		94	91
796-802	44	40	43	42

Table 3. 27 DRIFT spectral data of extracts of soil samples collected from Moorabool sites 1 to 3, in July 1999.

Moorabool S1		Sites		
Douglas fir	1,2	3,4	5,6	7,8
3805-3907	0	0	0	0
3395-3417	100	100	100	100
2961-2974	60	61	57	57
2917-2930	58	58	56	53
1931-1950	0	0	0	0
1626-1642	77	82	92	77
1400-1433	83	80	82	75
1356		82		
1104-1142	112	77		103
1038-1062	87		91	93

Moorabool S2				
Calif. redwood	1,2	3,4	5,6	7,8
3794-3929	0	0	0	0
3362-3397	100	100	100	100
2961-2966			48	62
2925				58
1928-1988	0	0	0	
1627-1631		72	76	87
1410-1428	110	78	100	87
1345	111			
1095-1141	61	113	82	121
1035-1038		116	90	142

Moorabool S3				
P. radiata	1,2	3,4	5,6	7,8
3788-3826	0	0	0	0
3381-3419	100	100	100	100
2957-2968	65	64	60	69
2914-2930	61	59	58	64
1991-2072	0	0	0	0
1600-1627	107	107	111	86
1399-1410	78	84	85	62
1111-1128	134	151	109	100
1024-1046	158	138	101	112

Table 3. 28 DRIFT spectral data of extracts of soil samples collected from Moorabool sites 4 and 5, in July 1999.

Moorabool S4				
Native trees	1,2	3,4	5,6	7,8
3788-3805	0	0	0	0
3370-3386	100	100	100	100
2952-2979	62	58	64	60
2924-2936	62	53	61	
1937-1980	0	0	0	0
1605-1616	113	116	92	90
1399-1415	88	87	75	76
1084-1133		107	86	95
1041-1046	117	90	90	88

Moorabool S5				
P. radiata	1,2	3,4	5,6	7,8
3832-3843	0	0	0	0
3370-3402	100	100	100	100
2947			45	
2920-2925	45	51		39
1937-2012	0	0	0	0
1633-1643	73	60	62	64
1356-1372	70	71	70	76
1084-1106	127	122	97	94
1024-1035	143	131	99	95

Table 3. 29 DRIFT spectral data of extracts of soil samples collected from Wartook, in July 1999.

All eucalypts		Sites		
Wartook S1	1,2	3,4	5,6	7,8
3777-3799	0	0	0	0
3359-3381	100	100	100	100
2920-2930	66	65	64	61
1953-1991	0	0	0	0
1611-1633	97	90	94	107
1388-1405	81	71	78	88
1030-1041	132	132	112	103
Wartook S2	1,2	3,4	5,6	7,8
3777-3853	0	0	0	0
3213-3386	100	100	100	100
2968-2963	61		65	65
2925-2940	58		64	65
1910-2143	0	0	0	0
1627-1649	87	76	92	69
1405-1421	68	83	66	51
1035-1046	180	165	200	91
Wartook S3	1,2	3,4	5,6	7,8
3777-3799	0	0	0	0
3381-3403	100	100	100	100
2957-2963	61	61	60	42
2925-2941	60	62	61	62
1942-2018	0	0	0	0
1600-1621	102	108	111	104
1399-1405	87	91	84	87
1030-1046	121	100	111	92

Table 3. 30 DRIFT spectral data of extracts of soil samples collected from West Gellibrand, in July 1999.

W. Gellibrand S3		Sites		
Native trees	1,2	3,4	5,6	7,8
3783-3842	0	0	0	0
3300-3359	100	100	100	100
2952-2963	63		61	70
1914-2268	0	0	0	0
1616-1638	75	78	78	100
1404-1420		113		85
1361-1388		124	95	
1095-1106	98	64	100	130
1024-1057	115	64	106	146
905-921	86		53	90

W. Gellibrand S4				
Native trees	1,2	3,4	5,6	7,8
3771-3821	0	0	0	0
3370-3386	100	100	100	100
2968-2985	59	63	64	59
2925-2936	57	64	61	58
1931-2002	0	0	0	0
1611-1627	95	91	94	88
1405-1415	77	77		73
1100-1106	97	108	96	103
1035-1041	95	120	102	109
915-921	42		52	71

W. Gellibrand S5				
P. radiata	1,2	3,4	5,6	7,8
3772-3799	0	0	0	0
3288-3365	100	100	100	100
2963-2985	67	74	62	63
2924-2936	64	54	63	62
2040-2241	0	0	0	0
1605-1633	97	99	81	82
1405-1415	94	91	69	69
1111	180	168	121	126
1030-1035	169	171	125	130
910-916	92	101	61	69

Table 3. 31 Summary data of DRIFT spectra of extracts of soil samples collected in July 1999.

(Mean and standard deviations (n=4) of DRIFT peak heights of soil extracts between the spectral regions of 1600 – 905).

Moorabool	Mean s1	S.D. s1	Mean s2	S.D. s2	Mean s3	S.D. s3	Mean s4	S.D. s4	Mean s5	S.D. s5	Total Mean
1605-1643	82	7.3	78	7.8	103	11.4	103	13.5	65	5.9	86
1356-1433	80	3.8	94	14.0	77	10.4	81	6.8	72	3.0	81
1084-1142	97	18.1	94	28.0	124	23.4	96	10.6	110	16.8	104
1024-1062	90	3.0	116	25.9	127	25.7	96	13.9	117	24.0	109

Wartook	Mean s1	S.D. s1	Mean s2	S.D. s2	Mean s3	S.D. s3	Total Mean
1600-1649	97	7.0	81	10.7	106	4.2	95
1388-1421	79	6.9	67	13.4	88	2.7	78
1030-1046	120	14.5	159	47.7	106	12.8	128

W. Gellibrand.	Mean s3	S.D. s3	Mean s4	S.D. s4	Mean s5	S.D. s5	Total Mean
1605-1638	83	11.6	92	3.4	90	9.4	88
1404-1420	99		76	2.6	81	13.7	85
1361-1388	110						110
1095-1111	98	26.9	101	5.3	149	29.9	116
1024-1057	108	33.5	107	10.7	149	24.6	121
905-921	76	20.1	55	14.6	81	18.7	71

3.4 Conclusions:

Distinctive differences were found in UV-vis data in relation to vegetation and sample type. The 254nm/456nm ratio tended to be in the order of ; reservoir~litter layer>soils. The mean ratio (254nm x 456nm x1000 / DOC^2) values appeared to have the following order: soils>reservoir~litter layer. These ratios may be interpreted on the basis of the degree of biodegradation of organics and a measure of humification. This ratio of organics from a grass site tended to have lower values than those of treed sites, indicating less lignin and/or tannic acid input to NOM.

DRIFT spectroscopy of organics extracted from soils and litter layers from Moorabool, Wartook and West Gellibrand catchments indicate relative differences in chemical functionality, particularly at about 1610 WN/cm and 1400 WN/cm. These appear to be due to differences in vegetation. The grass site from Moorabool tended to have the lowest absorbance bands at the above two wavelengths, reflecting lower aromaticity. Absorbances at these wavelengths, particularly at ~ 1610 WN/cm may be distinguishing features between grass and treed sites.

CHAPTER 4

SURVEY OF NATURAL ORGANIC MATTER IN SOILS AND WATERS OF MT BOLD AND MYPONGA, SOUTH AUSTRALIA.

4.1 Introduction

In this chapter is described the results of surveys performed to assess the diversity of natural organic matter in raw surface waters, in soils and in soil litter layers in South Australia. This data is an extension of that presented in Chapter 3. Its partitioning is somewhat arbitrary and was done in an attempt to simplify data compilation and reading.

The main aim in the collection of data presented here was to examine the diversity in the characters of NOM in catchment soils and waters over various climatic conditions in South Australia. Comparison could then be made to NOM isolates from other sources such as from Victoria, as described in Chapter 3. Potential comparisons could be made on the basis of geographical distance differences, vegetation in the catchment and season/climate differences.

4.2 Methods

Techniques used for the characterisation of NOM in the work reported here were predominantly UV-vis spectrometry and infrared analyses (DRIFT). Dissolved organic carbon and total Kjeldhal nitrogen were also determined and from these an organic carbon to nitrogen ratio (C/N) was calculated. Physico-chemical analyses performed on soil sample extracts also included pH measurement, metals (eg Al, Fe, Mg), total dissolved solids and alkalinity.

Data obtained varied between the three collections, for example, DOC analysis was not performed on any samples collected in October 1998. The basis for this was to implement an initial rapid and inexpensive survey procedure to assess the potential diversity in the character of NOM, to be followed by more detailed examinations.

The sampling locations, sampling sites and sampling methods are described in Chapter2. The procedures used for isolation of NOM from soils and litter layers and the analytical methods applied to extracts and Nom isolates are also described in Chapter 2.

4.3 Results and discussion

4.3.1 Organic and inorganic chemistry data of NOM isolates collected in October 1998.

In October 1998, the analyses performed on soil extracts and water samples were UV-vis scans and pH measurements. UV-vis data of Mt Bold and Myponga soil samples collected in at that time are shown in tables 4.1 and 4.2, respectively.

At Mt Bold, the 254nm/456nm ratios of samples from the top of the slope were greater than those from the bottom site. This may be due to the organics at the top of the slope being less bio-degraded overall, due to lower average residence times in the soils, compared with organics transported through the soil and present in the middle and bottom of the slope ie. the organics are comprised of a greater proportion of components such as carbohydrates, proteins, fatty acids and others which impart little or no colour, ie. humification is lower. This data correlates with that of soil litter and soils extract samples from Victoria, where it was found that this ratio was greater for litter compared with soil extracts. It would be expected that the overall degree of biodegradation would be higher in soils than in the litter layer. Data of this ratio for samples collected from the middle of the slope tended to vary with two, one on the right side and one in the centre of slope, consistent with the trend (top versus bottom of slope) while the sample from the left side was inconsistent. E4/E6 ratios tended to follow the trends of the 254nm/456nm ratios.

The 0 and A-horizons of soils in the Mt Bold catchment study site are generally likely to have greater impact on the character of organics than the corresponding horizons in the Myponga site due to differences in soil types ie clayey versus sandy loam, respectively. Sandy soils have less holding capacity for organics than clay soils ie. the greater cation concentration in clay soils form more ligands with the negatively charged functionalities of organics. Hence, where only the top soil layer is studied (as was the case here, A horizon, top 10-15 cm), and it is sandy then less difference should occur between the top and bottom of a slope. Where the B-horizon is of a higher clay content, organics predominantly move through the soil until they reach the A/B interface and are then transported along this interface. Table 4.2 gives UV-vis data of A- horizon soil extracts from Myponga, and as might be expected no trend in organic character change was evident. Further studies on this issue for this location might be extended to examine the B-horizon, C-horizon and B/C interface.

The pH levels of soil extracts and water samples are shown in Figure 4.1**.** Most pH values were between 6 and 7, except for samples collected from a pond and sludges that were between 7.5 and 8. Differences in these pH values are likely to be due to a greater concentration of carbonates/bicarbonates in the pond site and sludges. A speculative possibility but needing further investigation, is that the construction material used for the road raised the pH of the pond water.

DRIFT spectra of extracts of soils collected October 1998 are shown in figures 4.2 to 4.17. Spectra of individual sample extracts are shown for visual comparison only, of variations within and between sampling sites. The results of previous analyses of spectra of samples collected from Victoria were judged to not warrant numerical evaluation of the South Australian samples, for the purposes of this report and in the context of the large number of spectra taken.

General key features of the spectra as follows:
1. a broad peak at about 3300 to 3400 WN/cm, attributed to O-H stretching,
2. one or two shoulder peaks between 2900 and 3000 WN/cm, attributed to CH_2 and CH_3 stretching,
3. three prominent peaks at about 1650 WN/cm, 1450 WN/cm and 1100 WN/cm, attributed to 1. C=C and/or carbonyl band and/or primary amide, 2. C=C, CH of aliphatics, C-O of carbohydrates, 3. carbohydrates, respectively,

where assignments are made on the assumption that peaks are predominantly due to organics.

Visual inspection of DRIFT spectra of extracts of soil samples from Mt Bold (top-left, Fig. 4.2 and middle-left, Fig. 4.3) indicates differences in the relative peak ratios between 1000 and 1650 WN/cm. These differences within samples (eg. Fig. 4.3 ML1 and ML5) would seem to preclude determination of any difference between top, middle and bottom samples. Figure 4.4 shows the similarity of lower left samples, perhaps indicating less variation in the character of organics at this site/slope location.

The sample collected from Mt Bold – centre transect (Fig. 4.5 to 4.7) also showed variations in relative peak heights between 1650 to 1000 WN/cm, and in the presence of a smaller peak at about 1230 WN/cm (eg. Fig. 4.6, MCG-5, middle-centre; possibly due to carbonic acids, aliphatic alcohols, aryl ethers).

In contrast to the lower-left site, the spectra of the lower-centre differed between the samples collected, again indicating that variation within specific sites precludes determination of differences between the sites. Further, a single sample DRIFT spectrum may be unrepresentative of the general functionalities of organics at a specific site.

DRIFT spectra of soil extracts from the Mt Bold right transect are shown in figures 4.8, 4.9 and 4.10. Spectra of the extracts from the middle and bottom transects are similar as indicated by them being overlayed. Some differences can be seen in the comparative heights of the peak at ~ 1050 WN/cm in samples from the top-right, a shoulder peak at ~1550 WN/cm and a small peak at ~1200 WN/cm. Spectra of the pond samples (Figure 4.11) differed to those of the soil extracts by having (in 3 of 5 samples) a large peak at ~ 1150 WN/cm, particularly P1 and P5. This peak may be due to carbohydrates, being in greater relative abundance in the receiving water of the soil permeates. This may be due to high production of autochthonous organics via high microbial activities.

DRIFT spectra of extracts of soil samples collected from the Myponga catchment in October 1998 are shown in figures 4.12 to 4.17. Spectra of both Series 1 and Series 2 transects appeared similar with some differences in the peak at ~ 1150 versus 1050 WN/cm, (eg. Figure 4.17, M-RB5 and M-RB2). This may be attributed to differences in relative abundances of C-C of aliphatic, aromatic CH groups, phospholipids and carbohydrates. In this case the bottom site appears to show the greatest variation in relative peak heights and in peak numbers.

Table 4. 1: UV-vis data of extracts of Mt Bold soil samples collected in October 1998.

Location	BR Mean	BR SD	MR Mean	MR SD	TR Mean	TR SD
254nm/456nm	14.7	2.74	15.1	0.86	17.9	2.07
E4/E6	5.70	1.22	6.70	0.67	8.67	1.41

Location	LC Mean	LC SD	MCG Mean	MCG SD	TC Mean	TC SD
254nm/456nm	14.0	1.27	16.5	1.28	17.7	1.79
E4/E6	5.72	1.28	8.86	1.03	8.56	1.52

Location	LL Mean	LL SD	ML Mean	ML SD	TL Mean	TL SD	TGL Mean	TGL SD
254nm/456nm	16.1	1.09	14.3	0.83	18.4	1.48	16.0	3.77
E4/E6	7.07	0.31	6.24	0.64	8.38	1.82	10.87	2.45

Location	P Mean	P SD	road sludge	road sludge	reeds sludge	tree sludge
254nm/456nm	17.7	3.55	14.8	17.4	20.3	18.8
E4/E6	8.62	1.50	6.31	9.85	6.93	n.a.

B: bottom	R: right	T: top	G:gum
L: lower/left	C: centre	M:middle	P:pond

Table 4. 2: UV-vis data of extracts of Myponga soil samples collected in October 1998.

Location	LB Mean	LB SD	LC Mean	LC SD	LT Mean	LT SD	LTG Mean	LTG SD
254nm/456nm	16.0	1.47	14.7	1.18	14.2	1.35	15.7	1.37
E4/E6	6.57	0.89	5.74	0.72	6.21	1.29	6.30	1.15

Location	RB Mean	RB SD	RC Mean	RC SD	RT Mean	RT SD
254nm/456nm	14.8	0.73	15.1	0.98	14.2	1.81
E4/E6	4.47	0.59	6.83	1.02	5.68	1.79

L: left
B:bottom
C:centre
T:top
R:right

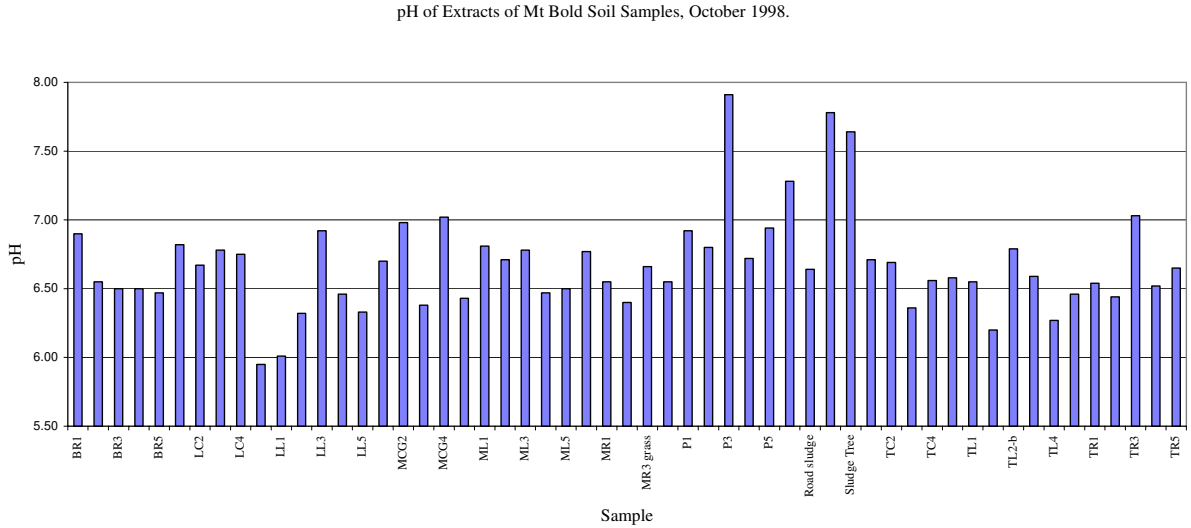


Figure 4. 1: pH levels of extracts of soil samples collected in October 1998. (Codes: B: bottom R: right, T: top, L: lower/left, C: centre, M:middle, G:gum, P: pond, numbers are sample replicate).

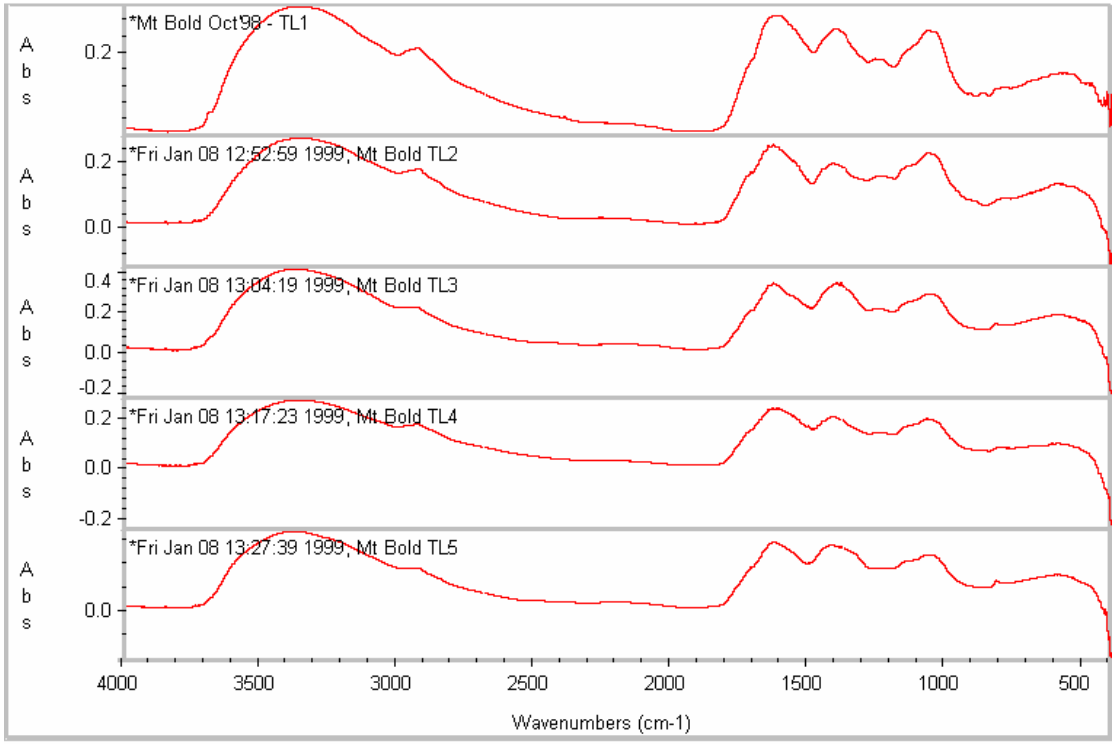


Figure 4. 2 DRIFT spectra of Mt Bold soil samples (top-left) collected in October 1998**.**

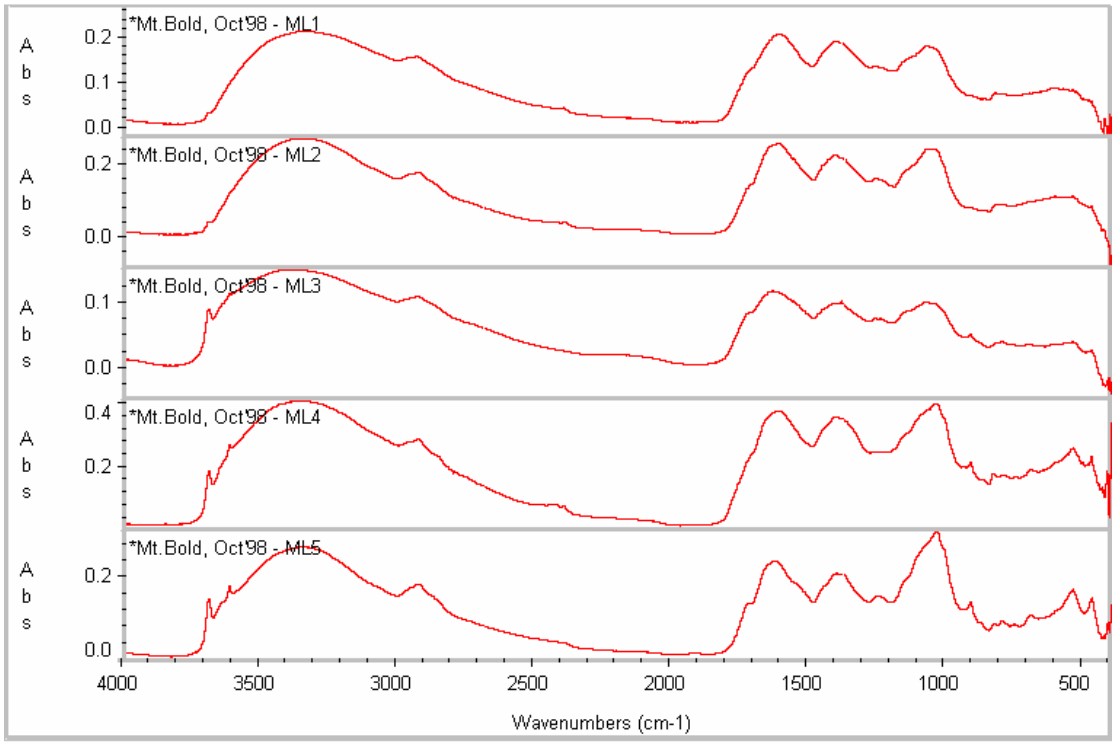


Figure 4. 3 DRIFT spectra of Mt Bold soil samples (middle-left) collected in October 1998.

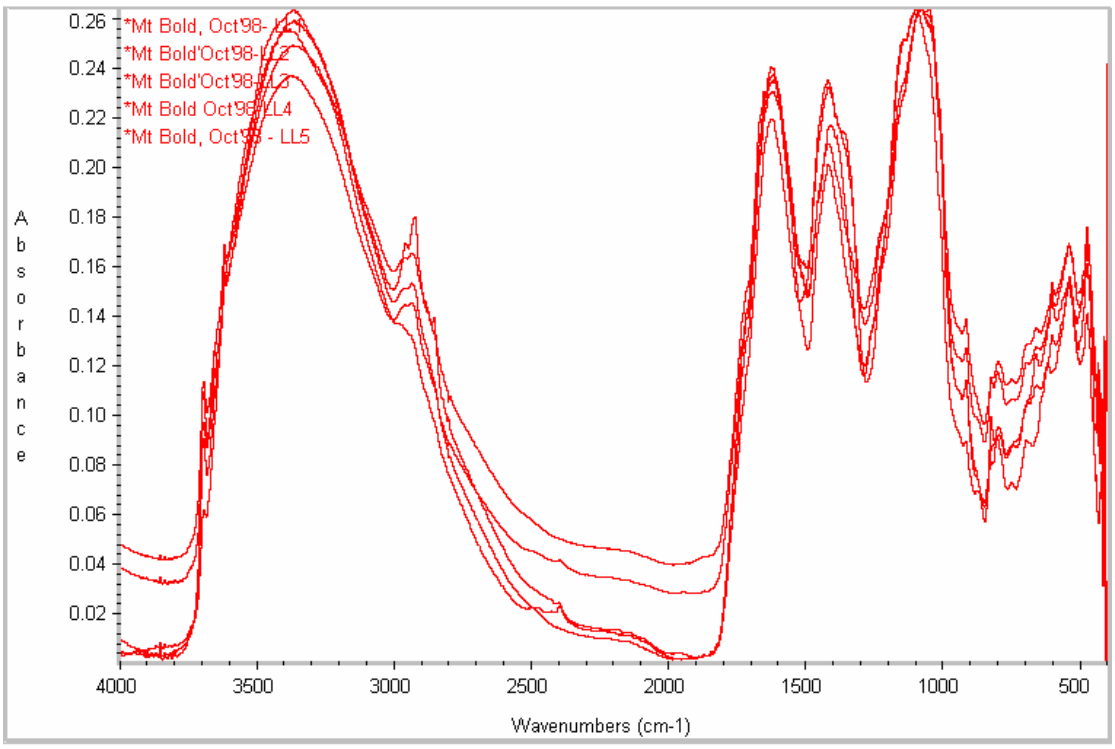


Figure 4. 4 DRIFT spectra of Mt Bold soil samples (lower-left) collected in October 1998.

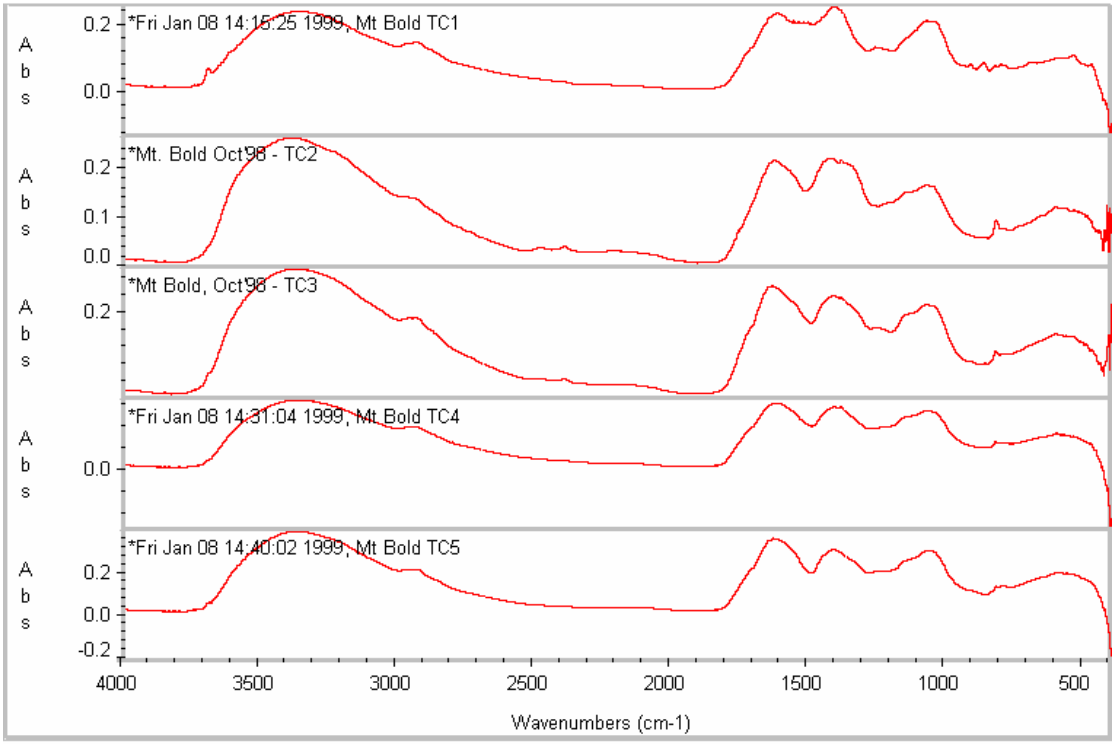


Figure 4. 5 DRIFT spectra of Mt Bold soil samples (top-centre) collected in October 1998.

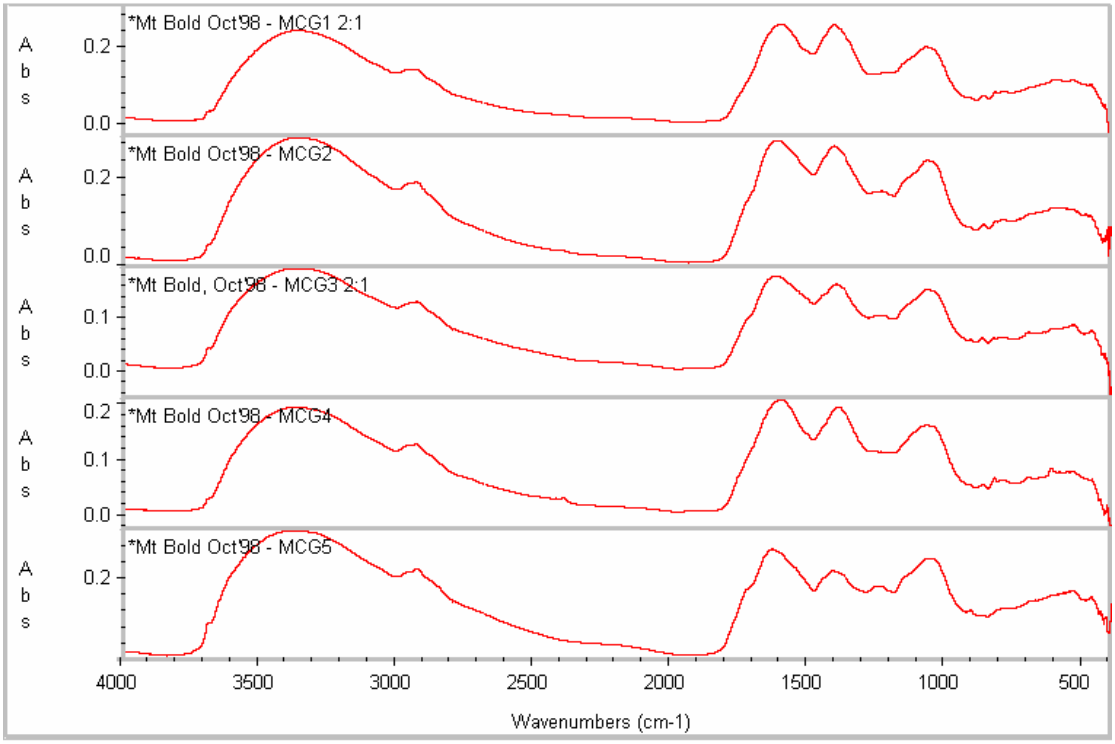


Figure 4. 6 DRIFT spectra of Mt Bold soil samples (middle-centre) collected in October 1998.

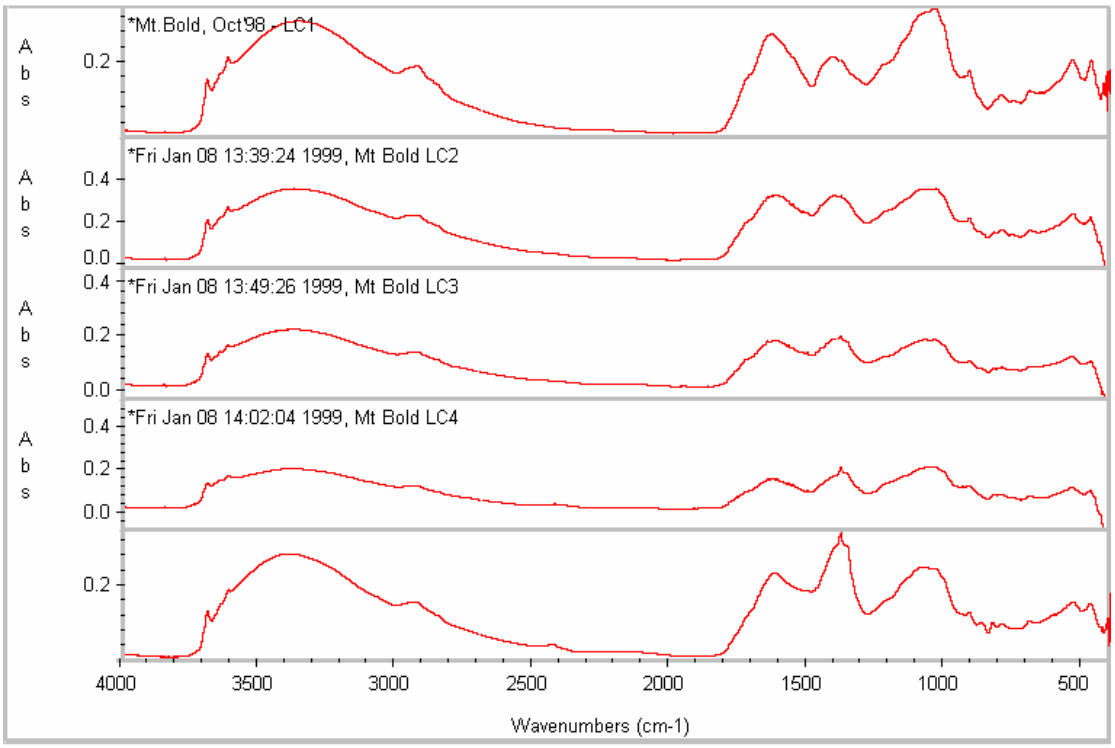


Figure 4. 7 DRIFT spectra of Mt Bold soil samples (lower-centre) collected in October 1998.

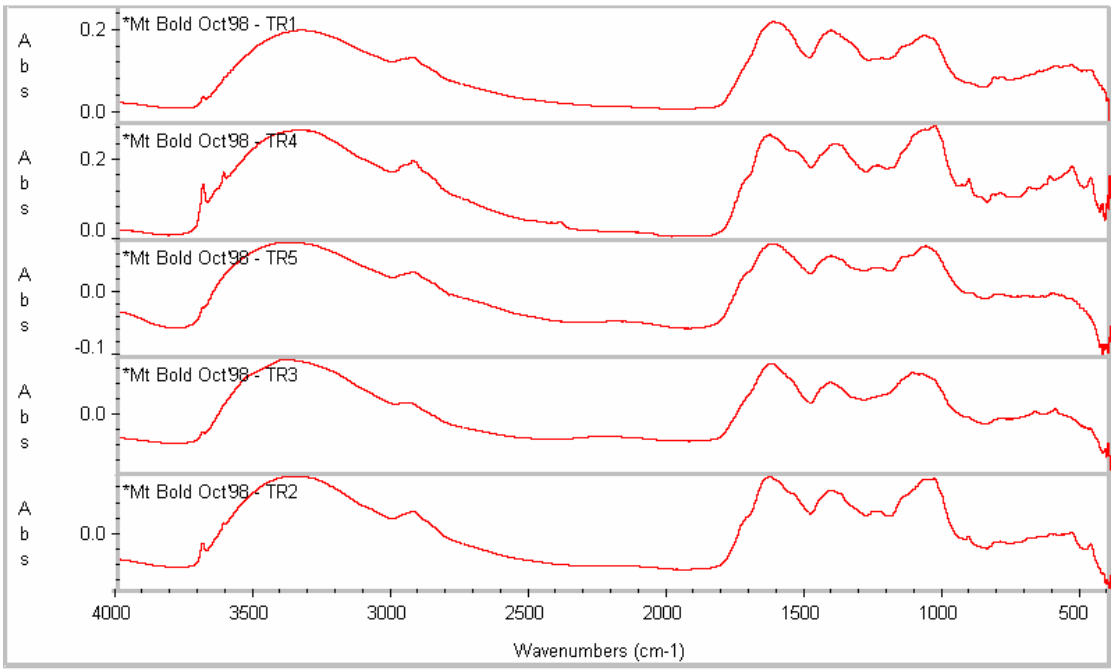


Figure 4. 8 DRIFT spectra of Mt Bold soil samples (top-right) collected in October 1998.

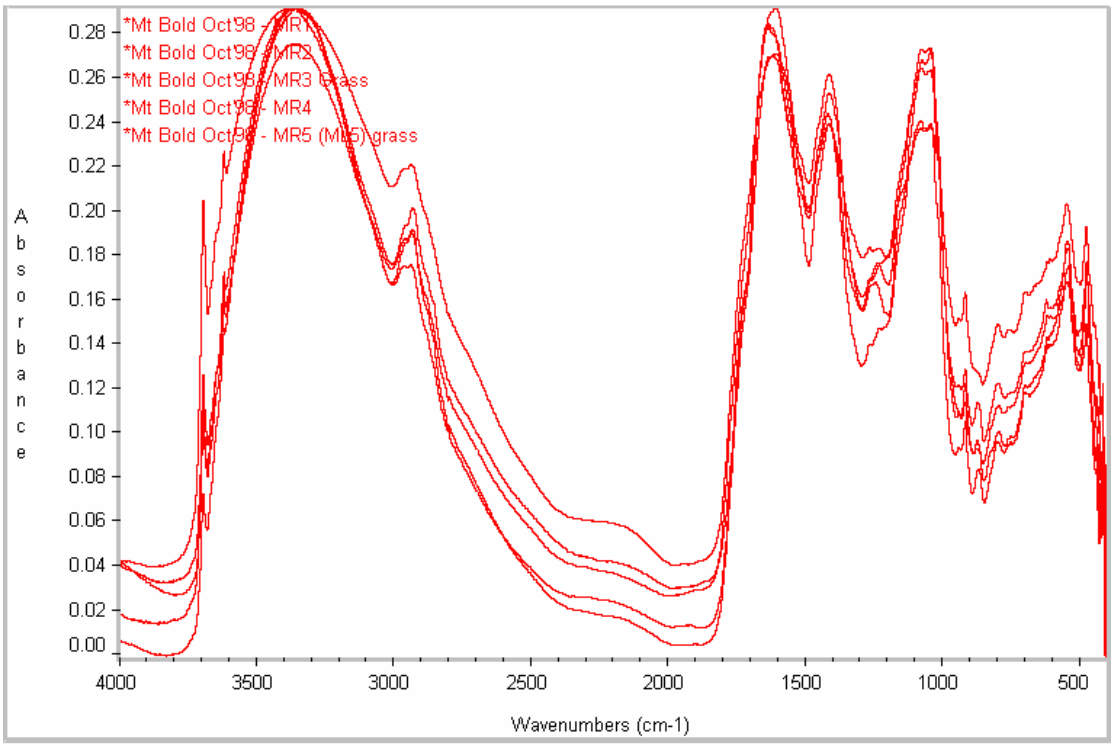


Figure 4. 9 DRIFT spectra of Mt Bold soil samples (middle-right) collected in October 1998.

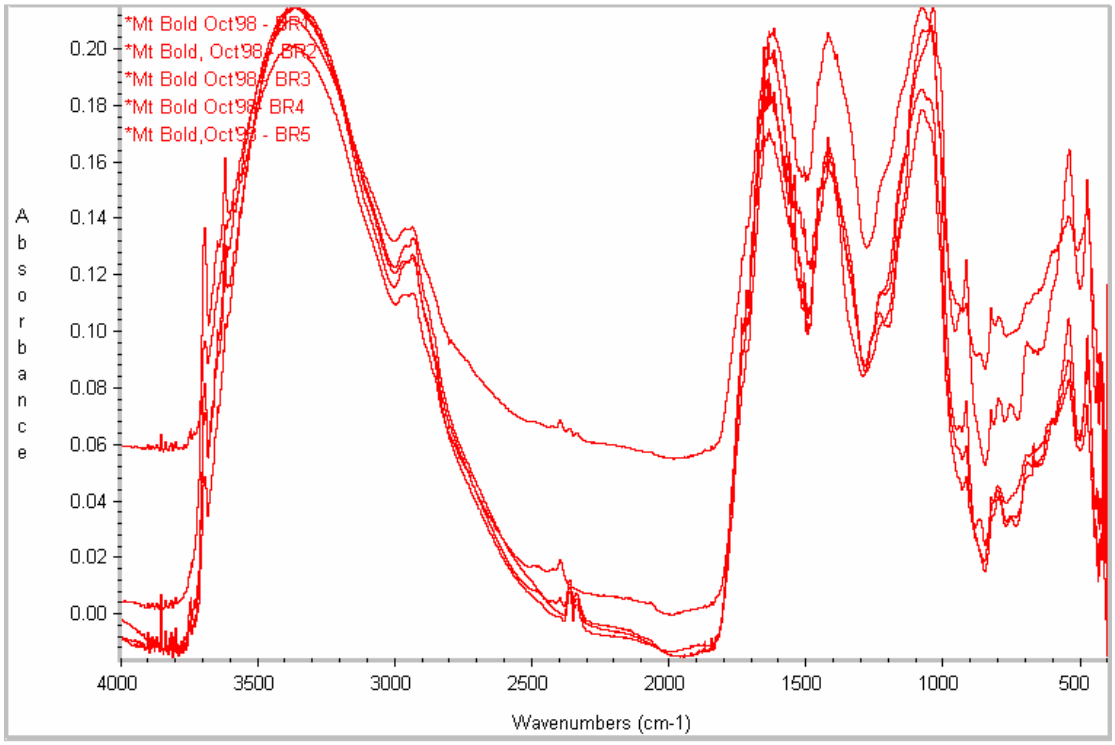


Figure 4. 10 DRIFT spectra of Mt Bold soil samples (bottom-right) collected in October 1998.

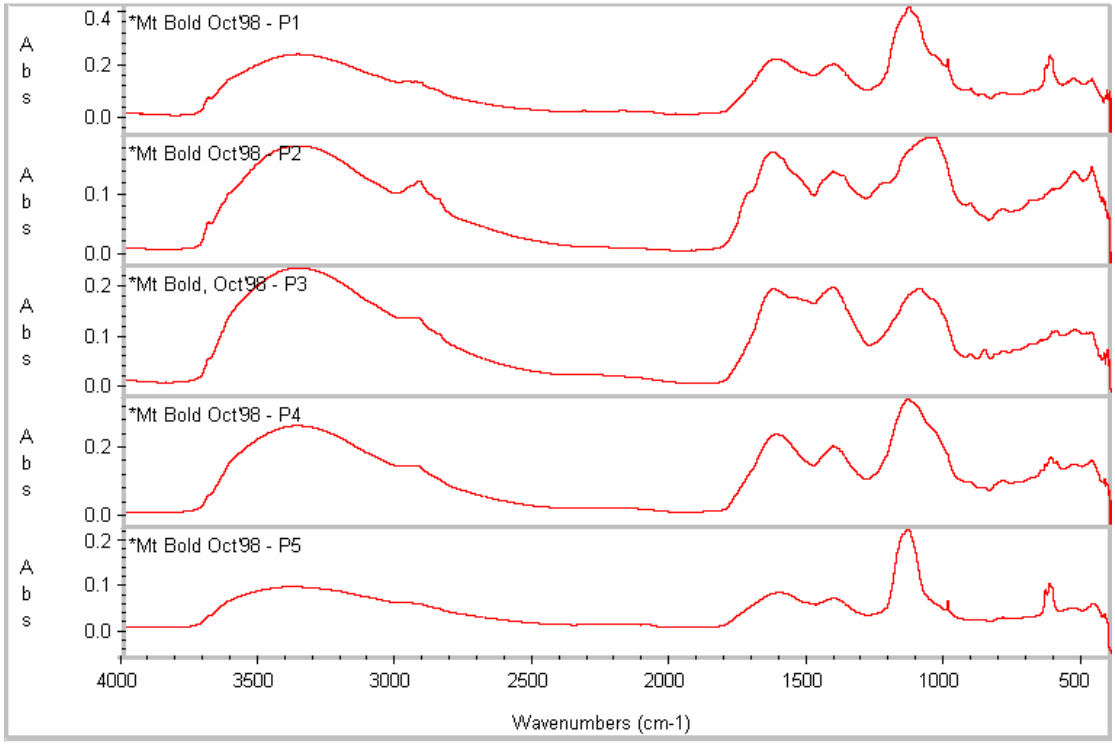


Figure 4. 11 DRIFT spectra of pond samples from Mt Bold catchment collected in October 1998.

DRIFT spectra of Myponga soil samples collected in October 1998.

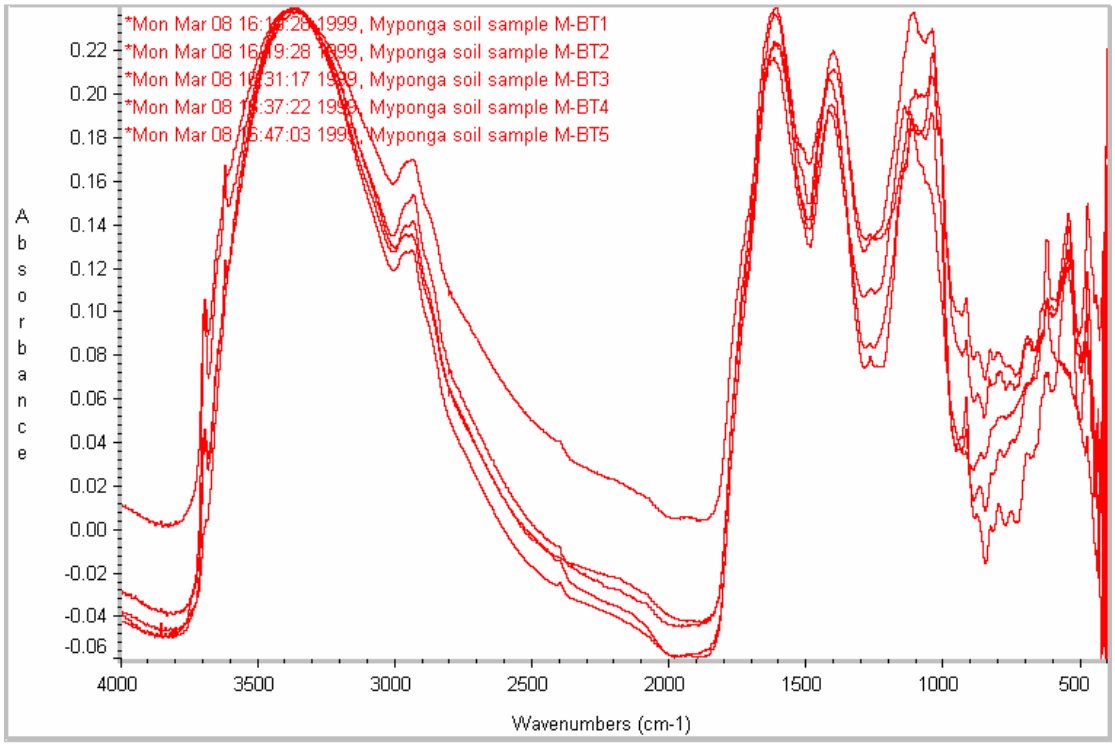


Figure 4. 12 DRIFT spectra of Myponga soil samples (Series 1, top, October 1998).

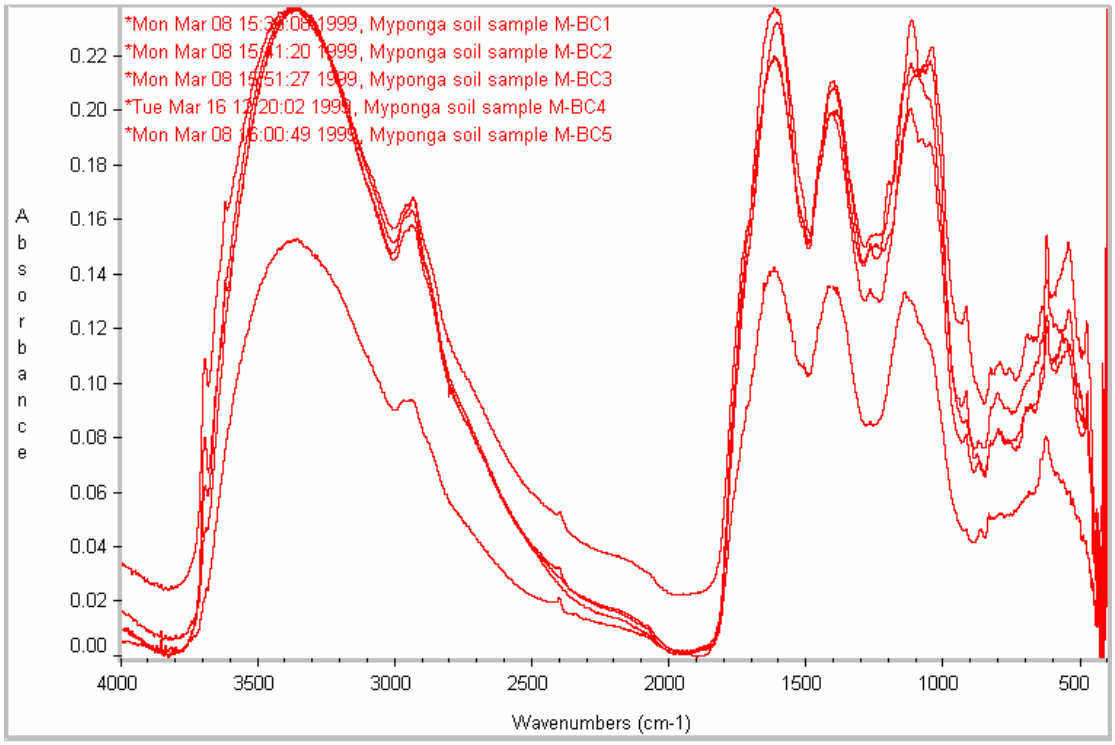


Figure 4. 13 DRIFT spectra of Myponga soil samples (Series 1, centre, October 1998).

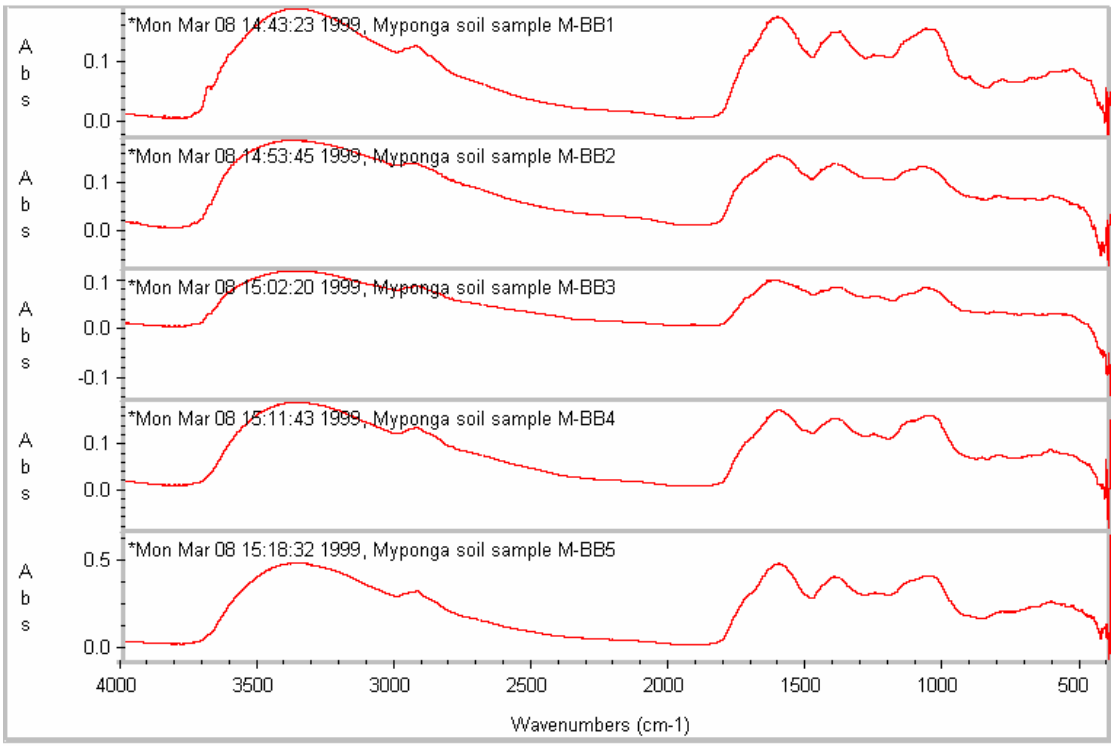


Figure 4. 14 DRIFT spectra of Myponga soil samples (Series 1, bottom, October 1998).

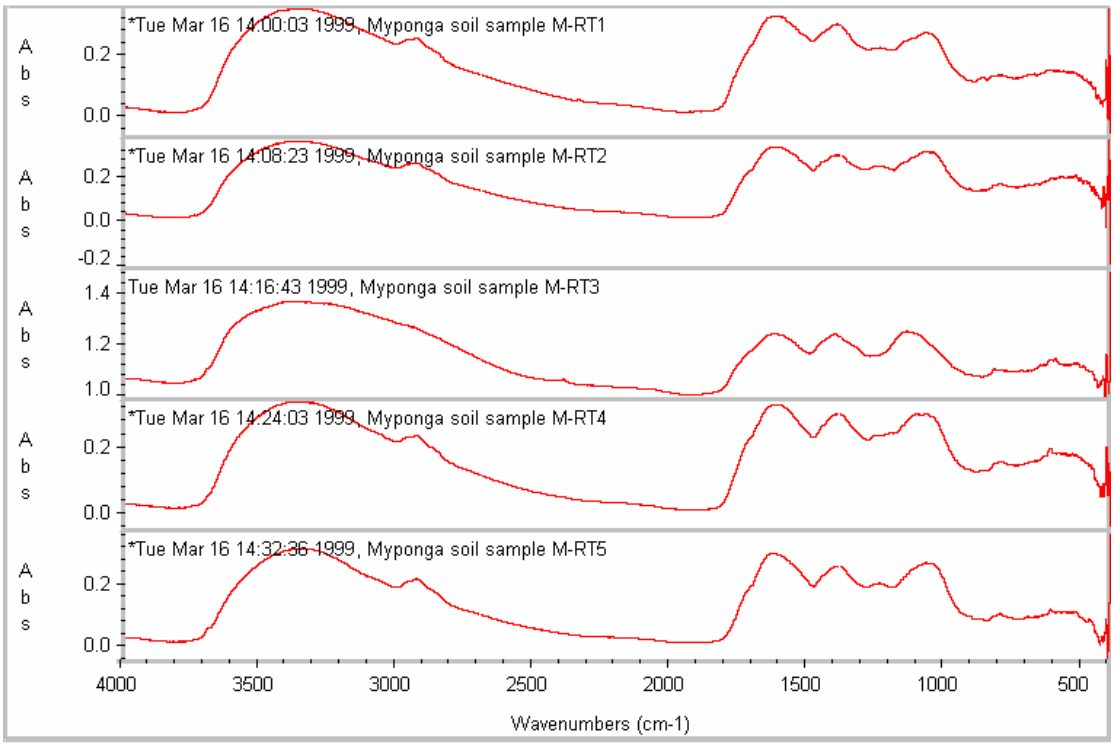


Figure 4. 15 DRIFT spectra of Myponga soil samples (Series 2, top, October 1998).

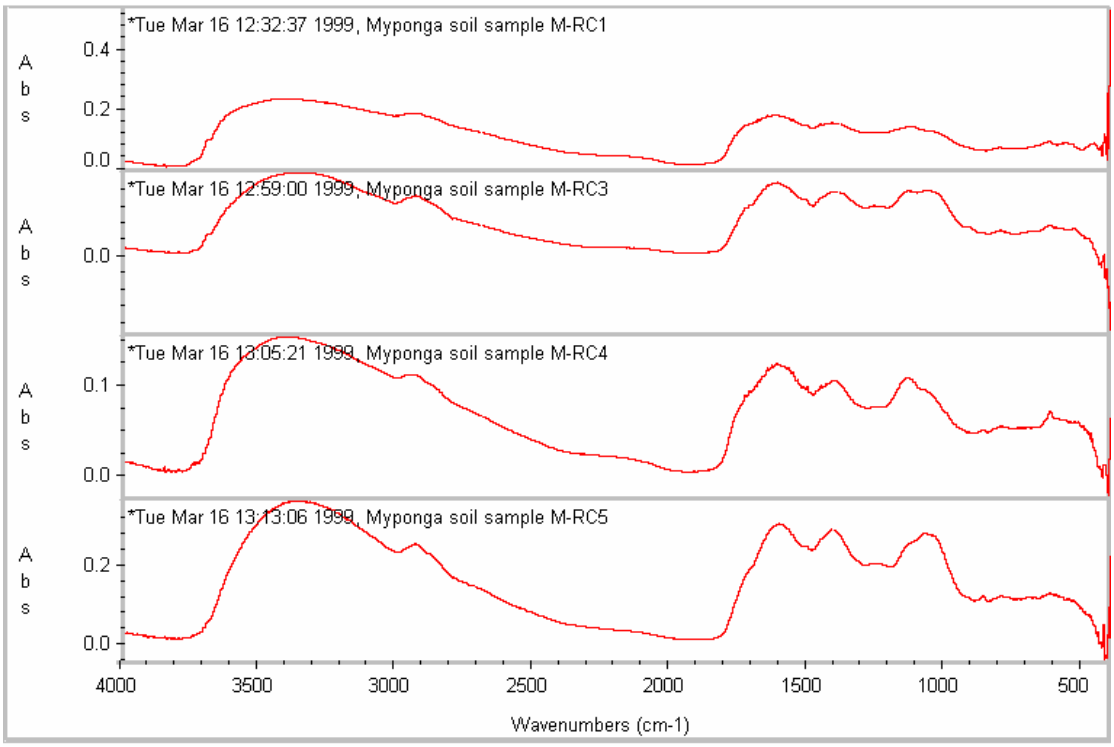


Figure 4. 16 DRIFT spectra of Myponga soil samples (Series 2, centre, October 1998).

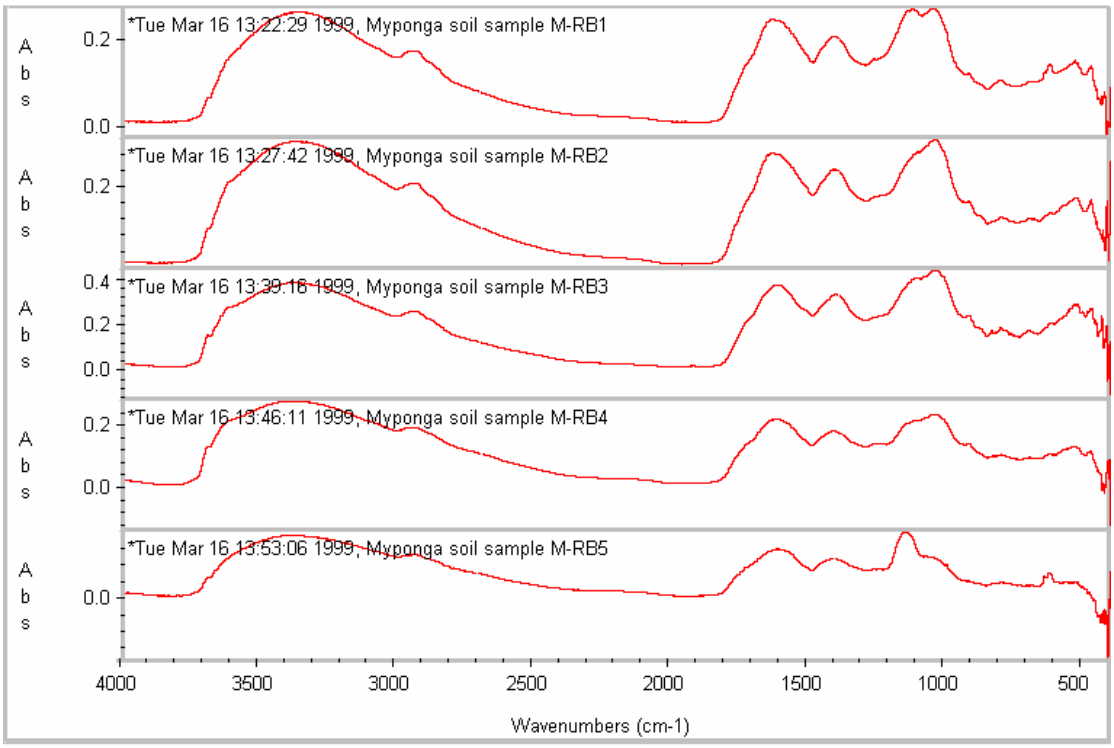


Figure 4. 17 DRIFT spectra of Myponga soil samples (Series 2, bottom, October 1998).

4.3.2 Organic and inorganic chemistry data of NOM isolates collected in March 1999.

Data from inorganic analyses of dried soil samples collected from Mt Bold in March 1999 are shown in Table 4.3. Clear trends can be seen in the composites of samples collected from the top, middle and bottom sites. The divalent cations, Al, Fe, Ca and Mg increase in concentration going down the slope, which should lead to greater ligand formation between clay particles and dissolved organic matter. Hence, a greater holding capacity for organics in soils should exist in the soils at the bottom of the slope. Countering this is the greater concentration of the monovalent cation sodium, which may form a salt with the carboxylic anions of DOM. The overall degree to which binding is enhanced by the increase in these cation concentrations was not investigated.

Organic chemistry and pH data of soil leachates from samples collected from Mt Bold in March 1999 are shown in Table 4.4. DOC concentrations were the lowest in leachates of samples collected at the bottom of the slope, which indicates that soils at this location has a higher capacity for binding organic compounds and may be postulated to be related to the higher divalent cation concentrations. The percentages of clay were highest in soils collected from the bottom of the slope at Mt Bold, compared with the middle and top sites. C/N ratios appear to be directly related to the DOC concentrations of the leachates. Inorganic chemistry data of leachates from soil samples collected from Mt Bold in March 1999 are shown in Table 4.5. The lowest mean concentration of extracted Ca was found in the samples from the middle section, as was the total concentration in the dried soil sample. The order of highest to lowest concentration in Ca is bottom, top and middle. Interestingly, the order of highest to lowest DOC in the extracts is the reverse ie. middle, top and bottom, which is as might be expected. Magnesium followed a similar trend as calcium. Neither Al nor Fe concentrations were related to those of DOC concentrations in leachates.

Data of UV-vis spectra of extracts of soil samples collected in March 1999 are shown in Table 4.6 (Mt Bold) and Table 4.7 (Myponga). For the Mt Bold sites, the order of highest to lowest mean 254nm/456nm ratios was middle, top, bottom, which correlates with the DOC concentrations. The basis for this may be that because of lower Ca and Mg concentrations, binding is reduced and the organics present have been transported in the soil to this site more recently. Hence less biodegradation/humification has occurred and a higher relative proportion of low-colour imparting organic compounds is present. The organics in soils with a higher 254nm/456nm ratio being more related to soil litter leachates. The data of C/N ratios also correlates with this, in that the higher C/N ratios (low nitrogen containing organics-low microbial input) were directly related to higher DOC concentrations. The mean E4/E6 trend was the same as that of the 254nm/456nm ratios.

The UV-vis spectral ratios of samples collected from Myponga in March 1999 are shown in Table 4.7 for comparison with those of Mt Bold.

Data of key cations in dried soil samples from the Mt Bold pine site are shown in Table 4.8. Similar trends in concentrations were found as for Mt Bold, with the bottom sites having the highest concentrations. However, the differences between the top and bottom sites in the Myponga soils were much less than for Mt Bold.

Data of extracted organics from soils of the Myponga pine site are given in Table 4.9. The mean concentrations of extracted DOC are related to the mean 254nm/456nm ratios (Table 4.7) as found for samples from Mt Bold. Similarly, the mean C/N ratios are related to the mean DOC concentrations. This data again indicates that where higher extractions in

organics can be achieved from soils, these have undergone less microbial degradation, possibly due to lower retention times in the soils. The significance of this is high in relation to such analysis as Bacterial Regrowth Potential (BRP), where an apparent relation between growth rate and DOC concentration is confounded by the different relative proportions of "readily assimilable" organics by common soil and aquatic micro-organisms. Data from this part of the study indicates that the relative proportion of assimilable organics in DOC increases with higher concentrations of DOC extracted. This may be a phenomena observed in some reservoir waters in certain seasons, such as with heavy rainfall where a catchment has high amounts of live and decaying vegetation.

Inorganic chemistry data of extracts from soil samples collected from the Myponga pine site in March 1999 are given in Table 4.10. Ca and Mg concentrations in extracts do not show the same inverse trend with DOC concentration, as was found for Mt Bold, opposing the hypothesis previously stated.

Inorganic chemistry data (key cations) of dried soil samples collected from the Myponga grass site are shown in Table 4.11. The slope of this site was gentle, in contrast to the Myponga pine and Mt Bold transects, which are steep. No consistent trend is present in relation to the topography of this site. Results of organic analyses are shown in Table 4.12. A relationship between DOC concentration and C/N ratio again appears evident.

DRIFT spectra of soil extracts collected in March 1999 are shown in figures 4.18 to 4.21 (Mt Bold), 4.22 to 4.25 (Myponga grass site) and 4.26 to 4.28 (Myponga pine site). In contrast to the spectra of samples collected in October 1998, a common feature found was sharp single peaks (eg. Figure 4.18-Top 2) and as shoulder peaks (eg. Figure 4.19 and 4.23).

These sharp peaks indicate a high influence by inorganics and therefore diminishes confidence in peak assignment and assessment of relative abundances of functionalities to the organic components. Features of spectra are as follows;

1. a general presence of three broad peak bands between ~ 900 WN/cm and ~1700 WN/cm, as previously found.

2. the peak band at ~ 900 to 1200 WN/cm is comprised of a range of individual peaks, which, having sharp apexes, indicates inorganic influences.

3. Some sites show variation in spectra of different samples (eg. relative heights of peaks of Mt Bold top and middle, figures 4.18 & 4.19), while others demonstrate a high level of similarity (eg . figures 4.22 and 4.23). The worth of these data may therefore be, not to extract potential information on organics alone, but as an empirical fingerprint of the inorganic and organic matrix, from which comparisons could be made. For example, DRIFT spectra could then be used to monitor overall changes in soils and waters over time. Potentially, artificial neural networks might be applied where DRIFT data and extensive inorganic and organic data exist. DRIFT data might then be applied to assess a broader range of parameters otherwise required to be determined by specific analytical techniques that might be much more expensive.

4. The Myponga samples at any one site appeared to have similar spectra, indicating greater homogeneity in soils than those of Mt Bold (top and middle), although inorganic and organic analyses points to diversity. At Mt Bold, the bottom site selected in March 1999 was covered with grasses and was at a level that was likely to be flooded by

reservoir water in winter. Samples from this site also had similar spectra, with a prominent peak at about ~1000-1100 WN/cm, possibly due to carbohydrates. In contrast to Mt Bold, the bottom sites at Myponga (both grass and pine) were above the maximum water height of the reservoir, though the bottom samples also showed the same prominent peak at ~1000-1100WN/cm. This feature can be seen in Figure 4.25 and might be due to greater leaching of carbohydrates through the soil. This is conceptually feasible where these organics are uncharged or have lower relative charges than other organic components, though they would be expected to be readily degraded by micro-organisms.

Table 4. 3 Inorganic data of dried soil samples collected from Mt Bold in March 1999.

	Total (mg/kg dry).				
Sample Description	Al	Fe	Na	Ca	Mg
Top 1,2,3	2170	2300	47	638	201
Middle 1,2,3	2840	5280	66	550	386
Bottom 1,2,3	18100	14300	191	1030	3140

Table 4. 4 Organic chemistry data and pH values of extracts of Mt Bold soil samples collected on 31st March 1999.

Sample Description	pH	DOC (mg/L)	TKN (mg/L)	C/N
Top 1	6.23	13.0	2.20	5.9
Top 2	6.14	10.9	1.54	7.1
Top 3	6.03	11.8	1.36	8.7
Mean	6.13	11.9	1.70	7.2
SD	0.10	1.1	0.44	1.4
Middle 1	5.87	28.3	2.42	11.7
Middle 2	5.92	11.2	1.20	9.3
Middle 3	5.71	26.7	1.64	16.3
Mean	5.83	22.1	1.75	12.4
SD	0.11	9.5	0.62	3.5
Bottom 1	5.48	4.6	1.32	3.5
Bottom 2	6.22	10.4	1.26	8.2
Bottom 3	5.68	8.6	1.32	6.5
Mean	5.79	7.8	1.30	6.1
SD	0.38	3.0	0.03	2.4

Table 4. 5 Inorganic chemistry data of extracts of Mt Bold soil samples collected on 31st March 1999.

Sample Description	Cond. [µs/cm]	Bicarbonate (mg/L)	TDS (by EC) (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Al (mg/L)	Fe (mg/L)	Alkalinity $CaCO_3$ (mg/L)
Top 1	110	6	60	4.2	2.4	5.0	1.40	0.728	4
Top 2	130	6	72	5	2.6	8.4	0.398	0.204	4
Top 3	80	6	44	1.8	1.0	5.6	0.738	0.348	4
Mean	107	6	59	3.7	2.0	6.3	0.846	0.427	4
SD	25	0	14	1.7	0.9	1.8	0.511	0.271	0
Middle 1	102	8	56	1.8	1.6	7.0	12.5	4.76	6
Middle 2	64	8	36	0.6	1.0	5.4	3.18	1.85	6
Middle 3	104	8	58	1.0	2.2	12.4	2.80	1.79	6
Mean	90	8	50	1.1	1.6	8.3	6.17	2.80	6
SD	23	0	12	0.6	0.6	3.7	5.52	1.70	0
Bottom 1	174	6	96	5.6	4.0	17.8	3.68	2.14	4
Bottom 2	90	8	50	1.6	1.4	11.8	3.52	2.12	6
Bottom 3	144	8	78	4.6	3.4	15.0	3.80	2.30	6
Mean	136	7.3	75	3.9	2.9	14.9	3.67	2.19	5.3
SD	43	1.2	23	2.1	1.4	3.0	0.140	0.099	1.2

Table 4. 6 UV-vis data of extracts of Mt Bold soil samples collected in March 1999.

Location	n=3	Bottom	Middle	Top
254nm/456nm	Mean	10.3	13.5	11.6
254nm/456nm	SD	0.53	3.06	1.46
E4/E6	Mean	4.13	5.79	5.32
E4/E6	SD	0.10	1.07	0.80

Table 4. 7 UV-vis data of extracts of Myponga soil samples collected in March 1999.

Location Vegetation		Bottom Grass	Middle Grass	Top Grass	Bottom Pine	Middle Pine	Top Pine
254nm/456nm	Mean	7.96	10.3	13.7	12.3	7.67	15.7
254nm/456nm	SD	0.64	3.36	7.45	6.49	1.51	10.7
E4/E6	Mean	6.52	18.9	17.8	7.74	5.60	7.16
E4/E6	SD	0.46	19.3	18.1	3.89	0.96	3.63

Table 4. 8 Inorganic chemistry data of dried soil samples collected from the Myponga pine site on 31st March 1999.

Sample Description (combined)	Total (mg/kg dry weight) Al	Fe	Na	Ca	Mg
Top 1,2,3	7230	7390	203	1890	2450
Middle 1,2,3	9240	7950	170	1650	2370
Bottom 1,2,3	9880	12300	360	2930	3330

Table 4. 9 Organic chemistry data and conductivities of extracts of soil samples collected from the Myponga pine site, on 29th March 1999.

Sample Description	DOC (mg/L)	TKN (mg/L)	C/N	Cond. [µs/cm]
Top 1	251	22.4	11.2	444
Top 2	43.1	4.12	10.5	260
Top 3	47.2	3.52	13.4	208
Mean	114	10.0	11.7	304
SD	119	10.7	1.5	124
Middle 1	26.7	2.34	11.4	82
Middle2	15.1	1.54	9.8	110
Middle 3	15.0	1.74	8.6	292
Mean	18.9	1.9	9.9	161
SD	6.7	0.4	1.4	114
Bottom 1	23.8	2.46	9.7	640
Bottom 2	22.5	2.52	8.9	488
Bottom 3	27.8	2.06	13.5	206
Mean	24.7	2.3	10.7	445
SD	2.7	0.3	2.4	220

Table 4. 10 Inorganic chemistry data of extracts of soil samples collected from the Myponga pine site, on 29th March 1999.

Sample Description	pH	Bicarbonate (mg/L)	TDS (by EC) mg/L	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Al (mg/L)	Fe (mg/L)	Alkalinity $CaCO_3$(mg/L)
Top 1	5.50	28	240	16.2	7.8	43.0	3.8	2.0	24
Top 2	5.40	8	142	5.0	3.8	29.2	8.4	5.1	6
Top 3	6.09	12	114	4.0	3.0	24.4	5.8	11.6	10
Mean	5.7	16	165	8.4	4.9	32.2	6.0	6.2	13.3
SD	0.4	10.6	66.2	6.8	2.6	9.7	2.3	4.9	9.5
Middle 1	5.94	8	44	2.8	1.8	11.8	8.1	20.2	6
Middle 2	6.02	6	60	3.4	2.6	14.6	14.4	7.7	4
Middle 3	6.13	6	160	6.0	5.6	33.2	4.9	3.0	4
Mean	6.0	6.7	88	4.1	3.3	19.9	9.2	10.3	4.7
SD	0.1	1.2	62.9	1.7	2.0	11.6	4.8	8.9	1.2
Bottom 1	6.08	8	360	11.6	10.8	80.8	1.0	0.5	6
Bottom 2	6.13	8	260	9.2	7.6	63.8	4.4	2.8	6
Bottom 3	5.65	6	112	2.6	2.4	25.4	22.8	13.3	4
Mean	6.0	7.3	244	7.8	6.9	56.7	9.4	5.5	5.3
SD	0.3	1.2	125	4.7	4.2	28.4	11.7	6.8	1.2

Table 4.11 Inorganic chemistry data of dried soil samples collected from the Myponga grass site on the 30th March 1999.

	Total (mg/kg dry weight)				
Sample Description	Al	Fe	Na	Ca	Mg
Top 1,2,3	9410	21600	77.4	1160	4270
Middle 1,2,3	11500	25400	71.3	1120	4030
Bottom 1,2,3	11700	22200	153	987	3310

Table 4.12 Organic chemistry data and pH levels of extracts of soil samples collected from the Myponga grass site on 30th March 1999.

Sample Description	DOC (mg/L)	TKN (mg/L)	C/N	pH
Top 1	6.22	1.14	5.46	5.71
Top 2	8.46	1.60	5.29	5.81
Top 3	8.52	2.34	3.64	5.60
Mean	7.73	1.69	4.79	5.71
SD	1.31	0.61	1.00	0.11
Middle 1	8.04	1.60	5.03	5.83
Middle 2	9.46	1.44	6.57	5.77
Middle 3	4.74	1.40	3.39	5.37
Mean	7.41	1.48	4.99	5.66
SD	2.42	0.11	1.59	0.25
Bottom 1	9.52	1.22	7.80	6.22
Bottom 2	9.44	1.00	9.44	6.20
Bottom 3	12.4	1.36	9.12	6.35
Mean	10.45	1.19	8.79	6.26
SD	1.69	0.18	0.87	0.08

Table 4. 13 Inorganic chemistry data of extracts of soil samples collected from the Myponga grass site on 30th March 1999.

Sample Description	Cond. [µs/cm]	Bicarbonate (mg/L)	TDS (by EC) mg/L	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Al (mg/L)	Fe (mg/L)	Alkalinity $CaCO_3$ (mg/L)
Top 1	96	6	52	6.4	3.0	7.4	1.27	0.95	4
Top 2	94	6	52	5.0	2.4	7.4	1.20	0.92	4
Top 3	106	6	58	5.0	2.4	7.2	0.35	0.26	4
Mean	99	6	54	5.5	2.6	7.3	0.94	0.71	4
SD	6.4		3.5	0.81	0.35	0.12	0.51	0.39	
Middle 1	84	6	46	4.4	2.4	6.0	3.58	2.78	4
Middle 2	110	6	60	6.0	3.0	8.4	4.16	3.22	4
Middle 3	208	4	114	13.4	5.4	6.0	0.69	0.49	4
Mean	134	5.3	73	7.93	3.60	6.80	2.81	2.16	4
SD	65.4	1.2	36	4.80	1.59	1.39	1.86	1.47	
Bottom 1	130	6	72	3.0	3.6	22.4	9.26	5.32	4
Bottom 2	136	6	74	3.0	2.0	24.6	12.14	17.3	4
Bottom 3	126	6	70	2.8	3.4	23.4	15.44	9.10	4
Mean	130.67	6	72	2.93	3.00	23.5	12.3	10.6	4
SD	5.03		2.0	0.12	0.87	1.10	3.09	6.14	

DRIFT spectra of samples collected in March 1999.

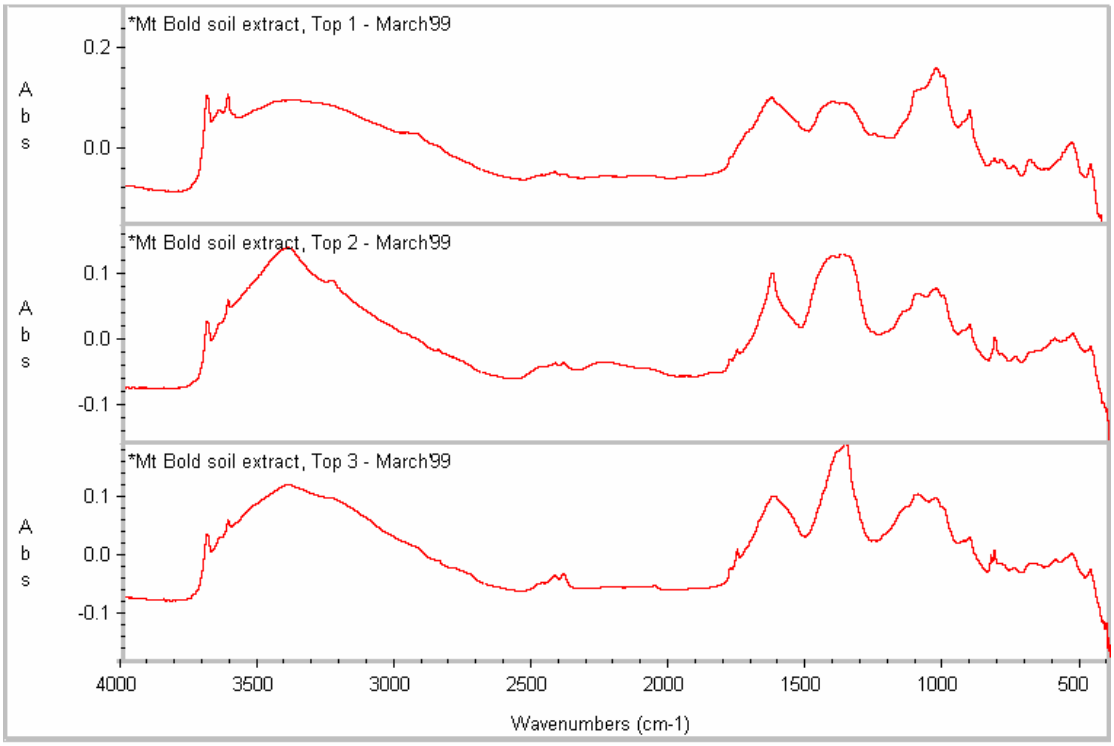


Figure 4. 18 DRIFT spectra of Mt Bold soil samples (top of slope).

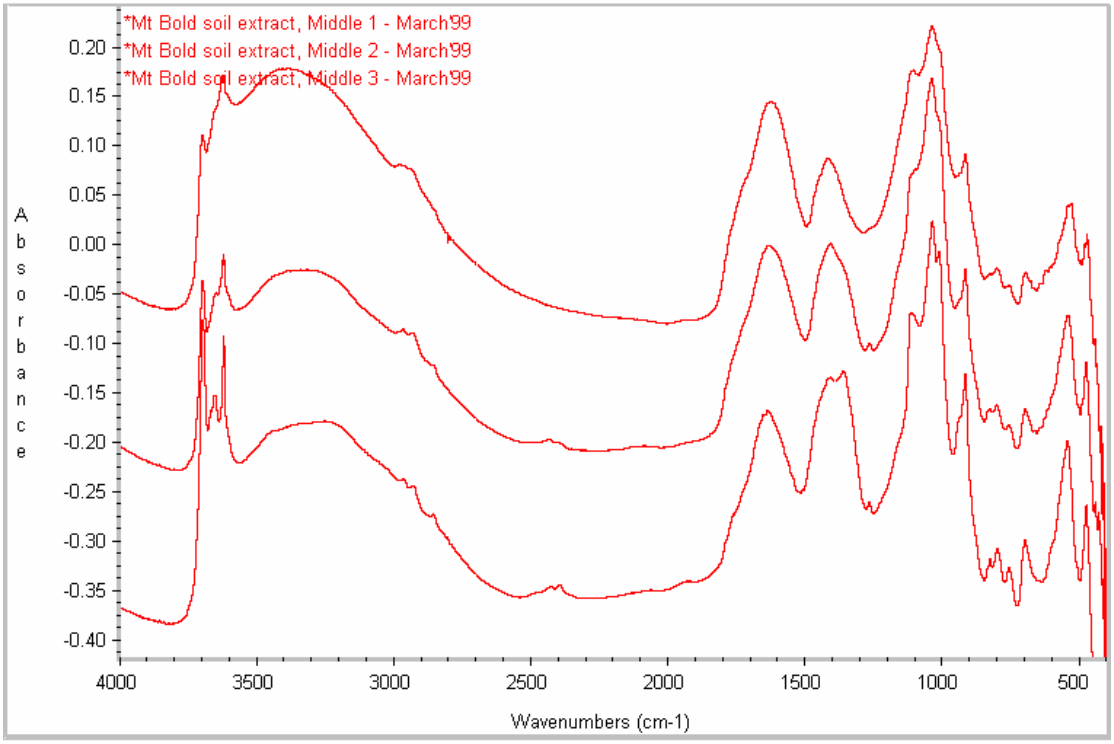


Figure 4. 19 DRIFT spectra of Mt Bold soil samples (middle of slope).

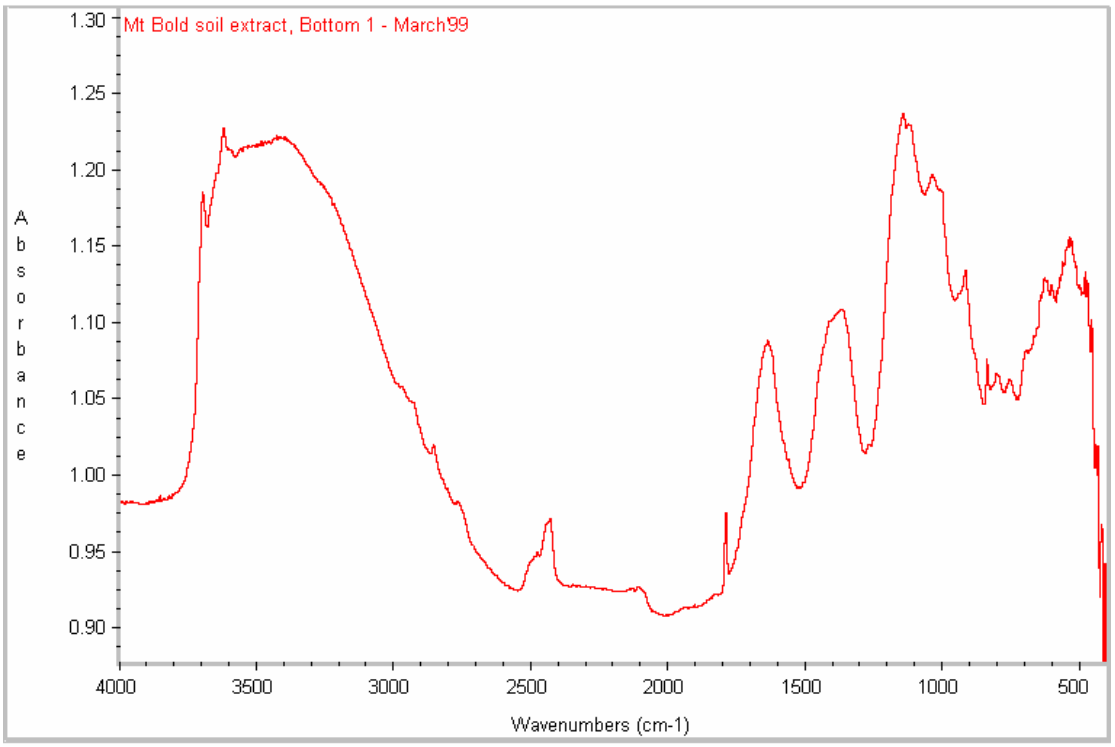


Figure 4. 20 DRIFT spectra of Mt Bold soil sample 1 (bottom of slope).

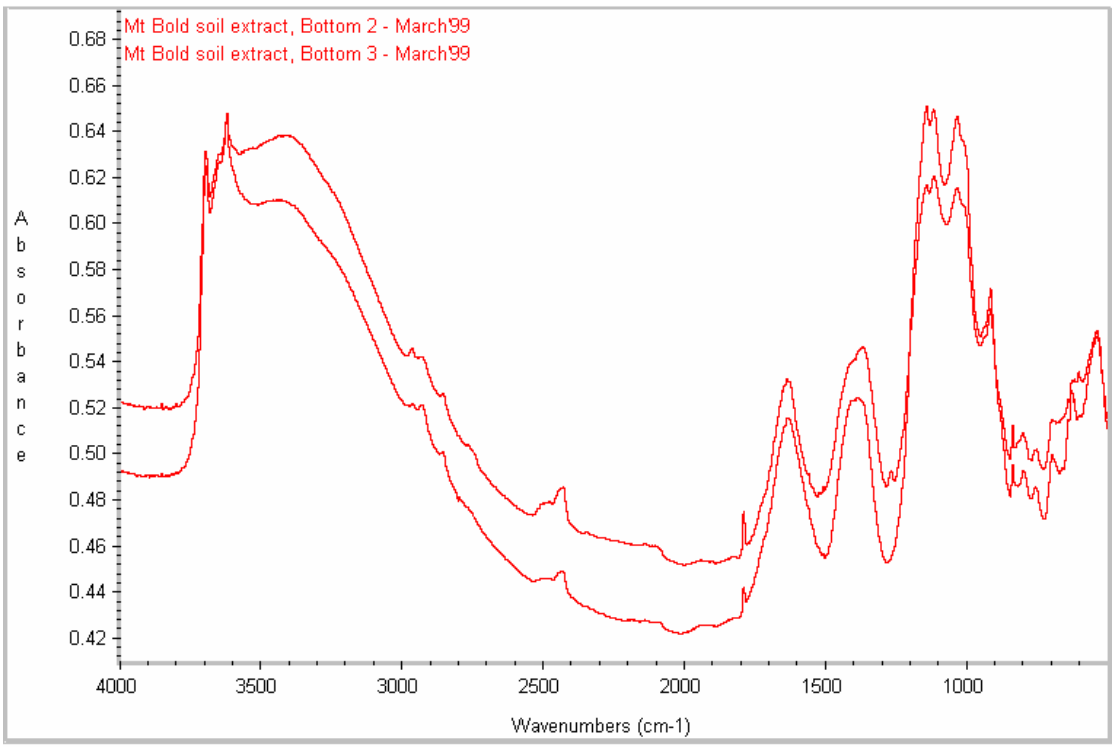


Figure 4. 21 DRIFT spectra of Mt Bold soil samples 2&3 (bottom of slope).

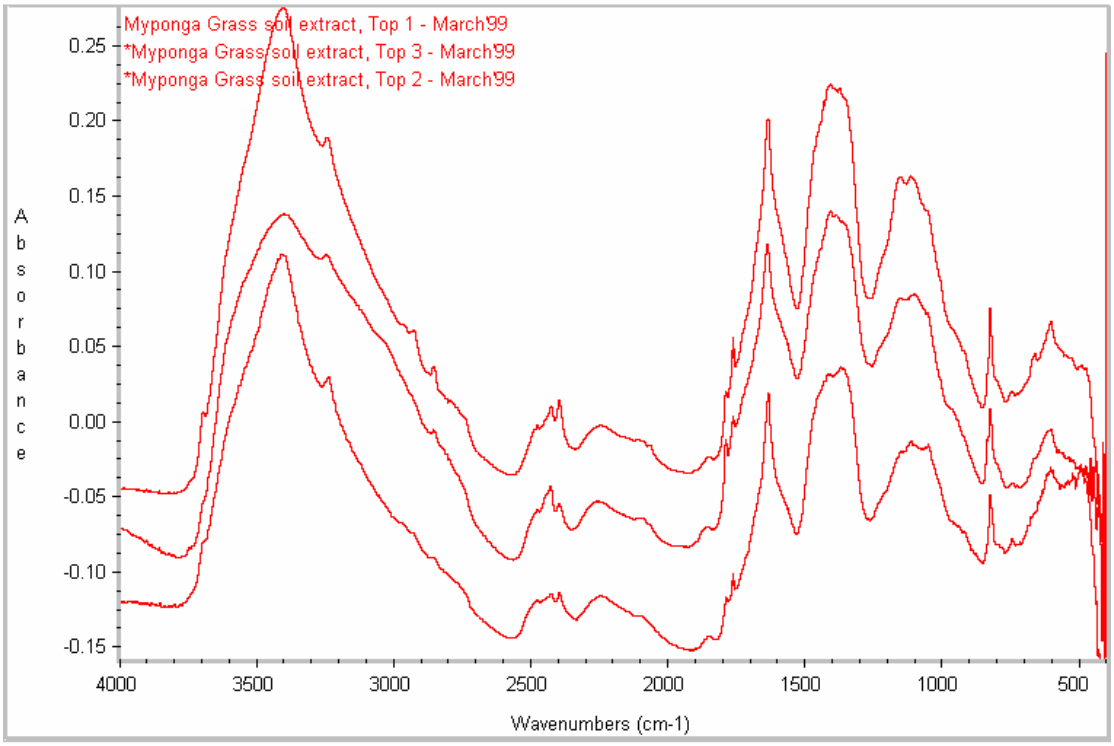


Figure 4. 22 DRIFT spectra of Myponga soil samples (grass site, top of slope).

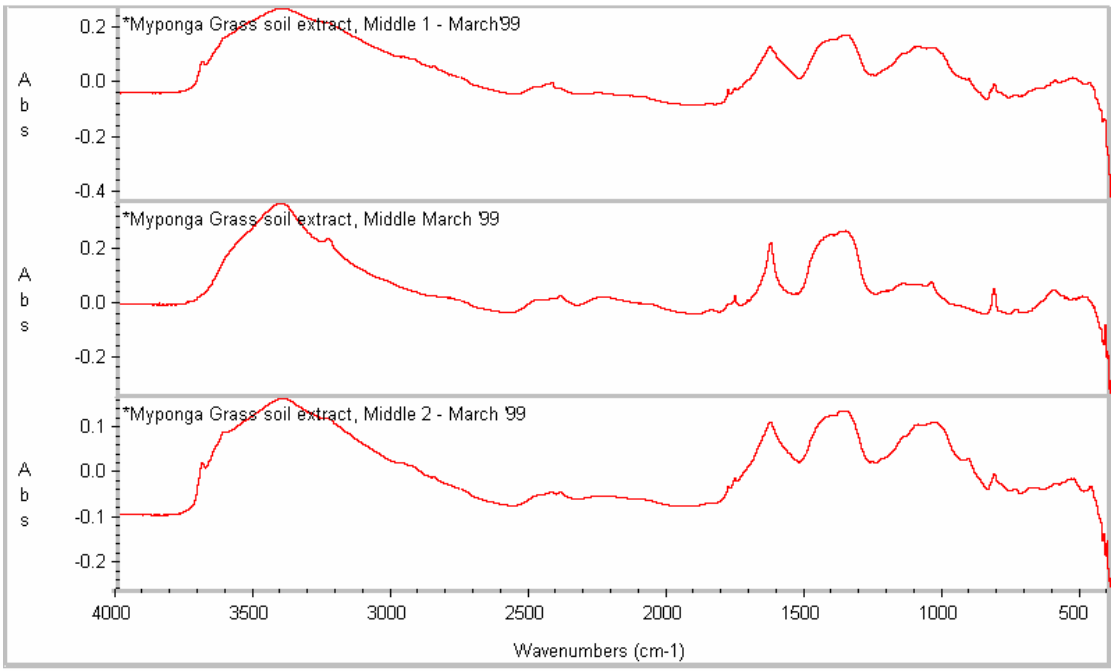


Figure 4. 23 DRIFT spectra of Myponga soil samples (grass site, middle of slope).

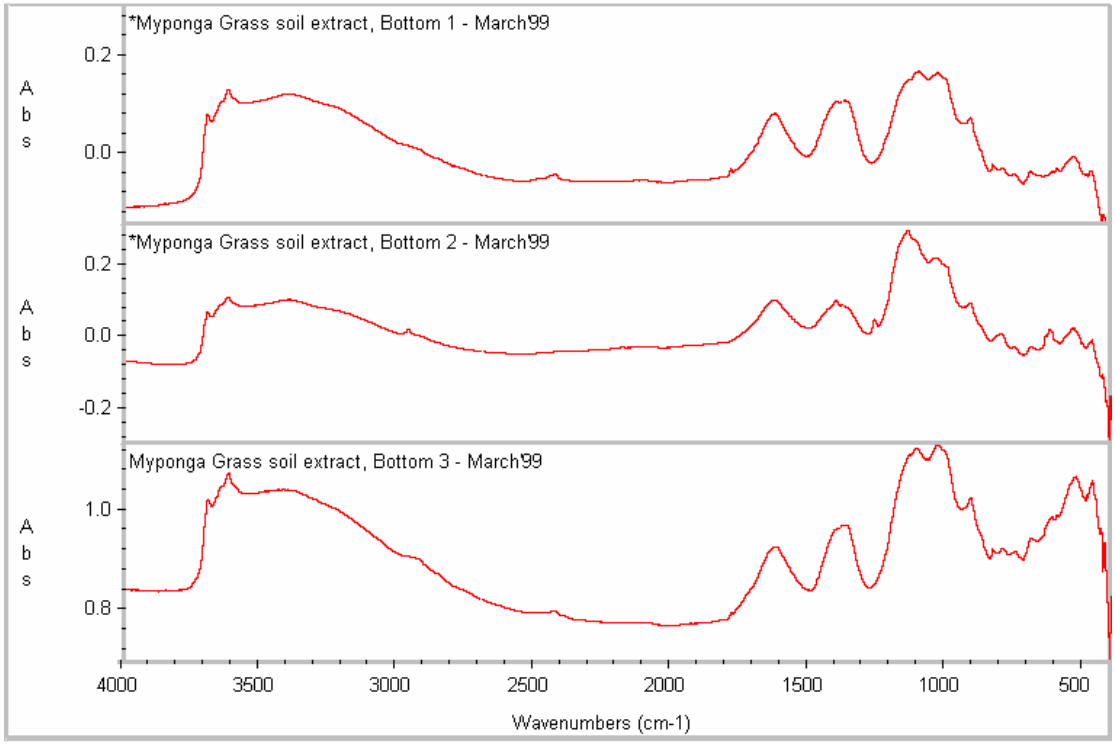


Figure 4. 24 DRIFT spectra of Myponga soil samples (grass site, bottom of slope).

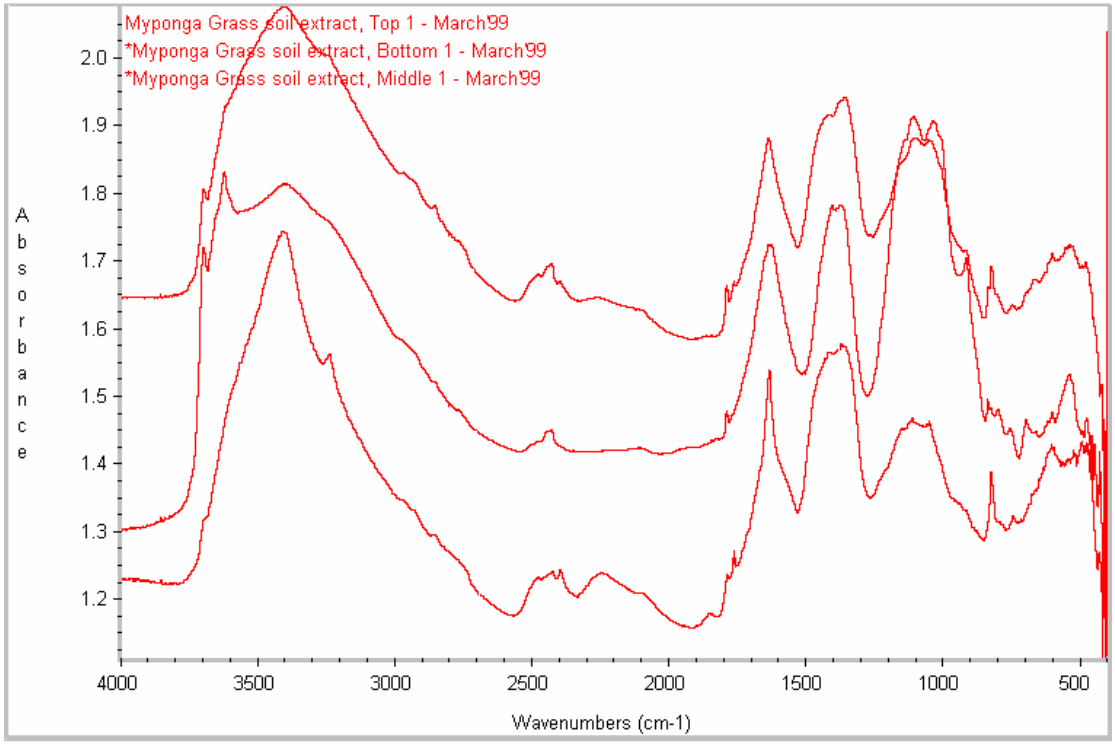


Figure 4. 25 Comparison of DRIFT spectra of Myponga soil samples (grass site, top, middle and bottom of slope).

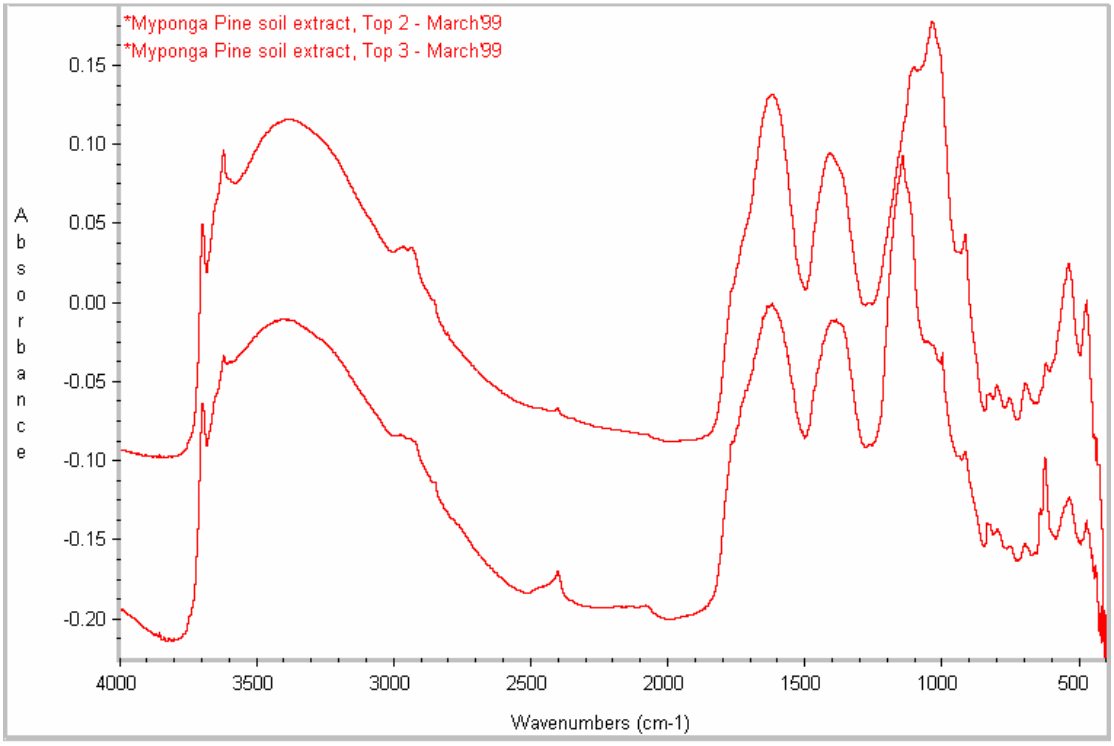


Figure 4. 26 DRIFT spectra of Myponga soil samples (pine site, top of slope).

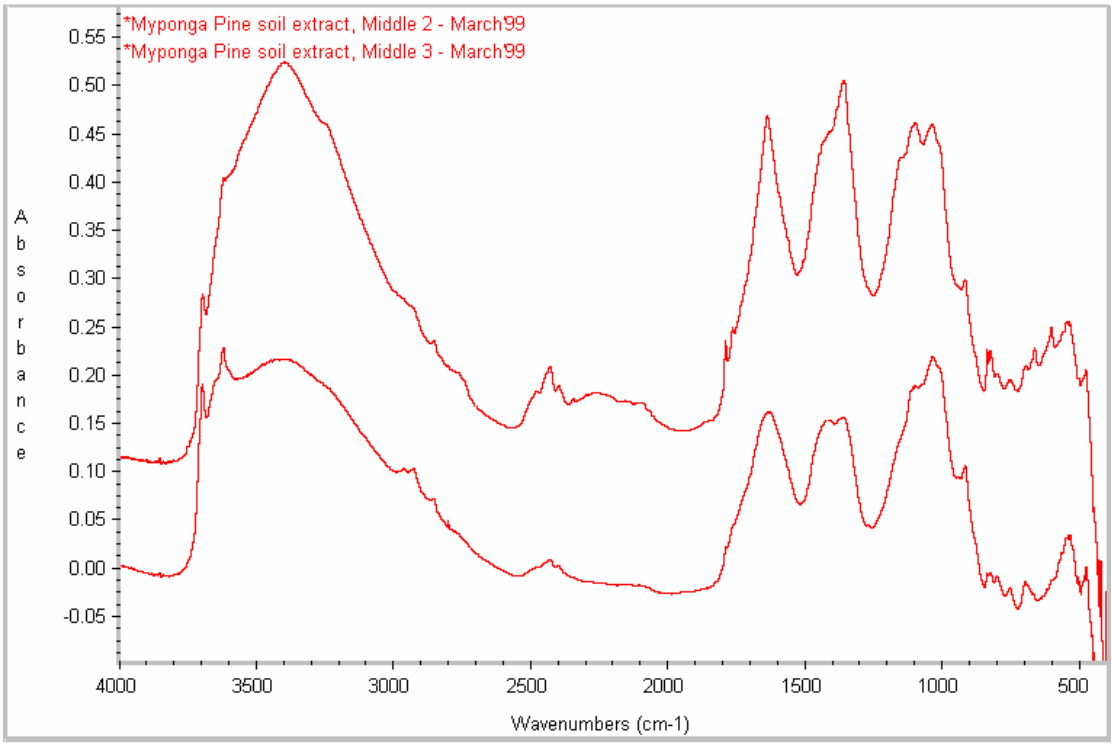


Figure 4. 27 DRIFT spectra of Myponga soil samples (pine site, middle of slope).

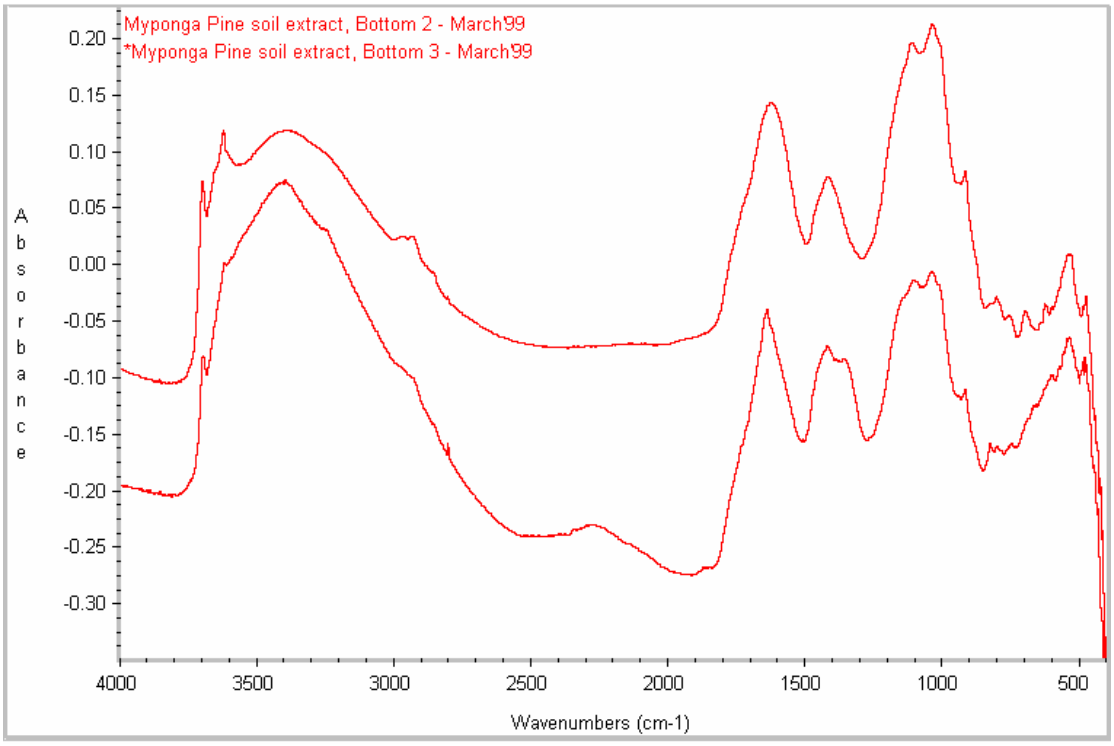


Figure 4. 28 DRIFT spectra of Myponga soil samples (pine site, bottom of slope).

4.3.3 Organic and inorganic chemistry data of NOM isolates collected in July 1999.

In July 1999 soil samples were collected from the Mt Bold and Myponga catchments and litter layer samples were collected from Myponga also. Sample sites were the same as those sampled in March 1999.

Data of UV-vis spectra of soil samples from Mt Bold are shown in Table 4.14, soil litter layers from Myponga in Table 4.15 and soil samples from Myponga in Table 4.16. The 254nm/456nm ratios show similar trends in relation to topography as previously with highest ratios occurring in samples from the top of the slope. Again, this infers less general degradation of organics at the top of the slopes than lower down. Of interest, are the high ratios of litter layer extracts compared with those from soil extracts. This feature is consistent with the organics extracted from the litter layers being less degraded than those in soils and points to this ratio being of value in characterisation of organic matter. E4/E6 ratios showed no consistent trends with topography. The ET/Bz ratio show interesting trends for the Mt Bold and Myponga grass sites with, lowest ratios at the top of the slopes, increasing going down the slope. This is in agreement with the basis for this ratio by Korshin *et al.* (1997), because at lower slopes, higher degrees of biodegradation would have occurred resulting in higher oxidation of organics, including higher oxygen substitution of aromatic rings. The bottom sites of these two slopes had smaller gradients than the middle and top sites, and would have been a sink for degraded organics. In contrast, the bottom site from the pine slope was much the same as that of the middle and greater than that of the top (near the crest of the hill). The trend of the ET/Bz ratios at the pine site indicates less difference in the aromatic functionality/oxidation levels at the different slope sampling sites. However, nitrate values in the soil and litter layer leachates were not taken, and therefore possible interference from this on the absorbance at 203 nm is not known. Hence, the ET/Bz ratios reported here should be viewed with this in mind, and the interpretations provided taken with caution.

Organic and inorganic chemistry data of an aqueous extract of combined litter layers collected from Mt Bold (top of slope) are shown in Table 4.17. Features are the high DOC concentration and high C/N ratio of the extract compared with extracts from soils. Organic chemistry data of extracts of soil samples from Mt Bold are shown in Table 4.18. On this sampling occasion three 7m x 7m blocks were selected at each site, from which duplicate samples were collected from each block. Concentrations of DOC extracted from each block were mostly similar but varied markedly between the different blocks, even at the same site. This variability occurred at the three sites, demonstrating the need for multiple sample collections for determination of organic loads in these soils.

For these samples, no relationship was found to exist between the DOC concentrations and C/N ratios (Table 4.18). The highest potential for microbial activity, based on the 254nm/456nm ratio would be at the top of the slope. SUVA values of extracts from the top and middle sites were mostly similar, ranging from about 1.5 to 4. In contrast to this the SUVA values of soil extracts from the bottom site were all higher, and up to 18.6.
Inorganic chemistry data of soil extracts collected from Mt Bold in July are shown in Table 4.19, as are soil moisture contents. This data shows the increase in moisture

levels going down the slope, which could be postulated to influence the C/N ratios ie. an increase in microbial activities.

Organic and inorganic chemistry data of extracts from soil litter samples collected from the Myponga pine site in July 1999 are shown in tables 4.20 and 4.21, respectively. The pine site litter layer was generally a thick layer of about 10 cm and generally uniform down the slope. DOC concentrations in extracts from combined block litter layer samples were similar at the three sites (Table 4.20), as were the C/N ratios and SUVA values. Inorganic chemistry parameters were also very similar except for the top site.

Organic chemistry data of soil extracts from the Myponga pine site are shown in Table 4.22. DOC concentrations in extracts varied greatly between blocks, but were mostly similar within blocks. The extracts of samples from the second block of the top site had very high DOC concentrations, indicating a very high organic load. C/N ratios were similar at the three sites, though the mean values were higher than those of Mt Bold. Myponga pine extract DOC concentrations were also higher than those of Mt Bold. At the three Myponga pine sites, the mean SUVA values were similar.

Inorganic chemistry data of extracts from soils from the Myponga pine site are shown in Table 4.23. No trends in relation to topography were observed.

Organic chemistry data of extracts of soils from the Myponga grass site are shown in Table 4.24. Of potential significance are the DOC concentrations of these extracts, which are all markedly lower than those of Myponga pine and mostly lower than those of Mt Bold. This data indicates that with intensive forestation bordering a reservoir, particularly with *Pinus radiata,* where thick litter layers are formed on top of the soils, then high amounts of organics can be leached from both the litter layers and the soils. In contrast to this the Myponga grass site had a very low load, (if at all), of decomposing vegetation and the soils released much less organics, under standard extraction conditions. The data obtained indicates that minimisation of leachable organics on and in soils may be achieved by coverage of catchment soils with grasses and natives rather than with *Pinus radiata.*

Inorganic chemistry data of extracts from soils collected from the Myponga grass site in July 1999 are shown in Table 4.25. Moisture contents of soils at collection are also given. At the time of collection at this site the soil pH increased going down the slope, which coincides with the bicarbonate concentration, TDS and alkalinity data of the extracts.

Comparisons of 254nm/456nm ratios of soil samples collected in March (tables 4.6 and 4.7) with those collected in July (tables 4.14 and 4.16) show a highly consistent trend in these ratios being lowest in March at each site/location. This infers that in March, after the summer period and before consistent rains had fallen, the organics in soils had generally undergone greater overall biodegradation than those present in soils in July. This is feasible as by July, greater and consistent rainfall had occurred, which would have leached fresh organics into the soils.

DRIFT spectra of extracts of samples collected in July 1999 are shown in figures 4.29 to 4.37. As with spectra obtained for samples collected in March, the impact of salt

appears high, based on the sharpness of peaks both shouldering other peaks or as separate peaks. Further, large variations in peak shape and relative sizes occurred between samples collected from the same site and between sites. With the apparent impacts of salts the spectra are better evaluated on the basis of empirical comparisons within and between sites. Large differences were found in the DOC concentrations of the extracts of soils from Mt Bold (Table 4.18) and DRIFT spectra obtained of these samples differed. However, some correlation appears to exist between them, ie. the Mt Bold top #3a extract sample (Table 4.18) had a much higher DOC concentration than those of top 1a and 2a. The DRIFT spectra of the top 3a samples shows broader peaks, indicative of the heterogenous nature of DOM from soils, that comprises a broad range of interacting chemical functionalities. In contrast, the spectra of the extracts of the top 1a and 2a samples show sharp peaks, indicating lower organic contents in the freeze-dried extracts used in the DRIFT analysis. This feature can also be seen in the samples collected from the bottom of the slope where the DOC concentrations of the #2 block samples are much lower than those of the top and the middle. The DRIFT spectrum of the 2a sample (Figure 4.31) is in clear contrast to the other two, by the sharpness of the peaks at ~ 3400 WN/cm and at ~1650WN/cm. For those with broader, less resolved peaks greater confidence can be made on these due to organic contents. The two peaks, at ~ 1600 WN/cm and ~1400 WN/cm of the middle and bottom sections of Mt Bold might be contributed by 1. C=O and C=C stretching and by 2. CH deformation, CH_2 and COO^- symmetric motion and C-O stretching.

Relative abundances of these appear to differ between samples from the same site, eg. Mt Bold bottom site, samples 1a and 3a.

The apparent relationship between DOC concentrations in extracts and the peak shapes is again evidenced by data of samples from the Myponga grass site (figures 4.32, 4.33 and 4.34). The DOC concentrations of the extracts were particularly low for extracts of soils from the top and middle sites (~ 5 to 13 mg/L) and about double that in the soils from the bottom site. The DRIFT spectra of samples from the top and middle sites of the Myponga grass location (figures 4.32, 4.33 and 4.34) had very sharp peaks at ~1650 WN/cm and ~ 1400 WN/cm compared with those from the bottom of the slope. The spectra of samples from the bottom site were also very similar to each other in terms of relative peak heights.

The extract DOC concentrations from the Myponga pine location were high in relation to the other locations (Table 4.22). The DRIFT spectra of these (Figures 4.35 to 4.37) do not show the sharp peaks as found for Myponga grass and Mt Bold locations. Spectra of samples from the different blocks of the Myponga pine sites were similar to each other, though differed between sites in relation to the peak height at about 1100 WN/cm. The spectra of samples collected from the middle of the slope appeared to be more influenced by salts, on the basis of peak sharpness and relative heights though the inorganic analyses indicate the reverse. Features of spectra from Myponga pine include the presence of CH_2 and CH_3 stretching and three major peaks between ~ 1700WN/cm and 800 WN/cm. The relative ratios of the ~1400 WN/cm peak to the ~1600 WN/cm peak increases going down the slope. This might be due to carbohydrates and carboxylate functional groups that are or cause the organics to be hydrophilic and more easily transported through the soil. This postulation is not

supported by the spectra obtained from the March collections (figures 4.26 to 4.28), though prior climate conditions could override topographical effects.

The data reported in this chapter should also be interpreted in relation to the study by Anstis (1999). In that study the activities of extracellular enzymes, presumed to be from micro-organisms, were determined in soil samples from the same sites as the March and July collections reported here. Cellulase, dehydrogenase and phenol oxidase activities were found to be influenced by catchment topography, vegetation and climatic conditions. Soil microbial activities, based on a soil respiration test was also measured as was the characterisation of NOM using high performance size exclusion chromatography, UV-vis data and pyrolysis-gas chromatography/mass spectrometry.

Table 4.14 UV-vis data of extracts of soil samples collected from Mt Bold in July 1999.

Location	Bottom Mean	Bottom SD	Middle Mean	Middle SD	Top Mean	Top SD
254nm/456nm	14.3	2.27	17.9	1.41	19.0	4.35
E4/E6	4.92	0.73	8.49	2.20	26.0	30.9
253nm/203nm	0.414	0.264	0.286	0.252	0.080	0.063

Table 4.15 UV-vis data of extracts of soil litter layer (pine forest) samples collected from Myponga in July 1999.

Location	Bottom	Middle	Top
254nm/456nm	26.8	28.7	29.1
E4/E6	8.74	9.59	9.91
253nm/203nm	0.399	0.401	0.413

Table 4. 16 UV-vis data of extracts of soil samples collected from Myponga in July 1999.

Vegetation Location	Grass Bottom Mean	Grass Bottom SD	Grass Middle Mean	Grass Middle SD	Grass Top Mean	Grass Top SD
254nm/456nm	10.7	0.62	11.5	4.74	15.0	4.89
E4/E6	8.54	1.27	9.58	3.44	7.81	4.74
253nm/203nm	0.598	0.037	0.074	0.054	0.038	0.015
Vegetation Location	Pine Bottom Mean	Pine Bottom SD	Pine Middle Mean	Pine Middle SD	Pine Top Mean	Pine Top SD
254nm/456nm	14.6	2.61	11.4	2.23	23.0	7.75
E4/E6	7.32	1.15	6.52	0.76	11.1	4.66
253nm/203nm	0.265	0.103	0.384	0.094	0.465	0.099

Table 4. 17 Organic and inorganic chemistry data of extracts of soil litter layers collected from Mt Bold on 20th / 21st July 1999.

Sample Description	DOC (mg/L)	TKN (mg/L)	C/N ratio	Abs. @254nm	SUVA	
Top 1,2,3	434	20.2	21.5	12.3	2.82	
Sample Description	pH	Cond. [µs/cm]	Bicarbonate (mg/L)	TDS (by EC, mg/L)	Na (mg/L)	Alkalinity $CaCO_3$(mg/L)
Top 1,2,3	4.77	240	10	130	<5	10.0

Table 4. 18 Organic chemistry data of extracts of soil samples collected from Mt Bold On 20th / 21st July 1999.

Sample Description	DOC (mg/L)	TKN(mg/L)	Abs.@254nm	C/N ratio	SUVA
Top 1a	5.8	2.72	0.116	2.1	1.99
Top 1b	3.4	1.06	0.057	3.2	1.65
Top 2a	7.3	1.22	0.139	6.0	1.91
Top 2b	3.3	0.6	0.053	5.4	1.61
Top 3a	28.6	6.22	1.101	4.6	3.85
Top 3b	42.1	6.96	1.364	6.1	3.24
Mean	15.1	3.1	0.472	4.6	2.4
STD	16.4	2.8	0.596	1.6	0.9
Middle 1a	44.7	4.76	0.881	9.4	1.97
Middle 1b	31.9	3.66	0.689	8.7	2.16
Middle 2a	11.3	4.36	0.211	2.6	1.87
Middle 2b	8.9	1.02	0.146	8.7	1.65
Middle 3a	32.0	2.16	0.654	14.8	2.04
Middle 3b	6.4	1.06	0.106	6.0	1.65
Mean	22.5	2.8	0.448	8.4	1.9
STD	15.8	1.6	0.332	4.0	0.2
Bottom 1a	15.5	1.48	0.872	10.5	5.63
Bottom 1b	24.2	2.66	4.135	9.1	17.09
Bottom 2a	1.7	0.28	0.074	6.2	4.28
Bottom 2b	2.5	0.38	0.195	6.5	7.88
Bottom3a	15.4	1.44	0.784	10.7	5.10
Bottom3b	34.9	4.04	6.493	8.6	18.59
Mean	15.7	1.7	2.092	8.6	9.8
STD	12.7	1.4	2.623	1.9	6.4

Table 4. 19 Inorganic chemistry data of extracts of soil samples collected from Mt Bold on 20th / 21st July 1999.

Sample Description	pH	Cond [µs/cm]	Bicarbonate (mg/L)	TDS (by EC, mg/L)	Na (mg/L)	Alkalinity $CaCO_3$ (mg/L)	Moisture content %
Top 1a	6.2	84	<2	46	<1	2	13.0
Top 1b	6.0	82	<2	44	14.6	2	11.5
Top 2a	5.7	66	<2	36	7.2	2	10.8
Top 2b	6.1	40	<2	22	5.2	2	10.8
Top 3a	5.3	102	<2	56	46.6	2	20.4
Top 3b	5.3	140	2	76	24.2	2	18.1
Mean	5.8	85.7	2.0	46.7	19.6	2.0	14.1
SD	0.4	33.8		18.3	16.9	0.0	4.1
Middle 1a	6.0	96	6	52	21.6	4	27.0
Middle 1b	6.2	86	4	48	18.4	4	23.0
Middle 2a	5.4	80	6	44	16.0	4	15.0
Middle 2b	5.4	130	<2	72	7.4	2	16.0
Middle 3a	6.1	64	<2	36	2.8	2	11.0
Middle 3b	5.1	92	<2	50	2.4	2	13.5
Mean	5.7	91.3	5.3	50.3	11.4	3.0	17.6
SD	0.5	22.0	1.2	12.0	8.3	1.1	6.1
Bottom 1a	6.2	40	<2	22	5.8	2	25.0
Bottom 1b	6.7	30	4	16	4.8	4	24.5
Bottom 2a	6.2	48	<2	26	2.8	2	15.0
Bottom 2b	6.1	40	<2	22	3.2	2	15.0
Bottom 3a	6.5	50	2	28	3.4	2	27.0
Bottom 3b	6.3	40	6	22	5.2	4	27.0
Mean	6.3	41.3	4.0	22.7	4.2	2.7	22.3
SD	0.2	7.1	2.0	4.1	1.2	1.0	5.7

Table 4.20 Organic chemistry data of extracts of soil litter layer samples collected from the Myponga pine site on 19th July 1999.

Myponga Pine-Litter layer (19/07/ 99)

Sample Description	DOC (mg/L)	TKN (mg/L)	C/N ratio	Abs. @254nm	SUVA
Top 1,2,3	219	9.8	22.3	7.0	3.21
Middle 1,2,3	160	6.9	23.2	5.3	3.29
Bottom 1,2,3	190	8.4	22.7	6.2	3.25

Table 4.21 Inorganic chemistry data of extracts of soil litter layer samples collected from the Myponga pine site on 19th July 1999.

Sample Description	pH	Cond. [µs/cm]	Bicarbonate (mg/L)	TDS (by EC) (mg/L)	Na (mg/L)	Alkalinity $CaCO_3$ (mg/L)
Top 1,2,3	6.39	160	40	90	23.0	30
Middle 1,2,3	6.46	130	40	70	<5	30
Bottom 1,2,3	6.43	180	40	100	<5	30

Table 4. 22 Organic chemistry data of extracts of soil samples collected from the Myponga pine site on 19th July 1999.

Sample Description	DOC (mg/L)	TKN (mg/L)	Abs. @ 254 nm	C/N ratio	SUVA
Top 1a	77.3	6.10	1.76	12.7	2.27
Top 1b	71.8	4.60	1.53	15.6	2.13
Top 2a	571	27.8	20.7	20.5	3.63
Top 2b	454	25.0	17.0	18.2	3.75
Top 3a	85.4	4.94	1.77	17.3	2.08
Top 3b	50.5	3.64	1.10	13.9	2.18
Mean	218	12.0	7.3	16.4	2.7
SD	231	11.2	9.0	2.9	0.8
Middle 1a	21.7	1.62	0.636	13.4	2.94
Middle 1b	34.2	2.14	0.824	16.0	2.41
Middle 2a	21.6	1.34	0.728	16.1	3.37
Middle 2b	24.3	1.58	0.689	15.4	2.83
Middle 3a	30.4	2.50	0.791	12.2	2.60
Middle 3b	30.5	2.88	1.00	10.6	3.29
Mean	27.1	2.0	0.8	13.9	2.9
SD	5.3	0.6	0.1	2.3	0.4
Bottom 1a	45.8	3.30	1.07	13.9	2.33
Bottom 1b	22.4	2.40	0.577	9.3	2.58
Bottom 2a	22.4	1.38	0.424	16.2	1.89
Bottom 2b	18.7	1.60	0.375	11.7	2.00
Bottom 3a	36.9	3.22	0.809	11.5	2.19
Bottom 3b	33.5	2.00	0.636	16.8	1.90
Mean	30.0	2.3	0.6	13.2	2.1
SD	10.5	0.8	0.3	2.9	0.3

Table 4.23 Inorganic chemistry data of extracts of soil samples collected from the Myponga pine site on 19th July 1999

Sample Description	pH	Cond [µs/cm]	Bicarbonate (mg/L)	TDS (by EC) (mg/L)	Na (mg/L)	Alkalinity CaCO3 (mg/L)	Moisture content %
Top 1a	5.9	122	8	66	10.6	6	36.9
Top 1b	6.3	128	10	70	16.4	8	31.5
Top 2a	5.1	398	32	220	28.6	26	37.3
Top 2b	5.8	364	48	200	30.2	40	40.1
Top 3a	6.3	120	12	66	13.8	10	29.7
Top 3b	5.7	136	4	74	16.0	4	27.2
Mean	5.8	211	19.0	116	19.3	15.7	33.8
SD	0.4	132	17.2	73.1	8.1	14.3	5.0
Middle 1a	6.6	74	4	40	6.0	4	24.4
Middle 1b	6.8	92	6	50	9.4	4	22.4
Middle 2a	6.8	58	4	32	4.6	4	21.1
Middle 2b	6.9	70	8	38	10.4	6	22.7
Middle 3a	6.2	78	4	42	10.2	4	34.2
Middle 3b	6.3	76	4	42	10.4	4	22.2
Mean	6.6	74.7	5.0	40.7	8.5	4.3	24.5
SD	0.3	11.1	1.7	5.9	2.5	0.8	4.9
Bottom 1a	6.1	160	6	88	28.0	4	29.0
Bottom 1b	6.2	130	4	72	19.4	4	28.6
Bottom 2a	6.7	90	6	50	12.4	4	29.6
Bottom 2b	6.7	140	6	76	24.2	4	24.0
Bottom 3a	6.6	206	8	112	36.8	6	22.5
Bottom 3b	6.3	210	4	116	36.8	4	32.8
Mean	6.4	156	5.7	85.7	26.3	4.3	27.8
SD	0.2	46.3	1.5	25.2	9.7	0.8	3.8

Table 4.24 Organic chemistry data of extracts of soil samples collected from the Myponga grass site on 19th July 1999.

Sample Description	DOC (mg/L)	TKN (mg/L)	C/N ratio	Abs. @254nm	SUVA
Top 1a	5.4	0.94	5.8	0.279	5.13
Top 1b	5.2	1.20	4.3	0.233	4.47
Top 2a	4.6	0.64	7.1	0.160	3.50
Top 2b	5.7	0.88	6.5	0.211	3.69
Top 3a	5.3			0.181	3.39
Top 3b	5.8	0.70	8.2	0.192	3.33
Mean	5.3	0.74	6.4	0.209	3.92
SD	0.44	0.37	1.5	0.042	0.723
Middle 1a	4.4	1.46	3.0	0.144	3.28
Middle 1b	6.8	2.50	2.7	0.666	9.80
Middle 2a	5.4	2.32	2.3	0.258	4.78
Middle 2b	8.2	4.32	1.9	0.433	5.28
Middle 3a	10.0	2.76	3.6	0.279	2.79
Middle 3b	12.8	3.36	3.8	0.543	4.25
Mean	7.9	2.79	2.9	0.387	5.03
SD	3.1	0.97	0.74	0.196	2.51
Bottom 1a	20.8	2.22	9.4	2.81	13.5
Bottom 1b	15.8	1.36	11.6	1.24	7.86
Bottom 2a	19.2	2.18	8.8	3.20	16.7
Bottom 2b	19.6	1.48	13.2	2.96	15.1
Bottom 3a	19.6	2.14	9.2	3.19	16.3
Bottom 3b	10.3	1.80	5.7	1.74	16.9
Mean	17.5	1.86	9.7	2.52	14.4
SD	3.94	0.38	2.58	0.829	3.43

Table 4. 25 Inorganic chemistry data of extracts of soil samples collected from the Myponga grass site on 19th July 1999.

Sample Description	pH	Cond. [us/cm]	Bicarbonate (mg/L)	TDS (by EC) mg/L	Na	Alkalinity $CaCO_3$	soil moisture content %
Top 1a	6.38	96	<2	52	18.4	2	25
Top 1b	6.27	104	<2	56	14.0	2	23
Top 2a	6.75	152	2	84	11.2	2	24
Top 2b	6.56	220	4	120	11.6	4	26
Top 3a	6.46	250	2	138	4.6	2	25
Top 3b	6.50	312	2	172	6.8	2	23
Mean	6.49	189	2.5	104	11.1	2.3	24
SD	0.16	86	1	48	4.95	0.82	1.3
Middle 1a	5.85	128	<2	70	7.0	2	22
Middle 1b	6.07	122	<2	66	7.4	2	23
Middle 2a	5.54	180	<2	98	6.0	2	22
Middle 2b	5.86	154	<2	84	7.0	2	22
Middle 3a	5.08	346	<2	190	12.6	2	23
Middle 3b	6.07	100	<2	54	5.4	2	22
Mean	5.75	172	<2	94	7.6	2	22
SD	0.38	90		49.6	2.6	0.00	0.63
Bottom 1a	7.34	46	14	26	9.4	12	20
Bottom 1b	7.17	46	10	26	8.0	8	22
Bottom 2a	7.18	58	12	32	11.8	10	19
Bottom 2b	7.24	78	18	42	13.6	14	20
Bottom 3a	7.30	52	16	28	11.2	14	18
Bottom 3b	6.45	56	14	30	11.8	12	19
Mean	7.11	56	14	31	11.0	12	20
SD	0.33	11.9	2.8	6.0	1.98	2.3	1.3

DRIFT spectra of samples collected in July 1999.

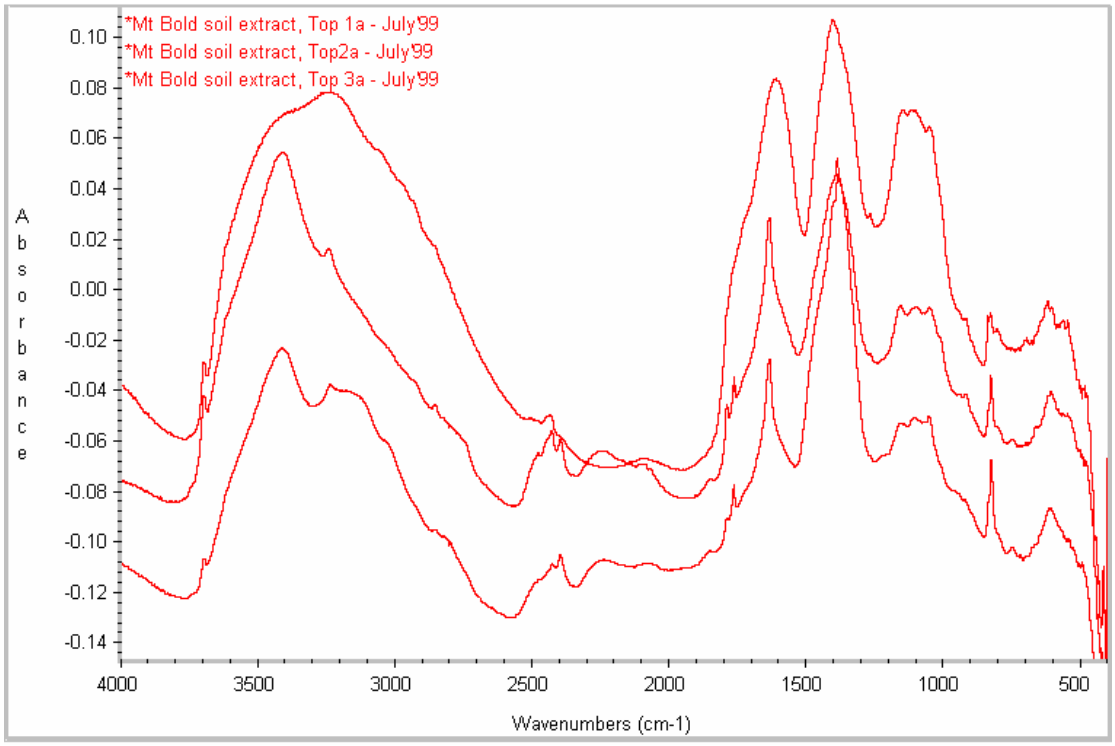


Figure 4. 29 DRIFT spectra of Mt Bold soil samples (top of slope).

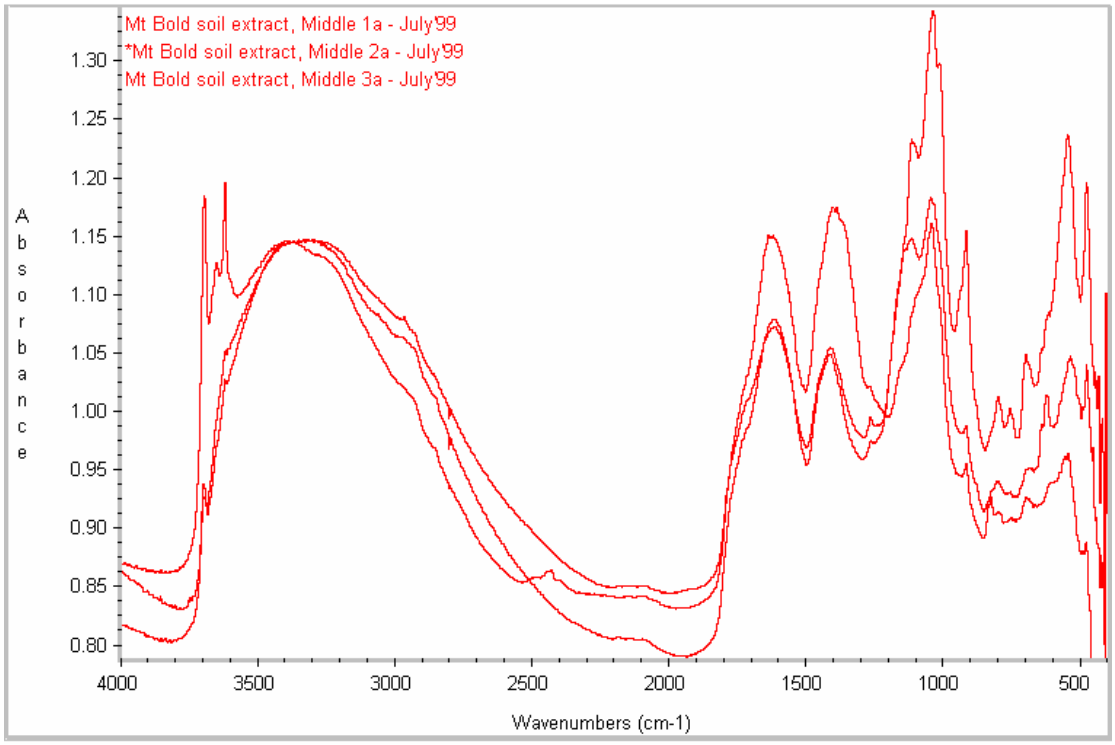


Figure 4. 30 DRIFT spectra of Mt Bold soil samples (middle of slope).

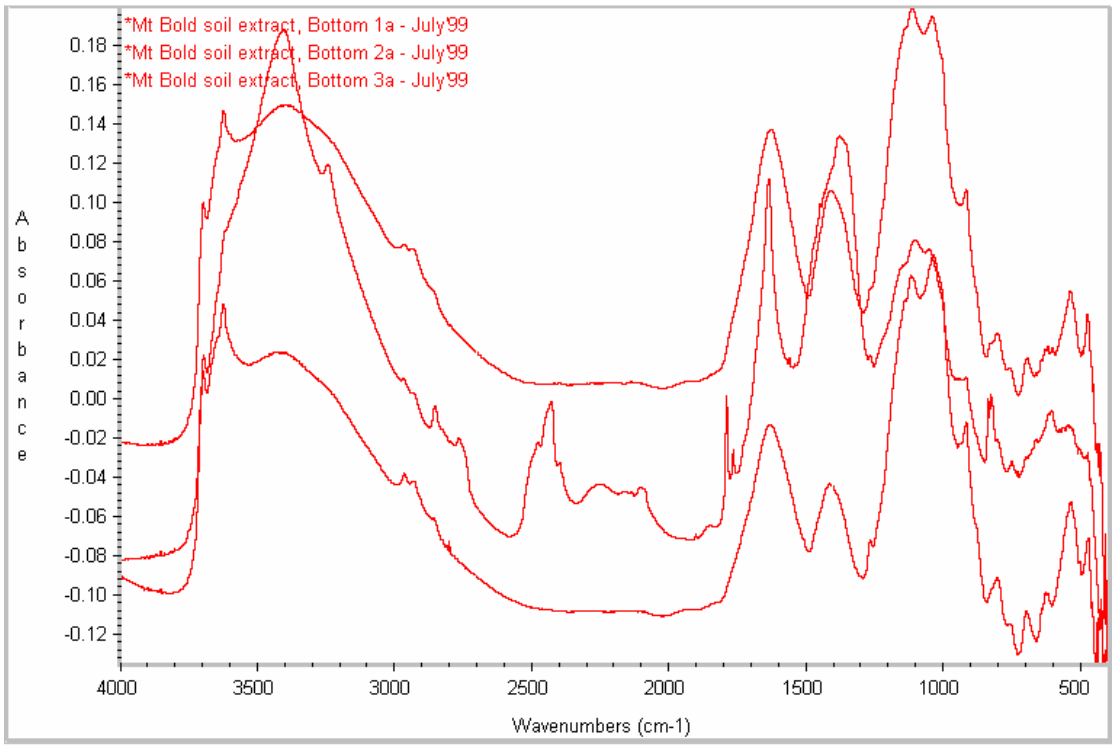


Figure 4. 31 DRIFT spectra of Mt Bold soil samples (bottom of slope).

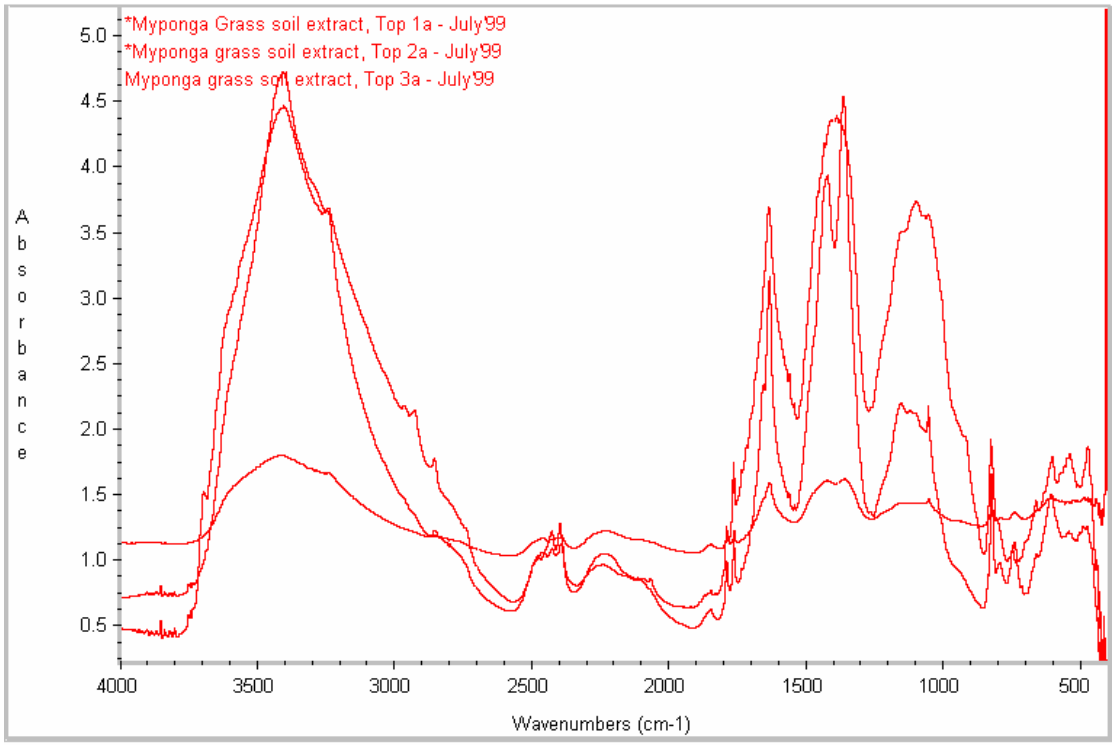


Figure 4. 32 DRIFT spectra of Myponga soil samples (grass site, top of slope).

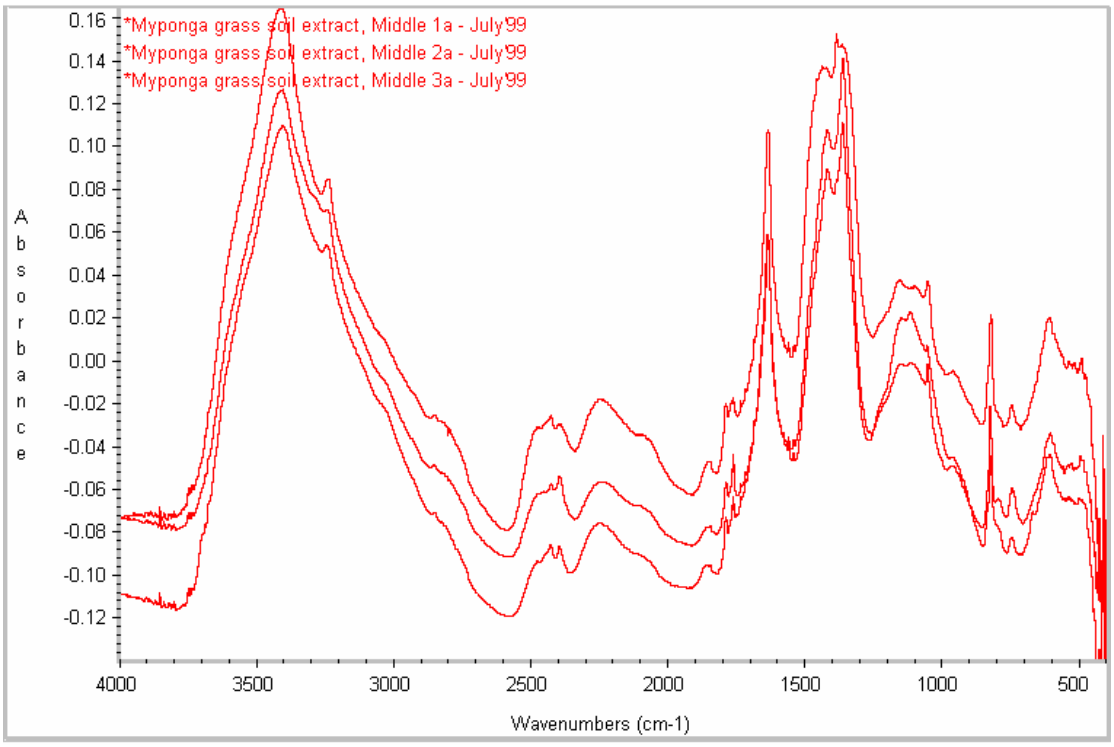


Figure 4. 33 DRIFT spectra of Myponga soil samples (grass site, middle of slope).

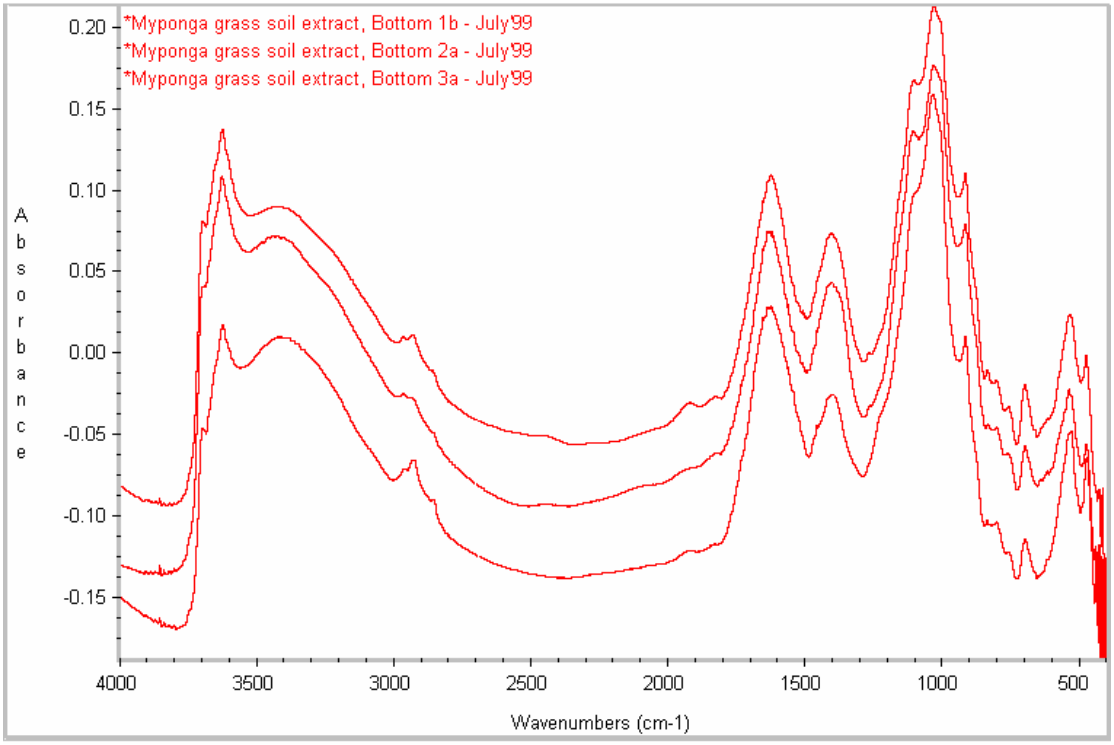


Figure 4. 34 DRIFT spectra of Myponga soil samples (grass site, bottom of slope).

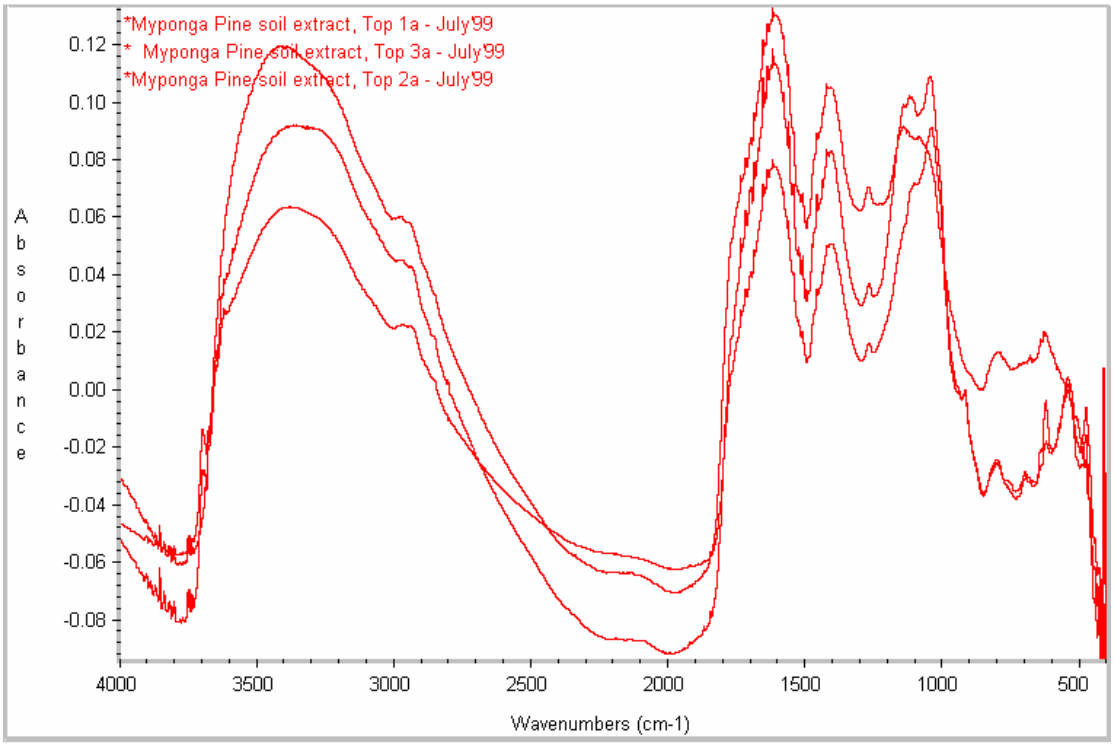


Figure 4. 35 DRIFT spectra of Myponga soil samples (pine site, top of slope).

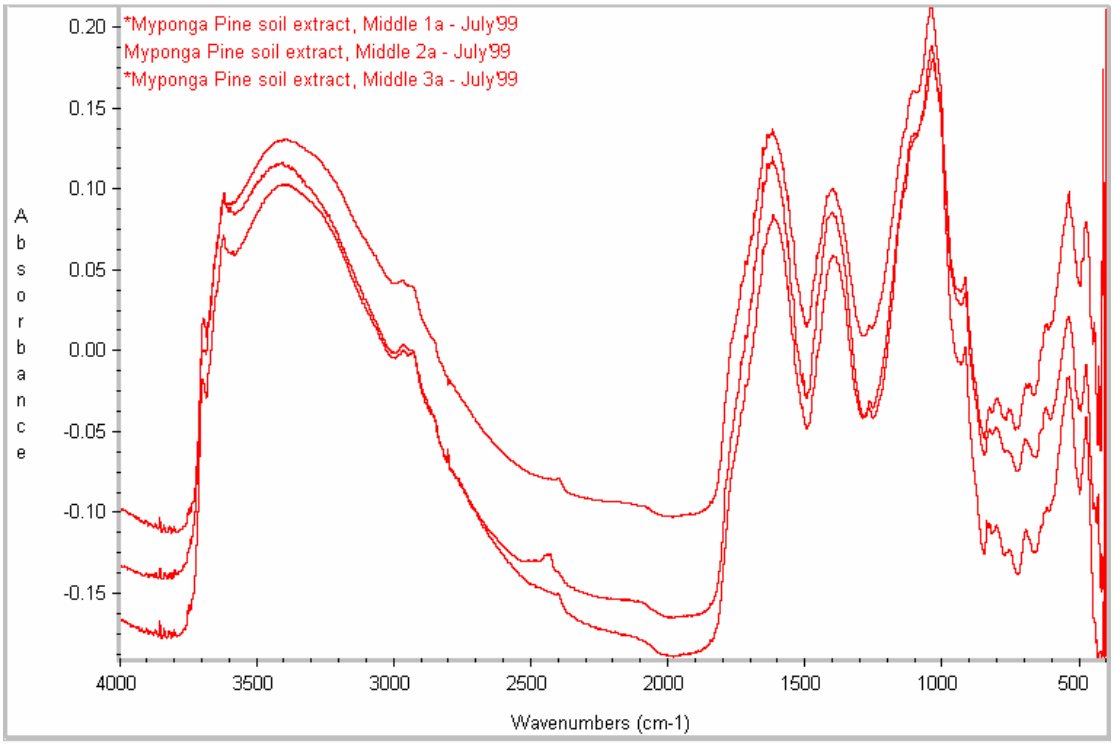


Figure 4. 36 DRIFT spectra of Myponga soil samples (pine site, middle of slope).

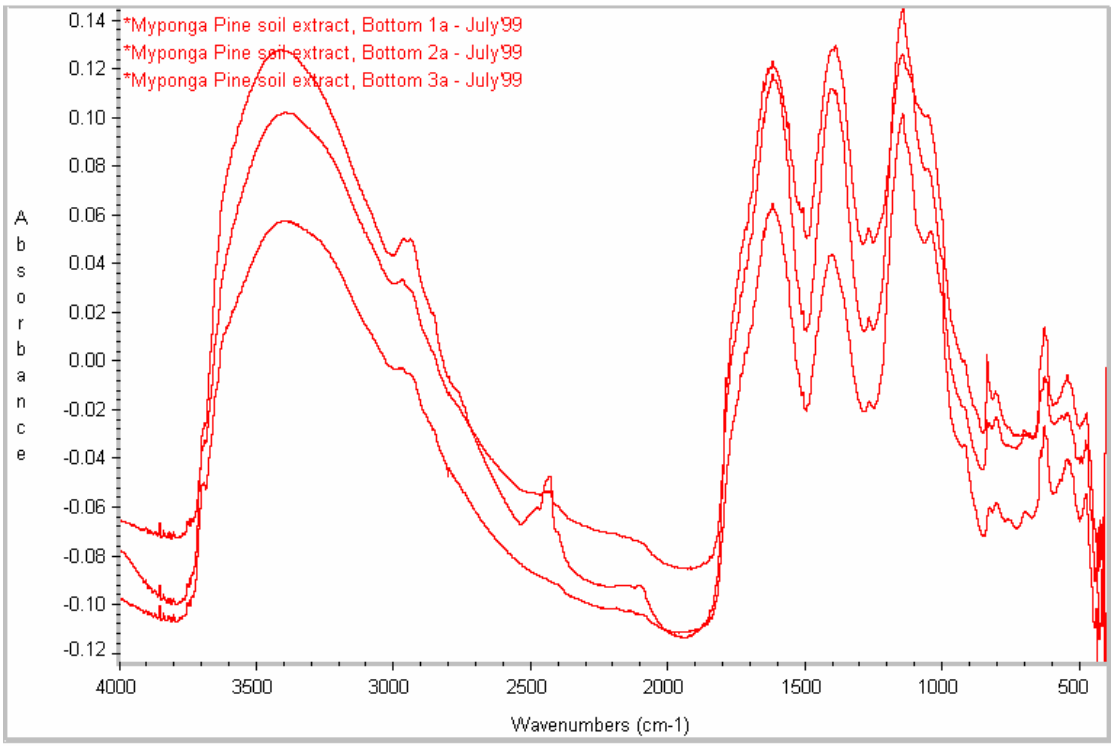


Figure 4. 37 DRIFT spectra of Myponga soil samples (pine site, bottom of slope).

The value of any analytical technique for characterisation of NOM is that the results give a clear indication of functionality, structural composition, and/or other factors such as the degree of degradation and enable differentiation between samples, either through simple or complex data analyses. In this part of the study it was evident that DRIFT spectra were influenced by inorganics, confounding assignment to the functionalities of organic compounds. Differences were found in salt interferences in the spectra of soil extracts where organic loads on the soils varied. Extracts from soils from the pine plantation had higher concentrations of dissolved organic carbon and the DRIFT spectra of these showed features that indicated less impact from salts. Nonetheless, few well resolved peaks were detected in the spectra, (between ~ 900 and 1800 WN/cm) consistent with other spectra of NOM isolates and references. Although DRIFT analysis is a rapid technique, the information from them for characterisation of NOM can be limited to comparisons of these major peaks and are based on assumptions of the relative contributions from organic and inorganic components. More advanced forms of statistical analyses and mathematical computations may provide better interpretation of DRIFT spectra. For example, peak fitting analysis may give insight into various minor peaks that contribute to the three broad peaks that generally occur between ~ 900 and 1800 WN/cm. However, the presence of these minor peaks should be firstly assessed through 1^{st} and 2^{nd} order derivatives to determine gradient changes and peak apexes.

Principal component analysis may also be adopted where confidence exists in the sources contributing to the absorbances. In this project, where PCA was trialed (by Downes, 2000), it was found that the inorganic components confounded interpretation of spectra for characterisation of NOM.

The use of the 254nm/456nm ratio is not conventional in NOM characterisation and is postulated here to be informative UV-vis spectral data. This is based on the consistency of the magnitude of this ratio from fresh vegetation and soil litter layer leachates and the generally distinctive difference to the ratio values of samples from soils and reservoirs. It is further proposed that this ratio gives an indication of the degree to which organics have undergone microbial degradation i.e. with lower ratios reflecting more bio-degraded organics. Clear trends in this ratio were found in relation to catchment topography.

Organics leaching from soils appeared to be related to organic loads on these soils with those of the pine forest contributing the most. This also appears to be important from a catchment management viewpoint, where there is a need to determine suitable vegetation cover in the catchment of a drinking water reservoir. Organic loads to soils and reservoirs are likely to be based on the vegetation production in a catchment, the rate of mineralisation in relation to that production, the leaching capacity of fresh and decaying vegetation and the soil type. Hence, high vegetation productivity in terrains that have a high potential for organic leaching and transport are likely to contribute more to organics in waterways. The data from this study indicates that large differences exist between pine and native forests and grassed areas. Other issues in catchment management, such as erosion control are highly important, and it may be that the minimisation of problems of one type exacerbates another. For example, replacement of pine trees from a steep slope with grasses may lead to erosion during high rainfall events. The thick litter, peat-like layer from the pine trees, as found in the Myponga catchment, might be retaining sediments during surface water flow. The

disadvantage of this however, is that organics are leached from this layer and transported to waterways. Site specific research work may be needed to predict actual outcomes of land management practices.

4.4 Conclusions :

UV-vis data, together with other water quality parameters, provided informative data on the characters of NOM from reservoirs, soils and litter layers from South Australian catchments. Trends in UV-vis data were found in relation to catchment topography and sample type (reservoir, soil and litter layer).

DRIFT spectroscopy provided some informative data for specific samples, particularly where the inorganic composition was known to be minimal. Its application should be on the basis of samples being known to contain minimal salts, to be desalted (eg. by ultrafiltration) or with salts present and then as an empirical fingerprint. In some samples, differences were found in relative peak heights that appeared to be based on different vegetation sources ie. grassed versus treed sites. A limitation of DRIFT spectroscopy for NOM characterisation was the relatively few well resolved absorbance bands that could be assigned to organic functional groups.

Marked differences were found in the organic loads in soils and in litter layers of areas vegetated by grasses, *P. radiata* and mixed native trees. It appears the high productivity of vegetation and their persistence in the environment leads to a greater potential for organics to be leached from the soils and litter layers. However, the perception of this as being of detriment to water quality needs to be tempered with the possibility that the heavy litter layer, trees, fallen branches and logs, minimises soil erosion, most especially on steep slopes that lead to waterways.

CHAPTER 5

EXPERIMENTAL WORK PERFORMED TO REMOVE INTERFERING SALTS FOR CHARACTERISATION OF NOM USING DRIFT ANALYSIS.

5.1 Introduction

DRIFT spectra of freeze-dried materials from raw surface waters and soils collected as part of the NOM survey work contained sharp peaks indicative of a strong influence from salts, also present in these matrices. The presence of salts therefore confounds the assignment of spectral peaks to organic matter. This reduces confidence in assignments of absorbance bands of DRIFT spectra, for characterisation of NOM in these matrices. A series of attempts were made to develop a procedure or procedures to remove or minimise salts in freeze-dried fractions which could then be analysed by DRIFT spectroscopy for characterisation of NOM.

5.2 Materials and methods

Two general methods were trialed,

1. Dialysis tubing to retain organics and separating inorganic salts by allowing them to pass across the dialysis tube membrane.
2. Ultrafiltration . These are detailed below. Other details on salt removal using ultrafiltration are given in Report 1 of CRCWQ&T project 2.1.

5.2.1 Dialysis tubings used in experiments conducted to remove salts from raw waters.

Two dialysis membranes were trialed, these being

1. Spectra/Por® 3 Regenerated Cellulose, Cat. No. 132 725, flatwidth: 54 mm, diameter :34mm, vol./length, mL/cm : 9.3 (Spectrum®, California, USA). Spectra/Por 3 has a molecular weight cut-off (MWCO) of 3,500 daltons (3.5k MWCO). This means organics whose molecular weights are less than 3.5k daltons can pass through the membrane,

and

2. Membra-Cel MD 34-14 x 100CLR, nominal dry flatwidth : 34 mm, dry diameter : 22 mm. (Viskase Corporation, Chicago, USA). The MWCO for this membrane is 14,000.

It should be noted that the MWCO sizes used would allow small molecular weight organic compounds to pass through the membranes and be lost with the salts.
The use of higher MWCO for dialysis compared with the commonly used smaller MWCOs in ultrafiltration is justified on the basis that dialysis separation is achieved only by diffusion through osmotic pressures. With the anticipated high desalting, DRIFT analysis would then be unambiguous in characterising isolated NOM. Further, although it is often presumed that organics remaining in water after treatment with conventional coagulants are of small molecular weight, this perception appears

incorrect. Work presented in Report 1 of this project shows a high percentage (35-42) of organics in water after enhanced alum treatment being greater than 1000 daltons, apparent molecular weight. It may be that, although the average molecular weight has been reduced (*importantly,* of the organics that could be detected by HPSEC with UV detector), some larger molecular weight, hydrophilic compounds (such as polysaccharides and aliphatic acids) are still present in treated water. A component of NOM may be of large molecular weight and still be recalcitrant to conventional water treatment. Py-GC/MS data, shown in Report 1, indicate that these comprise aliphatic compounds.

The basis for the work of this chapter was that the application of dialysis could potentially lead to valuable information on the character of NOM using DRIFT analysis, where the isolated NOM fraction is significant to the water industry and in reservoir catchment systems.

5.3 Experiments performed to test dialysis tubings under various conditions.

These are as follows:

5.3.1 Experiment 1. Dialysis of a raw surface water using the Spectra/Por membrane tubing**.**

Aim: to determine if dialysis can be applied for the removal of salts from raw reservoir water, in preparation for DRIFT analysis.

Materials and method: A sample of water (~0.5 L) from the Myponga Reservoir collected on 11th May 1999, was filtered through 0.45 μm and then adjusted to pH 7.

Three strips of dialysis tubing (15 cm) were soaked in RO water for 30 min. and then washed with RO water for 5 min. One end of each tubing section was tied into a knot, the tube filled with the sample water (100 mL) and the open end closed with a clamp. The three tubes were placed into a 5L beaker filled with RO water. The surrounding water was stirred using a magnetic stirrer and the time of stirring was recorded. Tubes were removed from the beaker after 2 hr, 6 hr and 22 hr. After removal of each tube, the RO water in the beaker was replaced with fresh RO water. After removal from the beaker, the tubes were opened, samples removed and freeze-dried. The freeze-dried samples were analysed by DRIFT spectroscopy. A sub-sample that had not been dialysed was used as a control.

Results and discussion : DRIFT spectra of the Myponga Reservoir water sample before and after dialysis are shown in Figure 5.1. Sharp peaks are present in the spectra of the control and samples dialysed for 2 hr and ~6hr. Further, in these spectra, no minor shouldering peaks at about 2930 to 2980 WN/cm (indicative of CH, CH_2 and CH_3 stretching) is evident. In contrast to this the spectrum of the sample dialysed for 22 hours shows the anticipated broad, poorly resolved peaks that might be expected from a heterogenous mixture of organics with diverse functionality associated with it. In this spectrum, peaks in the range of about 2930-2980 WN/cm can be seen, reflective of a higher relative content of organics in the sample analysed. Although, dialysis appears to have reduced the concentration of salts in the sample matrix, the resultant spectrum contained a limited amount of information, ie three

broad peaks in the region of 1000 to 1800 WN/cm. No data is available on the amount or types of organics removed with the salts in the dialysis process used. The results obtained indicate that this dialysis tubing may be suitable for desalting surface water samples, provided that dialysis is done for 22 hr.

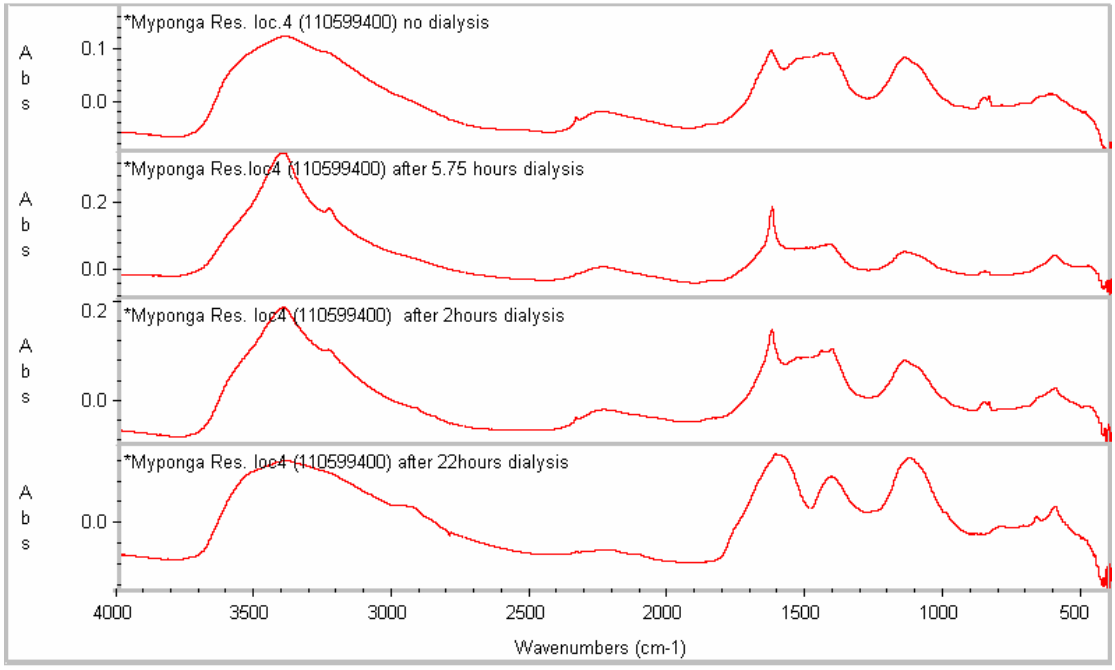


Figure 5.1 DRIFT spectra of a raw water sample from Myponga Reservoir before and after dialysis using Spectra/Por membrane tubing (54 mm).

5.3.2 Experiment 2. Dialysis of a raw surface water using Membra-Cel MD 34 - 14 x 100CLR 34mm

Aim : The aim of this experiment was to determine the suitability of the Membra-Cel MD membrane for desalting of reservoir water, particularly in relation minimising the time required for dialysis.

Materials and method : A combined sample (2 L) from Myponga Reservoir water samples was filtered through 0.45 µm and then adjusted to pH 7. Five strips of dialysis tubing (50 cm) were soaked in RO water for 30 min and then washed with RO water for 5 min. One end of each strip was tied into a knot and each tube filled with 100 mL of sample. The open end was then closed by tying a knot. These were placed into a 5 L beaker filled with RO water and stirred with a magnetic stirrer. At 90 min intervals, four samples were removed from the beaker, and the RO water replaced by fresh RO water. The fifth sample was left in the beaker overnight. A sub-sample that had not been dialysed was used as a control. Samples were then removed from the dialysis tubing, freeze-dried and analysed by DRIFT spectroscopy.

Results and discussion : DRIFT spectra of the Myponga Reservoir water sample before and after dialysis with the Membra-Cel MD tubing are shown in Figure 5.2.

Spectra of samples dialysed up to 180 min were similar to the control sample (except for the sample dialysed for 2 hr, which showed an unexpectedly high salt impact, the basis for this being unknown). At 270 min, the spectra show peak shapes that are intermediate to those from the 360 min sample. This latter sample having broad peaks that might be expected of a heterogenous mix of organics. With dialysis extended overnight, better resolution of peaks on either side of ~1500 WN/cm was found. Nonetheless, the spectrum provided limited information with only three notable peaks between ~1000 WN/cm and 1800WN/cm, though a small peak apex is evident at ~ 1250 WN/cm. The other feature that can be seen is the presence of very small shoulder peaks at ~ 2950, with longer dialysis times.

Similar to Experiment 1, the results of this experiment indicate that salt influences on DRIFT spectra can be reduced by dialysing, but the time required is in excess of 12 hours (overnight).

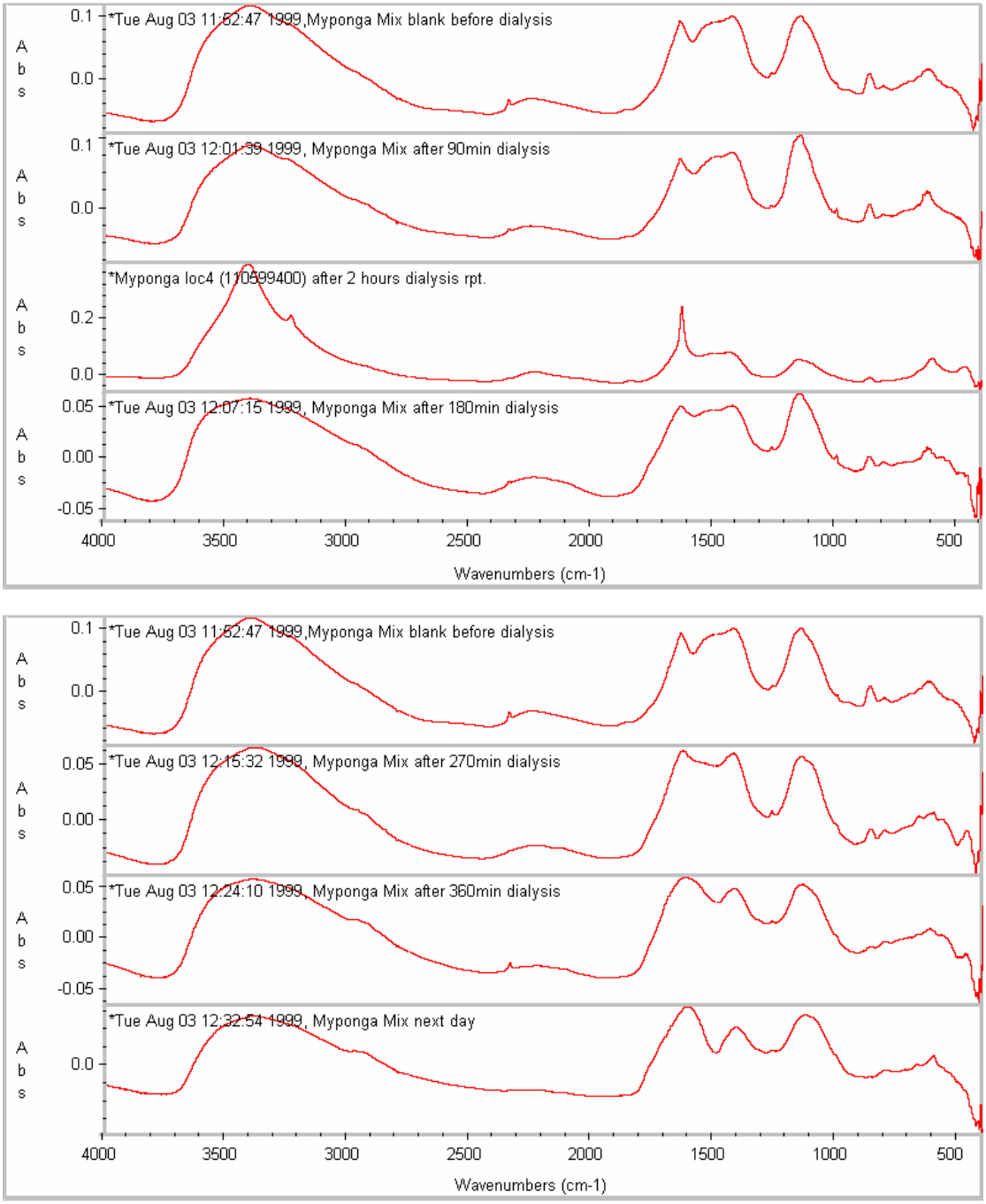


Figure 5.2 DRIFT spectra of a raw water sample from Myponga Reservoir before and after dialysis using Membra-Cel MD 34 - 14 x 100CLR 34mm.

5.3.3 Experiment 3. Dialysis of a raw surface water using Membra-Cel MD 34 - 14 x 100CLR 34mm, where two procedures were compared for permeation efficiency**.**

Aim : to determine a method that increases the rate of salt removal from a raw water sample when using dialysis.

Materials and method : Six strips of dialysis tubing, Membra-Cel MD 34-14 x 1000CLR, 50 cm long, were soaked in RO water for 30 min and then washed under running RO water for 5 min. One end of each tube was tied with a knot. Each of these was filled with 100 mL of a sample and the other end was then closed with a knot. The sample (2 L) was a composite of raw surface water samples collected from the Myponga Reservoir. The sample had been filtered through 0.45 μm and adjusted to pH 7. Three of these strips were placed into a 5 L beaker filled with RO water and gently stirred using a magnetic stirrer. The other three strips were placed into a long cylinder and running water was applied. At one hour intervals, one strip was removed from the beaker and another from the cylinder. After removal of a strip from the beaker, RO water was replaced by fresh RO water. One sample that was not dialysed, was used as a control.
Samples were then freeze-dried and analysed by DRIFT spectroscopy.

Results and discussion : DRIFT spectra of samples dialysed using the two methods and compared to a control sample are shown in figures 5.3 to 5.5. All spectra are very similar to each other. This indicates that there is no improvement in the rate of salt removal by placing the samples in a cylinder and running fresh RO water constantly though it, compared with placing dialysis tubing into a beaker and replacing the RO water periodically.

The spectra obtained for the samples dialysed at three hours were similar to those of experiments 1 and 2, where dialysis was done for the same or similar times.

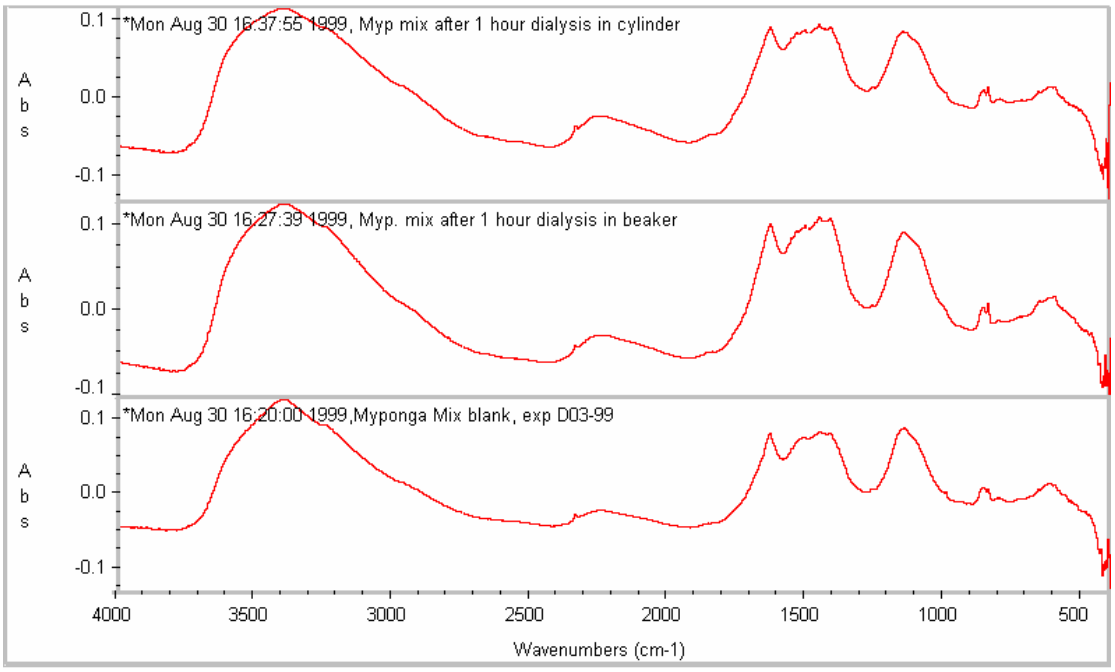


Figure 5.3 Comparison of DRIFT spectra of samples dialysed in a beaker, in a cylinder and not dialysed.

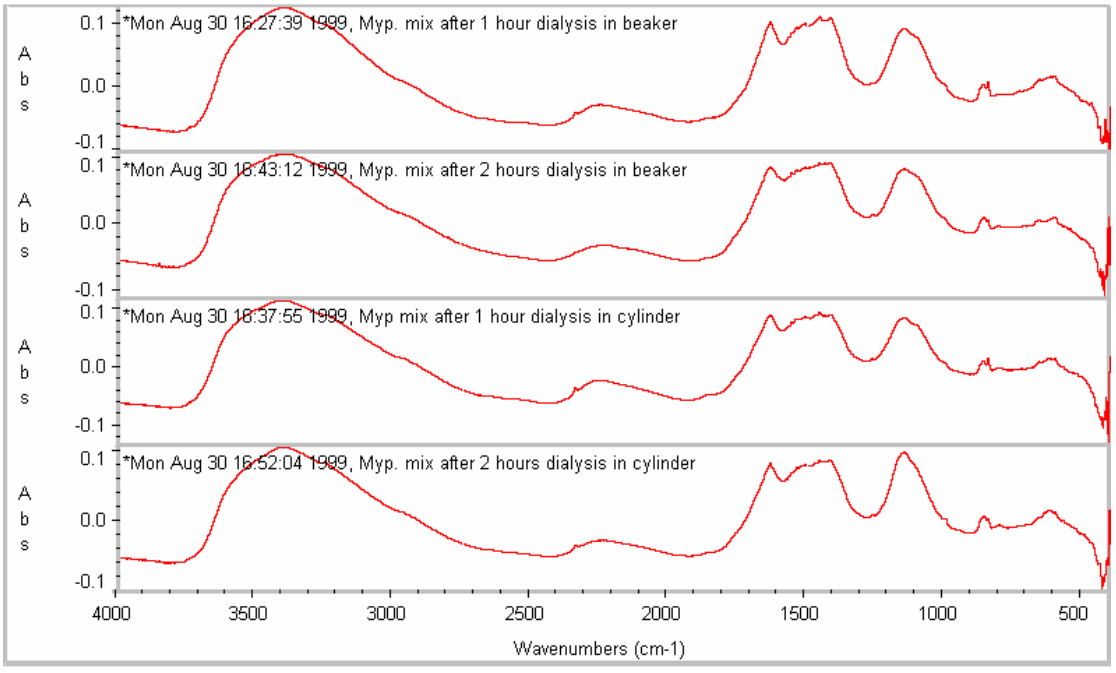


Figure 5.4 Comparison of samples dialysed in a beaker and in a cylinder for 1 and 2 hours.

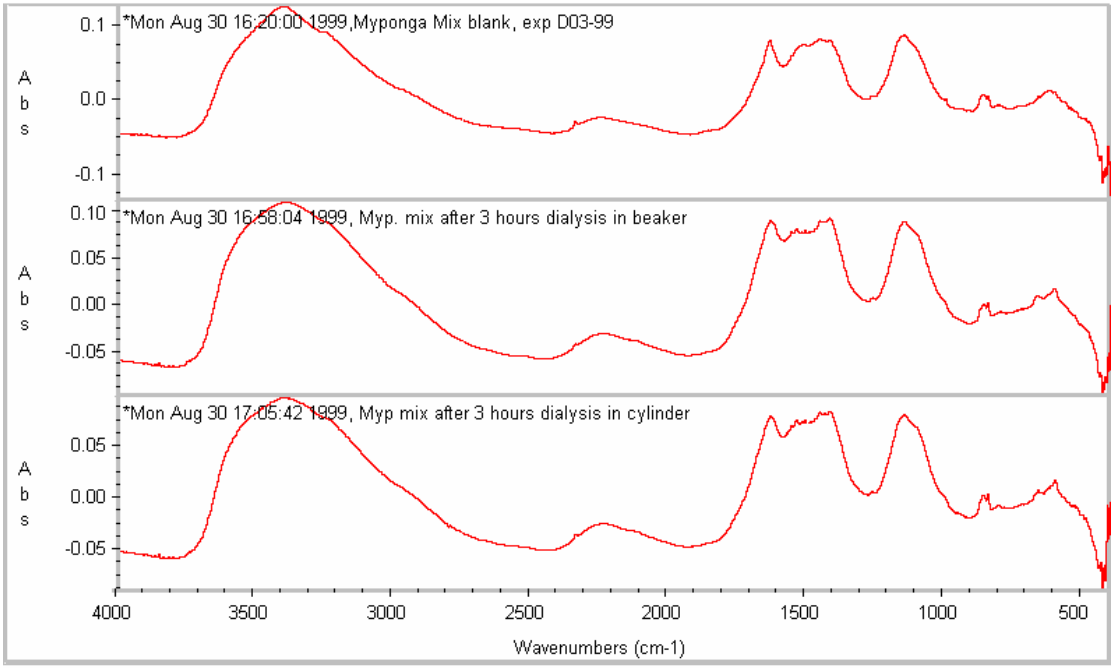


Figure 5.5 Comparison of samples dialysed in a beaker and in a cylinder for three hours and a sample that had not undergone dialysis.

5.3.4 Experiment 4. Dialysis of water samples from different locations using Membra-Cel MD 34 - 14 x 100CLR.

Aim: to determine the impact of dialysis in removing salts from different raw and alum treated waters and with frequent replacement of the RO water containing permeates.

Materials and method: Four water samples were selected for this experiment, these being:

1. Mt. Cole Reservoir, collected in February 1999.
2. A composite of waters from Myponga Reservoir.
3. Pankalak Reservoir, collected in February 1999.
4. Pankalak alum treated water collected in February 1999.

Samples were filtered < 0.45μm and adjusted to pH 7. Sub-samples (100ml) were freeze-dried to act as controls.

Four strips of dialysis tubing were cut, soaked in RO water for 30 min and washed under running RO water for 5 min. After tying one end of each tubing with a knot, test samples (4) were put into the strips, the other end tied as before and placed into a 5 L beaker. These were then stirred using a magnetic stirrer. The RO water was changed every 30 min and dialysis was allowed to proceed for a total of 4.5 hours.

After this, the samples were removed from the tubing and freeze-dried.

Results and discussion: DRIFT spectra of samples from the four waters with and without dialysis for 4.5 hours are shown in figures 5.6 to 5.9. Differences in peak numbers, relative peak heights and peak resolution can be seen between the spectra in the region of about 2000 to 1000 WN/cm. Dialysis resulted in greater resolution of peaks in the spectral region of about 1800 to 1300 WN/cm. This resolution of peaks was not to the same degree as found for the Myponga samples dialysed for the extended periods used in experiments 1 and 2. The large peak at ~ 1100 WN/cm in the spectra of the Pankalak treated water is typical of post alum treated waters. This peak may be due to carbohydrates and carbohydrate-derived compounds and/or sulphate from the alum coagulant, being prominent in treated water.

The results indicate that dialysis can be useful in preparing different water samples for DRIFT analysis, though the time required to maximise salt removal appears likely to be longer than 4.5 hours, based on the data obtained for Myponga water in experiments 1 and 2.

Conclusions: The data of the experiments performed show that attempts to remove salts by dialysis can markedly impact on the DRIFT spectra of samples from natural waters. Based on the assertion that reductions in the sharpness of peak apexes and improvement of peak resolution relate to a reduction in salt interferences, it appears that dialysis will reduce salt interferences. This should improve characterisation of NOM by DRIFT analysis, although the numbers of informative peaks are few. Further, no information is available on the organics which are lost with salt removal and from the point of view of drinking water quality, it is these organics that are likely to be important. However, organics isolated by dialysis may also be important,

though the degree to which they represent organics recalcitrant to conventional treatment needs further study. The duration required for maximising removal of salts, based on DRIFT spectra interpretation only, appears to be in the vicinity of 12 to 22 hours. An improved procedure could involve analysis of retentates for salt content and appears warranted in future investigations on dialysis of natural waters, in preparation for DRIFT analysis.

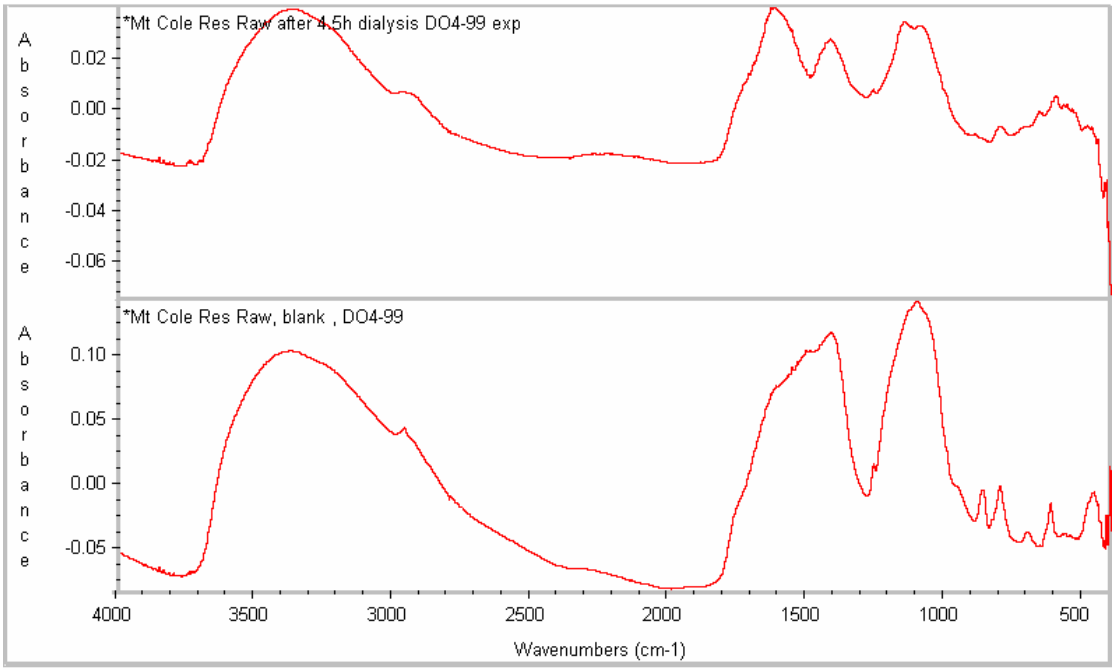


Figure 5.6 DRIFT spectra of the sample from Mt Cole Reservoir, Victoria, with and without dialysis for 4.5 hours.

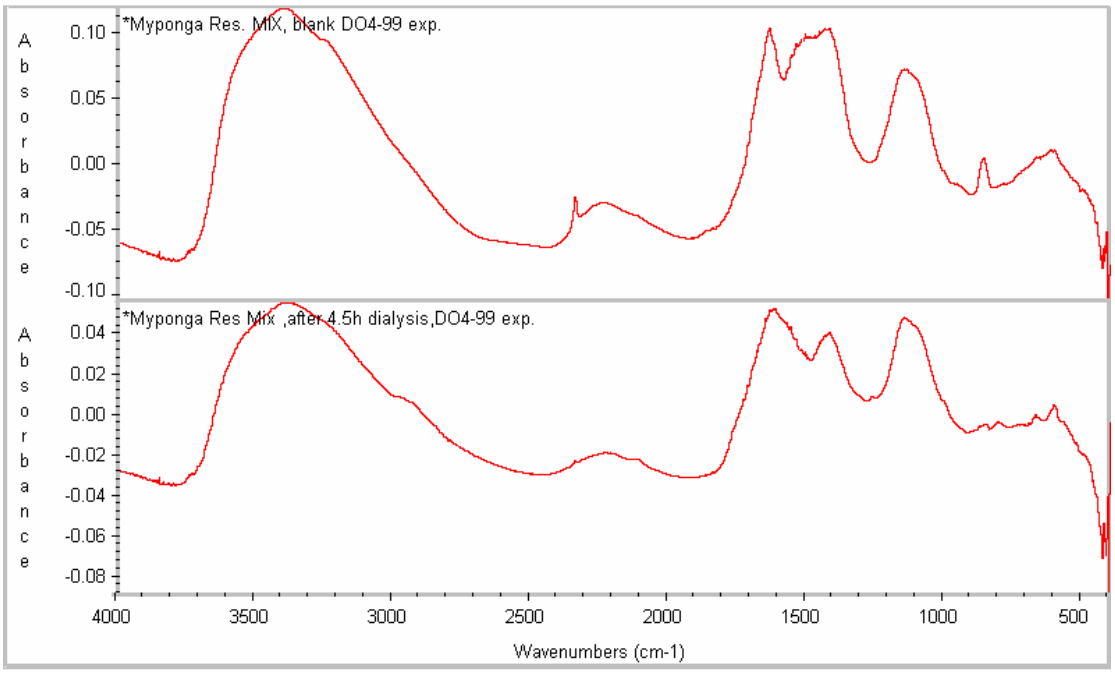


Figure 5.7 DRIFT spectra of the sample from Myponga Reservoir, South Australia, with and without dialysis for 4.5 hours.

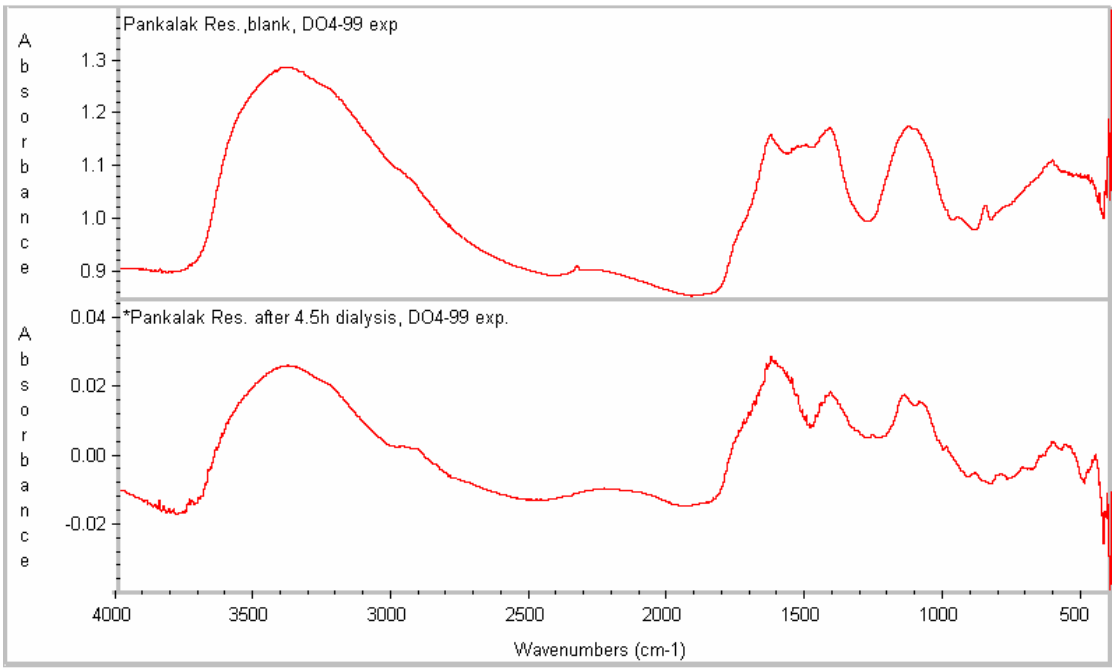


Figure 5.8 DRIFT spectra of the sample Pankalak Reservoir, Victoria, with and without dialysis for 4.5 hours.

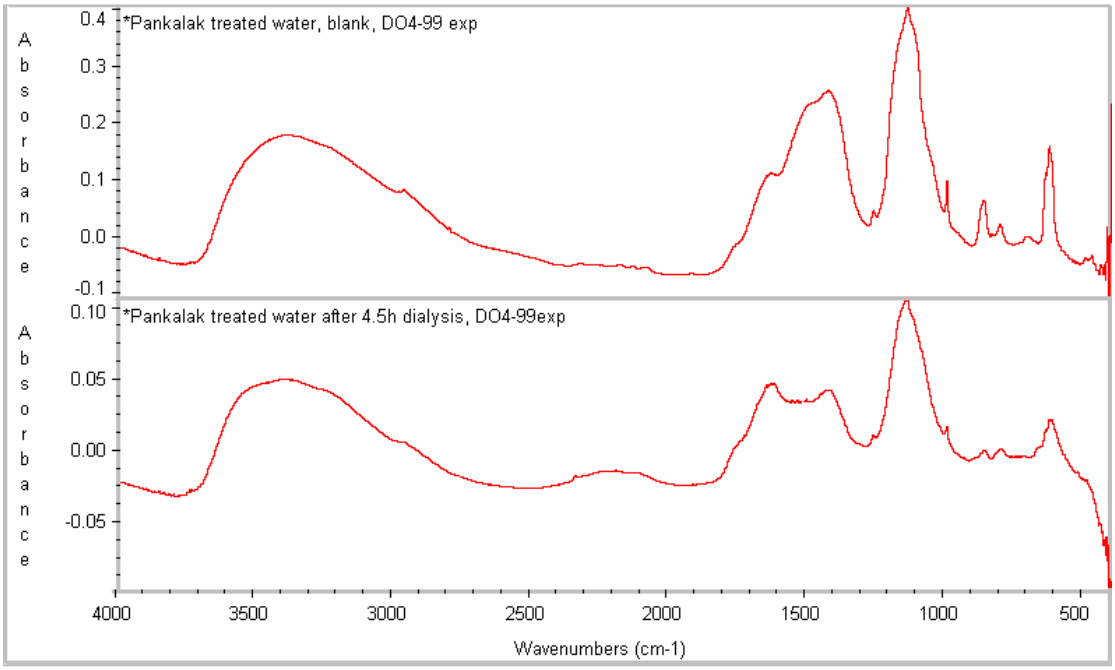


Figure 5.9 DRIFT spectra of the sample Pankalak Reservoir, Victoria, with and without dialysis for 4.5 hours.

5.3 Experiments conducted to remove salts from raw waters using ultrafiltration.

During the course of this project, ultrafiltration using 2L sample jars was attempted for the removal of salts from NOM isolated using MIEX treatment on a raw water sample from the Hope Valley Reservoir. It was intended that DRIFT spectroscopy would be performed to compare the ultrafiltered and non-ultrafiltered samples. However, the outcomes of these attempts were initially judged to have been unsatisfactory for the purposes of using this type of sample for DRIFT analysis.

In subsequent experiments, samples of raw and alum treated waters were ultrafiltered using a 500 mL (76 mm membrane) Amicon cell with a 1000 daltons nominal molecular weight membrane filter. After extensive rinsings, the retentates only were analysed by both DRIFT spectroscopy and pyrolysis-gas chromatography/mass spectrometry. The results of this work are reported in Report 1 of this project, which deals with characterisation of NOM in relation to water treatment processes. From this work it appears that ultrafiltration is suitable for isolating a fraction of the total NOM and salts, which can then be analysed using DRIFT spectroscopy.

CHAPTER 6

CHARACTERISTICS OF THE SORPTION OF DISSOLVED ORGANIC MATTER BY SOIL MINERALS.

6.1 Introduction

The water-soluble fraction of NOM is a dynamic pool of carbon in the natural environment. The soluble form of the NOM is the most readily mobilised form and hence likely to have the greatest impact on the nature of the organic matter in reservoir water for drinking purposes. The nature of the components in the dissolved fraction of the organic matter (DOM) impacts directly on the treatability of the water (Krasner and Amy, 1995; Owen *et al*., 1995; Chow *et al*., 1999). Improving the understanding of the parameters in the catchment affecting the components in this fraction is important to the water treatment industry.

The character of DOM is dependent on the source of the organic matter (Abbt-Braun *et al.* 1989; Bruchet *et al*., 1990; Hempfling and Schulten, 1991; Page *et al*., 2001) a range of degradation processes involving microbial and physico-chemical reactions) and transformations (Kogel-Knabner, 1993; Kogel-Knabner *et al*., 1997; Shindo and Kuwatsuka, 1976; Shevchenko and Bailey, 1996). In particular, a limited amount of research reported in the literature has focussed on the sorption of DOM by minerals changing the nature of, i.e. fractionating, the DOM by sorbing the components selectively (Kaiser *et al.,* 1997; Meier *et al*., 1999; Specht *et al*., 2000; Chorover and Amistadi, 2001).

The hydrophobic and aromatic fractions of a brown-water DOM were found to be preferentially sorbed by kaolinite, montmorillonite and goethite suspensions at pH 4 (Specht *et al.,* 2000; Meier *et al.,* 1999), with the NOM showing a greater affinity for the goethite surface (Meier *et al.,* 1999). In the latter study it was found that the large and intermediate molecules were sorbed first, with the result that the molecular weight of the DOM in solution decreased with increase in extent of sorption for both goethite and montmorillonite, with goethite suspension showing the greater effect.

Similarly, Chorover and Amistadi (2001) found that high molecular weight, and aromatic components of a forest floor leachate were preferentially sorbed by goethite at pH 4. However, these workers reported that sorption by montmorillonite showed a preference for components of lower than average molecular weight with no preference for any particular functional group.

A more extensive study using a range of aqueous extracts from an O-horizon found that carbonyl and aromatic C were sorbed preferentially, no preference was observed for O-alkyl, and alkyl C less readily sorbed from solution (pH 4), by soils (iron rich Inceptisols) and by the individual minerals goethite, ferrihydrite and amorphous $Al(OH)_3$ (Kaiser *et al.,* 1997). Similar results were found by McKnight *et al*. (1992) using a sample of dissolved organic matter (DOM) from a stream. However the DOM analysed in both studies were extracted using XAD resins and so were not "whole" samples but were fractionated in that the smaller molecular weight and neutral

components would have been discarded in the sample preparation. The fractionation of whole samples, including the recovery of neutrals, has been described by Croue *et al.* (1993, 1994) and Bolto *et al*. (1999). These latter components are thought to impact heavily on treatability (Chow *et al.,* 2000), and hence, are important to the water treatment industry.

The above studies concur in that fractionation of the DOM during sorption processes shows a preference to remove high molecular weight, aromatic and hydrophobic moieties. However, very little has been reported on how these fractionating processes are related to pH and the nature and concentration of the background electrolyte. The aim of this research was to carry out an overview of the sorption characteristics by a range of soil minerals of various DOM samples representative of catchment input into a reservoir. This sorption process was studied using a range of pH and electrolyte type and concentrations, typical of the natural environment. The results from this study improve the understanding of how the characteristics of the soils and streams in a catchment may impact on the nature of the DOM transported to reservoirs.

6.2 Materials and methods

Minerals

The methods used for the determination of the surface area, surface charge density and the micro-electrophoretic mobility of each of the four minerals, goethite, α-alumina, silica and kaolinite and more detailed analysis of these results are given in Spark *et al.* (1995 a, b). The surface areas of the mineral substrates goethite, α-alumina, silica and kaolinite, determined by the BET method using a Quantasorb instrument, were 62, 57, 117 and 10.9 m^2/g respectively. The point of zero charge values of these minerals were 9.3, 8.6, <4 and <4.5 respectively (Fig. 6.1), and the iso-electric point values were 9.1, 8.7, <3 and <3 respectively.

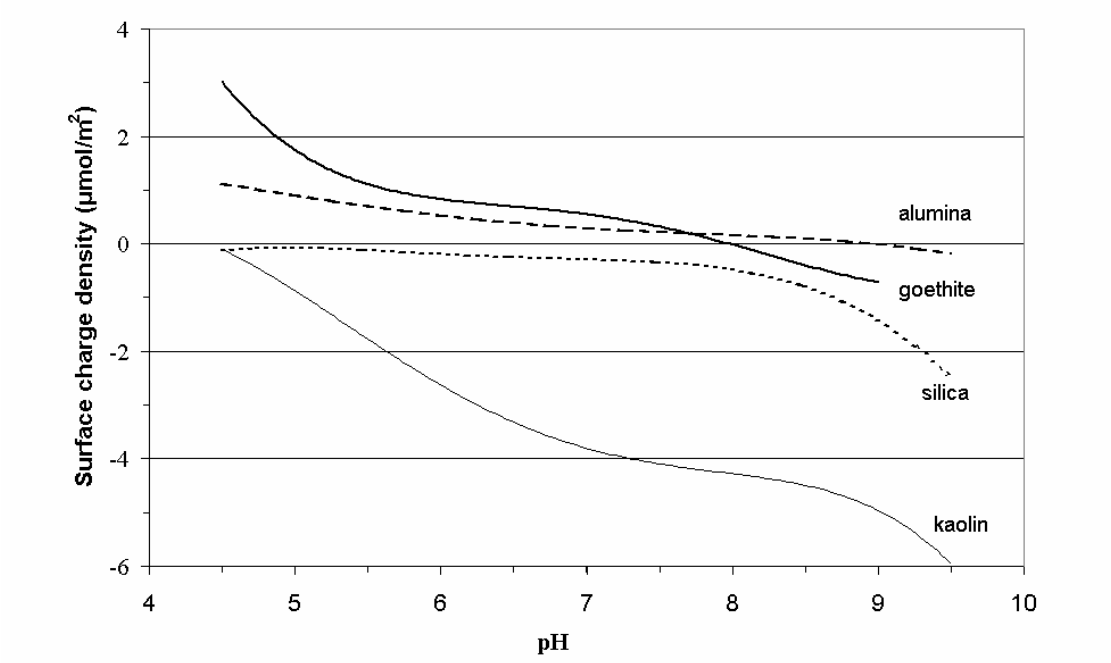


Figure 6. 1 Effect of pH on the surface charge density of the minerals in solution (0.01 M KNO_3).

The adsorption studies were all carried out using a mineral suspension containing 45 m^2/L and pH of either 5, 7 or 9. From Fig. 6.1 it is apparent that silica has a negatively charged surface at all three pH settings with the magnitude of the charge being relatively greater at pH 9 than at the other two pH. The surface of goethite in solution is positively charged at all pH settings with the magnitude decreasing with increase in pH to be almost zero at pH 9. Relative to the charge of goethite, α-alumina has a significantly greater positive charge at pH 5, is similar in magnitude at pH 7 and is negatively charged at pH 9. Kaolinite shows an overall negative charge at all three pH due to the permanent negative charge associated with this mineral. From the shape of the curve for kaolinite in Fig. 6.1, the pH dependent charge associated with the alumina surface and edge sites will be positive at low pH, becomes zero around pH 5-7 and is negative at higher pH.

Dissolved organic matter

The three samples of DOM used in this study were a plant litter extract (*Eucalyptus spp.*, Mt Bold catchment), a soil O-horizon extract (Mt Bold catchment upper slope) and from a reservoir (Happy Valley), (Happy Valley and Mt Bold are approximately 15 km S and 20 km south and east of Adelaide in South Australia). The samples were all collected in the spring of 1997. The plant, litter and soil DOM was extracted by soaking the organic material in a 1:1 suspension using reverse osmosis water. The characteristics of these DOM are shown in Table 6.1. The concentration of DOM used in the sorption studies as shown in Table 6.1, was selected as being representative of that associated with the source of the sample in the catchment. The DOC was measured using a total organic analyser (Model 820, Sievers Instruments Inc., USA), the TKN was measured using automated flow colourimetry and the apparent molecular weight (AMW, *Mn; number average molecular weight*) was determined using high performance size exclusion chromatography (HPSEC) based on the method by Chin *et al.* (1994). The HPSEC system consisted of a Waters 501 pump, 717 autosampler, 484 UV detector, InterAction column oven, set at 30^{o}C and a Shodex KW-802.5 column (Shoko Co., Ltd). Detection was based on UV absorbance at 260 nm.

Table 6.1 Characteristics of the DOM used in the sorption studies

Source	Leaf Litter (LL)	O-Horizon (OH)	Reservoir Water (RW)
DOC[#] (mg/L)	20	12	5
C/N ratio	14	8.0	6.8
pH of extraction	6.4	6.8	6.3
Apparent Molecular Weight (Dalton) (Mn)	2800	2200	1800

[#]*Concentration of DOM used in adsorption studies*

Instrumental analysis

The DRIFT spectra were determined using a Nicolet 750 Magna Spectrometer. Peak assignments were based on those reported by Gressel *et al*., 1995; Baes and Bloom, 1989; Painter *et al*., 1981; Socrates, 1994. The ^{13}C-NMR analysis was performed by Dr Ron Smernick, Dept. of Soil and Water, University of Adelaide, Glen Osmond,

South Australia. The UV-Vis spectra were determined using a UV/VIS 918 spectrometer (GBC, Australia) with a 1 cm square quartz cell. Scans were typically determined from 189 to 700 nm.

6.3 Results and Discussion

6.3.1 Characterisation of the DOM

The spectra of the samples of DOM, analysed using UV-Visible, ^{13}C-NMR and DRIFT spectroscopy, are shown in Figs 6.2, 6.3 and 6.4 respectively. The UV-Visible absorbance (Fig. 6.2) of the OH and LL DOM is similar but that of the RW DOM was significantly greater in the lower wavelength region compared with that at the higher wavelengths. This would suggest that the RW DOM has a larger proportion of aromatic groups than the other two samples (Croue *et al.,* 1999)

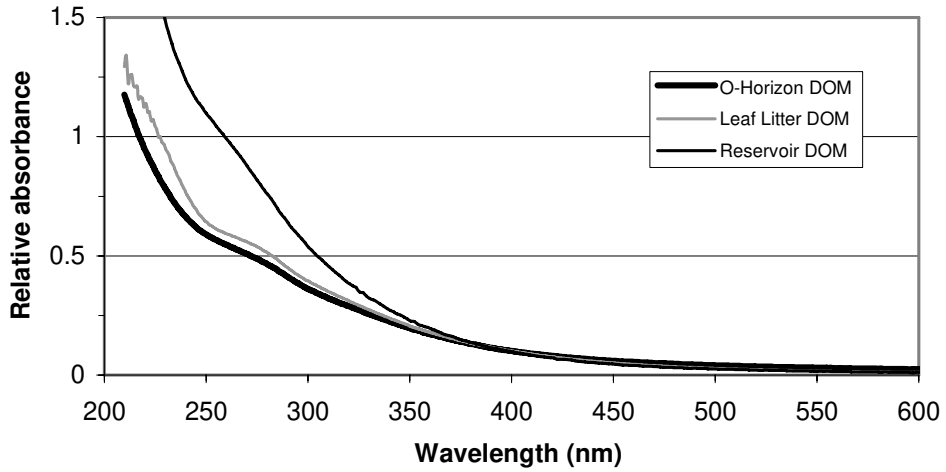
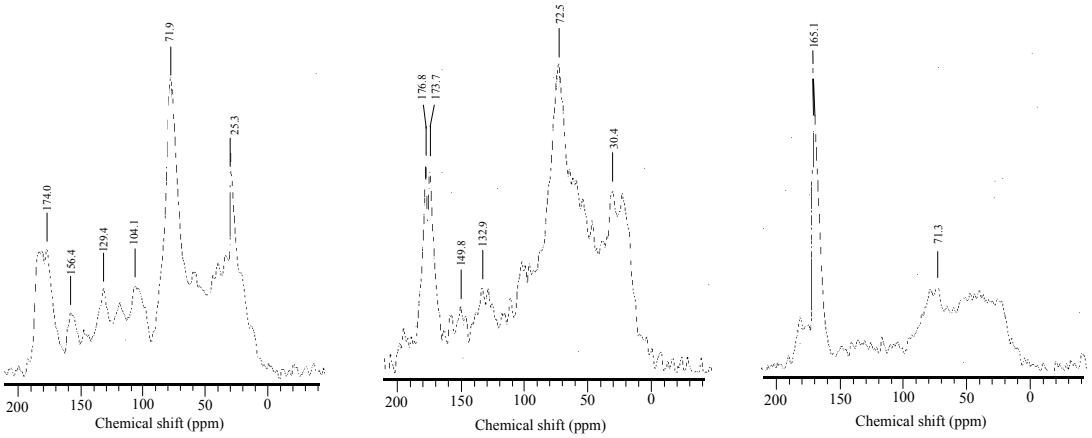


Figure 6.2 Absorbance spectra of the DOM samples optimized to 0.1 at 400 nm.



Leaf litter DOM O-Horizon DOM Reservoir Water DOM

Figure 6. 3 ^{13}C-NMR spectra of the DOM samples

Table 6.2 Integrated values of the peak areas for the chemical shift regions associated with the functional groups of natural organic matter.

	LL	**O-H**	**RW[#]**
190-165 (Carbonyl)	15	13	(19.0)
165-110 (Aromatic)	15	12	(19.9)
110-45 (O-Alkyl)	43	46	(29.4)
45-10 (Alkyl)	27	29	(31.3)
Carbonyl/O-Alkyl	0.35	0.28	(0.47)

[#]The RW sample is dominated by a peak at 165 associated with the presence of carbonate salts which would affect both the carbonyl and aromatic integrated values. Adjusting the integration to exclude the carbonate peak gives new values for the regions as shown in brackets in the table above.

The values from peak-integration of the NMR spectra (Table 6.2) indicate that the DOM samples from LL and OH are similar for all regions. The RW sample is similar to the other two in the carbonyl region, but has a higher alkyl content and aromatic content (which is in agreement with the data associated with the UV-Visible spectra in Fig. 6.2 above), and a significantly lower O-alkyl content.

The DRIFT spectra of the LL DOM samples (Fig. 6.4) has major peaks indicative of OH- and CH- (3300 cm^{-1}), alkyl (~2900 cm^{-1}), carboxylate (1600, 1400 cm^{-1}) and aliphatic alcohol 1100 cm^{-1}. The general broadness of the peaks reflects the diversity of the chemical environments of these functional groups

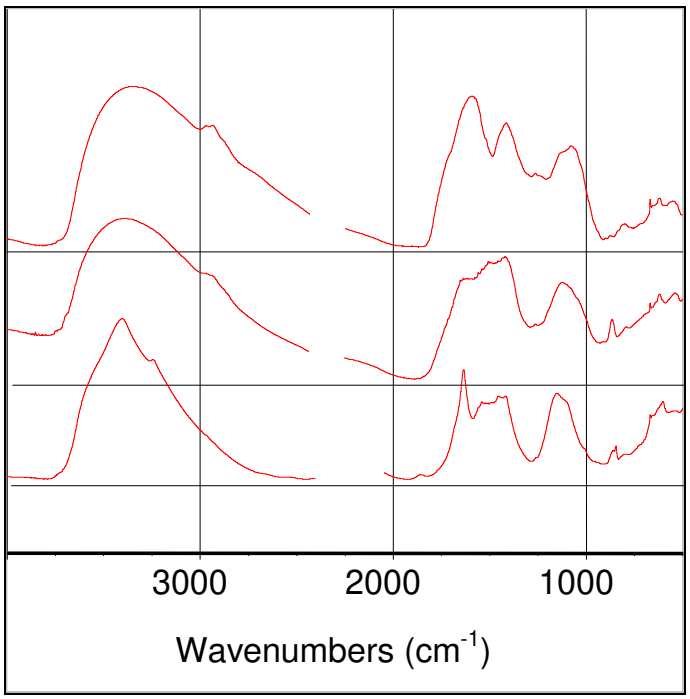


Figure 6. 4 DRIFT spectra of the DOM samples isolated from the Leaf litter (top), O-Horizon (middle) and Reservoir water (bottom).

The DRIFT spectra of the OH DOM shows the 1590 cm^{-1} peak assigned to carboxylates is considerably smaller relative to the absorbance in the 3300 cm^{-1} region than that for the LL DOM spectra. The relative increase in the absorbance in the 1400-1500 cm^{-1} region, assigned to amide groups in the absence of strong peak at 1600 cm^{-1}, is verification that this sample contains a greater proportion of these types of functional groups than the former sample, which is consistent with the lower C/N ratio in Table 6.1.

The DRIFT spectra of the RW DOM is dominated by peaks associated with the presence of salts, at 1640, 3250 and 3400 cm^{-1} (Page, 2001). The strongest peaks attributable to the organic matter are those in the region between 1400-1500 cm^{-1}. As for OH DOM, the absorbance in this region for this sample is most likely due to nitrogen groups such as amines and amides, which is consistent with this sample having the lowest C/N ratio (Table 6.1).

The differences in the nature of the DOM samples reflect the environment from which they were sampled. The leaf litter from which the DOM sample was extracted had had minimal contact with soil particles and so would be expected to be dominated by the breakdown products of cellulose, hemicellulose and lignin. The major functional groups associated with these two sources of organic matter are alcohol, alkyl, aliphatic ether, aromatic and carboxylate groups.

The O-horizon soil from which the OH DOM had been extracted was the grass root zone at the bottom of a hill slope. It would be expected to contain DOM originating mainly from breakdown of the cellulose material associated with the grass. Hence, this DOM will have a high proportion of aliphatic alcohol from cellulose degradation

products as well as those mentioned above for lignin. The proportion of microbial/plant input of OH and RW samples of DOM will be greater than for the LL DOM and a large part of these DOM will have already been in contact with a range of minerals and solid state organic matter.

6.3.2 Effect of pH and ionic strength on sorption of DOM

An indication of the extent of sorption of the DOM by the minerals was determined by comparing the absorbance at 350 nm of the supernatant solution before and after the adsorption process. The nature of the components not sorbed was determined by analysing the freeze-dried sample of the supernatant liquid using DRIFT spectroscopy and ^{13}C-NMR spectroscopy.

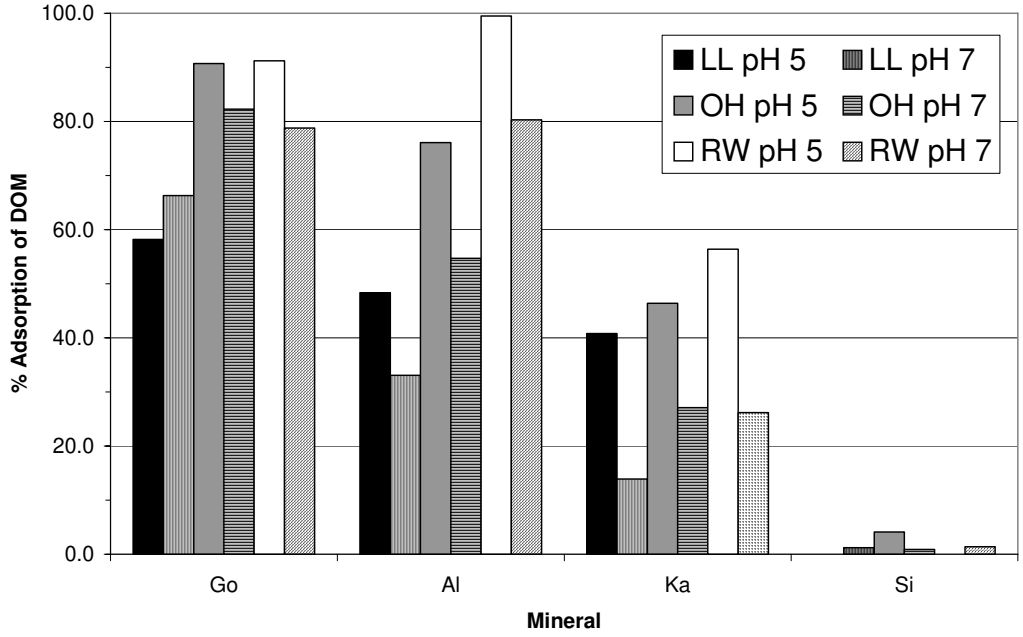


Figure 6.5 The percentage adsorption of 350 nm absorbers by the minerals goethite, α-alumina, kaolinite and silica from solutions of pH 5 and 7 for background electrolytes 0.001 M NaCl.

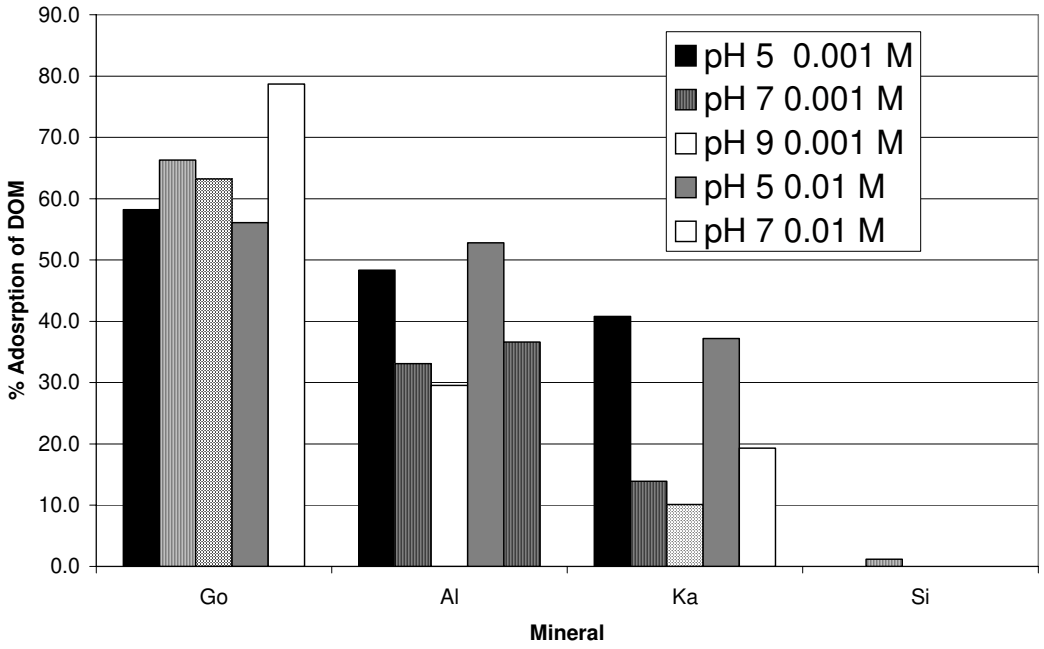


Figure 6.6 The percentage adsorption of leaf litter DOM by the minerals goethite, α-alumina, kaolinite and silica from solutions at pH 5, 7 and 9 for background electrolytes 0.001 and 0.01 M NaCl**.**

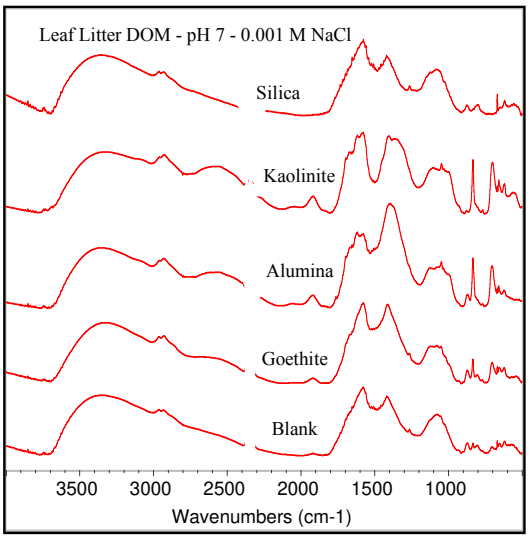
The sorption characteristics of each of the DOM by the four minerals are shown in Fig. 6.5, as a function of the type of DOM, and in Fig. 6.6 as a function of the ionic strength. The general order of the extent of adsorption of the DOM by the minerals is silica < kaolinite < α-alumina < goethite, with the adsorption by silica being almost negligible. The order is similar to that found by others including (Kaiser *et al.,* 1997; Meier *et al.,* 1999; Sprecht *et al.,* 2000; Chorover and Amistadi, 2001). The only exception to this is that the order for goethite and α-alumina is reversed for the RW DOM.

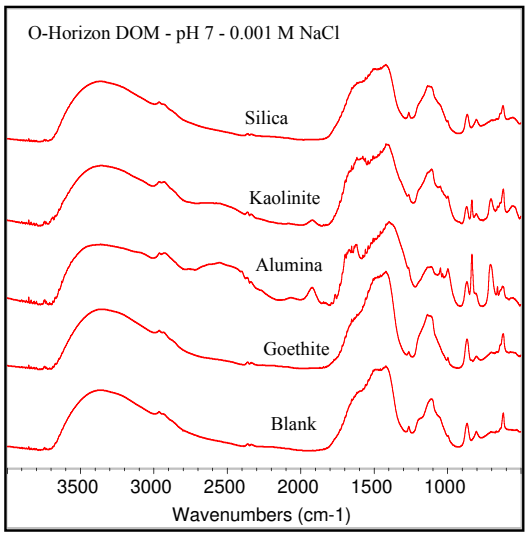
As the surface area of the four minerals was kept constant for all adsorption systems, the variation in the extent of adsorption on the minerals must be due to differences in the nature of the surfaces of these minerals solution. All the minerals have a smaller positive surface charge at pH 7 than pH 5 which is consistent with a lower extent of sorption of the negatively charged DOM components at the higher pH, except for that on goethite which showed the opposite effect.

The effect of higher ionic strength at a constant pH was not large in these studies (Fig. 6.5). Increasing the ionic strength from 0.001 to 0.01 at pH 5 resulted in a slight decrease in sorption of the LL DOM by both goethite and kaolinite, and a slight increase for α-alumina. Increasing the ionic strength from 0.001 to 0.01 at pH 7 resulted in an increase in sorption of the LL DOM by all three minerals, with goethite showing the greatest change. Kretzschmar *et al*. (1997) studied the pH dependence (between pH 3-11) of humic acid adsorption to kaolinite in a $NaClO_4$ background electrolyte using three different ionic strengths (0.001, 0.01, and 0.1M) and also found that sorption increased with increasing ionic strength. The random coil model of organic matter (Swift, 1989) would suggest that at higher ionic strengths the molecule would have a reduced overall size due to screening of the charged sites. A reduction

in size may allow more sorption of DOM to the mineral surface (Murphy *et al.,* 1994). This model also predicts an increase in sorption with a decrease in pH, as was generally observed in this study. The DOM would also be expected to reduce in size with a reduction in the number of charged functional groups. The nature of the adsorption process responsible for this effect is may be an electrostatic rather than a ligand exchange sorption process as it is pH dependent.

This reversal for goethite only occurred for the LL DOM and was not observed for the OH and RW DOM samples. Hence, this may also be related to the nature of one of the major types of sorbing species in the LL, which is not in the other two samples. One major group of readily sorbed species which is influenced by pH and has a significantly higher concentration in the LL sample relative to the other two DOM samples is the carboxylate group. The majority of the carboxylate groups in NOM samples deprotonate in the region pH 5-6 (Perdue, 1985) and hence it can be assumed that more are negatively charged at pH 7 and hence more readily sorbed to the positively charged goethite surface at the higher pH. This suggests that the carboxylates are hindered from sorption at pH 5 or enhanced at pH 7. The fact that α-alumina and kaolinite do not show a similar trend may be due to the fact that they sorb less DOM overall and hence sorption would be limited more by available sites on the surface of the mineral rather than species available for sorption.





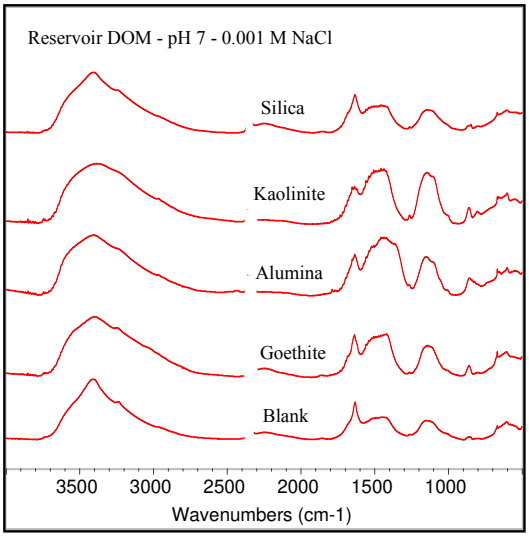


Figure 6. 7 The percentage adsorption of leaf litter DOM by the minerals goethite, α-alumina, kaolinite and silica from solutions at pH 5, 7 and 9 for background electrolytes 0.001 and 0.01 M NaCl

The DRIFT spectra of the DOM not sorbed by the minerals for suspensions at pH 7 and containing 0.001 M NaCl background electrolyte, are shown in Fig. 6.7. Silica showed very little adsorption of the DOM for all sets of conditions and hence, there was little expected change in the nature of the DOM left in solution following adsorption, verified by the similarity in the DRIFT spectra of the supernatant solutions for this mineral adsorption system with that blank DOM system (absence of minerals). Goethite exhibited the strongest adsorption for all three samples of DOM (around 70-85 %) but the DRIFT spectra of the freeze dried material of the supernatant solution after the adsorption was, in most systems, very little different to that of the DOM in the absence of mineral adsorption.

Even though the DOM showed a lesser extent of sorption on α-alumina than goethite there was a significant change in the DRIFT spectra of the supernatant solution as a result of adsorption. The spectra of the LL and OH DOM after adsorption by α-alumina at pH 7 in low NaCl concentrations (Fig. 6.7) show a relative increase in the peaks assigned to carbonyl groups (2500, 1680, 830 and 710 cm^{-1}). In addition all of the spectra associated with α-alumina adsorption, particularly those for LL DOM, exhibit a stronger peak at 1400 cm^{-1} relative to the blank DOM spectra. The presence of an associated peak at 1680 cm^{-1} suggests a strong possibility of an increased relative absorbance of amide and amine groups. There are similarities in the adsorption properties of kaolinite to that of α-alumina, which is not unexpected as it has been previously found that these two minerals have similar adsorption characteristics for ionic species (Spark *et al.,* 1995b and 1997).

The C/N ratio of the LL DOM not sorbed by the goethite and kaolinite minerals at both pH 5 (15, 14 respectively) and 9 (16, 15 respectively) was not significantly different to that of the initial LL DOM (16). However, the C/N ratio of the LL DOM not sorbed by the α-alumina at pH 5 and 9 (8, 10 respectively) was significantly reduced. These results suggest that α-alumina shows a low preference for adsorbing N-containing functional groups. Others have also found that sorption by clays such as

kaolinite, of N-containing functional groups such as amines and amides are less likely than the sorption of acidic groups (Dashman and Stotzky 1982, 1984).

A theoretical analysis of the crystal surface structures of these minerals (Varadachari *et al.,* 2000) suggests that there is a residual charge carried by O or OH on surfaces of various minerals; the charge on gibbsite is -1/2, and on goethite are -4/3, -2/3, or -1/3. Varadachari and co-workers predicted that these sites would sorb DOM components via cationic bridging. They also showed that the surfaces of these two minerals also contain octahedral sites in which one O/OH position is vacant, and concluded that on the surface of gibbsite, cation bridging links would be weak and surface coordination sites would be the dominant bonding sites, whereas on the surface of goethite both cation bridging and surface coordination sites would be present. The two types of sorption sites on goethite would enable a wider range of species to be adsorbed, and hence less specificity in the nature of the sorbed species. Hence the wider range of adsorption sites on the surface of the goethite compared with that on gibbsite would explain why in this study the sorption by α-alumina (and therefore kaolinite as well) showed a greater selectivity than did goethite for some components within the DOM sample.

6.3.3 The effect of the nature of the electrolyte on DOM sorption

The effect of the background electrolyte on the sorption of the DOM by the minerals is shown in Fig. 6.8. Sorption from suspensions containing 0.001 M KNO_3 was very similar to that containing 0.001 M NaCl; both solutions have an ionic strength (I.S.) of 0.001. However, sorption of the DOM from suspensions containing 0.0003 M $CaCO_3$ was much greater even though the ionic strength of this solution (0.0012) was similar to that of 0.001 M NaCl and KNO_3. This is confirmation that at least some of the sorption of the DOM by the minerals involves cationic bridging in which Ca^{++} participates by forming a bridging bond between the mineral and the DOM which is in agreement with others**.** The enhancement of sorption by the presence of multivalent cations would be expected to be less for DOM in which carboxylates are a lesser proportion of the overall sample. For example, Specht *et al*. (2000) found that the presence of Ca^{++} had no effect of sorption of DOM onto kaolinite, most likely because the DOM was from a lake and so would have a lower concentration of carboxylate groups and a higher concentration of N-containing amines and amides.

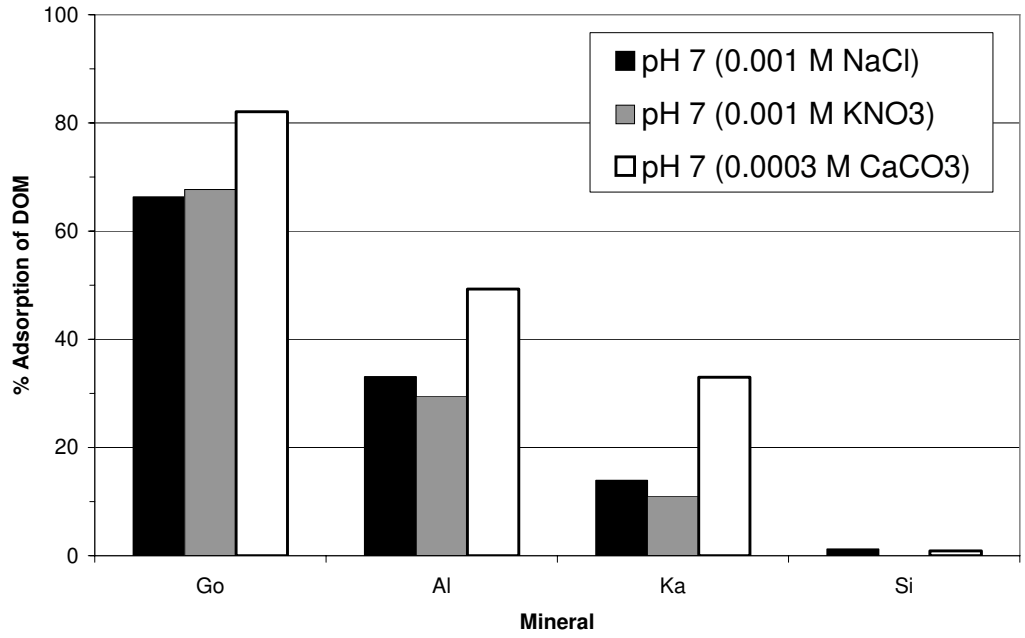


Figure 6.8 The percentage adsorption of the Leaf litter DOM by the minerals goethite, α-alumina, kaolinite and silica from solutions of pH 5, 7 and 9 for background electrolytes 0.001 and 0.01 M NaCl.

6.4 Conclusions

The general findings from this work indicate that the amount of DOM transported in a catchment is dependent on the nature of the minerals, and the conditions associated with the soil solution or stream chemistry. It has been found that order of adsorption of DOM is goethite > α-alumina > kaolinite > silica. Adsorption also generally increases with decrease in pH, increase in ionic strength and increase with the presence of multivalent cations such as Ca^{++}. Hence, the amount of DOM in runoff water from soil environments and turbid streams would be dependent on the pH of the water and the nature of the minerals with which it comes into contact.

In addition, the results of this study has shown that the interaction of the DOM in the runoff water with the aluminium based minerals (such as α-alumina and kaolinite) may fractionate the DOM by selectively removing the carboxylates and leaving behind the nitrogen containing components such as amides and amines which tend to be in the low molecular weight, neutral fraction of DOM. Others, as discussed in the introduction, have shown that sorption by minerals selectively removes the higher molecular weight, aromatic and hydrophobic components, the components which are most easily removed by flocculation with alum. Hence, the transport of DOM from catchments into reservoirs would have minimal effect on the treatability of the DOM in the reservoir water. Hence, the degradative processes resulting in the production of low molecular weight nitrogen-containing components linked to microbial activity would be expected to have a much greater impact on the treatability of reservoir water.

CHAPTER 7

7.1 General Discussion, Conclusions And Recommendations.

In this part of the study, UV-vis, DRIFT, HPSEC and C/N ratios were mostly used for characterisation of NOM from litter layers, soils and from waters, isolated from a range of locations and sources. Other techniques were also applied in the course of addressing objects for CRCWQT Project 2.1.1, including ^{13}C NMR, pyrolysis-gas chromatography/mass spectrometry, thermochemolysis and HPSEC. The results of their application are described more in Report 1 and in the theses of Page (2000) and Anstis (1999).

Ratios of selected UV-vis data appeared to enable differentiation of organic matter from various sources, particularly litter layers, soils and reservoirs. The ratio of 254nm/456nm (254/456) is simply that of conjugated double bonds to the colour imparted by organics. The standardised ratio of 254nm X 456 nm X 1000)/DOC^2 (254*456/DOC^2) is also an index of UV absorbing and colour imparting organics; presumably mostly from aromatic and coloured organics which are humic substances and/or lignin and tannins and derived compounds. These are proposed as ratios of some value in characterisation of NOM, particularly to the water industry, though they have not be previously reported in the literature as such (at least to the awareness of the authors of this report). A combined factor of absorbances at 254 nm and 456 nm was applied in studies detailed in Report I where it was used to indirectly measure the amount of organics "coaguable" and removable with alum treatment. The use of this parameter enabled prediction of alum doses for enhanced coagulation for the waters tested, which also provides evidence that parameters based on these UV-vis values are correlated to the amount/character of natural organic matter in water.

The 254*456/DOC^2 ratio data for soils tended to be higher than those of reservoirs and litter layers. Although not tested for in this project, low (254*456/DOC^2) values for natural waters might be an indicator that the organics are highly assimilable, while in natural waters post coagulant treatment, an indicator of the removal of dissolved humic substances. The commonly used UV/DOC ratio as SUVA was also determined for most samples collected in this study. These also appeared to be influenced by source type (litter layer, soil, reservoir), by catchment topography (influenced by the catchment slope height and location, see Anstis, 1999) and in relation to before and after treatment with alum. The absorbance at 254 nm in soils and natural waters might be due to aromatics from lignin derived compounds, lignocarbohydrates, tannins and humic substances and also from aromatic groups of amino acids of proteins and nucleic acids. The latter two impart little or no colour, but their character, particularly in relation to their microbial assimilable capacities, would be greatly different than say, solubilised lignin-derived compounds or dissolved humic substances. Hence, a measure of the natural colour appears to provide further information, in tandem with UV absorbance and DOC concentration.

A UV absorbance ratio reported in the literature to be of potential value, 254nm/203nm, was not extensively used in this study because of the possible confounding effects by nitrate. E4/E6 ratios tended to vary between and within sample types, precluding its use for characterisation of the NOM isolates. According to Gressel *et al*. (1995), this ratio for DOM is probably indicative of molecular size or

degree of complexation but a decrease in the ratio is not necessarily indicative of DOM humification. In this study, no relationship was found between this parameter and organics isolated from the slope heights of Myponga and Mt Bold. Assuming that greater humification of organics exists at the lower slopes, the data support the contention made by Gressel *et al.* (1995).

DRIFT spectroscopy provided some useful, though often limited information on the character of NOM isolates for soils, litter layers and waters. Attempts to apply this technique for characterisation of NOM functional groups were however, confounded by the presence of inorganics, particularly in raw water samples. DRIFT spectra of freeze-dried materials from soil samples appeared to be different in relation to the vegetation cover, with grass sites showing lower relative absorbances at wavelengths assigned to aromatic compounds. Although the surveys conducted to assess the diversity in the characters of NOM in catchments of Victoria and South Australia are reported separately here, the separation on the basis of location is arbitrary. Differences in these studies were more on the basis of what could be logistically achieved at these two locations. In the case of Victoria, the variables were sampling locations, vegetation and season while in South Australia, it was to a lesser extent sampling location with only two catchments studied, but these were studied in much greater detail eg. topography, contrasting vegetation and in association with soil microbiology activities by Anstis (1999). The type UV-vis trends found for the samples collected from Victoria were similar to those found for South Australia, based on the data available. Averages of all available data of the 254/456 ratio from the Victorian surveys are 26.6 for litter layers, 24.9 for reservoirs and 16.0 for soils. Averages of all the $254*456/DOC^2$ ratio data were as follows, 0.075 for litter layers, 0.332 for reservoirs and 5.34 for soils. Only the 254/456 data was obtained for the samples collected in South Australia. The ratios for soils, shown in Table 7.1, are comparable to those of soils from Victoria. Interestingly, the South Australian samples showed a further relationship of this ratio to slope height. Lower ratio values indicate higher colour per UV absorbance, which is postulated to be a result of higher humification of organics. These lower ratio values were generally found at the lower points of slopes, these being a sink for organics. At these slope locations the organics present would have undergone more overall bio-degradation, transport and be older than organics at the top of catchment slopes. An alternative explanation is that the higher colour at the base of slopes was not so much due to humification of organics but more due to higher concentrations of iron present in soils and then leached out with organics. The data of tables 4.3 and 4.8 show substantially higher iron concentrations in soils at the base of slopes than at the top for Mt Bold and Myponga pine site. In contrast to this is the data shown in Table 4.11 of the Myponga grass site, had very consistent iron concentrations (determined on dried soil samples) along the slope transect, with the highest at the middle sampling site. The trend of the 254/456 ratio at this site in March 1999 was as follows: 8.0 (bottom site),10.3 (middle site), 13.7 (top site). However, in the leachates of these soil samples, iron as well as aluminium, showed a clear trend of increases in concentrations from the top sites to the middle and bottom sites (figures 4.5, 4.10 and 4.13). Iron and aluminium as cations, may however, also be part of the humification process, and not be interferences to measurement of the 'colour' parameter alone. These cations may be acting as ligands between the overall negatively charged organics in water and soils, leading to the formation of higher molecular weight compounds, in a similar manner as in water treatment using these bases as inorganic coagulants. Higher

concentrations iron in leachates may therefore be associated with organics as opposed to being simply an interference. Accepting even that iron interferes and confounds the 'colour' measurement, a high ratio value could still be interpreted as being of fresh organics/and or having undergone a lower degree of humification. Further work on this parameter for characterisation of NOM, especially in soils, should incorporate an investigation to determine at which concentrations of iron, and their chemical speciations, and other compounds lead to inorganic interference in the visible region of the electro-magnetic radiation spectrum.

The average ratio for samples of pine site litter layers collected in July 1999 was 28.2 which is clearly different to that of the soils. This ratio is very similar to that of the corresponding Victorian samples (26.6). 254/456 ratio data of the South Australian soil samples, grouped according to the month of collection is shown in Table 7.2. It appears that there is also a season affect, with higher ratio values at times when soils would have consistently experienced rainfall events and probably were wetter, ie October 1998 and July 1999. Available rainfall data for these catchments from 1997 to 1999 are shown in figures 7.1 to 7.4. The 254/456 ratios were higher at the wetter times probably due to more recent leaching of organics from fresh vegetation and litter layers. As found in this study the ratios of litter layers were markedly higher than organics from soils. After an extended dry period, organics in soils would have undergone overall greater degradation and humification. Hence, the samples collected in March had lower 254/456 ratios.

Data of UV-vis ratio values for individual samples varied between and within sample types, though the averages point to distinct differences in the characters of NOM from the various sources. The impact of inorganic compounds on these ratios, such as ferric compounds that could contribute to 'colour' is yet unknown. Nonetheless, a wide range of soil types were encountered in sample collections and the ratios determined were mostly similar. Of interest would be the changes in ratio values of organics leached from soils in association with rainfall events and seasonal changes. If relationships between UV-vis parameters and changes in NOM/DOM/DOC characters hold, then this might provide a simple means of rapidly obtaining parameters that are empirically related to such features as the degree of humification of organics and readily assimilable organic carbon, at a site specific location.

Table 7. 1 Summary of the 254nm/456nm ratio data (means) of soil extracts collected from Mt Bold and Myponga in 1999.

Vegetation type	Sampling location on slope			
	Bottom	Middle	Top	Mean
Native vegetation	13.9	15.5	16.8	15.4
Pine forest	13.5	9.5	19.4	14.1
Grass	9.3	10.9	14.4	11.5
Mean	12.2	12.0	16.8	13.7

Table 7. 2 254nm/456nm ratios of extracts of soil samples from Mt Bold and Myponga, 1998 and 1999.

Location	Date	254/456
Mt Bold	Oct-98	16.1
Myponga	Oct-98	15.0
Mt Bold-native	Mar-99	11.8
Myponga-grass	Mar-99	10.7
Myponga-pine	Mar-99	11.9
Mt Bold-native	Jul-99	17.1
Myponga-grass	Jul-99	12.4
Myponga-pine	Jul-99	16.3

In the work reported here DRIFT spectroscopy was adopted on the basis that it provided an inexpensive, rapid and potentially informative method for characterisation of NOM. With the large amounts of samples collected in the course of the studies conducted, other techniques, such as pyrolysis-gas chromatography/mass spectrometry, ^{13}C NMR, fractionation studies would not have been possible, based on the high cost of analyses and the time required for these to be conducted. These techniques were only able to be performed on representative samples, and the results of these analyses have been reported in Report 1 and theses.

DRIFT spectroscopy is a technique that is very commonly used for elucidation of chemical functionality of organic compounds. Advantages are that it is a non destructive method and does not rely on extraction techniques which may have different efficiencies for different compounds.

The technique has been applied to determine the hydrophobicity of soil organic matter in arable soils by Capriel *et al*. (1995) and Capriel (1997). In these studies it was found that sandy soils contained more aliphatic C-H units (in the region of 3000 to 2800 WN/cm) than clayey soils. Capriel used a hydrophibicity index which he determined by dividing the area of the C-H bands by organic C.

In this study shoulder peaks at about 2850 and 2950 WN/cm were clearly observed for many samples from both the Mt Bold and Myponga catchments. This was also the case for samples collected in October 1998, when Myponga samples were taken from sandy soil sites and the Mt Bold samples were from clay soils. However, hydrophobicity indices, as detailed by Capriel were not determined, so direct comparison between these soil types, based on hydrophobicity, cannot be made.

Other examples of DRIFT having been applied for characterisation of natural organic matter in soils are the studies by Gressel *et al*. (1995), Kaiser *et al*. (1997), Woelki *et al*. (1997) and Francioso *et al*. (1998). Gressel *et al*. (1995) presented DRIFT spectra of pine litter extracts using KBr, in the pH range of 3.5 to 6.0. These results are not directly comparable to those of this study because of the standard use of pH 7. Nonetheless, comparison of results using pH 6 and 7 show a number of similarities; these being:

1. Clear absorbance bands at ~ 2950 WN/cm and,
2. the presence of three strong peaks at about 1600, 1400 and 1100 WN/cm.

The relative abundances of these three peaks in spectra of extracts from Mt Bold and Myponga soil samples were markedly different, though their assignment to organic functionality is speculative. Woelki *et al*. (1997) used DRIFT to characterise fractions of humic acids. They found strong absorbance bands at ~1650 WN/cm, 1450WN/cm and 1100-1270 WN/cm, particularly at ~ 1650 and all of these were much higher than the peak height at ~ 3350 WN/cm. The band at ~ 1650 WN/cm is attributable to carbonyl and C=C of aromatics and this might be anticipated from humic acids. The humic acids studied for this project also showed a prominent peak at ~ 1600 WN/cm relative to bonded OH absorbance. This indicates that hydroxyl functionality is comparatively low in the humic acid fraction compared with whole NOM isolates, correlating with their nature being hydrophobic. Francioso *et al*. (1998) characterised peat fulvic acid fractions, (to different molecular sizes using ultrafiltration) using DRIFT spectroscopy, and as in the study of Woelki *et al*. (1997) found these fractions to have very strong absorbance bands at about 1600 WN/cm. Using ^{13}CNMR, they also found very weak resonances at 150 and 165ppm indicating the presence of only a small amount of phenolic compounds.

DRIFT spectroscopy has also been applied characterisation of organics binding to soils and minerals (Kaiser *et al*. 1997); for the study of coals (Cai and Smart, 1994 and Cagniant *et al*. 1994); for characterisation of lignins (Heitz *et al.* 1995) and in novel ways, such as for the identification of bacterial species (Goodacre *et al*. 1996).

In all of these applications, the relative abundance of organics in the sample matrix might be expected to have been high. In contrast to this, the percentage of organics present in freeze-dried materials of natural waters studied for this project and reported here were comparatively very low, particularly as waters from South Australia are highly buffered. Further, waters that were treated with alum, as required in the various experiments performed for this project, caused what appears to be significant inorganic interferences in the spectra. Hence, the application of DRIFT for characterisation of organics in natural waters and in relation to treatment strategies requires desalting, despite the risk of losing some key organics. As found in the present study and detailed in Report 1, ultrafiltration can be used to desalt natural

waters before and after treatment with an inorganic coagulant. In that work, and using several waters, it was found that the organics which are highly recalcitrant to removal with alum comprises a substantial fraction (about a third) which is greater than 1000 nominal molecular weight (NMW). This fraction, although possibly being structurally different than those of the fraction less than 1000 NMW, have similar chemical behaviours.

This preparative procedure is both costly and time consuming but gives spectra that have no evidence of salt interference. Therefore its application is recommended in a restrictive and targeted manner.

DRIFT spectroscopy is a valuable tool for characterisation of organics from a range of sources, though its interpretation should be performed and qualified in the context of known details of the sample matrix. The amount and type of information desired by application of DRIFT then dictates the preparative methods that are required.

7.2 Recommendations:

1. DRIFT spectroscopy can be applied for the characterisation of natural organic matter in matrices where interferences from inorganics are likely to be minimal. These include leaf litter extracts, soils and soil extracts. Where interferences from inorganics are likely or apparent, their removal or reduction from the sample matrix is then required. Ultrafiltration can be used though some loss of organics will result. Ultrafiltration should be applied with sufficient rinses to enable removal of salts from the retentate.
2. DRIFT spectroscopy was extensively applied to most samples collected or generated through experimentation in the studies reported here. The application of DRIFT should be considered in the context of the likelihood of the type and limitations of information that will be obtained. DRIFT data could be further explored using newer data handling methods such as artificial neural networks, which might give useful information in relation to changes in sample matrices.
3. 254/456 and 254*456/DOC^2 ratios can be applied for characterisation of NOM based on these ratios being related to source type (vegetation, litter layers, soils and waters), topography and possibly climate. The cause for absorbance in the visible region may be contributed by iron and further work to determine its impact on these ratios is recommended.
4. The data on the reservoir-catchment systems obtained through the studies of this project should be considered when selecting reservoir-catchment systems in future Australian based studies. The data given in this report should be of some historical significance with respect to the water qualities of soils, rivers and reservoirs of these catchments and provide a comparison for any future changes.
5. Future studies dealing with characterisation of NOM from the same catchment environments should consider application of different characterisation techniques, such as field flow fractionation, resin based fractionation and isolation, measurement of specific monomers and bio-polymers using wet chemical methods, enzymology and greater use of ^{13}C NMR. Their consideration being made in the context of cost, equipment and human resource availability and suitability of information generated.
6. The relationships between the amount of organics in catchment soils and their potential for leaching and transport to waterways with catchment features such as

vegetation type and density, topography and climate should be considered in land management practises. For example, the results of our studies show that dense pine forests in catchments can cause high organic loads above and in soils, leading to a high potential for organics to be transported to waterways. These issues should be further considered in relation other requirements of such as minimisation of soil erosion, and protection and preservation of native fauna and flora.

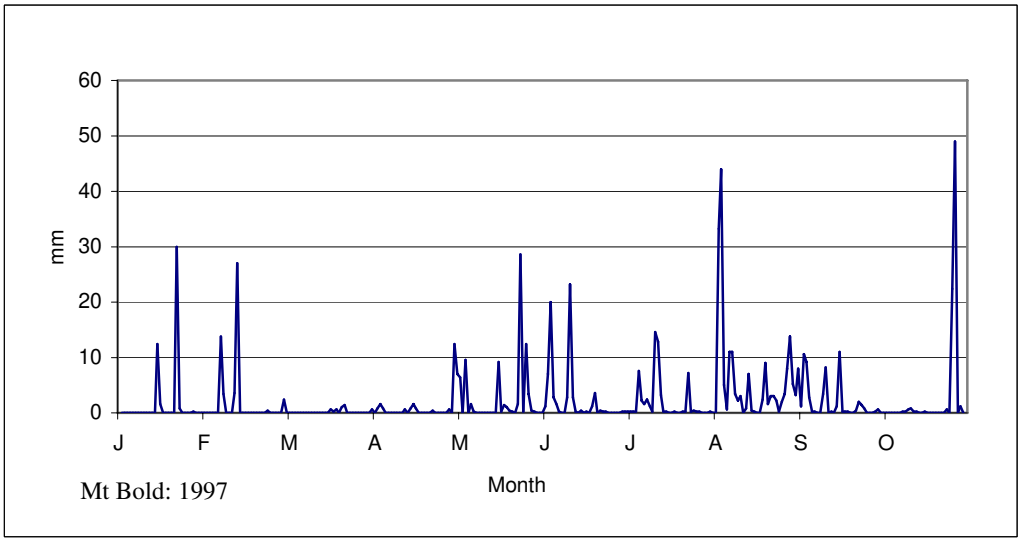


Figure 7. 1 Rainfall data for Mt Bold catchment, 1997

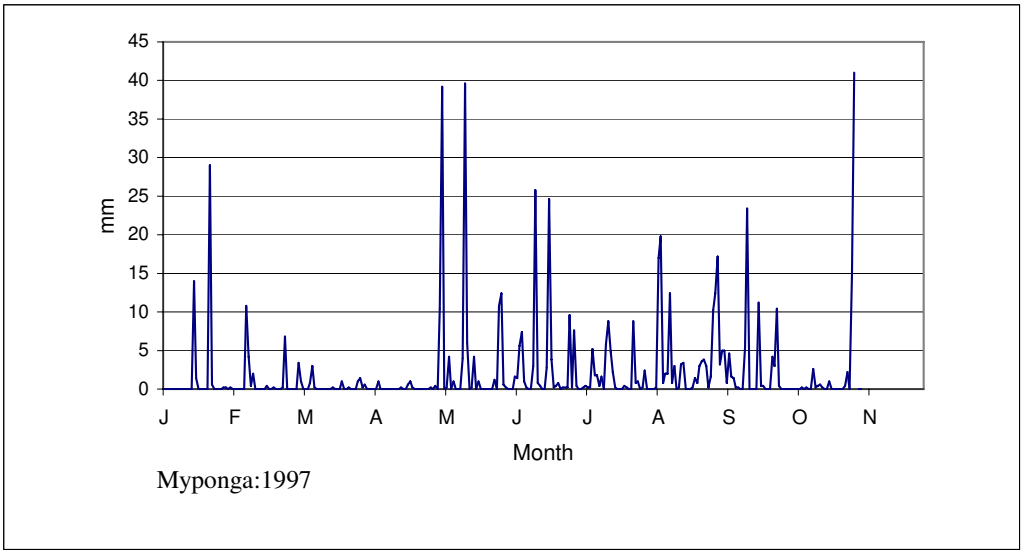


Figure 7. 2 Rainfall data for Myponga catchment, 1997.

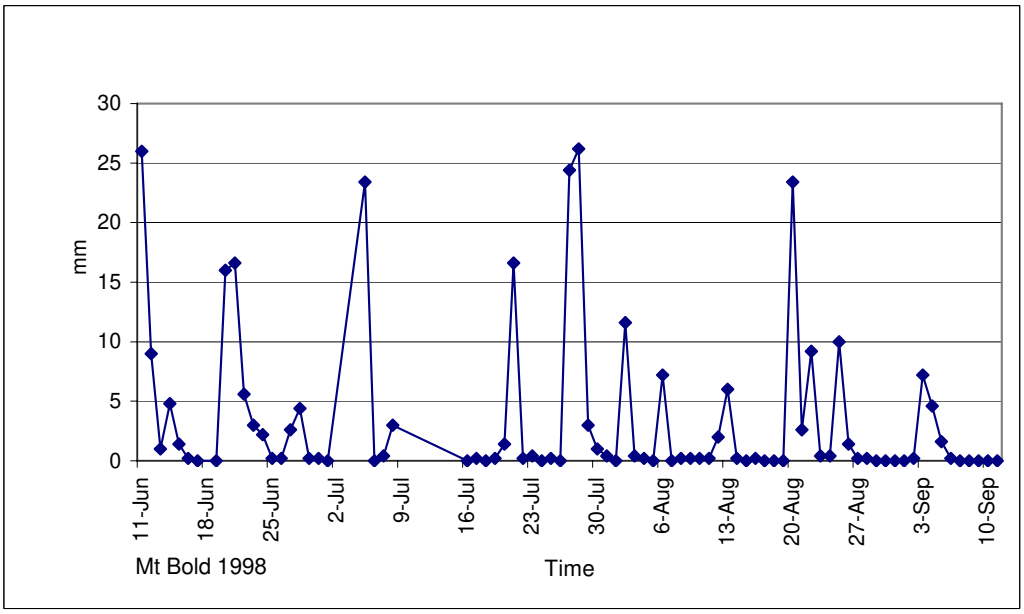


Figure 7. 3 Available rainfall data for Mt Bold, 1998.

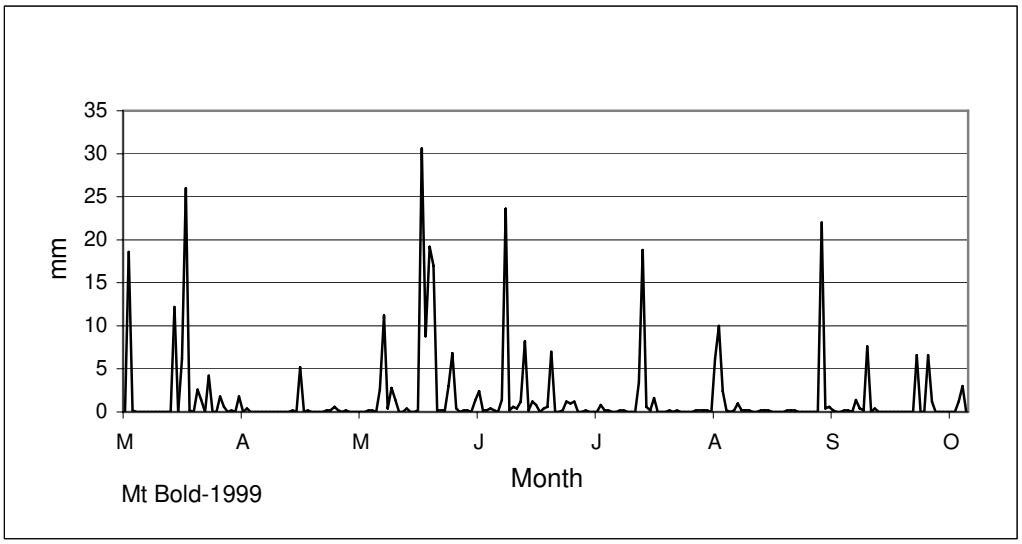


Figure 7.4 Available rainfall data for Mt Bold, 1999.

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