Carbon Storage in an Artificial Soil

STEWART, LAURA

Carbon Storage in an Artificial Soil

Laura Stewart

Department of Earth Sciences

Durham University

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Abstract

As we strive to find new technologies to dispose of our municipal solid waste, compost-like outputs (CLOs) are becoming more widely created. As a product of both aerobic and anaerobic digestion, they provide a potentially important carbon store and some have proven to enhance existing carbon stores when added to brownfield sites and agricultural land. However, the CO_2 flux from this artificial soil is relatively high when compared to natural soils. The aerobic digestion process under which it is produced lasts only 9 days, producing a material which is still comparatively unstable and yet to mature. The CLO is laid in windrows where it is hoped that it will stabilise and mature; if the humification process at this stage can be optimised, would an even greater carbon store be achieved?

This thesis seeks to answer this question, through the research into humification in both natural and artificial systems; through the measurement of CO_2 flux to assess the stability of CLO over time; using adapted methodologies to gauge the maturity of this artificial soil by analysing the amount of humic acids present; by adding proposed catalysts to the material in fully factorial lysimeter studies; and by examining the affects of different physical environmental conditions under which CLO product humifies.

The results of a series of experimental trials, undertaken over a three year period, are presented. Manganese-coated sand and char, both currently 'waste' products were both used as potential catalysts for the humification process of CLO. Temporal trends were seen in most samples using infra-red gas analysis, an alkali extraction technique, UV photospectrometry, fluorescence and a novel pseudo-thermogravimetric analysis. The waterlogging of the samples appeared to have an effect on the humification process and a great deal of concurrent data was seen upon the addition of Mn-coated sand and char to the CLO. Both appeared to have a stabilising effect on the CLO, reducing flux rate and increasing humification as compared to a control.

An overriding theme present throughout this thesis is the heterogeneous and contaminated nature of the non-source-segregated CLO tested. It is therefore recommended that similar studies be undertaken on a purer, more homogenous CLO in order to assess whether promising results seen could be elucidated in order to gauge the efficacy of biochar and Mn in encouraging the production of humic substances. A field trial would allow the unified soil system to be considered, rather than the CLO alone.

Declaration and Copyright

I confirm that no part of the material presented in this thesis has previously been submitted by me or any other person for a degree in this or any other university. Where relevant, all material which is the work of others has been acknowledged.

Signed:

Date:

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Abbreviations

AD	Anaerobic digestion
ADE	Amazonian Dark Earth
ANOVA	Analysis of variance
BMW	Biodegradable municipal waste
C	Carbon
CLO	Compost-like output
CO_2	Carbon dioxide
CH_4	Methane
DECC	Department for Energy and Climate Change
DEFRA	Department for Farming and Rural Affairs
DOC	Dissolved organic carbon
EA	Environment Agency
EC	European Community
EFW	Energy from waste
ETS	Emissions Trading Scheme
EU	European Union
FA	Fulvic acid
GHG	Greenhouse gases
H	Hydrogen
HA	Humic acid
HIX	Humification Index
HS	Humic substances
HCl	Hydrochloric acid
IPCC	Intergovernmental Panel for Climate Change

IR	Infra-red
IRGA	Infra-red gas analyser
LA	Local Authority
LATS	Landfill Allowance Trading Scheme
LCA	Life Cycle Analysis
LGA	Local government association
MBT	Mechanical biological treatments
MHT	Mechanical heat treatment
Mn	Manganese
MnO_2	Manganese oxide
MSW	Municipal solid waste
MW	Molecular weight
MT	Million tonnes
N	Nitrogen
NaOH	Sodium hydroxide
NTDP	New Technologies Demonstrator Programme
N_2O	Nitrous oxide
O	Oxygen
OM	Organic matter
PARC	Premier advanced recycling centre
PAS	Publically Available Standard
PCA	Principal component analysis
ppm	Parts per million volume
PVC	Polyvinyl chloride
PWM	Premier Waste Management
PTGA	Pseudo Thermo-gravimetric analysis
RDF	Refuse-derived fuel

rpm	Revolutions per minute
SOC	Soil organic carbon
SOM	Soil organic matter
SVS	soil screening values
TGA	Thermo-gravimetric analysis
UN	United Nations
UNEP	United Nations Environmental Programme
UV	Ultraviolet
WMO	World Meteorological Organisation
WRAP	Waste and Resources Action Plan
WT	Weight

“The constant effort towards population, which is found even in the most vicious societies, increases the number of people before the means of subsistence are increased.”

Thomas Malthus, 1798.

Chapter 1

Introduction

The aim and objectives of this research are presented in this chapter. The global context, legislative drivers and the commercial aspects are also introduced.

1.1 Climate change: summary of IPCC report 4

The rise in atmospheric carbon in the form of greenhouse gases (GHG) has led to an increased rate of global warming. There is much debate as to whether this is a natural phenomenon or whether the change is directly linked to anthropogenic processes. Since the industrial revolution the large-scale occurrence of the mining of fossil fuels and changes in land use, particularly deforestation, has meant that this natural balance has been disturbed; carbon is being removed at a far greater rate than it is sequestered. This has led to depletion in terrestrially-stored carbon and an increase in atmospheric carbon over the past two centuries.

As the effects of global warming become ever more apparent, leading scientists from around the world have called for urgent action to be taken. In 1988, the Intergovernmental Panel for Climate Change (IPCC) was established as a joint venture between the World Meteorological Organisation (WMO) and United Nations Environmental Programme (UNEP). Scientists from all country members contributed research to provide three reports on the causes and impacts of global climate change.

These reports state that the dominant factor in radiative forcing during the industrial era is increased GHG emissions. Several GHGs occur naturally but have risen over the past 250 years due to human activity whilst other GHGs are entirely as a result of human activity.

Carbon dioxide (CO_2) and methane (CH_4) are both naturally occurring and being chemically stable they are persistent over decades, centuries or longer. Any GHGs already released into the atmosphere will have long term impacts on climate. Current atmospheric concentrations of CO_2 far exceed pre-industrial values found in polar ice core records of atmospheric composition dating back 650 years, increasing from 280ppm (pre-industrial) to 379ppm by 2005 (IPCC). Even in recent decades, emissions of CO_2 have continued to increase. The primary source of CO_2 emissions is from the burning of fossils fuels and from the effects of land-use change on plants and soil carbon, as shown in Figure 1.1

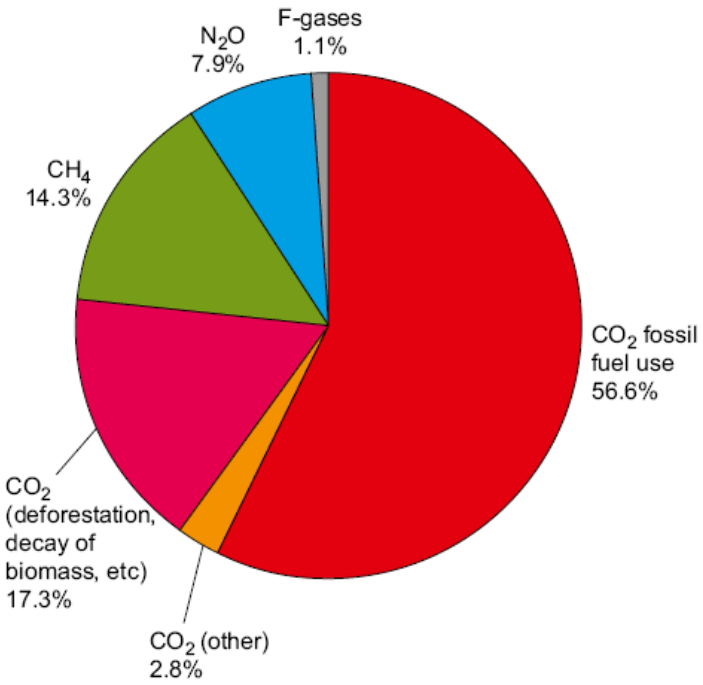


Figure 1.1: Global anthropogenic GHG emissions in 2004 (IPCC Fourth Assessment Report (AR4)). The nitrous oxide (N_2O) is mainly from agriculture, as is the methane. Other significant sources of methane are energy and waste.

Whether or not the current period of global warming is anthropogenically-enhanced climate change, the fact that certain measures could be introduced to foster a more sustainable future is irrefutable. The natural carbon cycle is being distorted to the detriment of the atmosphere, the oceans and the terrestrial carbon stores. As such, several of the United Nations (UN) Member States have committed to reducing their GHG emissions following the Kyoto Protocol in 1997. Ahead of the Copenhagen Climate Summit in December 2009, the United Kingdom (UK) has committed to further reduce its GHG emissions to 80% of 1990 levels by 2050.

In order for this to happen, it is necessary to analyse the main sources of GHG emissions. Figure 1.2 shows the UK's GHG emissions from 2008 by sector. Energy supply, business, transport and residential use account for the largest emissions, but several other sectors are significant, including agriculture and waste management.

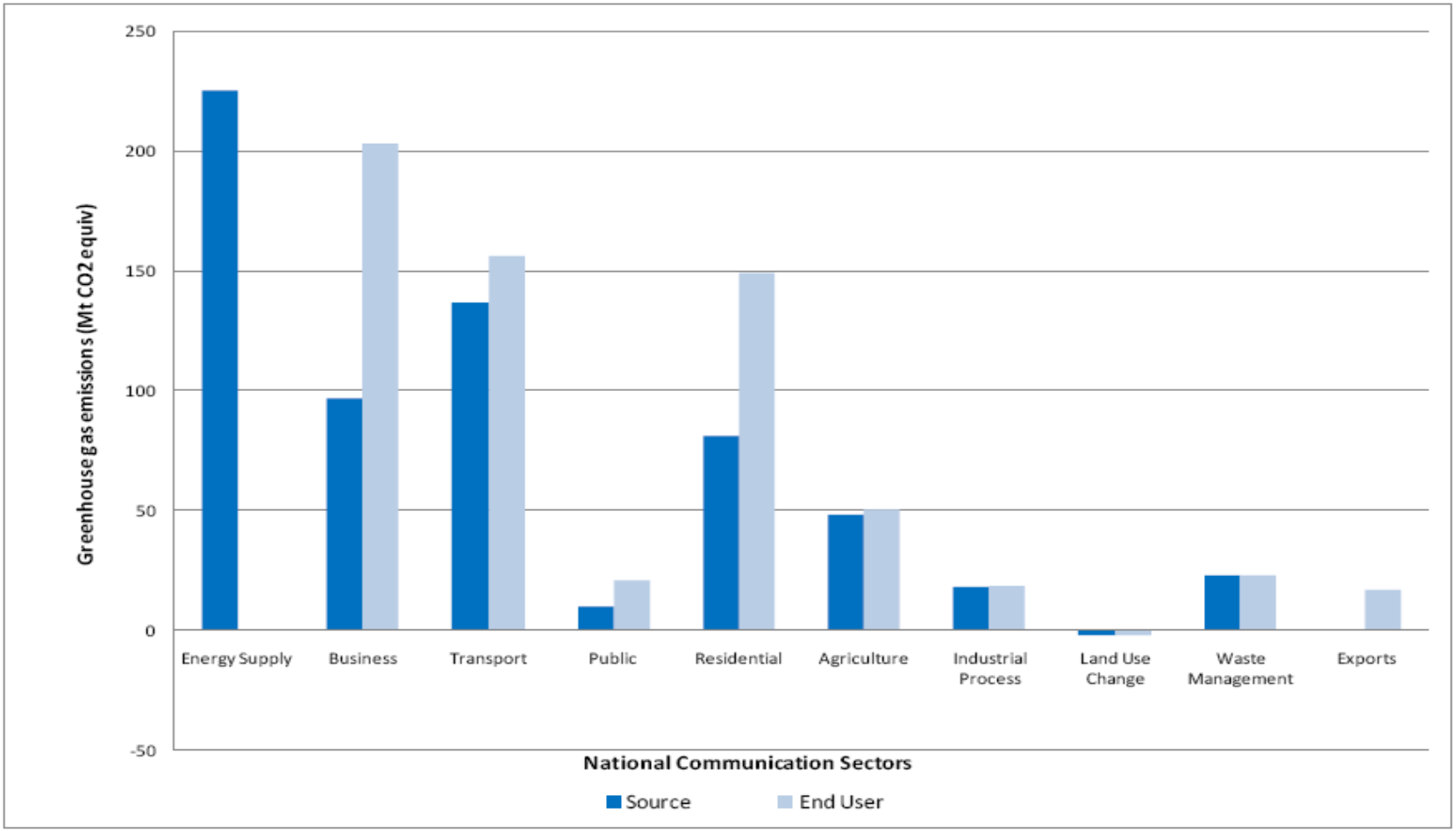


Figure 1.2: 2008 UK greenhouse gas emissions, provisional figure by sector (Department of Energy and Climate Change (DECC)).

1.2 Tackling the problem

Although waste disposal and treatment only contributed 3.4% to the total global annual greenhouse gas emissions, as calculated by the IPCC, it was still identified as being a key problem area. It is believed that this figure could indeed be higher but problems with a lack of agreement in data of locally managed waste disposal practices make it difficult to accurately measure (IPCC).

In December 2002, the European Commission (EC) published the European Union (EU) Waste Statistics Regulation (EC 2150/2002). This outlines standard nomenclature for waste categories and disposal and it requires each Member State to present the Commission with their country's statistics on waste generation, recovery and disposal every two years.

The IPCC summarises that although CH_4 emissions have largely been stabilised in developed countries, certain measures should be enforced to encourage this positive trend. Firstly, the primary reduction in waste generation through the promotion of recycling and re-use to conserve raw materials and energy ought to be encouraged. It is held that waste management should be managed at a local level to minimise problems created through variation in waste quantity and characteristics; cost and financing issues; regulatory constraints and infrastructure requirements. Landfilling-diverting technologies should continue to be developed, including large-scale composting, anaerobic digestion, mechanical biological treatments (MBTs), and incineration. Secondly, gas could be recovered from existing and new back-up landfills. Such strategies can also bring co-benefits such as improved public health and safety, pollution prevention, local energy supply and soil protection.

In 2006, the EU produced a waste framework based upon EC Council Directive 75/442/EEC of 1975 and its amendments (1991, 1996, and 2003). Its purpose was to address the issue of waste from an environmental perspective, taking a similar stance to the recommendations laid out by the IPCC. The report outlines the need for member states to develop clean technologies that do not rely on natural resources; recover waste to extract re-useable raw materials; and to promote the production of energy from waste management (2006/12/EC Article 3).

For these objectives to be achieved, it was proposed that each Member State should draw up a waste management plan which addressed the following:

i. The type, quality and origin of waste to be recovered or disposed of.
ii. General technical requirements.
iii. Suitable disposal sites or installations.

It also suggests that the persons legally responsible for waste management should be listed, along with estimated costs for schemes and the appropriate measures to encourage rationalisation of the collection, sorting and treatment of the waste (2006/12/EC Article 6).

This waste management plan was to be transposed into national law within two years of the publication of the directive. As a result, The UK Department for the Environment, Food and Rural Affairs (Defra) updated England's Waste Strategy in 2007. Targets already laid out in the European Landfill directive had already been incorporated into the Waste Strategy published in 2000.

1.3 Waste management in the UK

1.3.1 Defra's Waste Strategy 2007

Around 100 million tonnes of waste is being produced annually from households, commerce and industry in the UK. The 2008/2009 figures show that 50.3% of this waste is being landfilled (Defra). As a result, large volumes of CH_4 are still being produced from the biodegradable fraction (40% of total CH_4 emissions). Although a small amount of residual material must be landfilled, much of the waste is recyclable. The government has sought to reduce the amount of biodegradable municipal waste (BMW) going to landfill in accordance with Article 5.2 of the European Landfill Directive.

The Government has introduced several key measures to drive this progress. The Landfill Allowance Trading Scheme (LATS) was introduced in 2005. Local authorities across England will be allocated an allowance to landfill BMW each year. These allowances can be traded, banked and borrowed to encourage the development of cost effective strategies. When the Waste Strategy was published in 2007, all 121 waste disposal authorities were within their limits and none were liable to penalties. This coupled with the Landfill Tax Escalator (a scheme which sees an incremental increase of £8 per tonne per annum in the charge paid for landfilling) have proven to be successful schemes to bring about the positive changes illustrated in Figure 1.3.

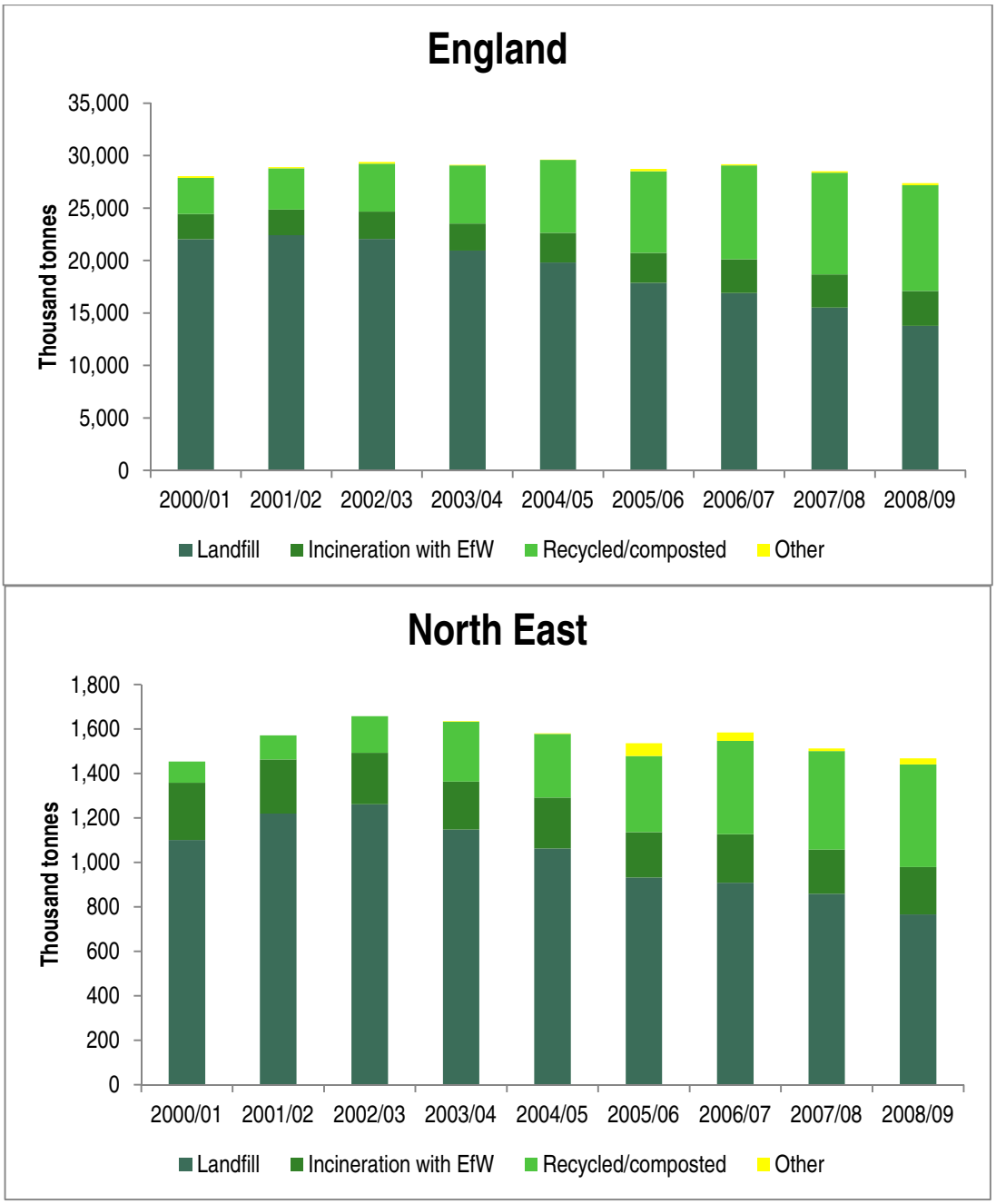


Figure 1.3: Defra 2009/2008 Municipal waste statistics.

The government met its landfill targets for BMW in 2010 and now aims to meet and exceed targets for 2015 and 2020. It is proposed that 53% of municipal solid waste (MSW) will be recovered by 2010; 67% by 2015 and 75% by 2020. Within the same timeframe, it is hoped that recycling and composting will increase to 40%, 45% and 50% respectively.

Based upon data from 2001, Figure 1.4 shows how far the UK had to go. It can be seen that the UK remained behind its European counterparts.

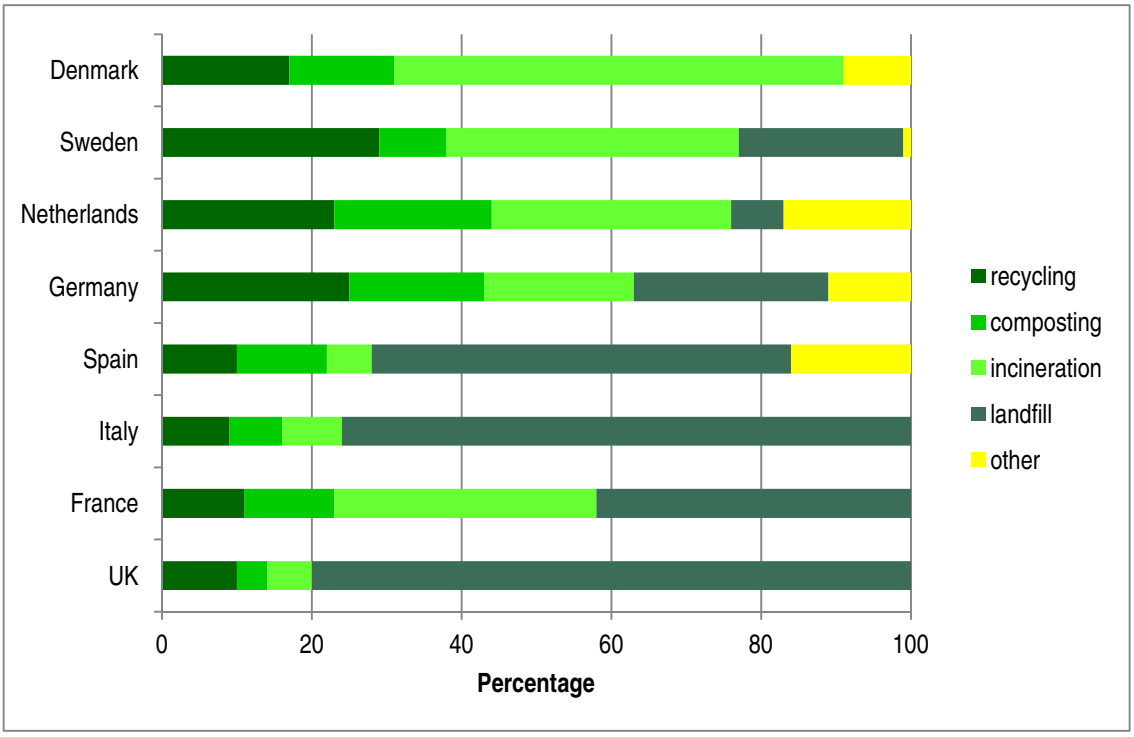


Figure 1.4: Treatment and disposal of MSW, Adapted from Eurostat (2001).

Eurostat reported in 2011 that by 2008, although a 20% increase was seen in recycling and composting and a corresponding decrease in waste sent to landfill, there were still significant differences seen between the different member states. Figure 1.5 shows the UK still lying behind Germany, The Netherlands and Sweden in its ability to divert from landfill.

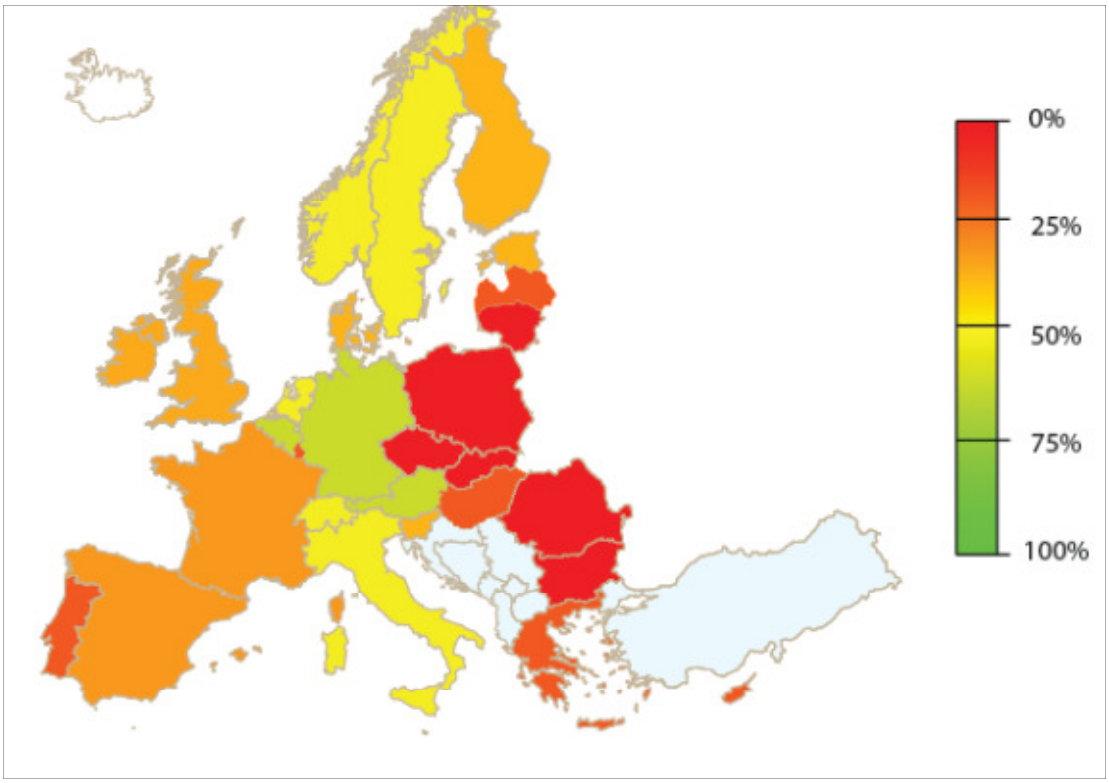


Figure 1.5: Recycling rate of MSW in 2007 (ec.europa.eu/environment/waste/strategy,2011).

Several EU member states impose legal restrictions on the types of waste that can be landfilled, a strategy that DEFRA is considering implementing in England. In Denmark, for example, landfill is only to be used for waste which cannot be re-used, recycled or incinerated. Landfilled material accounts for only 10% of all waste and it includes asbestos, non-recyclable polyvinyl chloride (PVC), impregnated wood, contaminated soil, residues from car shredding and the bottom ash from municipal incinerators (EEA 2002).

The UK needs to secure investment in infrastructure if it is to meet the high targets set (Maynard and Chemett, 2006; Jones *et al.,* 2006; Waste strategy, 2007). New technologies must be cost-competitive to landfill to attract investment. The government have suggested that private finance initiatives could encourage a variety of energy recovery technologies. However, because many waste management projects are still in the early stages of development and therefore perceived as high risk; coupled with the fact that little clarity is given about the acceptable end uses for recovered materials, many are reluctant to invest. Financiers need more assurance which would come from a degree of certainty over the implementation of legislation (Jones *et al.,* 2006).

In the main elements section of the Waste Strategy, regulation reform is discussed but it is mainly focused on imposing further restrictions on the type of waste that can be landfilled and clamping down on illegal dumping of waste. There is little discussion about new legislation for recovered materials. For example, anaerobic digestion (AD) is one of the new technologies which are strongly supported in the report as it combines the separation of recyclables, the production of CH_4 which has energy-producing capability through energy from waste (EfW) and a compost-like output (CLO) which could potentially be used as a soil conditioner. This CLO, however, still remains in the early stages of development and research is hindered due to strict legislation which prevents it being laid to land.

Public opinion also hampers technological advancements. Waste is largely viewed as a negative entity and its potential benefits are little understood. Government initiatives such as Act on CO_2 and the Waste and Resources Action Programme (WRAP), a non-profit organisation backed by the government, promote public awareness surrounding waste issues. However, proposed schemes such as introducing taxes on waste will only foster negative attitudes towards waste. A carrot rather than stick approach, whereby people are rewarded for recycling could be more successful.

WRAP also work alongside the Local Government Association (LGA) in helping Local Authorities (LA) to meet their recycling targets. It is the local authorities who are responsible for the collection, treatment and disposal of municipal waste. The Environment Agency (EA) monitors this to ensure that the LAs are meeting their targets for recycling and composting.

To ensure that these targets are met, further policies will be implemented and current policies upheld. An increase in investment for local government waste collection and disposal is to be seen. This is the stance taken by LGA who believe that the landfill allowance trading scheme (LATS) and a lack of funding to establish new green technologies makes it impossible for some Councils to successfully divert from landfill and meet targets.

Ultimately, it is important that waste is viewed as a potential resource with financial benefits, as well as an environmental problem in need of a solution. If investment in good, robust research is made now, a long-term, sustainable approach could be formulated to meet and exceed imposed targets.

1.3.2 Alternatives to landfill

As approved in the IPCC reports and highlighted in the Waste Strategy, the UK uses a variety of new technologies in its drive to divert BMW from landfill. Currently, the LAs' approaches are diverse with some very much more successful than others. Statistics from Defra for 2009/2010 show counties such as Devon, Dorset and Somerset recycling and composting 49-53% of their household waste; Middlesbrough, Sunderland and Sheffield only recycle and compost 23-27%. It is widely believed that the most beneficial approach would be to use a mix of technologies; the solution must be appropriate to its specific location and supply of materials (Jones *et al.,* 2006). It is also important to assess each component of a waste stream independently using life cycle analysis (LCA) to best deal with each material. For example, one tonne of aluminium recycled saves eleven tonnes of CO_2 based upon this approach (Waste strategy, 2007).

Green waste is often added to household waste, which increases the biodegradable fraction. In County Durham, the fraction of biodegradable waste is estimated to be around 54% of waste collected and the recyclable materials around 60 % (Premier Waste Management).

1.3.3 The New Technology Demonstrator Programme

As 400 mega-centres are needed by 2015 in order to meet the landfill targets in the next 10 years (Jones *et al.,* 2006), many of the new waste disposal technologies need thorough investigation to weigh up each of their individual benefits.

The New Technologies Demonstrator Programme (NTDP) was established by Defra in 2003, to trial several potential treatment methods for BMW. This was achieved through the establishment of nine pilot schemes, each of which demonstrated the viability of a particular process. The research resulting from the programme was designed to enable local authorities to make impartial, informed decisions when forming their individual waste strategies.

The budget for the NTDP was £30 million and all bids made for grants were assessed on a number of criteria including technical feasibility, emissions, marketable outputs, energy, tonnage and value for money.

Project Name	Location	Summary
Biocycle	Ludlow, Shropshire	Anaerobic digestion of green and kitchen waste
Bioganix	Leominster, Hereford & Worcester	In-vessel composting of green waste
ENVAR	St Ives, Cambridgeshire	In-vessel composting
Premier Waste	**County Durham**	**Aerobic digestion of Municipal Solid Waste (MSW)**
Novera	Havering, Essex	Gasification of refuse-derived fuel (RDF)
Merseyside Waste Disposal Authority	Merseyside	Complex materials recycling facility /mechanical heat treatment (MHT) processing
Waste Gas Technology UK Ltd	Isle of Wight	Gasification of RDF
Compact Power	Bristol	Gasification/pyrolysis of MSW
Scarborough Power	North Yorkshire	Gasification of MSW

Table 1.1: A summary of the nine successful projects chosen by DEFRA

1.4 Premier Waste Management (PWM)

One of the nine NTDP funded projects is the aerobic digestion of MSW by Premier Waste Management in County Durham.

Currently, County Durham has two landfill sites which are leased to PWM. Historically, most of the county's waste was disposed of here, being that it was the cheapest option. With annually increasing landfill tax, this is become less viable. The company now operates kerbside collection of household waste, garden waste and recyclables (paper, cardboard, glass, plastics and metals) and manages a recovery scheme - Premier advanced recycling centre (PARC) based in Thornley, County Durham.

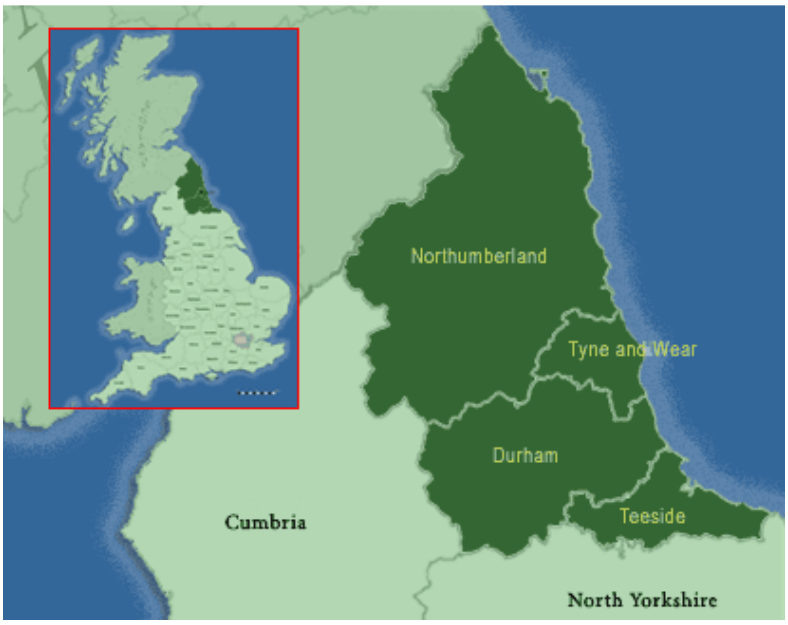


Figure 1.6: County Durham, area serviced by PWM

In 2002, an in-vessel aerobic digestion plan was opened at the PARC waste transfer station. Initially, the plant consisted of two aerobic digestion towers with a combined capacity of over 30, 000 tonnes per year. Since 2007, a third tower has been in commission, which facilitates an extra 22, 500 tonnes. With reductions in waste collected, this combined capacity should be sufficient to process all the waste brought to the transfer station.

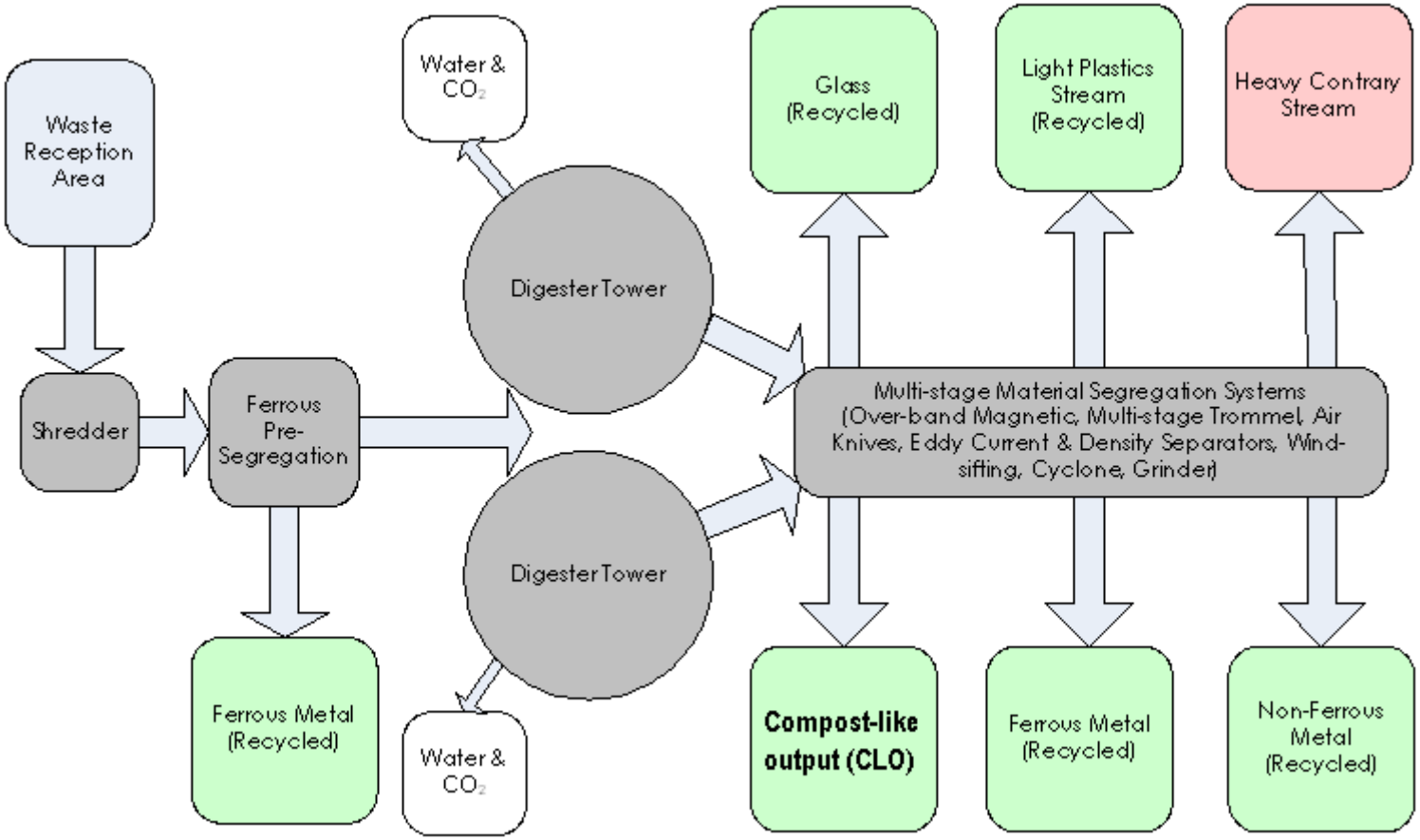
1.4.1 Aerobic digestion process

In-vessel aerobic digestion allows the composting process to happen on a much larger scale, under carefully controlled conditions and over a much faster timescale. Unlike anaerobic digestion, which also produces a CLO, this process allows the composting process to occur with relatively low GHG emissions.

Once the MSW has been shredded, the ferrous metals are removed and the remaining material is fed into a digester at the rate of 100 tonnes every six days. Under carefully controlled conditions, the BMW is digested for a period of six days, after which CLO is produced. Other recyclable materials are separated via a mesh, over band magnet, density separator and eddy currents. The remaining fraction (around 25%) goes to landfill. The CLO produced is transferred to windrows for six weeks before a final washing stage to extract any remaining plastic film.

During the process, air is added to maintain aerobic conditions and water to increase microbial activity. The water is recycled as a seed for subsequent batches. Moisture content and batch temperature are monitored and logged throughout. As the process is fully aerobic, the system is also constantly observed for the presence of CH_4. As yet, however, no CH_4 has been detected during the digestion process at the Thornley plant.

Figure 1.7: The aerobic digestion process summarised (PWM).



As the MSW contains raw and cooked meat, it was necessary for the towers to be approved by the State Veterinary Service division of Defra to process category 3 animal by-products and catering waste. To meet this requirement, the process has to be monitored to ensure that the material reaches and exceeds temperatures of 70˚C for at least one hour. This is to guarantee that any potential microbial biohazards are eliminated and no pathogens are present.

Figure 1.8: a PWM digester tower

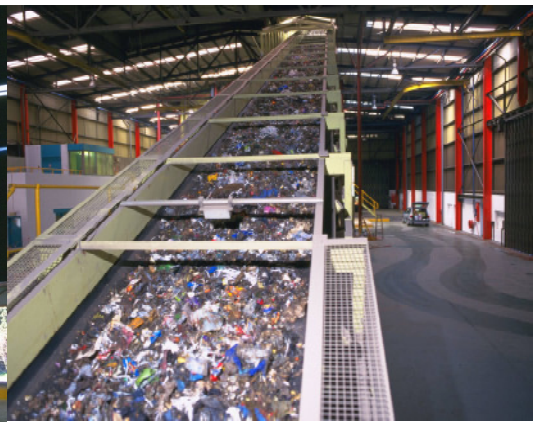


Figure 1.9: a hopper, transporting MSW from the shredder to the digester tower.

During the first seven months of 2006, there were 21,300 tonnes processed of which 15,600 tonnes were diverted from landfill:

Recycled Compost	31.4%
Recycled Plastics	2.8%
Recycled Glass	4.0%
Recycled Metals	3.8%
Process Losses	31.2%
Landfill Diversion Efficiency	**73.2%**

1.4.2 The carbon budget model

An earlier collaboration between PMW and Durham University in April 2006 produced a carbon budget model which sought to calculate the potential for carbon storage in CLO. The carbon lost during aerobic digestion, incineration and landfilling of MSW was compared and the graph in figure 1.10 produced.

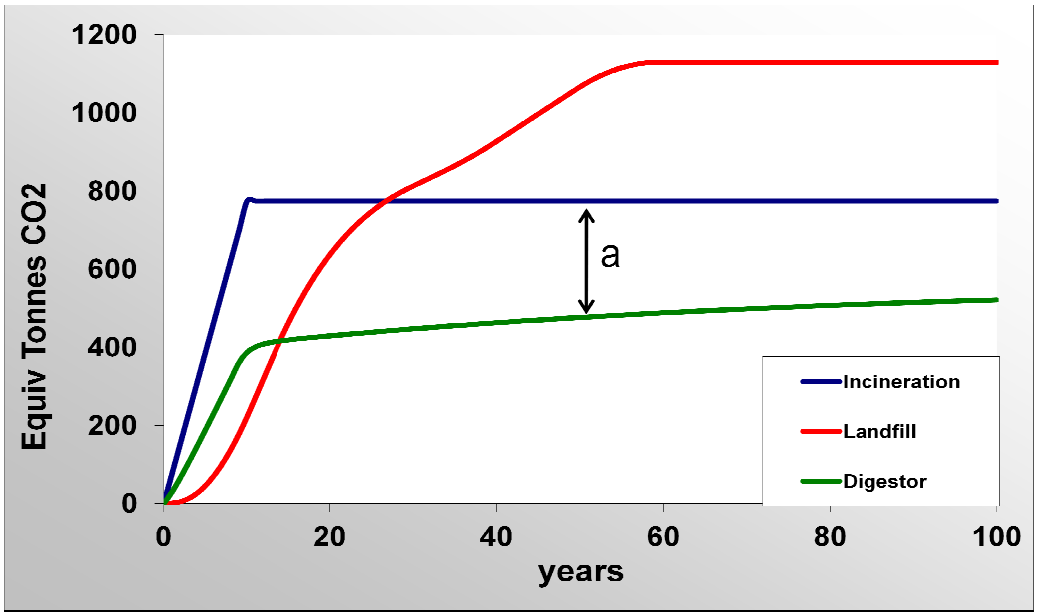


Figure 1.10: The Carbon budget model for aerobic digestion, incineration and landfill (Worrall and Johnson, 2006). This model was further developed by Eunomia in June 2007 and amongst other improvements, allows the composition of the MSW to be varied. This is increasingly necessary as the kerbside collection initiatives and public perception of waste changes.

It can be seen that aerobic digestion, shown by the green line (figure 1.10), is the waste disposal option which gives the lowest release of carbon to the atmosphere. This research project is based upon this premise and seeks to carry the idea further. Arrow 'a' shown on the graph indicates the difference between aerobic digestion and incineration; the wider this margin, the more economically viable aerobic digestion would be as a recovery scheme. In order for this gap to be widened, carbon would need to be further stabilised at some point during the process.

This project seeks to stabilise the CLO produced during the process, thus locking up carbon and reducing carbon flux. If this could be achieved, the carbon released throughout the process' life cycle would be decreased and the gap between incineration and aerobic digestion increased. Premier waste management stand to gain through the EU Emissions Trading Scheme (EU ETS), which Defra describes as:

"...a market-based mechanism to incentivise the reduction of GHG emissions. The scheme operates through the allocation and trade of greenhouse gas emission allowances throughout the EU – one allowance represents one tonne of carbon dioxide equivalent." (Defra 2006).

If carbon emissions could be reduced effectively during the composting process, PWM would in theory be able to sell their surplus allowances by trading directly with other companies or through a broker. The EA regulates the scheme in England and Wales.

Another source of income from aerobic digestion of MSW are the recyclable materials and the compost-like output.

1.4.3 The product: Compost-like output

In addition to diverting organic waste from landfill, CLO could potentially prove to be an important carbon store. With the UK losing 13 million tonnes of carbon per year (mtC/yr) from its soils (Loveland and Bellamy, 2004), restoring this carbon store is of significant importance. If the CLO is returned to the land, it could be used to plant crops which in turn would remove carbon from the atmosphere. In earlier studies the potential of CLO as a marketable soil improver has been explored and has been found to enhance crop yield, soil organic matter (SOM), moisture content and decrease erosion in urban soils (Martinez- Blanco *et al.,* 2009; and references therein).

Compost-like outputs are still a relatively new product but the government and WRAP have developed a publicly-available specification (PAS) BSI PAS 110 for source-separated anaerobic digestates in 2010. This provides an industry specification against which producers can verify that the product is of a consistent quality against certain parameters and therefore fit for purpose for use as a soil improver. It is vital that products from 'waste' are properly regulated to help to dispel any mistrust and scepticism that potential buyers might have.

The CLO manufactured by Premier is not produced from a source separated feedstock so does not meet the requirements for PAS110. It is currently used as topsoil for landfill, but it is hoped that with further research, it can be remediated and sold as a soil improver. A synthetic soil mix called Parcgro™ has combined CLO with garden waste (tree prunings, grass clippings and leaves collected from parks and gardens within County Durham) and it meets the British Standard for topsoil (BS3882). It is laid at a brownfield site in Willington, County Durham, where willow (which can withstand metal-contaminated soils) it is being grown as a feedstock for biofuel. The willow is harvested every three years and fulfils the long-term demand for a local biomass-fuelled power station in Teesside.

Figure 1.11: The Willington site showing increased crop growth on the Parcgro™ treated soil.

However, this product is still in the early stages of development. Research already carried out has shown the initial separation process to be inefficient, in that the CLO retains a high level of plastic, metal and glass (Simpson, 2008). This gives a highly heterogeneous product which causes problems aesthetically and unless pre-treatment occurs, also has the potential to cause environmental issues. Furthermore, the material in its current heterogeneous, contaminated state is difficult to analyse in terms of the organic carbon content.

This research project will be carried out under the assumption that the CLO will be refined through on-going research which includes enhancements to the post digester separation process to maximise the recovery of recyclables and to remove the contaminants from the CLO.

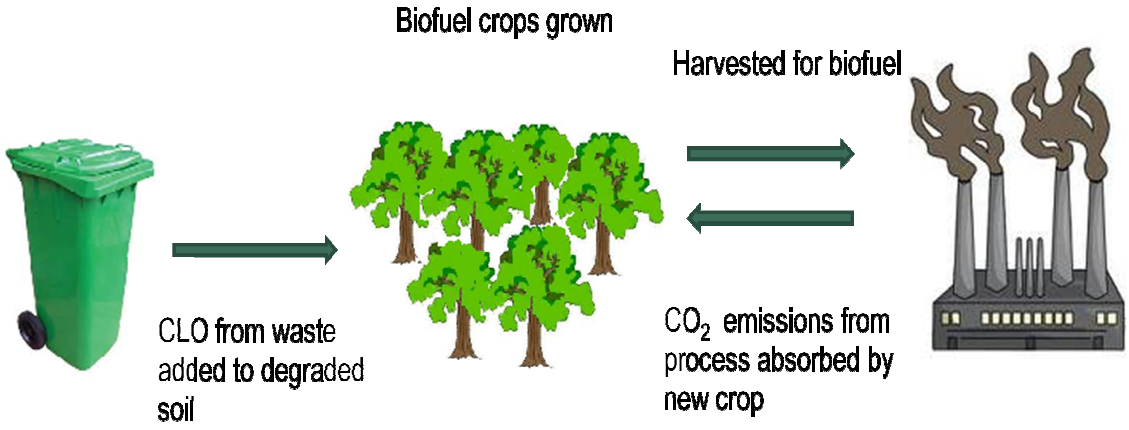


Figure 1.12: from waste to energy and carbon store.

If these initial problems can be addressed, the aerobic digestion process could be one sustainable way of reducing waste and feeding back into the terrestrial carbon store. Figure 1.12 shows a scenario whereby waste could be transformed into an artificial soil which could be used to regenerate brownfield soils, providing a feedstock for biofuel crops. Essentially, this would restore otherwise redundant land into cropland and facilitate the production of a more sustainable fuel for energy.

1.5 Land management

The pedosphere holds around 4% of the global carbon store, four times the amount in vegetation and currently two thirds that held in the atmosphere (Smith, 2004). This may be significantly less than the oceans hold but it is by far the most easily managed (Janzen, 2004). However, global soil organic carbon (SOC) stocks are difficult to estimate which gives a range in variation of between 1000-3000Gt (Schwartz and Namri, 2002). Different variables (temperature, pH, soil moisture, terrain, land-use) are responsible for this but the variation must be overcome in order to protect and preserve areas of high-carbon storage and to enhance areas of low-carbon storage (Bell and Worrall, 2009).

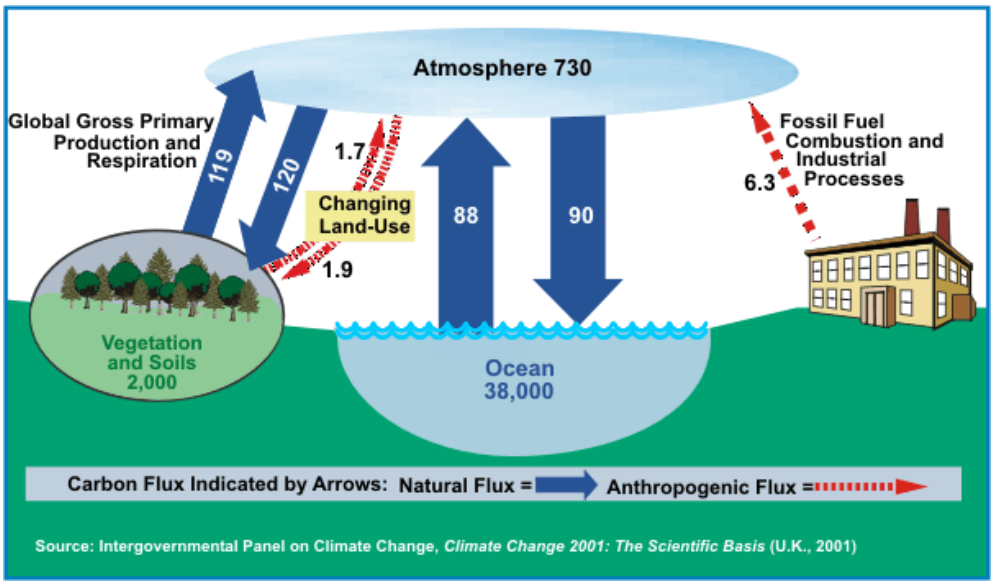


Figure 1.13: Land use change (Janzen, 2004).

Increasingly, more carbon is being taken from the land and is being released into the atmosphere. Currently, it is thought that global soil stocks are being eroded around 10-40 times as fast as it can regenerate (Pimentel, 2005). This is not only having a huge impact on global climate, but also on global carbon stores. Poor land management including vegetation removal and over grazing has seen the rapid depletion of carbon from soils. The expansion of agriculture has had the largest influence on the carbon cycle; an estimated 55PgC has been lost to the atmosphere through the cultivation of agricultural soils (Thompson *et al.,* 2006).

It is becoming increasingly more important that land is managed effectively to preserve and, if possible, enhance the carbon stores that remain. Good, fertile topsoil is essential for 97% of the world's food supply.

Artificial soils could prove to be the solution to the problem of enhancing existing carbon stores. Currently, natural soils are being removed for use in urban landscaping and construction. If artificial soils could be used instead, the natural soils would be preserved. As previously mentioned in the case of PWM, damaged, nutrient-depleted soils (such as ex-colliery and other industrial sites) could potentially be improved with the addition of CLO, subsequently allowing them to be planted with CO_2-absorbing woodland or biofuel crops, and thus reducing the need for farmland to be used. If the amount of carbon stored in vegetation and soils increased, changes in terrestrial carbon storage observed over recent years might be reversed (Janzen, 2004). In order for this to happen, the nature and behaviour of SOC must be understood. This will be addressed in the following chapter.

1.6 Project Aim and Objectives

1.6.1 Aim

The aerobic digestion process yields a potential carbon store in the form of an artificial soil: compost-like output (CLO). The research seeks to reduce carbon emissions from this artificial soil by optimising the humification process. This would increase the stable carbon store within the soil, thus reducing carbon released to the atmosphere. If by producing CLO, PWM could cut their carbon emissions and return solid, stable carbon back to the earth, two problems might be addressed with one solution. Ultimately, the research should enable the company to maximise their profits via the carbon credits trading scheme.

1.6.2 Objectives

In order to fulfil this aim, there are five main objectives which are detailed below.

1. To complete a literature review in order to better understand the conditions needed for optimised humification and to investigate methods available for humic acid analysis
2. To adapt and develop suitable methodologies for the analysis of CLO.
3. To explore the effects of manganese coated sand, sand and biochar on the humification process: the net gain or loss in humic acid and the resulting carbon flux.
4. To investigate the effects of waterlogging on the carbon flux and humic acid production in CLO in order to establish the ideal conditions for the material to be laid.
5. To attempt to characterise the CLO in order to begin to understand the chemical reactions involved during the humification process to further explain the optimum conditions for the production of stable carbon.

1.7 Thesis outline

Chapter 1 summarised the global context in which the project lies; discussing the legislative drivers that have encouraged the use of green technologies when dealing with MSW in the UK. The merits and potential problems associated with CLO product derived from an aerobic digestion process have also been introduced. Finally, the project's aim, objectives and thesis outline have been described.

Chapter 2 gives a comprehensive study on natural soil organic matter and how this might compare to the artificial soil and its organic component. Particular attention is paid to the composition, genesis, structure, humification process; some of the commonly used analytical methods available for the determination of stable carbon compounds are also introduced. The purpose of this chapter is to address project objective 1.

Chapter 3 outlines the materials and methods used in each experimental trial. The adaptation of traditional methods of soils analysis for use on an artificial soil will be described and results from preliminary trials presented. Also, the experimental equipment and tools for data analysis will be discussed. This has been undertaken in order to fulfil objective 2.

Chapter 4 describes the experimental design, execution and results the first full experimental trials, the first of the three investigations into the humification process of CLO. Manganese dioxide-coated sand is added to the soil in a fully factorial experimental design. Objective 3 is tackled here.

Chapter 5 explores the potential to adapt the accepted and well-used traditional thermogravimetric analysis (TGA) method to accommodate larger, more heterogeneous samples. The process of Pseudo TGA is detailed with standard reference material to provide a matrix with which to compare samples. This chapter straddles both objectives 2 and 3, compounding research in earlier trials.

Chapter 6 presents the results from a follow-on trial to that presented into chapter 4 where char is added to both CLO and peat samples. The latter is introduced as a standard soil. Here, the waterlogging of the materials is also introduced. Objectives 3 and 4 are covered here, again developing earlier research.

Chapter 7 addresses the final objective, where micro-scale study of the interactions between four components of CLO; a lipid, a protein, lignin and cellulose along with the three amendments used in previous trials; manganese dioxide, sand and char is given.

Chapter 8 draws together the main conclusions from each trial so that recommendations might be made to PWM and possible further studies suggested. This chapter will report whether or not the project aim and objectives were met.

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Trial 1	This trial was orchestrated in order to determine appropriate experimental techniques for assessing the maturity and stability of the CLO. These procedures, ordinarily used for natural soils were then optimised to suit CLO.	*Discussed in chapter 3*
Trial 2	Building upon the results from trial 1, trial 2 was the first fully factorial long trial where lysimeters were constructed and different parameters introduced. The effect of time and addition of a proposed catalyst on the stability and maturity of the CLO were assessed.	*Discussed in chapters 4/5*
Trial 3	Trial 3 expanded the themes explored during the previous trials. The parameters measured during trials 1 and 2 were repeated but the lysimeters were housed indoors in order to control temperature and moisture content. New parameters were introduced: peat was included in the trial matrix as a control soil; waterlogged versus free-draining samples of each was studied; a char was added to the matrix as another potential catalyst. It was hoped that this trial would run for 12 months but due to technical problems at PMW, the CLO was unavailable for testing for several months so the trial was postponed and conducted over a shorter timescale of 26 weeks instead.	*Discussed in chapter 6*
Trial 4	It was intended that this would be a field trial to re-employ the factors assessed in trials 1, 2 and 3 in order to ascertain whether the same effects witnessed in the laboratory would be seen when the material was laid to land. However, owing to the legal implications of laying various waste materials to land and the time remaining, this was not viable. Instead, a micro-study was undertaken, examining the relationship between the four main components of CLO: protein, lignin, lipids and cellulose. Samples were left for 10 weeks, to make it comparable to trials 2 and 3 and the same experimental techniques were used to assess the humic acid content after this time period.	*Discussed in chapter 7*

Table 1.2: The relationship between the four experimental trials and in which chapters they are discussed.

Chapter 2

Soil Processes

Discussed in this literature chapter will be the composition and genesis of soil organic matter along with the principle reaction processes involved in the formation of humic substances. Furthermore, the structure of these stable carbon compounds will also be explored. A review of current literature relating to the composting of MSW and other waste materials to produce artificial soils will also be presented.

2.1 Organic matter in natural soils

Before artificial soils are considered, it is important to understand the processes that occur in natural soil systems.

Natural soils are comprised of both inorganic and organic carbon. The former is derived from eroded bed-rock material; the latter from the decomposition of plant matter, microorganisms and small animals. It is this, the soil organic matter (SOM) that is of interest to the research presented in this thesis. However, it is necessary to appreciate the interactions between the organic components, minerals and microorganisms, so that can SOM's place within the unified soil system can be fully appreciated (Huang *et al.,* 2004).

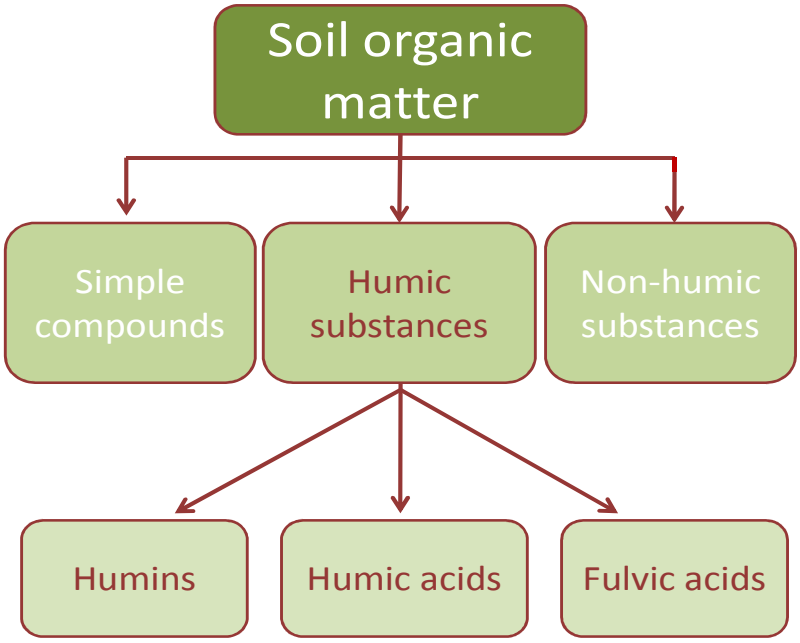


Figure 2.1: the composition of SOM, adapted from Berth et al (2008).

Simple compounds are represented by unaltered biomolecules and can be defined by discrete categories of biopolymers (polysaccharides, proteins, lignin, and lipids). These biopolymers are low molecular weight (MW), easily degraded molecules. Humic substances (HS) are biomolecules which are considered to be altered by biological oxidation or chemical reaction are not placed in discrete categories. They are characterised by compounds with higher MW and a greater degree of aromaticity and are relatively rich in carboxyl and hydroxyl groups (Garcia –Gill *et al.,* 2004). The third group are the non-humic substances that satisfy neither of the other two categories and are largely hydrophilic acids which are expected to constitute a substantial fraction of dissolved organic carbon (DOC) (Vergnoux *et al., 2011*).

SOM is a complex heterogeneous mixture of these organic particles and molecules exhibiting a variety of stages of decomposition, ranging from fresh, unaltered material through to thoroughly decomposed states. The mean residence time of SOC varies over several orders of magnitude between leaf litter and the various humus fractions with turnover of resistant plant residues adsorbed onto soil particles being in the order of years; fulvic acid (FA) approximately 100 years; humic acids (HA) can remain in the soil for thousands of years (Huang *et al.,* 2004). Although HAs and humins constitute the majority of SOC, they only contribute a small amount to carbon cycling within the soils due to their recalcitrance.

2.2 Humic substances

Humic substances are difficult to define, although several definitions exist:

Being *"...amorphous, polymeric, colloidal, polydispersed substances with high molecular weights."* (Sposito, 1989).

They are *"...covalently linked aromatic and aliphatic residues, carrying carboxyl, phenolic and alkoxy groups with sulphate esters, alanine and semiquinones, phosphate ester and hydroquinone moieties"* (Jones and Bryan, 1998).

They are ubiquitous in both terrestrial and aquatic environments: in natural waters, sediments, soils, peats and other natural ecosystems and they are the largest SOC pool (70-80% of soil carbon) (Nichols and Wright, 2006). They can comprise between 40 and 70% of dissolved organic matter in rivers and streams (Lu *et al.,* 2000). They form highly complex, heterogeneous, amorphous molecules and are extremely resistant to degradation: their half life can amount to thousands of years (Grinhut *et al.,* 2007).

Their recalcitrant nature makes HSs an important carbon sink. They represent a dynamic system which is subject to continual change; a system which is dependent upon plant cover; activity of microorganisms and animals; climate; chemical, physical, physicochemical properties, and also mans' activity. All of these factors determine the amount, composition, distribution in soil profile, nature of the HSs and their complexes with minerals (Kononova, 1961). They play an important biogeochemical role in the ecosystem, having influences on redox reactions; the sorption, complexation and transportation of pollutants, minerals and trace elements; soil structure and formation (Lafrance *et al* 1989). Their dark colour aids the regulation of soil temperature and they also improve a soil's water and nutrient holding capacity (Smidt *et al* 2008).

Humic substances comprise three operationally defined compounds: humic acids, which are soluble in alkali solution; fulvic acids, which are soluble in alkali and acid solution and humin which is soluble in neither. It is widely believed that humic acids are compounds with MW somewhere between several hundred to several hundred thousand daltons (Da) (Shin *et al.,* 1999) and fulvic acids less than 10,000 Da (Reemtsma *et al.,* 2008). However, no sharp division exists between these different fractions as all are extremely structurally heterogeneous (Nichols and Wright, 2006).

2.2.1 Structure

In order to appreciate the reactivity of HS, their structures must be considered. Being that they are such a heterogeneous mixture of materials formed from a variety of sources, elucidation of their precise structure is a challenge. There is no one universally accepted structure, though several researchers have attempted to define one. However, Stevenson's proposed structure of humic acid is the most commonly quoted.

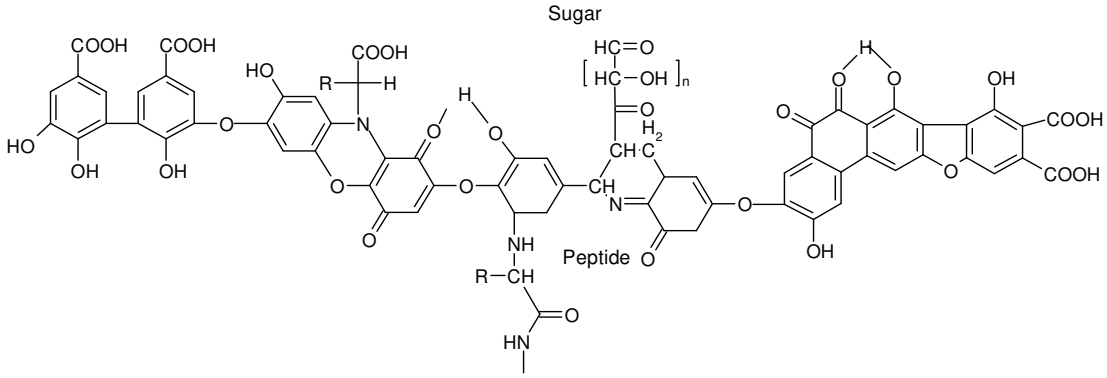


Figure 2.2 Stevenson's (1994) proposed structure of humic acid

It is generally accepted that HAs are part of a product of heteropolycondensation of carbohydrates, proteins, fatty acids, lignins... depending upon their origin (Li *et al.,* 1996). They are multifunctional molecules chiefly built up from an aliphatic hydrocarbon skeleton core (Osterberg *et al.,* 1993) and are substituted with oxygen-containing functional groups including carboxyl > phenol > alcohol > quinone and ketonic carbonyl > amino > sulphydryl alkoxyl**,** hydroxyl (Sposito, 1989; Pehlivan and Arslan, 2006). Generally, fulvic acids contain more functional groups and have a typical composition of 40-50% carbon (C) and 40-50% oxygen (O). Humic acids tend to be more advanced in the humification stages, therefore more polymerised with a slightly higher ratio of C (50-65%): O (30-40%) (Stevenson 1994).

Quantitative studies using elemental analysis can illustrate elemental composition of humic structures. This is also the most sensitive method for assessing changes in HA structure (Bernal *et al.,* 2009). Table 2.1 shows the results from a study by Li *et al.* (2004) whereby bulk HA was separated into 8 fractions based on MW via ultrafiltration. Elemental compositions of each fraction are shown along with the E_4/E_6 ratios (see section 3.6.2)

Fraction (1,000Da)	Mass	MW	Elemental composition (weight %)				Atomic ratio			E_4/E_6
			C	N	H	O	C/H	C/O	C/N	
<1	1.90	1.07	48.7	4.0	3.3	44.0	14.76	1.11	12.18	13.6
1-3	1.60	1.18	48.5	3.6	3.5	44.3	13.86	1.09	13.47	13.2
3-5	1.70	1.49	48.4	3.4	3.8	44.4	12.74	1.09	14.24	12.6
5-10	2.00	1.77	49.9	3.9	3.9	42.3	12.79	1.18	12.79	11.7
10-30	15.5	3.24	53.6	4.4	4.1	37.9	13.07	1.41	12.18	7.0
31-100	22.2	5.25	54.0	4.6	4.3	37.1	12.56	1.46	11.74	6.1
100-300	9.40	6.29	54.7	4.6	4.4	36.2	12.43	1.51	11.89	5.7
>300	45.8	18.56	57.0	5.3	5.3	32.4	10.75	1.76	10.75	4.5
Bulk	-	10.68	56.1	3.9	5.0	35.0	11.22	1.60	14.38	5.8

Table 2.1: various molecular weight fractions of HA, their mass, MW, elemental composition, atomic ratio and E_4/E_6 ratio (*Li et al.,* 2004).

During this study, aromaticity decreased as MW decreased; smaller MW molecules tend to contain a greater number of oxygen-containing functional groups, possibly deriving from lignin materials. It was thought that the higher MW fractions may originate from lipid rich biopolymers.

For a more qualitative picture, spectroscopic techniques can be employed to establish connectivity and specific functional groups present. Pyrolysis gas chromatography/mass spectrometry (Py-GC/MS) determines skeleton structure of HA, whilst nuclear magnetic resonance (NMR) has highlighted the existence of primary structures in HSs with major molecular structural units including aliphatic acids, ethers, esters, alcohols, aromatic lignin-derived fragments, polysaccharides and polypeptides (Simpson *et al.,* 2002). Thermochemolysis provides detailed structural information on the building blocks of SOM. This non-destructive and highly selective method cleaves ester and some ether linkages in macromolecules. It also renders many of the polar products volatile enough for gas chromatography (GC) analysis (Chefetz *et al.,* 2002). With the development of such analytical techniques, our understanding of these complex molecules has been further elucidated.

Recent studies using these sophisticated forms of analyses have evolved new proposed structures of humic substances (Sutton and Sposito, 2005). Based upon their studies, Grinhut *et al* proposed the following revised structure in 2007.

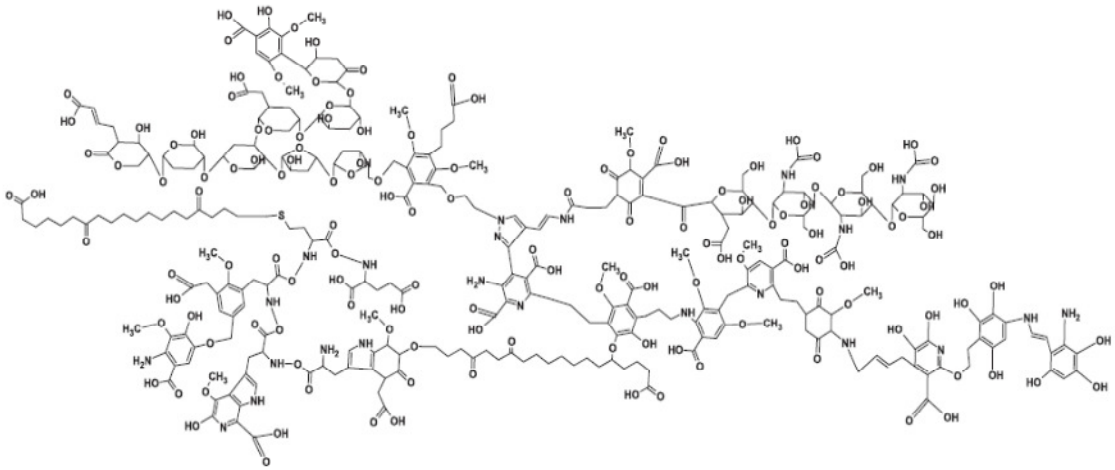


Figure 2.3: Proposed structure of humic acid by Grinhut et al 2007

However, this large macromolecule is considered to be inconsistent with evidence from several other studies (Schaumann, 2006a; Simpson *et al.,* 2002; Li e*t al.,* 2003). The more widely accepted view is that humic substances are collections of diverse, relatively low MW components which form dynamic associations, stabilised by hydrophobic interactions and H-bonds (Sutton and Sposito, 2005). Such an arrangement is referred to as a supramolecular assembly, and is defined by Schaumann and Bertmer (2008) as:

" ...a multi-component system of atoms, metal ions and/or molecules which are held together by non-covalent interactions such as hydrogen bonds, Van der Waals forces, π-π interactions and or electrostatic effects."

Tetraethyl ammonium acetate pyrolysis (TEAAc-pyrolysis) has shown that these weak bonds play a key role in the make-up of humic substances and induce the retention of low MW molecules (fatty acids and alkanols) (Guignard *et al*., 2005). Diffusion ordered spectroscopy (LC-NMR) has also determined that they are largely low M_W molecules (2000 Da) that can be easily separated (Simpson *et al* 2002). Coordinated cross-links can form between humic substances and multivalent cations which may increase apparent molecular weight (Schaumann, 2006).

A study by Li e*t al* (2003) concluded that two sub-units of HA may in fact exist: an aliphatic with a larger MW and a smaller aromatic group, thus consolidating the heterogeneous nature of HS.

Different source material can lead to different HA within the same environment *(*Li *et al.,* 2004) but there is little conclusive data available concerning specific biological sources of HS from different environments. However, a study by Lu *et al* (2000), showed that differences occurred between samples of soil, swamp sediment, peat and coal; the first two having more carbohydrate and carboxylic components and a greater loss of polysaccharide and lignin fractions. Peat had a greater proportion of aliphatic biopolymers and in coal, aromatic compounds dominated. A similar study on agricultural soils showed that they contained a large proportion of lignin in their structure, shown by py-GC/MS and Tetramethylammonium hydroxide (TMAH). Smaller particles are expected to have more lignin-derived units in the final stages of oxidation (Chefetz *et al* 2002).

With this considered, if the source material in the BMW fraction of MSW is significantly different to that seen in natural systems, it is probable that differences will be seen in HA produced. What affect might this have on the genesis of HAs during aerobic digestion and subsequent maturation as compared to the humification processes seen in natural soils?

2.2.2 Humification process

Humic substances are formed via the degradation and transformation of biomolecules/plant and animal litter (particularly plant cell wall compounds such as lignin and structural polysaccharides, together with lipids and proteinaceous materials). This assembly of organic "leftovers" provide vital properties to soils (Sutton and Sposito, 2005). The SOM is degraded both biotically and abiotically, usually giving a more stable product than the original component materials (Lu *et al.,* 2000). The precise nature of the chemical process is little understood. Mechanisms can be placed into one of two categories:

i. Either the biotic polymer degradation where integrity of the biopolymer is not destroyed but modified by enzymatic degradation such that it forms the backbone of the altered biomolecules (Hedges, 1988).
ii. The abiotic condensation polymerisation in which simple products of biopolymer degradation (phenols, quinones, sugars, amino acids) repolymerise to form altered biomolecules (Hedges, 1988).

These two options are not necessarily mutually exclusive (Huang *et al* 2002). Major components of HS include:

Lignin is a phenolic monosaccharide polymer which is highly aromatic and insoluble in water, making it hydrolysis-resistant and therefore protected from microbial attack (Adani and Ricca, 2004). It ultimately forms peat, coal and oil, under the right conditions. It is thought that aromatic compounds in HSs should mainly derive from lignin (Kogel-Knabner 2002; Akim *et al.,* 1998).

Lipids are almost always a minor component of natural SOM but can provide important information on sources of humic material (Guignard *et al* 2005). Three main lipid types (aliphatic, aromatic and sterols/terpinols) were identified by a study by Allard in 2005 in forest and agricultural soils.

Proteins are condensation polymers of amino acids which comprise one of the largest classes of nitrogen-bearing substances in soils (30-45%). Containing an amino group ($R\text{-}NH_2$) and a carboxyl group (R-COOH) which can form a cation and an anion respectively, the molecules can exhibit both acidic and basic properties. Protein amino acids dominate but non protein amino acids can also be present such as hydroxyl proline and amithine (Allard, 2005). Glomalin, a glycoprotein produced by fungi, is found in high concentrations in temperate soils due to its resistance to degradation, heat and low pH (Nichols and Wright, 2006).

Carbohydrates comprise up to half of organic carbon in soils and the common monosaccharides include: glucose, galactose, mannose, xylose, glucuronic acid, glucosamine. They polymerise to form polysaccharides; for example, glucose plus glucose gives a cellulose repeating unit.

The four biomolecules above (lignin, lipids, proteins and carbohydrates) (and shown in figure 2.4) are the most abundant organic compounds produced by living organisms in the soil environment but this list is not exhaustive.

Sposito (1989) identified four stages encountered by soil biomass on its formation to humus.

1. Decomposition of biomass components including lignin into simple compounds

2. Microbial metabolism of simple compounds

3. Cycling of C, H, N, O between SOM and microbial biomass

4. Microbially mediated polymerisation of cycled organic compounds

Stages 1 and 2 form phenolic polymers, whilst 3 and 4 form humic substances. These are converted readily into reactive quinone compounds which polymerise readily. As humification progresses, these residues are metabolised (Hayes *et al,* 1999). Although the residues have short lifetimes (perhaps hours), they are produced continuously (Sposito, 1989).

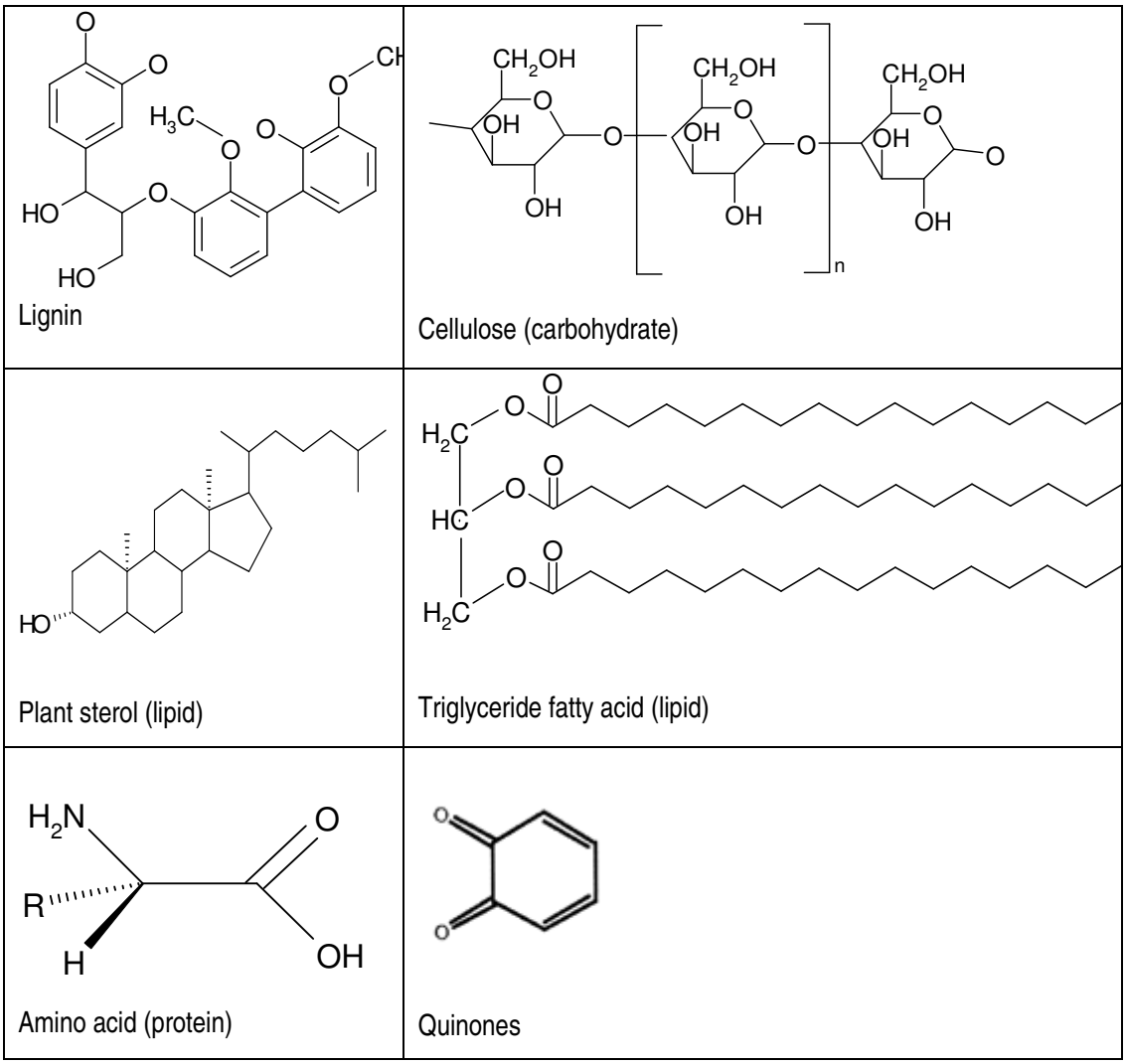


Figure 2.4 some of the biomolecules and biopolymers that undergo degradation and transformation to form HS.

The specific reaction pathways involved in the production of humic material are much disputed. Figure 2.5 summarises proposed pathways that may occur to produce humic substances. Many studies cite lignin as the main contributor of aromatic compounds that define HA (Kalbitz *et al.,* 2003; Kogel-Knabner 2002; Akim, *et al.,* 1998) but as many other reagents all co-exist in soils, it is probable that this is not the only possible pathways that occurs independently, but rather one of several that occur closely and interact (Jokic *et al.,* 2004). Furthermore, a variety of proposed methods may account for diversity in functional groups, presence of different monomeric species and inclusion of biopolymer-like materials in HS (Huang *et al.,* 2002).

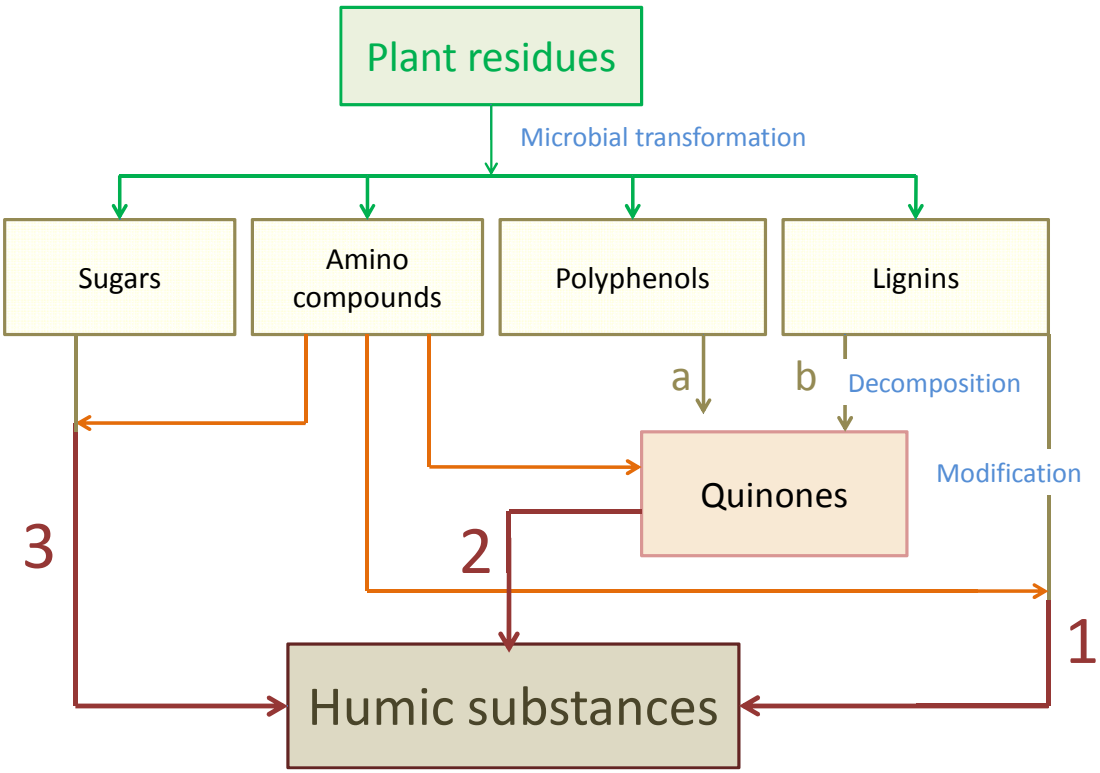


Figure 2.5: Proposed reaction pathways of the humification process, adapted from Stevenson (1994).

Route 1 (Figure 2.5): Lignin decomposition

Lignin is one of the most abundantly present and most recalcitrant biopolymers present in SOM. Following microbial attack, lignin undergoes demethylation which exposes hydroxyl phenols that can then be oxidised to form quinones (Filley *et al.,* 2002). Subsequent oxidation of the aliphatic side-chains produces carboxyl groups (Saiz-Jimenex, 1994)). Condensation with N-containing compounds, then further re-polymerisation occurs, to give an insoluble humic material (Czechowski *et al* 2004).

Route 2a and 2b (Figure 2.5): Polymerisation of quinones

Route 2 (b) involves the polymerisation of quinones from lignin derivatives in the presence or absence of amino acids (Stevenson, 1994). The alternative for this route is 2 (a) where polyphenols are synthesised by microorganisms from non-lignin carbon sources (e.g. cellulose) then are oxidised to quinones (Stevenson, 1994).

Extra-cellular enzymes attack lignin and cellulose to produce polyphenols which give low MW organic acids and aldehydes on oxidation. As with the previous pathway, quinones form as a decomposition product of polyphenols on further enzymatic attack from bacteria, actinomycetes or fungi (Kirby, 2006). Quinones then recombine, attach to other molecules or undergo self-condensation (especially in the presence of amino acids) (Huang *et al.,* 2005).

Route 3 (Figure 2.5): Maillard reaction

The Maillard reaction (Maillard, 1913) involves a sugar-amine condensation. The aldehyde group on the sugar reacts with the amine to produce an N-substituted glycosylamine. This then undergoes dehydration to form highly reactive compounds. In the presence of amino acids, these compounds polymerise to form a brown humic type substance at moderate temperatures (Stevenson 1982).

Yamamoto and Ishiwatari (1992) demonstrated that the Maillard reaction also occurred when using proteins other than amino acids. This is important in deeper soil layers as the abundance of amino acids is seen to decrease with soil/sediment depth (Yamamoto and Ishiwatari, 1992).
The mechanisms and rate of the Maillard reaction remain vague and it may occur slowly under ambient conditions, despite the abundance of sugars and amino acids in soils (Hedges, 1988). Natural soil processes such as freezing and thawing or wetting and drying may speed this process.

It is thought that soil mineral processes play a vital role in the catalysis of abiotic formation of HS (Huang *et al* 2004; Jokic *et al*., 2001). Manganese makes an effective catalyst due to its high oxidation potential, high specific surface area and high surface reactivity (Wang and Huang 2000). In common soils, manganese dioxide (MnO_2) acts as a Lewis acid by accepting electrons from phenolic compounds to produce semiquinones and then produces humic substances via oxidative polymerisation (Hardie, 2007). The catalytic effects of MnO_2 on the humification process will be further investigated in Chapters 4 and 7.

With the major organic constituents of natural soils being plant residues (transformed to amino acids, sugars, polyphenols and lignins), what happens in artificial soils when new components are added and different proportions of the above are seen?

2.3 Artificial soils

With the global carbon store diminishing through processes of poor land management, it is increasingly important to find ways to enhance and add to the soil. The obvious way to do this is to return organic wastes back to the earth. Furthermore, this may become mandatory in the near future with the European Soil Strategy (Banks and Stentiford, 2007). Despite the high levels of CO_2 released during mineralisation of composted wastes, the humification stage contributes to carbon sequestration (Smidt *et al.,* 2008).

Adding green wastes and composts to damaged soils can improve vegetation establishment, reduce compaction, protect against soil erosion and bind toxic trace metals (Beesley *et al.,* 2010; and references therein). With evidence pointing to the fact that urban soils may be a greater carbon sink than neighbouring native soils, it becomes increasingly more relevant to treat the management of carbon storage and the environmental pollution issue as a holistic concern rather than two separate issues (Beesley and Dickinson, 2010).

The ability of composts to improve soil quality and fertility is well understood. They also reduce the risk of pathogens, weeds and parasites that are abundant in uncomposted manures and other organic wastes that are also put to land. It is important, however for the CLO to be fertile enough for planting. A continuous input of organic materials, principally through plant production is required to maintain or enhance the structural stability of the soil (Huang *et al.,* 2002). Soils should be an open system with energy and matter flowing in and out (Bear, 1964).

The major organic biodegradable component in MSW is holocellulose (cellulose plus hemicellulose) with lignin also being highly significant, especially with regards to its resistance to anaerobic degradation. The cellulose/lignin ratio can be used to assess degree of decomposition in landfilled wastes. Old landfill (8yrs) would have a ratio of around 0.8; fresher refuse samples would be around 4 (Zheng *et al.*, 2007). This ratio can be used as a relatively accurate indicator for compost maturity with values of <0.5 being seen for fully degraded substrates (Komilis and Ham, 2003).

2.3.1 Compost-like outputs

As previously discussed in Chapter 1, CLOs are produced via aerobic and anaerobic digestion. These technologies not only offer a diversion from landfill but produce a soil product which can potentially be returned to the ground as a carbon store. Many European countries such as the Netherlands and Austria favour aerobic digestion (Veeken *et al.,* 2000; Smidt *et al.,* 2008). The UK government in its 2007 Waste Strategy championed anaerobic digestion for its ability to produce energy**.** Little is known currently about the differences in the CLOs produced via each method but before any compost material is used, it must fulfil certain criteria. The PAS 100 standard, introduced by WRAP in 2007, is used to assess the suitability of compost made from greenwaste only. In 2010, PAS 110 was introduced for source-separated anaerobically digested wastes. Currently, no standard exists for CLO produced from a co-mingled waste source which is something that would need to be addressed if this product was to be marketed.

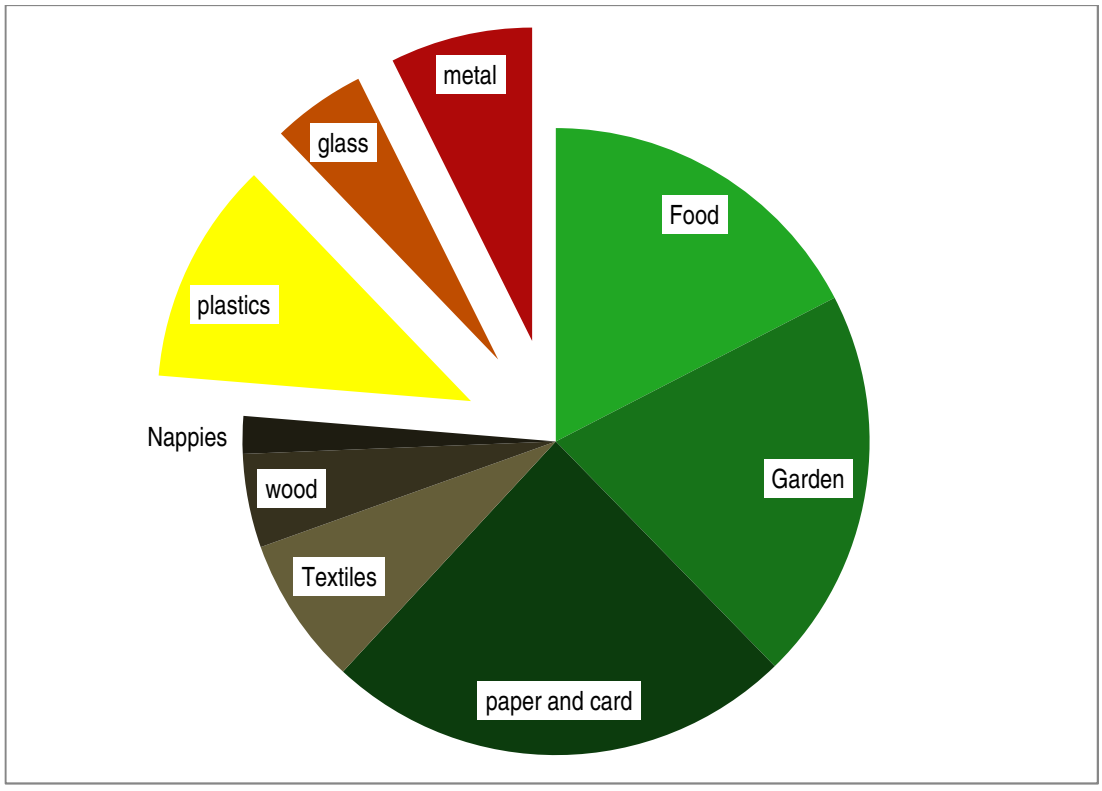


Figure 2.6 Adapted from WRAP (2003) and Eunomia (2007): typical composition of MSW in England and Wales.

Defra is currently working on a new report (WR0119) which aims to provide current and reliable information on the composition of municipal waste. Figure 2.6 gives the most up to

date information for England and Wales on the average composition of household waste. Changes in composition will be seen both geographically and temporally with the evolution of waste management techniques and different cultural habits. For example, food waste varies from 40% of MSW in the United States of America (USA) and 20-45% in Asia (Chang and Hsu, 2008).

The green and brown segments show the contributory fractions to biodegradable municipal waste. It can be seen that paper, garden and food wastes make up the largest percentage. Improvements in source-separation may see a greater reduction in contaminants (metal, glass and plastics) entering the biodegradable municipal waste stream.

Figure 2.7, taken from the same sources shows the average biochemical composition of the BMW fraction of MSW.

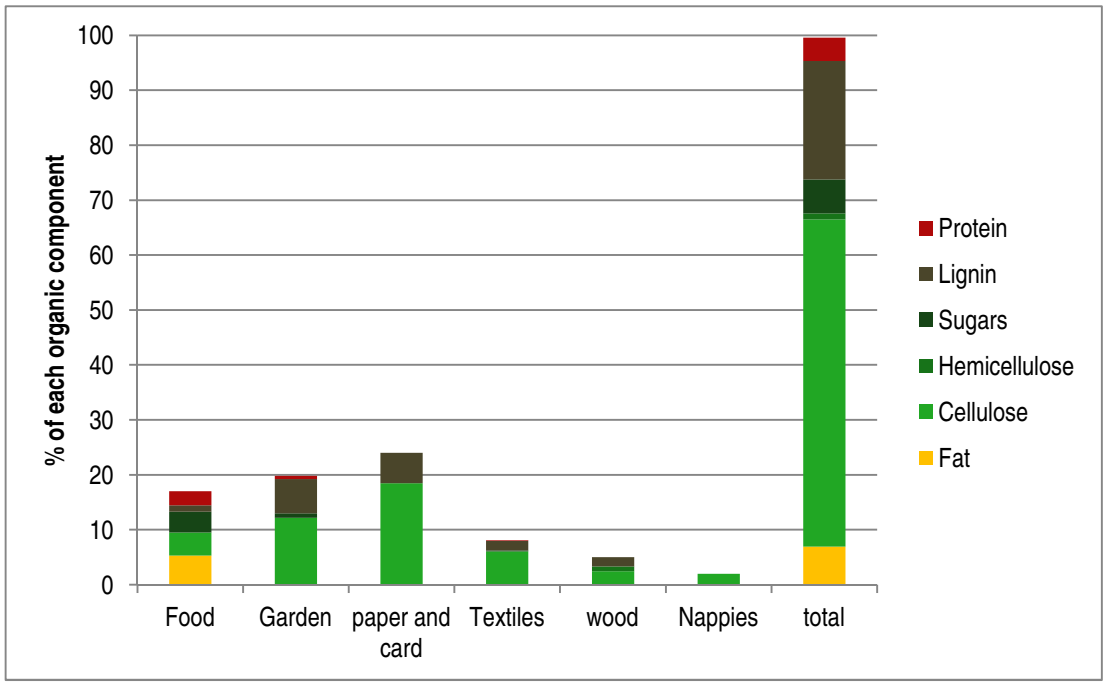


Figure 2.7: Adapted from Eunomia (2007) and wrap (2003): % biochemical composition of each waste fraction (% dry matter).

From these sources, it can be seen that cellulose at around 60% is the principal contributor to the degradable fraction of BMW, followed by lignin at around 20%. Proteins, sugars and fat contribute around 4, 6 and 8% respectively. The large proportion of fat from food waste is one

of the key factors that set CLO apart from natural soils. This fraction also contains a great deal of water; 50% of meat and 95% of vegetables such as lettuce, cabbage and tomatoes are comprised of water. This high moisture content and loose physical structure make it a good feedstock for composting (Chang and Hsu 2008). Furthermore, kitchen and garden wastes yield more humic substances than other organic waste feedstocks, possibly due to the high content of aromatic compounds in plant materials (Smidt *et al* 2008). A study carried out on other organic wastes such as sewage sludge, beer brewery sludge, raw tea compost and tobacco dust found a lower degree of humification than in natural soils (Unsal and Ok, 2001).

Composting is thought to yield HA with chemical and structural characteristics similar to more humified soil HA (Sanchez-Monedero *et al.,* 2002). Carbon contents are calculated at 41.1-63.2% which is similar to those seen in humic acids from natural soils (Unsal and Ok, 2001). The chemical properties will differ though, depending upon the composition of the organic waste source.

As compositions of the substrate material fed into digesters present so much variation, it is important that engineering designs can accommodate these changes. When the conditions appropriate for traditional feedstocks are applied to food waste-rich feedstocks, the process usually performs poorly or fails (Chang and Hsu 2008). Food waste has a particularly high potential for CH_4 production, partially due to the high fat content (Neves *et al*., 2009). This is beneficial for anaerobic process but not so for aerobic.

Few studies have investigated the effects of composition variation on the composting process (Chang and Hsu 2008). However, several studies have been undertaken to investigate the effect that fat has on the process. Fats from animal and vegetable origins are almost completely degraded when co-digested with the organic fraction of MSW but can have inhibitory effects initially (Neves et al 2009).

Chang and Hsu (2008) found that protein was the major factor that controlled the rate of composting, due to its requirement by bacteria to gain nutrients for their cell structures; fat was the most difficult to decompose.

Bulking agents are often used in food-waste composting to improve structure, enhance aeration, to absorb excess liquids and to provide microorganisms with an extra energy source to balance the normally high N content. Composting without litter can lead to anaerobic conditions. Different bulking agents yield different effects on the composted product; leaf litter

and paper give the shortest half life for C whilst peat gives the longest (Eklind and Kirchmann, 2000). This is possibly due to the fact that excessive aeration that the large surface area of litter provides can push the decomposition process preferentially towards mineralisation (Smidt *et al* 2008). It is also believed that lignin addition can also improve HA yields on its integration into the molecule (Smidt *et al* 2008).

The acidity of food waste due to the presence of short-chain organic acids may also pose problems. These may be present in initial materials but also generated during the initial stages of the composting process (Yu and Huang, 2009). This generation can further reduce the pH which eventually inhibits microbial activity (Beck-Friis *et al.,* 2003)**.** This can be controlled, however, by the addition of an alkali amendment. Yu and Huang (2009) advocate the use of sodium acetate as a buffer salt which combines with the acetic acid to form a buffer solution. This has been found to have positive effects on degradation, although ammonia loss was increased also.

In order to consider CLO as a potential carbon sink the nature, decay processes and decomposition products of its organic matter must be considered. The four proposed reaction pathways discussed in section 2.2.2 are based on natural systems. Components and proportions of such components are very different in artificial soils. As kitchen waste is a major component of CLO, the fat and protein levels in the soil are much higher than in natural soils. The lipids and proteins from food wastes are likely to differ from the waxes and amino acids found in leaf litter.

Veeken *et al.* (2000) suggest that the polyphenol/condensation route and lignin theory/degradative pathway both had a significant contribution to HA formation in biowastes. This is based upon the fact that the feedstock mainly comprises plants with many types of lignin groups in the HA fraction. Figure 2.8 offers a typical composition of MSW-derived compost; shown are the degradation times for each component over a 60 week period.

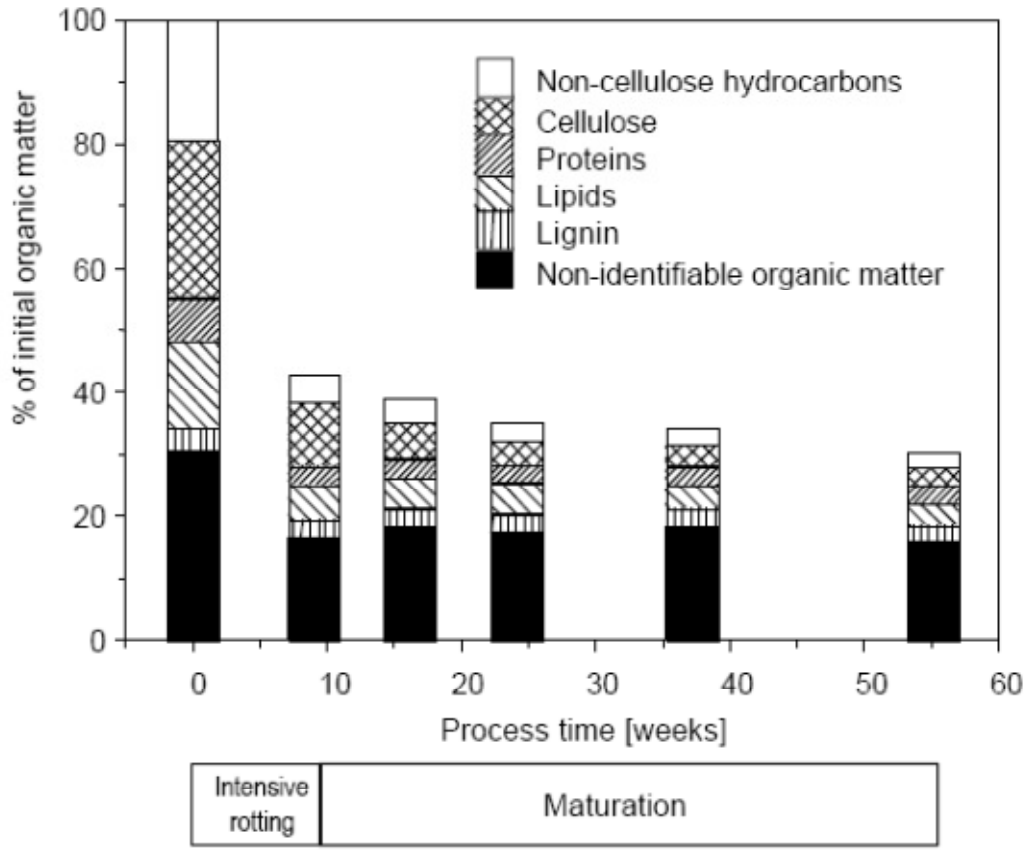


Figure 2.8: typical composition of MSW-derived compost with each component's degradation times (Soyez & Plickert 2002)

If the humification process could be engineered, the extra C that was being added to soils through various waste streams would have the potential to store more stable C. If the process could be studied and the possible reaction mechanisms catalysed, then more SOM could be converted to stable humic material and less mineralised. This would essentially mean less C flux and a greater tonnage of C stored.

Once HAs form, can conditions be manipulated to ensure their recalcitrance? Could the environment in which they are laid to earth be engineered to discourage degradation and prolong its residence time in the soil? The final section of this chapter will look briefly at the factors which make HA vulnerable to degradation.

2.4 Degradation of humic substances

Susceptibility to biodegradation depends upon the structural characteristics. The concentration of O-alkyl structures and phenolic-O are thought to be the most susceptible. As humification proceeds, a loss of O-alkyl (utilised by microbial population) and an increase of aromatic and alkyl carbons can be seen with decomposition (Chefetz *et al.,* 2002). Low biodegradability is seen in aromatic rich, complex molecules that are low in carbohydrates (Kalbitz *et al.,* 2003); dissolved carbohydrates and amino acids are preferentially degraded by soil microorganisms (Amon *et al.,* 2001). However, Almendros and Dorado (1999) state that the aromatic/aliphatic ratio is not thought to have any significant correlation with resistance of OM and that the disordered macromolecular structure seems to have a greater influence than the relative proportions of individual components. Because fulvic acids are possibly formed by the cleavage of polyphenols (Preston *et al.,* 1982) and tend to be more aliphatic in structure, they may be more vulnerable to degradation (Kalbitz *et al.,* 2003). However the humic fraction, may behave very differently in its reactions as a supramolecular structure as compared to the reactions of its individual components.

In a study by Qualls (2004) HA, being the most recalcitrant, saw 12.7% of its C mineralised after one year; FA 29.2% and leaf litter 38.8%. The depth of the material is also an important factor with decomposition in surface leaf-litter being much more rapid than in deeper, mineral soils (Qualls *et al.,* 2003). This may have implications for CLO when it is laid to land.

The process of microbial degradation of HS is largely undertaken by fungi. Bacteria may be more dominant in the environment but their ability to decompose stable macromolecules is limited to fulvic acids and other lower MW molecules. HA are too large to be taken in by microbial cells so are degraded by extra cellular enzymes. These enzymes vary from soil to soil, as do the species of fungi to which the enzymes belong (Huang *et al.,* 2004). Small pore sizes typically associated with clay soils can only be infiltrated by these enzymes.

Extracellular enzymes are rapidly sorbed to mineral and humic colloids, which can influence the ability of the enzyme to retain its catalytic effectiveness. When adsorbed, changes in the tertiary structure of the enzyme and its active site can decrease its activity or disable it altogether (Burns, 1986). However, different materials have different enzyme immobilisation capabilities. It has been demonstrated that high concentrations of humic-like polymers may inhibit enzyme reactions (Kang *et al* 2002). Conversely, low concentrations of humic acid

might enhance enzymatic transformations of phenolic compounds. Furthermore, it has been suggested that SOM can stabilise enzymes (Burns, 1986). Oxidative degradation of lignin components in HS carried out by fungi, some microorganisms and minerals and can break down these usually refractory molecules to CO_2 and water. In a study by Sunda and Kieber (1994), manganese oxides were found to split complex HS to form organic compounds of lower molecular.

Molecules adsorbed onto clay minerals decrease the rate of biodegradability rendering them unavailable for microbial attack. Spatial arrangement of molecules is also a factor, with the dispersion of soil particles aiding mineralisation. The soil matrix is compartmentalised: organic substrates can be locked up in pores to which microorganisms do not have access either because the pore necks are too small or because water pathways are discontinuous. Non-polar substrates (e.g. hydrophobic organic molecules) will tend to remain partitioned in hydrophobic regions of HS and not diffuse in aqueous solutions where microorganisms and their enzymes are located (Huang *et al.,* 2002).

Since bacterial populations can be preyed upon by protozoa, clay minerals can provide protection in small pores (<6µm) offering shielding from these protozoa. Pore-size distribution of a soil is critical in determining relative abundance of habitats with different sizes and water regimes (Huang *et al.,* 2002).

Abiotic degradation of a photophysico-chemical nature can occur in HA (Polewski *et al.,* 2005). Chromophores in HA absorb light which can lead to an alteration in structure and composition. Mekkaoui *et al.* (2000) however, found them to be photostable after 24 hours, and furthermore, that they can produce a screen effect on the photochemical degradation of other organic species. Lutzow *et al.* (2007) report that a proportion of SOM is resistant to UV oxidation, possibly due to spatial inaccessibility of OM within clay aggregates and chars.

The fact that CLO is a heterogeneous material means that a certain degree of flexibility in reaction to environmental conditions might be seen, as compared to homogenous material (Schaumann, 2006a).

2.5 Summary

In relation to natural soils, CLO has a high CO_2 flux rate, possibly due to its large organic carbon content. As the composition of the MSW and its corresponding CLO are inalterable within the scope of this project, the conditions under which the degradation of the OM occurs must be monitored and adapted instead.

Simple compounds are low MW molecules and easily degraded. HS, in contrast, can remain in the soil for thousands of years; although they constitute the majority of SOC, they only contribute a small amount to carbon cycling within the soils due to their recalcitrance making them an important carbon sink.

As many other reagents all co-exist in soils, it is probable that there are several pathways that occur closely and interact to form HS. Furthermore, a variety of proposed methods may account for the diversity seen in functional groups. Due to the lack of concurrent knowledge at a molecular level, there remains a certain level of debate as to whether or not HS even exist as a chemically distinct class or whether they are simply mixture of diverse classes of compounds, associated by intermolecular forces (Reemtsa *et al*., 2008).

Despite the high levels of CO_2 released during mineralisation of composted wastes, the humification stage contributes to carbon sequestration. Kitchen and garden wastes yield more HS than other organic waste feedstocks, containing chemical and structural characteristic similar to more humified soil HA. The chemical properties will differ though, depending upon the composition of the organic waste source.

Susceptibility to biodegradation depends upon the structural characteristics with low biodegradability is seen in aromatic rich, complex molecules that are low in carbohydrates dissolved carbohydrates and amino acids are preferentially degraded by soil microorganisms . The depth of the material is also an important factor with decomposition in surface leaf-litter being much more rapid than in deeper, mineral soils. Molecules adsorbed onto clay minerals decrease the rate of biodegradability rendering them unavailable for microbial attack. Perhaps when the CLO is added to natural soils, it ought to be well mixed to prevent rapid mineralisation of a potentially immature material.

Chapter 3

Development of analytical methods

The CLO used in the following experimental trails is of an unknown age; it must therefore be assumed that the degradation processes are ongoing. When determining the stability and maturity of a CLO, two parameters can be used: the measurement of microbial activity via respiration (CO_2 produced or heat evolved); or the determination of chemical factors, chiefly by examining the HS present (Veeken *et al.,* 2000).

Current literature outlines several viable methods for analysing HA in natural soils. Spectroscopic methods such as Py-GC/MS, Fourier Transfer Infra-red (FTIR) spectroscopy and ^{13}C Nuclear Magnetic Resonance (NMR) outlined in Chapter 2, give a wealth of information about the elemental composition and possible structure of these complex molecules. Analysis using these methods, however, can be expensive. Simpler, more cost effective methodologies are available to identify and quantify HS present in soils. This may not offer the same detail of elemental composition nor information about the functional groups present, but can be useful nevertheless.

Although methods for the identification and quantification of HS within natural soils are well documented, relatively little is known about the organic nature of CLO. Being a relatively new material and being produced from a variety of feedstocks by an array of different methods, it is difficult to define CLO in order to describe its structure and properties. In determining its composition, problems lie in the heterogeneity that can be seen not only geographically but also temporally. Different producers use various separation techniques to eliminate contaminating components (glass, plastics and metals for example); some are more successful than others. MSW composition also varies across the year, depending upon consumer spending. For instance, the proportion of plastic seems to increase in December and January due to the use of carrier bags for Christmas shopping (Pers. Comm., Tony Hitchens). It also varies from year to year as recycling strategies evolve. The implication is that it is difficult to formulate a robust method that can be used to assess HA contents of all CLOs when proportions of organic carbon differ greatly from batch to batch.

3.1 Trial aim

This chapter will provide details of the preliminary experimental methods carried out to assess their suitability for their use on CLO from MSW; their development and optimisation will also be presented. As most of the trials employ the same materials and basic methods, it is practical to outline them in this chapter to avoid repetition. Details of any differences in the experimental set up of each trial will be included in the relevant chapter.

The primary aim of this project is to assess CLO as a viable C store. In order to investigate this, two factors have been chosen. The degree of composting can be measured using two parameters:

Stability - which is directly related to microbial activity. Stable composts have a relatively low proportion of easily degradable OM; the more stable the material, the less microbial activity will be seen and thus, a lower carbon flux will be exhibited.

Maturity - which is associated with potential plant growth in the composted media and is measured by the presence of humic acid; and

With these parameters in mind, the evolved CO_2 has been measured using an Infra-red Gas Analyser (IRGA). Secondly, the production of HA has been used as an indicator for stable C.

3.2 Trial objectives

In order to ensure that the trial aim was met, the work was divided into five main objectives.

1. To find an appropriate method for the analysis of CO_2 flux from the CLO samples.
2. To optimise the alkali extraction methodology for the isolation of HA from the parent material.
3. To test the efficacy of using ultra-violet visible (UV) and fluorescence spectrometry to examine the nature of the humic acid extracted from the CLO.
4. To select apposite statistical analytical techniques to evaluate the data collected.

3.3 Experimental set up

3.3.1 Materials

The CLO used was supplied by PWM from their Thornley site in County Durham and was produced via aerobic digestion of MSW, as outlined in Chapter 1.4.1. The MSW fed into the digester varies in composition but Figure 2.6 in the previous chapter offers a typical composition for England and Wales. The resulting CLO is a heterogeneous material that is largely organic C, contaminated with ferrous and non-ferrous metals, hard and soft plastics and glass. Because the MSW is shredded before it reaches the digester, the small contaminating particles easily pass through the mesh filters.

Figure 3.1 typical sample of PWM's CLO

The composition of CLO sees regular changes as a result of evolving recycling initiatives and varying collection areas; more deprived areas tend to see less green food waste and more packaging (Pers. Comm., Tony Hitchens). As a result of this diversity, this CLO exhibits a very broad range in average C content of between 20 - 80% (PWM).

The variation in C content poses a problem when trying to acquire a representative sample for analysis. Property measurements often require the sample to be homogeneous in order that sub-sampling will be representative. In heterogeneous materials such as CLO, too small a sample and an insignificant or random characteristic could be magnified; too large a sample could mask non-homogeneities (Gao *et al.,* 1995). When testing batches of CLO, several replicate samples are needed to give as representative a picture as possible of the nature of the batch as a whole.

During trial 1, an attempt was made to manually extract the contaminating macro-components from the CLO, but this was found to be too time-consuming and would have required the collection and storage of more than double the sample material. The removed material collected was stored for subsequent control experiments.

The contamination level of various trace metals and salts from PWM's CLO was studied by Simpson (2008) and compared to similar soils. The main elements of concern found in its leachate were cadmium, lead, aluminium, iron and manganese which not only all breeched the EC drinking and surface water directive, but also landfill discharge consents. It was found that the most successful method of remediation was a simple five minute wash with tap water. This was found to remove a significant percentage of these contaminants, bringing them to within the environmental quality standards (EQS) mentioned. However, at the time of writing, this step had not been incorporated into the process so it can be assumed that as yet, the CLO is still heavily contaminated.

3.3.2 Lysimeters

Figure 3.2: Lysimeter used in trial 1

Lysimeters were constructed as shown in Figure 3.2. They were made of 8cm diameter polypropylene piping, cut into 30cm lengths. The diameter of the tube was chosen so that the chamber of the Infra-red gas analyser (IRGA) might fight directly over the top, creating the necessary seal. The bases were made of polypropylene end-piping, attached with super glue and sealed with water-resistant sealant.

The lysimeters were open at the top to allow aeration; some were free-draining at the bases and others sealed, depending upon the nature of the experiment. A mesh lining was included to ensure that the CLO material was held in place and not lost through drainage pipes. In earlier trials, the lysimeters were placed outdoors on gabions to prevent waterlogging and to allow exposure to the environment to mimic field conditions as much as possible.

For trial 1, they held a capacity of around 600g of CLO. Before the CLO was utilised, the dry weight was calculated; as some materials have a greater water-retaining capacity than others, it was necessary to establish this so that trial 1 was comparable to future trials. For all trials, the soil depth was a minimum of 30cm.

The trial was conducted outside of the Department of Earth Sciences, Durham University, and flux measurements were taken between October 2006 and December 2006. At low temperatures, a small change can have a great effect on flux rate (Chapman and Thurlow, 1996). This has significant implications for experimental trials carried out during the winter months in the north of England.

The measurements commenced after a two week delay period to allow the microbial community to establish. Flux was then measured up to three times a week for ten weeks. Once all of the flux measurements had been taken, the samples were sacrificed and frozen for humic analysis from January to April 2007.

Sample	CLO (g)	Sand (g)	MnO_2 (g)
1a	600	**0**	**0**
1b	600	**0**	**0**
2a	555	**0**	**5**
2b	555	**0**	**5**
3a	555	**5**	**0**
3b	555	**5**	**0**
4a	550	**5**	**5**
4b	550	**5**	**5**
5a	550	**0**	**10**
5b	550	**0**	**10**
6a	550	**10**	**0**
6b	550	**10**	**0**

Table 3.1: Proportions of CLO, sand and MnO_2 added to each lysimeter for trial 1.

The sampling matrix is shown in Table 3.1. All samples were duplicated and measurement of each sample was taken twice to give a total of four replicates. Control samples were taken for each test to calculated lower limits of detection for each method. Results that fall below this will be discounted.

3.4 Physical data

Temperature and soil moisture content are both considered to be important factors that control flux rate in natural systems. Decomposition is affected by these factors, with seasonal patterns observed in CO_2 flux (Pumpanen *et al.,* 2003). Preliminary trials were used to determine whether or not this was also the case for CLO. It was also useful to obtain some baseline data on levels of flux from CLO in order to plan further trials.

3.4.1 Ambient temperature

The temperature was measured using a min/max thermometer on each day that the samples are measured for CO_2 flux. This will be incorporated into the flux calculation when interpreting the data received.

3.4.2 Moisture content

A high water table can increase flux rates (Vouklitis *et al.,* 2000) so any waterlogging within the lysimeters may drive CO_2 efflux.

During future trials, moisture content will be determined by sub-sampling and drying a small amount of CLO for on each sampling day. The equivalent dry-weight will then be used in the flux calculation to make all samples comparable over the course of the trial.

3.4.3 pH

This measurement was not taken until subsequent trials. The leachate from the lysimeters was collected and stored below 4ºC in 30ml polycarbonate screw cap containers. On the day of analysis, the samples were equilibrated to room temperature and the pH values read using a Hanna instrument HI 9025 microcomputer pH meter which had been calibrated at pH 4 and

pH 7 ± 0.01 at 25ºC. The electrode probes were rinsed with Mili-Q de-ionised (DI) water between each sample reading to minimise contamination.

3.5 Carbon flux analysis

The method chosen for this parameter was a closed chamber method with CO_2 measurement via infra-red (IR) analysis, using an EGM-4 Environmental Gas Monitor and CPY-4 chamber from PP Systems (Massachusetts, USA). The principle of this involves the measurement of gases by determining the absorption of an internally emitted IR source by each sample. The technique offers high precision and an accuracy of < 1% of span concentration over the calibrated range. It is also portable for field sampling so measurements could easily be taken from samples based outdoors. The IRGA allows a relatively quick and simple collection of data compared to other methods, which is imperative when a large number of samples must be analysed within a tight time-frame. The collected data can be stored on the IRGA and transferred to PC or laptop for analysis.

Figure 3.3: EGM-4 Environmental Gas Monitor with canopy chamber. The piping is used as a hood to eliminate any photosynthetic effects.

3.5.1 Data collection

The conventional method of taking measurements directly from the lysimeters with the use of a canopy chamber was adapted for trials one and two, in order to overcome the problem of comparatively large fluxes associated with CLOs. The lysimeters were sub-sampled and a representative amount of CLO was measured into an air-tight container. Holes were drilled into the lid of the container for the air-in and air-out tubes of the IRGA and were made airtight with a sealant.

Samples remained outside until directly before their measurement as initial tests saw a significant increase in flux when they were brought inside and allowed to warm to room temperature. Following a number of pilot trials, the ideal amount of CLO was found to be 30g which was collected from throughout the lysimeter to give a representative sample. The sample was then weighed and the exact weight recorded and sealed into the airtight container. The ambient outdoor and room temperatures were also measured and recorded.

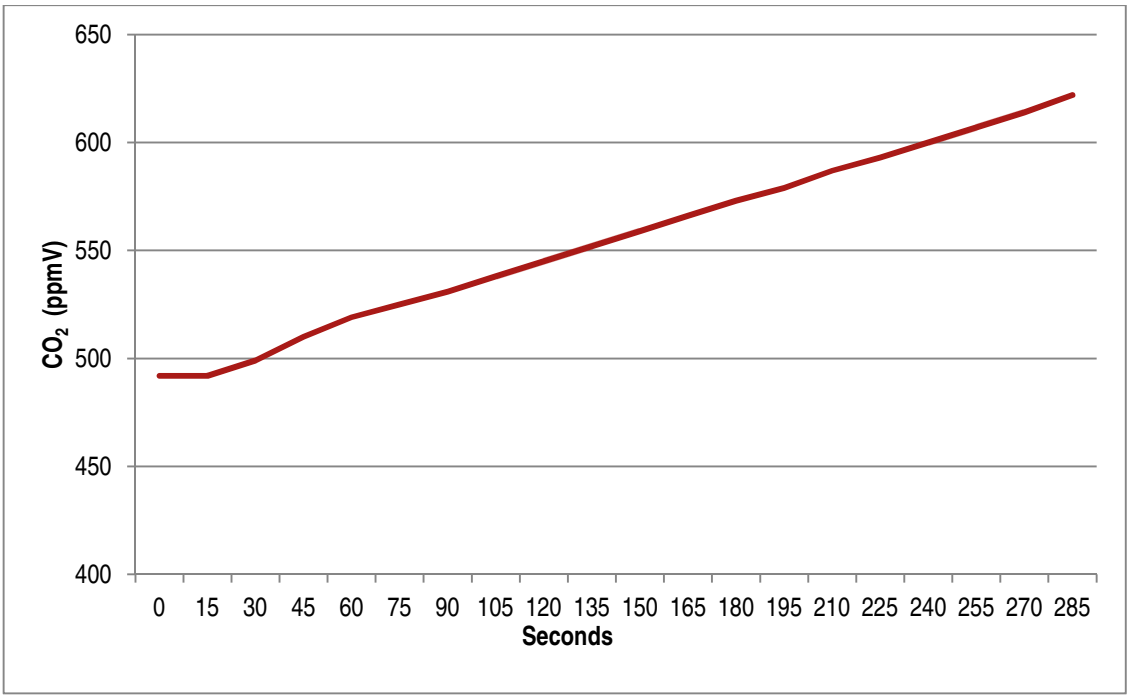


Figure 3.4: typical flux time series in part per million by volume (ppmV) of a CLO sample over five minutes. An initial delay period of 30 seconds can be seen followed by a steady increase of flux.

Initially, readings were taken every fifteen seconds (manually timed) over a five-minute time period for each sample, with the first minute disregarded to allow the flux to stabilise. The lag time can be seen in the first 30 seconds of the sample flux shown in Figure 3.4. On analysis of the results, the r^2 values were calculated between each minute of data collected. Little difference was observed between data collected at two minutes and five minutes. It was decided, therefore, to reduce the sampling period from five minutes to two minutes. The frequency of data collection intervals was increased from every fifteen seconds to every five seconds to ensure sufficient data points were obtained for analysis.

The IRGA was set to collect data at five second intervals over the period of two minutes. Once the tubing was fitted to the glass chamber, parameters such as chamber volume and maximum flux rate were set. Finally, the chamber was flushed for two minutes to allow the baseline to return to ambient CO_2 levels before the lid was sealed and the flux measurement commenced. After the specified measuring period the data was stored, the material removed and the chamber flushed again before the next sample. Several samples were measured in duplicate to ensure precision and blank readings were taken periodically to ensure the chamber was being flushed efficiently.

3.5.2 Data conversion

The data is recorded as carbon dioxide flux (ppmV) and can be uploaded directly onto a PC from the IRGA. A spreadsheet was created to convert the recorded values to grams of carbon per gram of CLO per hour. This was achieved by adapting the ideal gas law:

[3.1] $$Pv = nRT$$

Where P is the partial pressure; v is the volume measured; n is the number of moles; R is the Gas Constant and T is the absolute temperature. Ultimately, the required value is the flux of C so equation 3.1 is rearranged thus:

[3.2] $$n = \frac{Pv}{RT}$$

And then combined with:

[3.3] $$m = nM$$

Where m is mass of carbon fluxing, n is the number of moles and M is molecular mass. This give:

[3.4] $$m = \left(\frac{Pv}{RT}\right) M$$

In order to include changes in ambient temperature, the term 'b' for the measured air temperature at the time of analysis is also incorporated. The Arrhenius equation is often used to demonstrate the temperature dependence of flux but this is not ideal in natural systems, as microbes have an optimum temperature above which respiration no longer increases (Fang and Moncreiff, 2001).

[3.5] $$m = \left(\frac{Pv}{R(T+b)}\right) M$$

Finally, the flux value given by the IRGA in ppm (term 'a') is added. This is converted to a weight, rather than a dimensionless figure, by multiplying by 10^{-6}.

[3.6] $$m = (a \times 10^{-6}) \left(\frac{Pv}{R(T+b)}\right) M$$

Where **m** is the mass of carbon fluxing; and M in this case is **44** which is the molecular weight of CO_2 as shown in Table 3.2 along with other constants used.

Chamber volume (V)	1140 (ml)
Surface area (SA)	0.0184 (m^2)
Molecular weight of CO_2	44.1(g)
Zero Kelvin (K)	273.15 (°C)
Atmospheric pressure (P)	0.995 (atm)
Gas constant (R)	0.0820575 (L atm/mol K)

Table 3.2 the fixed values used in the flux calculation.

The IRGA data was uploaded into Microsoft notepad and then transferred into a Microsoft Excel spreadsheet. Flux was measured in four second intervals over a two minute period; the average flux for each sample was calculated and then equation 2.6 used to convert the ppm values given by the IRGA to grams of carbon per gram of CLO fluxing per hour (gC/gCLO/h) or gC/gCLO/day by multiplying by 24.

3.6 Humic acid analysis

The CO_2 evolved from the soil gives only one dimension to processes that may be occurring; in order to better understand C storage within the soil, it is important to look at the stable C compounds that are present as well as the CO_2 released when labile components are mineralised.

It is also important to use a suite of analytical techniques, rather than relying solely on one. This is particularly the case when dealing with new or modified methods or largely uncharacterised material. The amount of extractable C depends upon starting material of the soil (Veeken *et al* 2000) so with a material such as CLO, where the starting material is variable, it is vital to employ several robust methods.

In this section, the chemical techniques chosen to analyse the stable HA fraction of the soil are detailed.

3.6.1 Alkali extraction

The isolation of HS from soils has long been seen as a challenge, but the highest yields are often achieved with a conventional alkaline extraction process (Shirshova *et al.,* 2006) as proposed by The International Humic Substance Society (IHSS). This is a fairly standard and widely used technique for both natural and artificial soils that has been developed and modified over recent years (Osterberg *et al.,* 1993; Filip *et al.,* 2000; Cheftetz *et al.,* 2002; Khayet *et al.,* 2004; Li *et al., 2003;* Allard, 2005; Hayes, 2006; Brunetti *et al.,* 2008; Droussi *et al.,* 009; Vieyra *et al.,* 2009). As each artificial soil differs with regards to its source materials and method of production, it is necessary to treat it as an unknown material, rather than relying on methods used in other studies.

The organic C content is high for this artificial soil and any inorganic C that may be present should be negligible so ought not to require removal. This method has been modified for use with CLO through a series of preliminary experiments.

Current literature and the IHSS propose two main alkaline reagents with which to extract HA: sodium hydroxide (NaOH) and sodium pyrophosphate/sodium hydroxide mix ($Na_4O_7P_2$). These were each tested at increasing molar concentrations from 0.1-0.5 molar (M) with CLO samples weighing 2g, 5g, 10g, 20g and 100g (Table 3.3). Additionally, the contaminating components of the CLO (hard plastic, soft plastic, glass and metal) were also tested to ensure that they would not skew the results.

	Alkaline extractant	Concentration (Molar)	Acidification to pH 2
Protocol 1	Sodium hydroxide	0.1	HCl
		0.2	HCl
		0.3	HCl
		0.4	HCl
		0.5	HCl
Protocol 2	Sodium hydroxide. sodium pyrophosphate	0.1	HCl
		0.2	HCl
		0.3	HCl
		0.4	-
		0.5	-

Table 3.3: The two reagents used at various concentrations used for the preliminary alkali extraction procedures. HCl was always used for the acidification step.

Often, a pre-treatment with an organic solvent such as benzene or methanol is employed to remove amino acids, lipids, saccharides and other non-humic compounds that can be co-extracted (Shirshova *et al.,* 2006). However, if HSs exist as a supramolecular assembly, molecules that are held together by weaker forces within the molecule may also be removed, thus altering the nature of the ultimately extracted material. Wilson *et al.,* (1988) used ^{1}H-NMR to show that these low MW fractions were not, in fact contaminants during the extraction procedure but present from association with higher MW molecules. Hence, it was decided to omit this pre-treatment step.

The following method was used for each test:

1. The appropriate amount of CLO was weighed out into clean, labelled 100ml polyethylene centrifuge bottles.

2. The specified alkaline solution was added using a 10ml Gilson pipette. The bottles were placed on a shaker table and agitated for 24 hours.

3. The samples were then separated in a centrifuge (ALC multispeed centrifuge PK121) at 4000 rpm for 15 minutes and the supernatant decanted into a clean, labelled 250ml polyethylene bottle, sealed and stored below 4°C.

4. A further wash of extractant of the same concentration and volume as the first was added to each of the 100ml bottles and a second, third and fourth extraction taken.

5. These were again agitated on the shaker table for 24 hours and steps 2-4 were repeated.

6. The subsequent washes were added to the first and then the solution was filtered under vacuum filtration using 1.2mm filter paper[1].

7. The filtered solution was then divided between two clean, labelled 30ml polycarbonate screw cap containers and 100ml was transferred into clean, weighted and labelled 100ml polyethylene bottles.

8. The samples in the 30ml polycarbonate screw cap containers were stored in the fridge for further analysis (via UV spectroscopy and fluorescence photospectrometry) and the samples in the bottles acidified with Analar HCl to pH 2

9. The bottles were then returned to the shaker table for two hours to allow the humic and fulvic acid fractions to separate.

[1] Largest humic acid particles that exist in most soils and water systems are at most, 110nm (Osterberg *et al.,* (1993).

10. The 100ml bottles were then centrifuged at 4000 rpm for 15 minutes and the fulvic acid decanted off into clean, labelled 30ml polycarbonate screw cap container

11. The remaining humic acid was dried in an oven at 30°C for 48 hours and then the bottles weighed and yield calculated. The humic acid was collected and stored in clean, labelled sterilins for further analysis.

3.6.2 UV photospectrometry

UV spectroscopy has been used extensively in studying HS, with the ratio of absorbance at 465nm to 665nm giving the humic and fulvic acid ratio (E_4/E_6 ratio). This is a useful indicator for the degree of aromaticity and thus, humification of SOM (Ghosh and Schnitzer, 1979) and furthermore, is thought to be the best indicator of the degree of maturity of composts (Domeizel *et al.,* 2004).

Like alkali extraction, this is a well-used technique with several different methodologies advocated. Chen *et* al., (1978) for example is a much cited methodology (Trubetskya *et al.,* 1994; Unsal and Ok, 2001; Shirshova *et al.,* 2006; Wei *et al.,* 2007). However, this methodology utilises sodium pyrophosphate ($Na_4O_7P_2$) as the humic/fulvic extractant. For efficient analysis during this project's trials, the first stages of the alkali extraction and the UV photospectrometry could be combined. It was decided to trial the use of NaOH rather than $Na_4O_7P_2$, as used successfully by Fuentes *et al.* (2006), amongst others.

Samples were prepared as in stages 1-8 of the above alkali extraction method. The samples were then pipetted into clean, dry quartz cuvettes and analysed using a Jenway 6505 UV photospectrometer at wavelengths 465nm and 665nm. The E_4/E_6 ratio of the absorbance given for each wavelength gives an indication of the aromaticity of the organic compounds presents in the sample; the lower the ratio, the greater the degree of aromaticity.

It was suggested that a pH of between 7 and 8 was optimum for E_4/E_6 measurement (Chen et al., 1977). Some preliminary measurements were taken to assess whether or not an acidification stage in the procedure was necessary. Samples were acidified to pH 6, 7, 8, 9 and 10 and spectra run as per the method outlined above.

3.6.3 Fluorescence spectroscopy

Fluorescence spectroscopy is a useful tool for the analysis of HS. This form of analysis only became available to this project in September 2008, so only samples from trials 3 and 4 benefitted from this procedure (Chapters 6 and 7).

Again, as with UV photospectrometry, the same alkali-extracted material could be used for this method. Samples had to be run as soon as feasibly possible after extraction to ensure that any degradation of the humic material within the sample was avoided (Allard *et al.,* 1994).

The samples were pipetted into clean, dry quartz cuvettes and first run through a Jenway 6505 UV photospectrometer. Emission was read over a range of wavelengths from 200-500nm to assess the concentration humic material contained in the sample. All HA solutions were diluted to <0.3 at the excitation wavelength 337nm (Shirova *et al.,* 2006) with 0.2M NaOH x10, x100 or x1000 until an appropriate concentration was achieved. This was to avoid re-absorption or the inner filter effect (IFE) (Larsson *et al.,* 2007). The dilution factor was recorded for subsequent calculations. Once the concentration was correct, the samples were pipetted into a clean, dry quartz cuvette and placed in the fluorescence spectrometer. The parameters were set at 300-700 nm emission range and 200-800 nm excitation range with readings taken every 5 seconds. This ensured that the quality of the data was sufficient and that samples could be run within a suitable timeframe.

For each sample, the resulting data sheet was stored and then transferred into Microsoft Excel (2007) and SigmaPlot 10.0 for analysis. Contour maps of excitation-emission matrix (EEM) spectra were produced to provide qualitative information and humification indices (HIX) were calculated for quantitative analysis (Ohno, 2005). HIX was calculated using a comparison of the emission seen over two different excitation ranges (435-480 nm and 300-345 nm); the higher the index value, the greater the degree of humification.

3.7 Statistical analysis

Once the data was collected, it was of fundamental importance to assess whether relationships between sets of data were significant or not. For this research, the data has been collected and organised using Microsoft Excel (2007) and Minitab 13 software has been used for the statistical analysis.

Analysis of Variance (ANOVA) (General Linear Model) was chosen to assess whether there were any patterns present in the data. Throughout the experiments, several parameters are examined and with each form of analyses, a different responses. It is necessary to examine the relationships between these factors and all must be compared against each other in a sensible and logical way.

Variations will exist between not only the trials undertaken for this project, but similar trials carried out by other authors. Thus, it is necessary that a standard exists with which data can be effectively compared.

3.7.1 Analysis of Variance (ANOVA)

Data was entered into a master Microsoft Excel (2007) spreadsheet; data of interest was then chosen and transferred into a Minitab 13 worksheet. ANOVA (General linear model) was run, with the appropriate response variable (flux rate for example) and predictors (week or treatment for example) taken from specified columns on the worksheet. Tukey's simultaneous pair-wise comparison test was used to distinguish between means at $p < 0.05$ as a post-hoc test. All results used from this point will be quoted as being statistically significant if they satisfy this requirement.

3.8 Preliminary test results

3.8.1 CO_2 flux

Both temperature and moisture content were measured alongside carbon flux over a 36 day period. Both parameters were found to significantly affect CO_2 flux from CLO (Jarvis, 2007). This highlights the need for the ambient temperature to be recorded at the time of measurement and its inclusion in the flux calculation, outlined in equations 2.1-2.6.

This data also provide an expected baseline flux for means of comparison in subsequent trials. Flux was given at a rate of between 1-25 gC/gCLO/day, which was seen to decrease over the short trial period. Replicate samples gave low r^2 values, showing a large range in values.

3.8.2 Alkali extraction

First, optimum amount of sub-sample needed was established against varying concentrations of NaOH ranging from 0.1M – 0.5M. The results of this are shown in Figure 3.5. The yield is given as a percentage of the total amount of CLO for that particular sample.

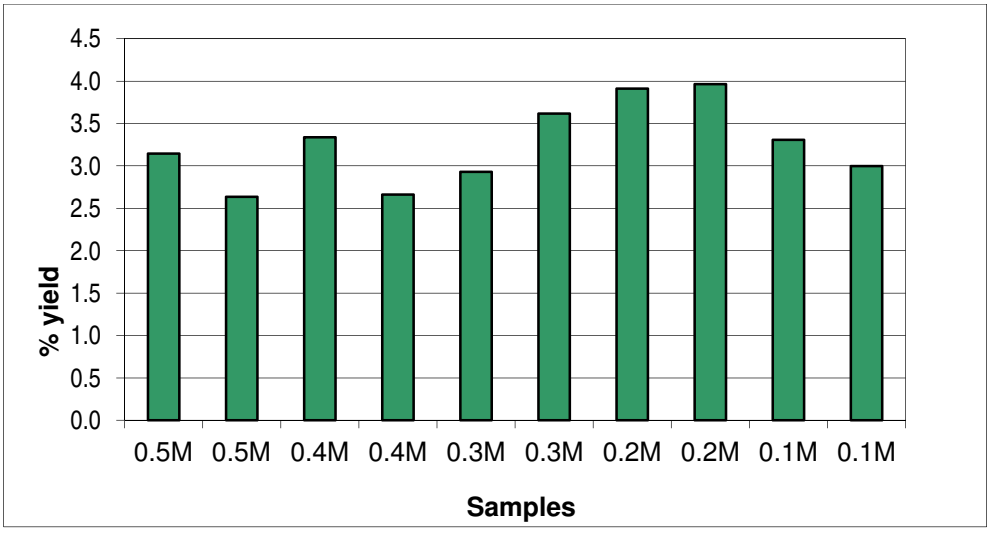


Figure 3.5: Percentage yield of HA (relative to the initial sample weight) determined by varying the concentration of NaOH used in the alkali extraction. Duplicate samples are included to demonstrate the lack of agreement between replicates.

The results show a high degree of variation between the replicate samples. A concentration of 0.2M appears to give the best yield; the difference between it and the other samples is statistically significant ($p<0.05$). A sample weight of 10g gave satisfactory yield of HA for comparison and would mean that enough of the sample remained for any further analyses.

The same tests were repeated with $Na_4O_7P_2$. The results of the multiple extractions and the comparison of its performance against the NaOH can be seen in Figure 3.6 and Figure 3.7 respectively.

The $Na_4O_7P_2$ extracted a greater yield than the NaOH; however, contaminating components appeared not to be affected by the latter but the former seemed to extract material from the contaminants, thus favouring NaOH as the extractant.

When making the alkali solutions, the $Na_4O_7P_2$ pellets took around 6 times longer than the NaOH pellets to dissolve. A less concentrated solution might have been necessary to accommodate a large number of samples (and therefore large volumes of solution) which may have been less effective at extracting the humic substances. Lutzow *et al.* (2007), found NaOH to be most efficient, giving yields of up to 80% OM (as compared to $Na_4O_7P_2$ which only yielded up to 30%). They also found the former much more effective than the latter on coarser SOM fractions. Hence, the NaOH is the preferred option.

So in summary:

- The NaOH solution is optimal over sodium pyrophosphate.
- A 10g sample of the CLO yields enough HA for measurement
- Two extractions with 100ml of 0.2M NaOH is sufficient for the purposes of this trial.

This method provides an adequate compromise between yield and time/resource constraints imposed by the number of samples that needed to be analysed. It also ensures that a minimal amount of the samples will be removed from the lysimeters throughout the trials.

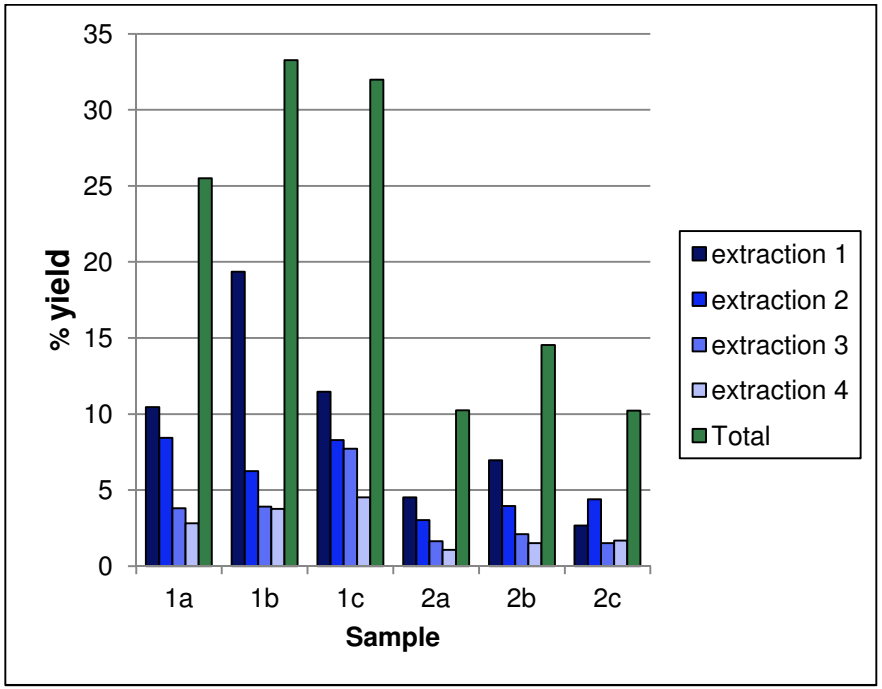


Figure 3.6: the percentage of humic acid extracted with each wash and the total amount for each sample; samples 1a-1c were extracted with 0.2M sodium pyrophosphate and samples 2a-2c with. 0.2M NaOH.

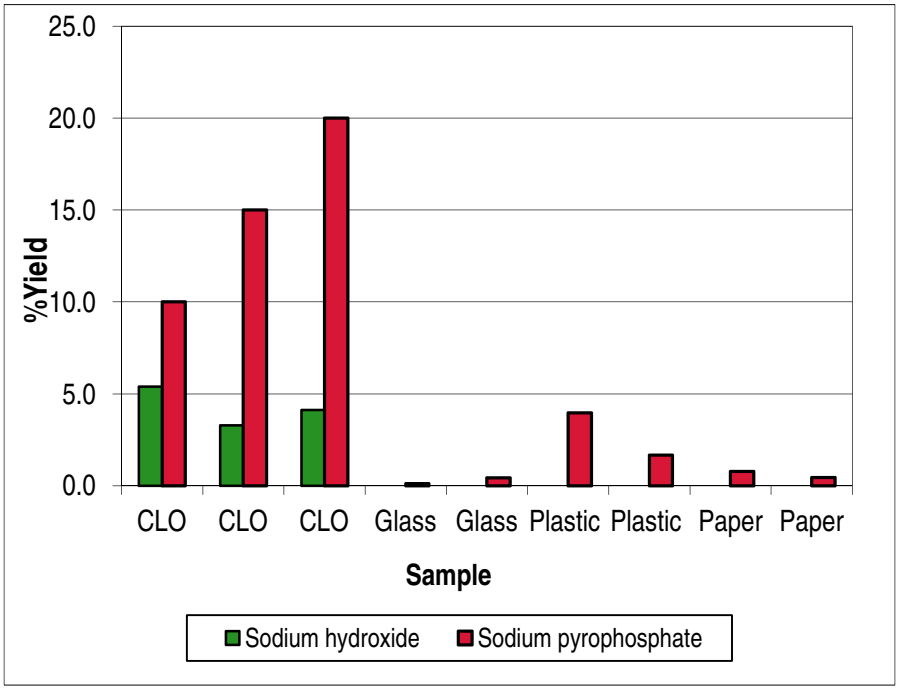


Figure 3.7: the percentage yields from both CLO and contaminants using 0.2M NaOH and 0.2M $Na_4O_7P_2$ as indicated in green and red respectively.

3.8.3 UV Photospectrometry

When samples were acidified, no statistically significant difference was observed between data sets. Moreover, on the addition of HCl, precipitation occurred which would render the sample less effective for analysis using this method.

For trial 1 samples, the typical E_4/E_6 ratio was in the range of 5-12. This is in agreement with similar studies conducted on other CLO material (Garcia-Gil *et al.,* 2003; Li *et al.,* 2004; Fuentes *et al.,* 2006; Wei *et al.,* 2007; Pedra *et al.,* 2008). For subsequent trials, it is hoped that this ratio will reduce if the optimisation of the humification process can be achieved and more stable C produced.

3.8.4 Fluorescence spectroscopy

From preliminary tests it was observed that the addition of acid to achieve a neutral pH could result in the separation of fractions within the extracted material so it was optimal not to alter the pH. Baker *et al., (*2006) concluded that the pH of the solution had a negligible effect on the results and so samples were not adjusted through acidification.

3.9 Conclusions

3.9.1 Revisiting the objectives

At the outset of the trial, five main objectives were outlined.

1. *To find an appropriate method for the analysis of CO_2 flux from the CLO samples*

This was achieved via the sub-sampling of the lysimeters to overcome the comparatively large flux of CLO. It was initially hoped that the IRGA's flu chamber could be fitted directly over the lysimeters so samples could be analysed quickly *in situ*. When this was attempted, the flux readings were above the upper limits of detection so another method had to be sought.

When sub-sampling, it is difficult to obtain a representative sample due to heterogeneity that would naturally exist at different depths within the lysimeter (Veeken *et al* 2000). This necessary adaptation to the preferred method also meant that the samples were turned every time the flux was measured. Would this, therefore, have been representative of the conditions under which the CLO would be laid? Thus, Sub-sampling was not only time consuming, but may not give a true representation of how a non-aerated soil would flux. However, Kuzyoukov *et al.,* (2009) found that mechanical disturbance of the test sites only had an influence on the CO_2 efflux for up to two weeks so given the trial period, this ought not to be a problem.

Ultimately, this method of analysis was relatively quick with little sample preparation needed. The IRGA was easy and convenient to use. This meant that all of the samples could be tested within the same day, which is important to maintain a certain level of control over the experiments. Flux rates seen were similar to those sited in a similar study by Tognetti *et al., (*2007) giving confidence in the results produced. There is, however, very little literature available on CO_2 flux from composted MSW using this particular methodology.

2. *To optimise the alkali extraction methodology for the isolation of HA from the parent material.*

Based on the IHSS method, 0.2M NaOH was chosen with two extraction stages. A sample size of 10g with 100ml of extractant appears to yield sufficient humic acid when the process is repeated twice. The sodium pyrophosphate, although it gave a greater yield of humic acid, was more time consuming to prepare. More importantly, it also gave extraction yields against the control samples of contaminating material. The implications being that if it were used, any contaminants present would skew the results.

A study by Li e*t al* (2003) showed that each extraction can remove humic acids of differing MW. The first six extractions appeared to select the components with the highest molecular weights**.** It is important to consider this when deciding upon the optimum number of extractions to carry out on each sample. Because of time constraints, the two extractions decided upon for this study may preferentially remove higher MW fractions and not give a true representation of the humic material as a whole.

The use of this method alone, however, remains unreliable as non-humic materials (such as amino acids, carbohydrates, lipids and metals etc) may be extracted with the NaOH and inflates the reported yield (Veeken *et al* 2000; Nichols and Wright, 2006). This basic extraction process may promote various chemical reactions within the humic substances such as oxidation, hydrolysis and cleavage reactions (Shirshova *et al.,* 2006). That said, if humic substances do exist as a supramolecular assembly, then these moieties would be part of the structure.

3. *To test the efficacy of using UV and fluorescence spectrometry to examine the nature of the humic acid extracted from the CLO*

These methods were successfully integrated with the first stage of the alkali extraction (pre-acidification), meaning that a great deal of time and materials were saved. Efficiency is imperative when many samples must to be analysed in a short period of time.

The data produced at this stage seemed to be in general agreement with similar studies. In conjunction with UV photospectrometry, it has the potential to provide information about the degree of humification of each sample.

4. *To select apposite statistical analytical techniques to evaluate the data collected.*

ANOVA has proven to be a suitable technique to analyse data sets produced, so will be used throughout this research for data analysis and the appraisal of results. Although the data sets seen thus far have been relatively small and many trends apparent to the naked eye, it will be interesting to use a larger amount of data to see whether more subtle differences between data sets may occur.

3.9.2 Trial limitations

The sub-sampling necessary to meet the requirements of the IRGA meant that the CLO may not have given flux rates that would be representative to those seen in a field situation. These preliminary tests meant that measurements were taken over a short space of time; a longer trial period might yield more interesting results.

Some compromises must be made when it comes to deciding over the number of replicate samples and washing steps (alkali extraction) in the consideration of time and resources available.

3.9.3 Implications for Trial 2.

These preliminary methods have provided a suite of analytical methods, with which the project can progress. In trial two, new experimental parameters will be introduced; these methods will be employed to give a coherent and comparable new data set within the boundaries of this research. Baseline data produced in trial one will add to the information collected in further trials to give insight into the reproducibility of the methods in terms of their robustness.

The need for replicate sampling was highlighted but this must be balanced with the time and resources available for each trial. With the inclusion of new parameters and possibly additional methods, each series of experiments will take longer and use more material. It is important, therefore to use the least number of replicates viable without any detriment to the trial.

Chapter 4

The effect of manganese-coated sand on the humification of CLO

This chapter details the design, implementation and results for the second experimental trial. Following the method development in trial 1, these experiments seek to test the effectiveness of a proposed catalyst of the humification process. It is hoped than manganese dioxide will encourage the production of humic substances in the CLO as it has been proven to do in natural soil systems (Yamamoto and Ishiwatari, 1992; Wang and Huang, 2000).

4.1 Catalysis: Manganese dioxide

Manganese is present naturally in most UK soils. Being a transition element metal, it has multivalent nature and can exist in many different forms. With a high specific surface reactivity and oxidation potential, manganese oxides are highly reactive as catalysts within soils (Brunetti et al., 2008).

As previously discussed in section 2.2, humic substances are polyelectrolytic macromolecules contain a variety of functional groups; of these functional groups, oxygen-containing carboxyl and phenolic hydroxyl groups offer acidic binding sites for such naturally occurring metals (Senesi and Calderoni, 1988; Jones and Bryan, 1998; Zhou *et al.*, 2005; Chien *et al.,* 2006; Chassapis *et al.,* 2009; Li *et al,* 2010). Manganese may act as a Lewis acid by accepting electrons from phenolic compounds, which form semiquinones and then humic substances via oxidative polymerisation (Huang, 2004). As well as humic ligands, carbonyl, alcoholic and water molecules are also arranged around Mn^{2+} ions to form an irregular octahedral configuration (figure 4.1).

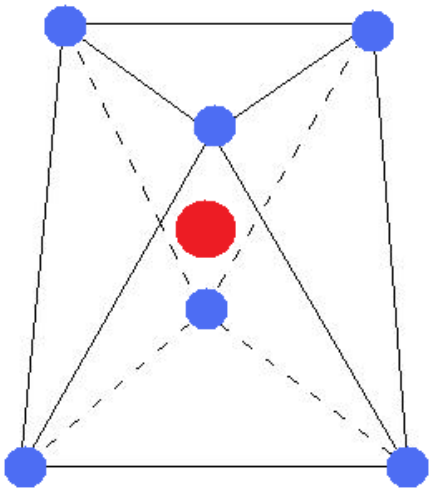


Figure 4.1: the irregular octahedron configuration of Mn^{2+} (indicated by the red circle) and its ligands (indicated by the blue circles).

Humic ionic groups undergo ligand exchange with H_2O or OH^- and by surface complexation between oxide OH_2^+ groups and humic ionic groups. Both require either the uptake of protons or the expulsion of hydroxyl ions by the oxide. This requirement for protons accounts at least partially for the dependence of adsorption on pH (Tipping and Heaton, 1983).

Metal-humic complexes can affect the concentration, mobility and bioavailability of these metals in soils and associated environments (Zhou et al., 2005; Chien et al., 2006). They can offer stability to metals, allowing them to remain in solution and are readily available, yet resistant to microbial attack (Chassapis et al., 2009). Metals can reduce intermolecular repulsion by reducing humic charge (Bryan et al., 2001).

The extent of metal-humic binding may vary with the source, MW and configuration of humic matter; pH conditions within the soil; ionic strength of the soil water, and chemical properties of the metal as well as the relative abundances of both the metal ions and humic substances (Chassapis et al., 2009). Li et al (2010) found that the effective pH range was greater than 6; considering the pH results given in the previous chapter CLO should offer a suitably operational environment although Tipping and Heaton (1983) state that adsorption decreases with increasing pH. Zhou et al., (2005) also suggest that pH is amongst the most critical parameters in controlling metal-humic complexation.

Even when humic acid concentrations are low, metal binding can still be significant (Zhou et al., 2005) so premature CLO could still potentially benefit from the addition of MnO_2. Metals with a high charge and a small radius are the most effective at inducing structural change (Bryan et al., 2001); again, Mn^{4+} satisfies this requirement: Mn (along with iron, aluminium, cadmium, copper, nickel, lead and zinc) binds particularly strongly to high molecular weight fractions (Jones and Bryan, 1998).

As metal binding plays an important role in the diagenesis (Senesi and Calderoni 1998) and aggregation (Bryan et al., 2001) of humic materials, this trial will incorporate MnO_2 into the CLO to investigate its effects on the humification process. It is hoped that it will act as a catalyst in this artificial soil, as observed in natural soils. Aggregation is thought to be due to the reduction of long-range electrostatic repulsion on the complexation of the metal ion. Conversely, Sunda and Kieber (1994) propose that manganese oxides can actually split humic substances to form lower molecular weight compounds, thus making them accessible to soil microbes. If this is the case, the addition of MnO_2 to CLO should see increased CO_2 flux when compared to control samples.

One possible source of MnO_2 is from the water treatment process. Rapid sand filters remove Mn from drinking water, leaving behind manganese-coated sand. These beds are refreshed every four weeks and the waste product is sent to landfill. This totals around 400 tonnes per

year in the north-east of England alone. Instead, it could potentially be utilised as a catalyst for the production of humic acid in soils.

In a similar study by Brunetti *et al*., (2008), humification in olive mill waste was analysed using MnO_2 as a catalyst. Although this waste product is potentially a good source of nutrients for cropland, it is insufficiently mature and stable to be added straight to natural soils. Doing so could increase the rate of mineralisation of the native soil so it is necessary for this material to be stabilised before addition. During this study, treatments increased the pH and electrical conductivity over the trial period. Over time, a loss of aliphatic material was observed, along with a corresponding increase in extraction yield, oxygenation, acidic functional groups, carbohydrates and aromaticity of the humic acid fractions. Ultimately, the addition of manganese did show an indication of increased humification when compared to the control samples.

Although CLO is a different substrate to the olive mill wastes (aqueous solution of sugars, phenols, nitrogenated compounds, organic acids, polyalcohols and residual soil emulsion); they have similar components. The MnO_2 is from a different source, meaning possible differences in crystallinity, grain size and mineralogy, but it was expected that a similar result would be seen during this trial; that the manganese dioxide-coated sand will increase humification of the CLO. The idea of mixing waste streams to potentially provide a solution to carbon emissions and carbon storage is an attractive prospect.

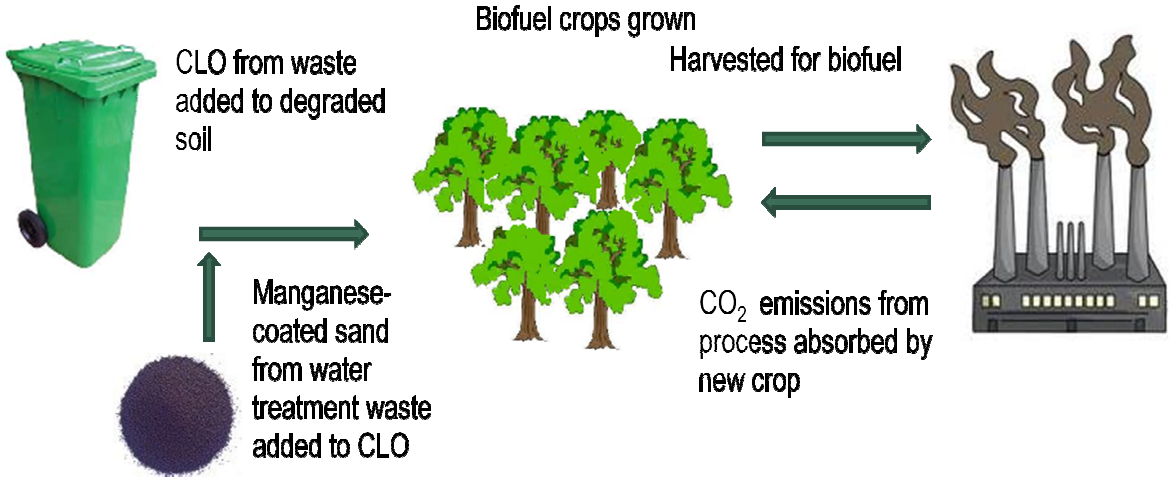


Figure 4.2: the 'waste to energy diagram re-visited with the addition of Mn, another material which could be diverted from landfill and potentially used to aid carbon storage in soils.

If CLO can provide a stable carbon store, enriched by manganese, two waste products could be used in a beneficial way. With many metals species, manganese is essential for life at low concentrations but becomes toxic above certain levels; therefore, the levels added to CLO could be a limitation.

4.2 Trial aims

To combine manganese dioxide-coated sand from Northumbrian Water with PWM's CLO in order to establish whether it has any effect on the humification process.

4.3 Trial objectives

1. To find the ideal proportion of Mn to use.
2. To identify the species present in the manganese dioxide coated sand.
3. To test the availability of the manganese within the given media.
4. To ensure that the manganese has no unexpected effect upon the analytical methods used.
5. To employ a sand control to exclude any physical effects.
6. To asses study length, looking at the humification of samples over time.
7. To examine the proposed catalytic properties of MnO_2 –coated sand when added to CLO through a fully factorial 10 week trial.

4.4 Materials used

A more detailed description of the materials (including the contaminating metals species present in CLO) and methods used can be seen in Chapter 3.3.1. This section will outline any additional methods used, and the experimental design specific to this trial.

The manganese oxide-coated sand was supplied by Northumbrian Water Ltd. In order to satisfyTrial objective 2, the MnO_2-coated sand was sent for comprehensive analytical tests to an independent laboratory (AES analytical and environmental services, Tyne and Wear). The material was found to comprise 90% quartz, 10% MnO_2 along with iron and a number of trace elements (Table 4.1).

Element	MnO_2-coated sand (mg/kg)	Soil guideline value[1]		Dutch Intervention levels[1]
		Residential without plant uptake	Commercial/ Industrial	
Arsenic[1]	2.8	20	500	60
Cadmium[1]	**32**	**30**	1400	**6**
Chromium[2]	4.0	200	5000	30
Copper[2]	42	-	-	74
Iron[2]	20000	-	-	-
Lead[2]	**44**	450	750	**75**
Manganese[2]	90000	-	-	-
Nickel[1]	**1600**	**75**	5000	**75**
Zinc[2]	**10000**	-	-	**800**

[1]www.environment-agency.gov.uk/subjects/landquality

Table 4.1: Summary of main contaminants present in MnO_2-coated sand used in the trial. The numbers in bold represent those values which exceed soil guideline values and/or Dutch intervention levels.

Cadmium, lead, nickel and zinc all show potentially concerning concentrations. The levels of cadmium were particularly high and exceeded the lower soil guideline value (SGV) given by the Environment Agency. Cadmium is a major toxic trace metal which reaches the food chain directly through crop intake and also indirectly through animal transfer (Liu et al., 2009). Zinc and copper are both toxic to plants above certain levels and although the Environment Agency does not have a SGV for either, they are both above the Dutch intervention levels. Mn exceeds the WHO drinking water guidelines, as discussed in Chapter 3....

This problem would have to be addressed if the trial proved to be successful. Simpson (2008) found that washing CLO was effective in the removal of certain trace metals; Manganese has been proven to show high stability against intense washing (Senesi and Calderoni, 1988), so this could be a possible solution. However, toxicity is largely associated with the free cation so if the species are complexed, then toxicity would be reduced (Jones and Bryan, 1998).

4.5 Experimental set up

Preliminary experiments were carried out to ensure that the Mn used was characterised to guarantee continuity throughout the project's trials.

A Tessier extraction (Tessier *et al.* 1979) was carried out on MnO_2 coated sand to assess its leachability/ availability, as specified in trial objective 3. The World Health Organisation (WHO) gives guideline values of 0.05mg/l as the safe limit for manganese in drinking water. The concentration of manganese found in leachate from this trial was 5.9mg/l (Jarvis, 2007). At the time of analysis, it was believed that the guideline value was 10mg/l, meaning that the manganese in the leachate would have been within the safe limit. However, knowing the correct guideline value, there could be a significant risk posed to surface and ground water associated with the addition of MnO_2 coated sand to CLO in its current concentration, should any leaching occur. If the MnO_2 does have a beneficial effect on the humification process, this would have to be addressed prior to it being laid to land.

In order to rule out any physical effects that the manganese coated sand may exhibit, sand was introduced as a control (objective 2). Both the MnO_2 coated sand the control sand were sieved to eliminate particle-size bias. The average particle size was determined to be 0.85mm.

Lysimeters were constructed as described in Chapter 3.3.2 and placed outside on gabions, as in trial 1. Each sample was set up in triplicate with a fourth sample for sacrifice after five weeks. The dry weight of the CLO was calculated and the appropriate amount weighted out and transferred to the lysimeters, with the 100% CLO samples weighing 600g. The sand and the manganese were added at either 5 or 10% of the dry weight of CLO. The relative proportions for each sample are shown in table 4.2.

Sample	CLO %	Sand %	Mn %
1	100	0	0
2	95	0	5
3	90	0	10
4	95	5	0
5	90	5	5
6	85	5	10
7	90	10	0
8	85	10	5
9	80	10	10

Table 4.2: Proportions of sand, CLO and MnO_2 added to each lysimeter for trial 2

The trial was implemented in January 2007 where the ambient temperature was considerably lower than in December when the initial trial was completed.

Consequently, little or no flux was observed at temperatures below 5°C. A study was devised to establish the minimum temperature at which flux could be observed. As a result of this study, it was decided that on the days where the ambient temperature fell below this minimum, three samples would be chosen at random to ensure that no flux could be measured and the experimental testing abandoned for that day.

Sub-samples were taken, as described in section 3.5.1. The soil was turned in the lysimeter in order to get a good, representative sub-sample and flux measurements were recorded, where possible, three times a week for ten weeks; samples for humic analysis were taken at weeks one, five and ten. These samples were transferred into clean, polythene sample bags; labelled with the sample batch, number and date, and stored in the dark at <5°C until they could be analysed. Alkali extraction and UV analysis were employed as described in Chapter 3.

As ambient temperature and soil moisture are known to have an effect on temperature flux (see previous Chapter), both parameters were recorded on the days that the flux was measured (section 3.4.1 and 3.4.2). It would have been useful to measure the moisture content of each individual sample to see whether any differences between different treatments could be observed. However, as each measurement required the removal of around 20g of material (a large enough sample being necessary to remove any effects of heterogeneity), the total amount of removed sample at the end of the trial would have been around 50% of the

total volume. Instead, three extra pots were set aside for this purpose, each containing 600g of CLO. The soil moisture was measured in triplicate on each sampling day.

The final physical parameter measured was pH (section 3.5.3). It was recorded at weeks 1, 5 and 10 in order to establish any differences that might be observed between samples or in the same samples over time.

4.6 Statistical analysis

As described in Chapter 3.4, all data collected were entered into a Microsoft Excel spreadsheet, formatted and then ANOVA (General Linear Model) performed using Minitab 13. The results are given as p-values and all < 0.05 show a significant relationship between the two parameters compared. All results used from this point will be quoted as being statistically significant if they satisfy this requirement.

4.7 Results

4.7.1. Physical data and observations

Over the ten week period, no plant growth was observed on the lysimeters despite being exposed to wind-blown seeds. In similar CLO lysimeter trial set up by Simpson in 2006, plant growth was seen on all samples (Simpson 2008). The lack of growth in this trial was possibly due to the fact that the samples were turned twice weekly (during the process of subsampling) so any seeds that might have germinated may have been too deeply buried within the lysimeter, making growth unlikely. It could also be due to the trial being carried out over the Winter months, therefore not conducive to plant growth. The immediate environment also differed, with the predominant species of flora being Beech trees; during the Simpson study, the site was surrounded by grassland.

Soil moisture

The moisture content was not controlled and therefore was dependent upon local climatic conditions over the 10 week period. This data is of little use alone, but when used in conjunction with the flux data, the differences can be incorporated into the flux calculations.

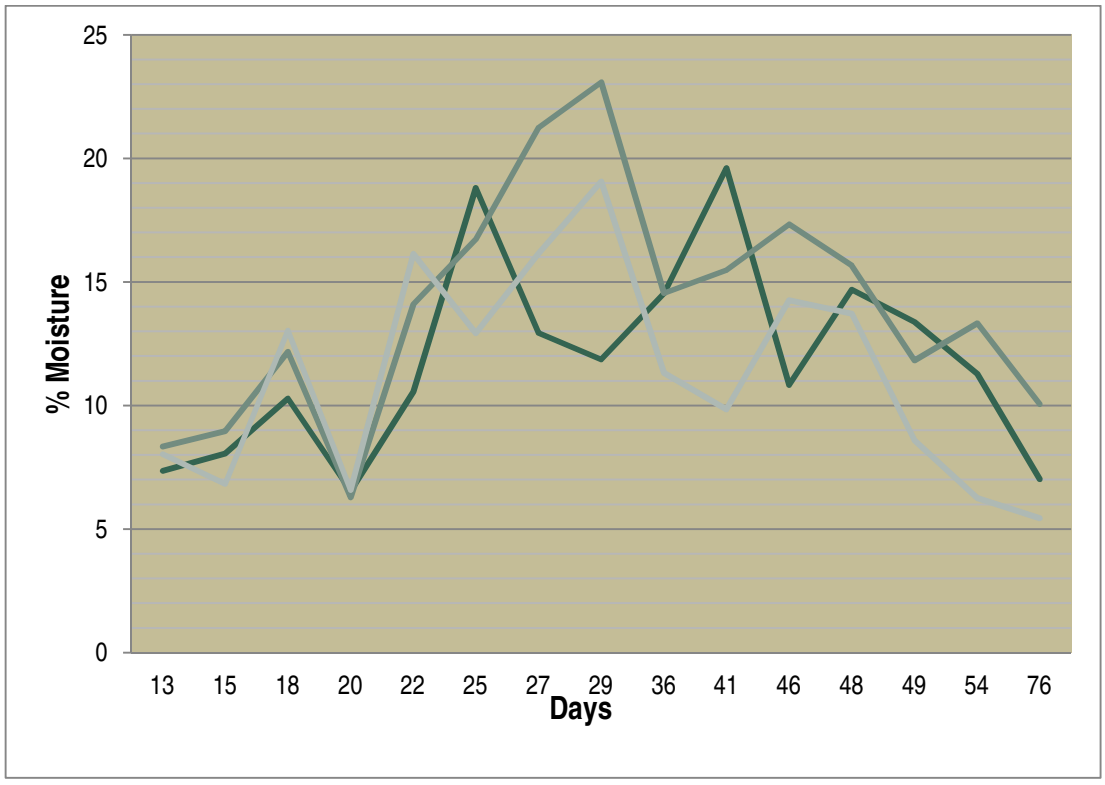


Figure 4.3. Gravimetric moisture content of the triplicate CLO samples over 74 days.

The soil moisture ranged between just over 5% to around 24% over the 74 days. The range between triplicates varied between 0 and 10%. This can only be due to the heterogeneous nature of the CLO. If, for example, a significant proportion of the subsample contains glass or plastic, this could lead to large differences seen in moisture content. This highlights the need for large sample sizes and replicates.

Further research would be needed to begin to understand the intermolecular reactions that link humic components into supramolecular associations and to establish the pathways which lead to such associations (Sutton and Sposito, 2005). However, Schaumann and Bertmer (2008) observed that water molecules bridge molecular segments of SOM which could further

adds weight to the supramolecular assembly model theory. Hence, soil moisture may not only be necessary for a healthy soil microbial community, but also to aid in the binding of humic acids.

Literature discussed in Chapter 2 suggested that during composting, the ideal moisture content would be >40 %; it is possible that the low levels seen in these samples over this 10 week trial have had an effect on the CO_2 flux rate.

pH

The pH of CLOs are generally mildly alkaline, being in the region of 7- 9.5 (Kaschl, et *al* (2002) and references therein). All samples from this trial measured between 7.4 and 8.3, which is comparable to this range, to natural alkaline soils and consistent with the study carried out by Simpson (2008) on earlier batches of the same material.

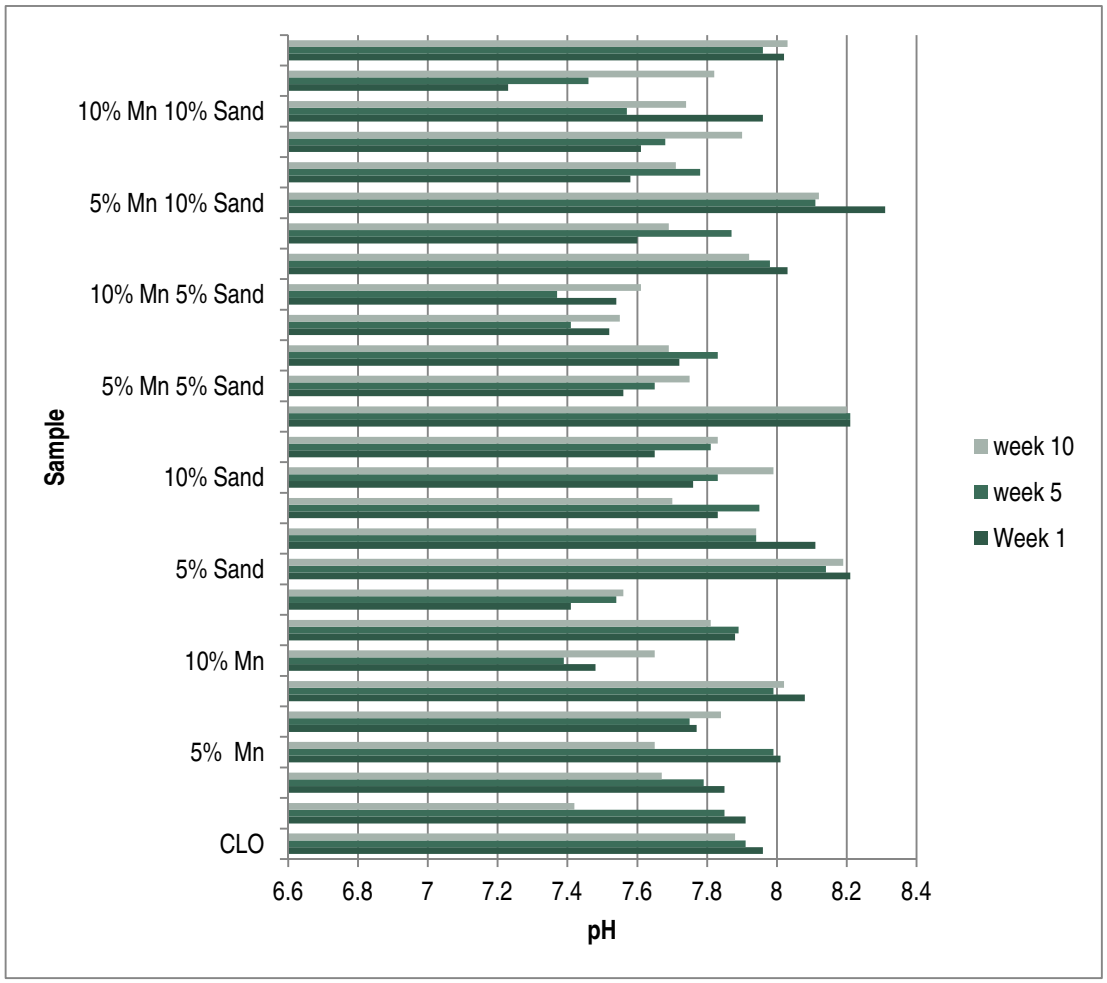


Figure 4.4: The pH for each replicate sample at weeks 1, 5 and 10.

A great deal of variance was seen between replicate samples; no significant differences seen either temporally, or between different samples ($p>0.05$). In a study by Brunetti et al (2008) pH increased on addition of Mn; however, the amended material was of a more homogenous material nature so perhaps any differences between samples were easier to detect.

The fact that no significant differences were seen between samples could mean that the behaviour of CLO and the manganese-amended CLO might be more easily predicted. With a pH of 7.4 - 8.3 which appears to be consistent through all samples over time, this mildly alkaline artificial soil could have a significant impact on soils to which it was added.

This soil parameter can be the chief factor in controlling phytoavailability in composts, more so than trace metal concentration (Smith 2009). This is of considerable importance when such composts contain high volumes of potentially toxic elements. Chu and Wong (1987) attributed the relatively low concentration of trace metals accumulated by vegetable crops, treated by an MSW-compost, on its higher pH and thus causing a liming effect.

One of the four principal structural characteristics of the HAs and FAs that influence their chemical reactivity is structural lability which means that they have a great capacity to associate intermolecularly, changing conformation in responses to pH value (polyfunctionality, anionic macromolecular charge, and hydrophobicity being the other three) (Sposito, 1989).

Humic acids have an amphiphilic nature as they have both hydrophilic and hydrophobic moieties in their structures. Thus, HAs are able to reduce surface tension in aqueous solutions and can form micelle-like (Figure 4.5) aggregates (Yates and Wandruszka, 1999; Kleber *et al* 2001; Sutton and Sposito, 2005). This micellar formation can be catalysed by the presence of metal cations and can arise from both intermolecular aggregation and intramolecular coiling (Yates and Wandruszka, 1999). A zonal structure is proposed by Kleber *et al* (2001) whereby OM is attached to a mineral surface and is segregated into more than one layer or zone of molecules, meaning that some of the adsorbed molecules will not be in contact with the mineral surface.

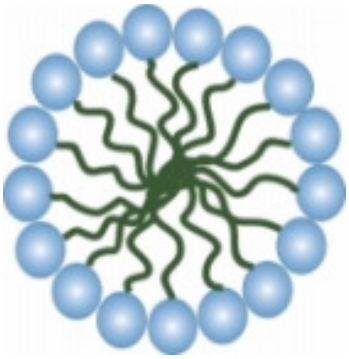


Figure 4.5: a micelle with its hydrophobic heads and hydrophilic tails *(Stewart, 2008).*

Surface activities of humic acid can play an important role in the transport, bioavailability and biodegradability of hydrophobic organic pollutants (Yates and Wandruszka, 1999) and the pH of the system can largely influence the extent to which this happens. Terashima *et al* (2004) found that an increase in pH within a soil can increase micelle-like aggregation and interfacial adsorption, thus having implications on the transport, bioavailability and biodegradability of hydrophobic organic pollutants (HOPs) which may be present. This is particularly pertinent if the CLO is intended to be used to remediate contaminated land.

Presence of functional groups (particularly carboxyl and hydroxyl groups) provides the capacity for interaction with inorganic cations, specifically in complexation of metals in environment (Pehlivan and Arslan, 2006). This metal-humic binding is influenced/controlled by pH (You *et* al 1999). Polar organic functional groups of amphiphiles interact via ligand exchange to form stable inner-sphere complexes, favouring particularly strong organo-mineral interaction (Kleber *et al* 2001). Complexation with a metal ion may also instigate the aggregation of compounds (Simpson *et al* 2002) increasing the stability of the soil and the availability of phosphorous through displacement. Many contaminated soils are degraded in terms of stability and nutrients so if the CLO was to be added to such a site, could the pH value of the soil be increased and the soil improved? Several studies have proved thus (Garcia-Gill *et al.,* 2004; Warman *et al.,* 2009). As pH increases, the amount of metal binding increases due to ionisation of functions groups (Lu *et al* 2000).

4.7.2. CO_2 flux

The carbon flux was recorded in ppm with an IRGA as described in section 3.6 and then converted into gC/gCLO/hour via the equation given in section 3.6.2; all data presented in this section are given in gC/gCLO/day, to make it comparable to similar studies (Jarvis, 2007; Tognetti *et al.,* 2007).

Control tests were carried out on the flux of both sand and MnO_2 coated sand where each was measured in turn into an air-tight container. Holes were drilled into the lid of the container for the air-in and air-out tubes of the IRGA and were made airtight with a sealant. The flux was measured using with an IRGA following the methodology outlined in Section 3.5.1. For each the flux was negligible with the concentration of CO_2 measured being around ambient level (around 460ppm).

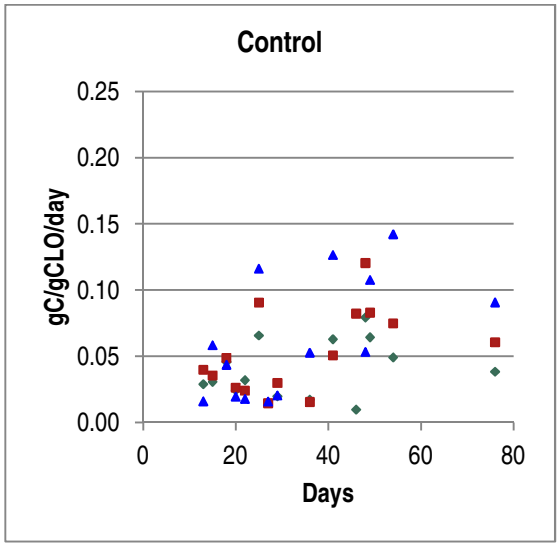


Figure 4.6: CO_2 flux observed over 80 days for the control sample; each replicate is represented by a different series on the chart.

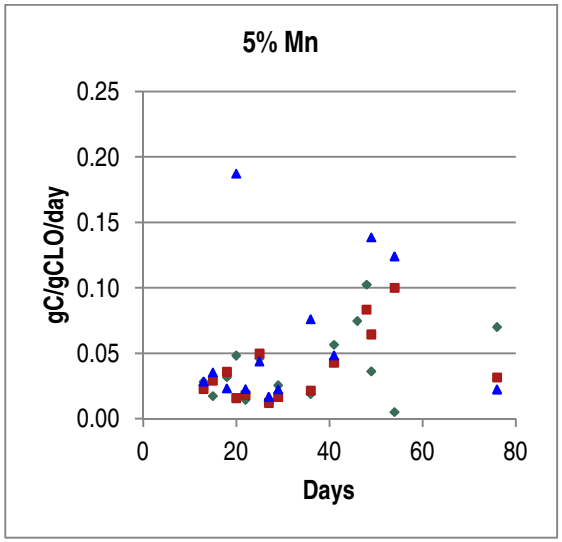


Figure 4.7: CO_2 flux observed over 80 days for the sample with 5% Mn; each replicate is represented by a different series on the chart.

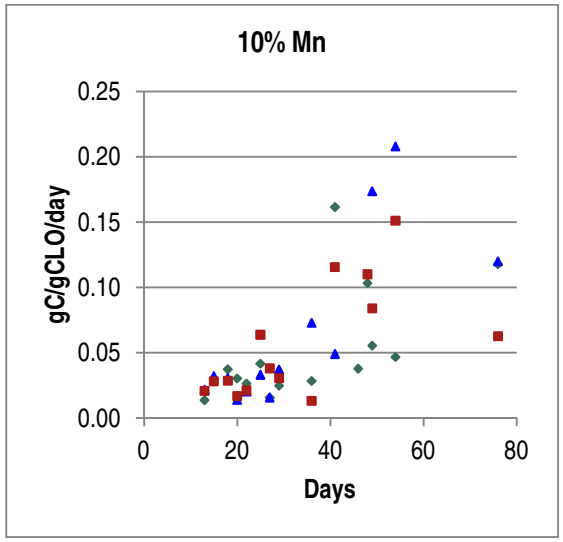


Figure 4.8: CO_2 flux observed over 80 days for the sample with 10% Mn each replicate is represented by a different series on the chart.

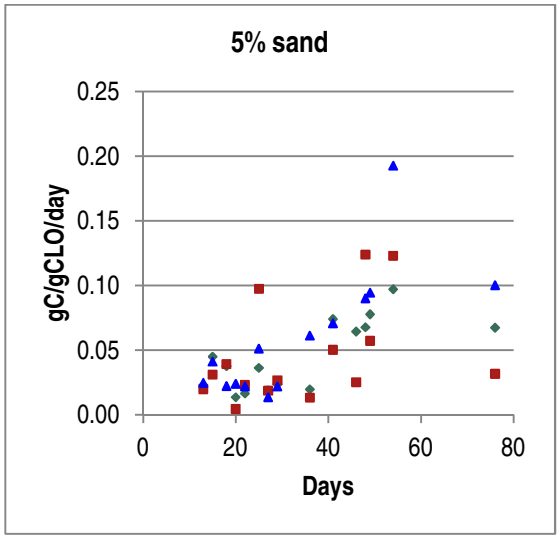


Figure 4.9: CO_2 flux observed over 80 days for the sample with 5% sand; each replicate is represented by a different series on the chart.

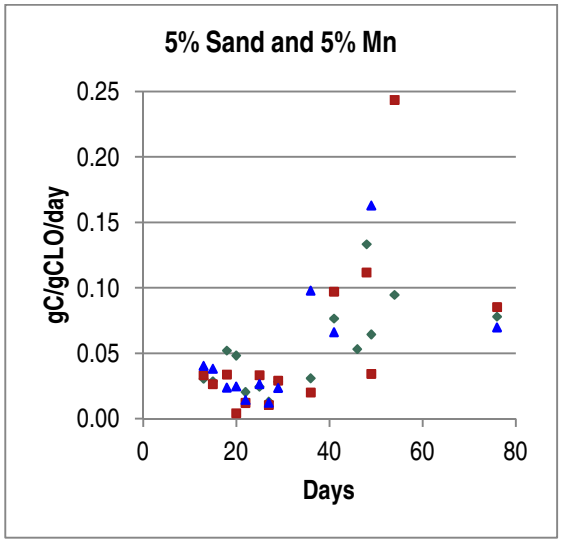


Figure 4.10: CO_2 flux observed over 80 days for the sample with 5% sand and 5% Mn; each replicate is represented by a different series on the chart.

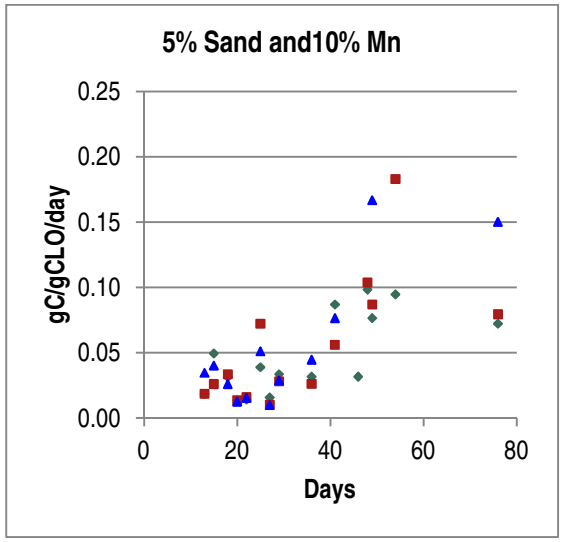
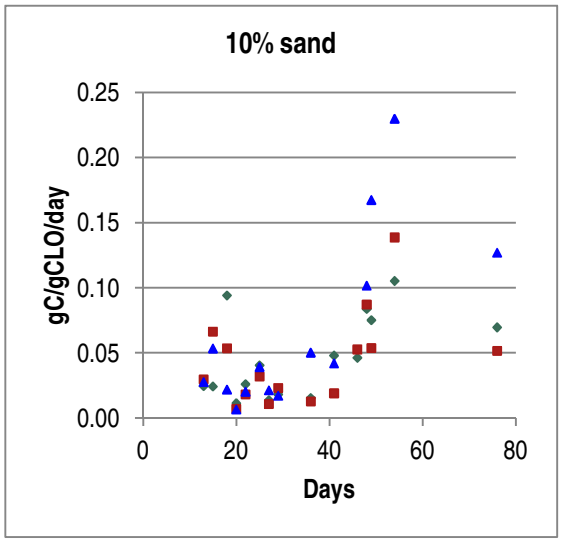


Figure 4.11: CO_2 flux observed over 80 days for the sample with 5% sand and 10% Mn; each replicate is represented by a different series on the chart.



Figures 4.12: CO_2 flux observed over 80 days for the sample with 10% sand; each replicate is represented by a different series on the chart.

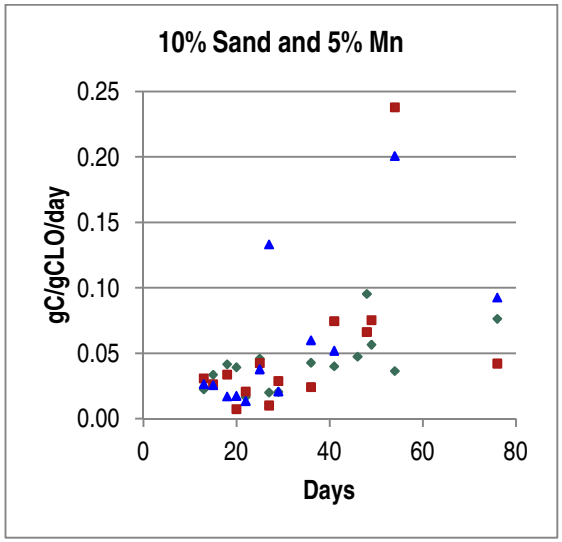
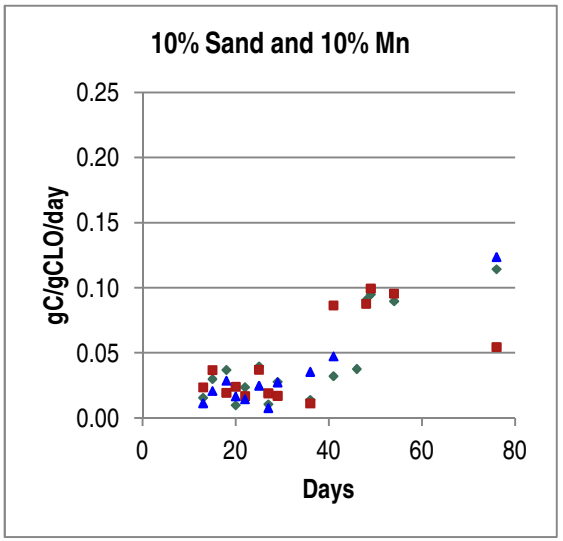


Figure 4.13: CO_2 flux observed over 80 days for the sample with 10% sand and 5% Mn; each replicate is represented by a different series on the chart.



Figures 4.14: CO_2 flux observed over 80 days for the sample with 10% Mn and 10% sand; each replicate is represented by a different series on the chart.

The samples showed no measurable flux for the first 11 days of the trial. This was thought to be due to a retardation of establishment of a microbial community due to the freezing temperatures (Blume *et al.,* 2002; Andersson and Nilsson, 2001).

The range of data for each day is apparent between the triplicate samples but a slight trend to increasing flux over time can be observed. In a study by Neves *et al* (2008), a similar lag time of 10 days was observed. Fats were added to cow manure and food waste in order to stimulate the production of methane in anaerobic digestion. The lag time observed during trial 2 could be due to the inhibitory effect of fats, as was witnessed in the Neves study but as the maturity of the CLO is unknown, it is difficult to know whether or not these initial stages of composition have progressed.

This initial period was particularly dry so the lack of moisture could account for the absence of microbial activity (Schimel *et al.,* 1999).

During the composting process, two main phases exist: The biooxidative phase and the maturing phase. The former sees the rapid degradation of the simple compounds and other organic matter. Once the organic substrate is largely depleted, the maturing phase begins. This stage is associated with the stabilisation and humification of the remaining organic matter. Humic acids with increasing MW, aromatic characteristics, oxygen and nitrogen concentrations increase in presence where as fulvic acids and water extractable organic carbon decrease (Bernal *et al* 2009). This stage requires several weeks to months (Veeken et al 2000). This period is necessary to ensure the formation of an end product which is stable with high nutrient content and a neutral pH. During aerobic digestion, the MSW feedstock is processed for only 9 days. Although the conditions are controlled during this time, the material that is produced has not reached maturity, nor is particularly stable, hence the large flux rate.

Each sample saw a rise in flux over the sampling period and the flux begins to decrease again towards the end of trial. The flux appears to be greater than the control for samples with 5% Mn and even more so with 10% Mn added. However, in order to rigorously examine these differences in data, the ANOVA procedure with Tukey post-hoc significance testing was used once again.

The control sample (Figure 4.6) , although there appears to be a trend of rising flux from 0.01 – > 0.12 gC/gCLO/day over ten weeks, the variation between replicate samples meant that no statistical significance was seen in the flux over time. The key period for flux change was at around day 50 when a significant increase in flux (0.04-0.24gC/gCLO/day) was seen in the all samples, excluding the control and the samples containing 5% and 10% Mn (Figures 4.6-4.8). Again, this lack of statistical significance could be due to the large spread in data between replicates as in each case, a similar qualitative trend can be seen in all samples. This increase in flux could be due to the Birch effect as described by Jarvis *et al.,* (2006) whereby the re-wetting of the soil after a period of dryness sees a corresponding burst of decomposition. Figure 4.15 illustrates the relationship between soil moisture (determined by largely by precipitation at the temperatures seen during the trial period) (Figure 4.16)) and flux.

At seventy days, the flux had decreased again in most samples, back down to a similar level seen at the beginning of the trial. This could indicate the depletion of the simple compounds present, corresponding to the mesophilic phase of the biooxidative stage of composting.

At this stage, the flux of the samples with 5% sand, and 5% Mn with 5% sand still remained slightly higher that recorded in the initial few days of the trial. Both samples containing 10% sand reached a flux of almost 0.25 gC/gCLO/day, 0.1 gC/gCLO/day more than the control sample at its greatest flux rate. As the proportion of CLO in each sample is taken into account for the flux calculations, this factor should have no bearing on the results seen. All samples with >10% of amendment added saw an increase in flux; could this be a physical affect due to increased aeration? If porosity in a composting pile exceeds 50%, the energy lost could exceed the heat produced, eventually meaning a slowing of the rate of degradation (Bernal *et al* 2009).

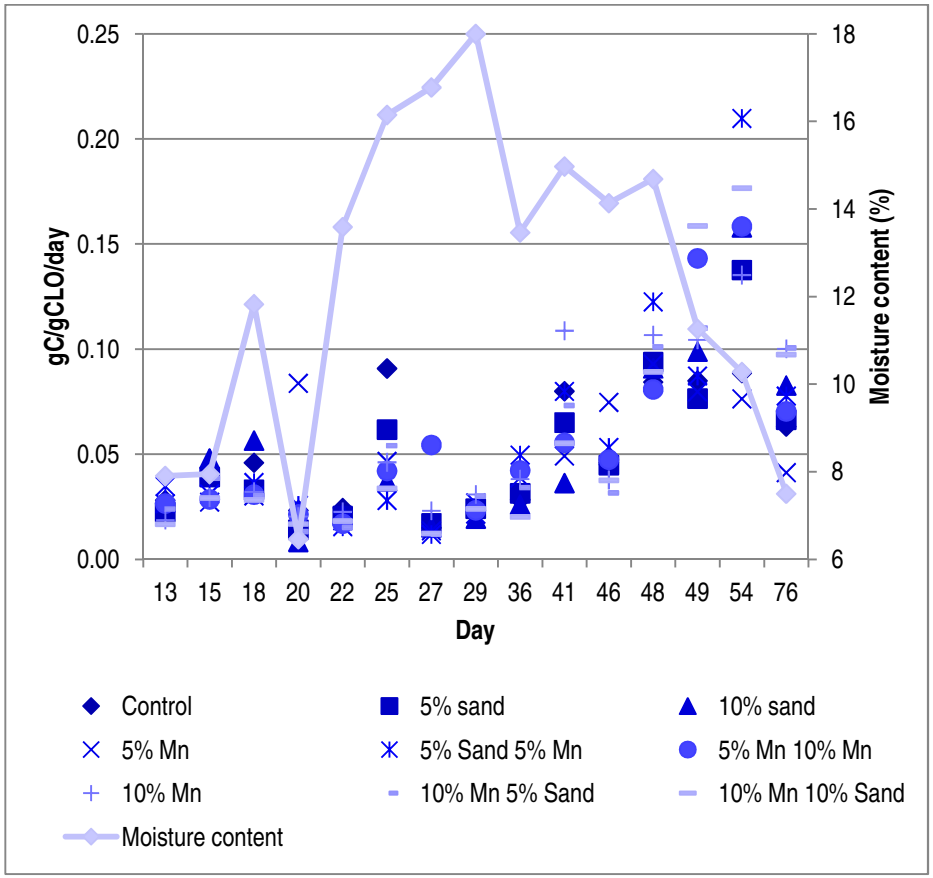


Figure 4.15: CO_2 flux against the soil moisture content over the 10 week trial period.

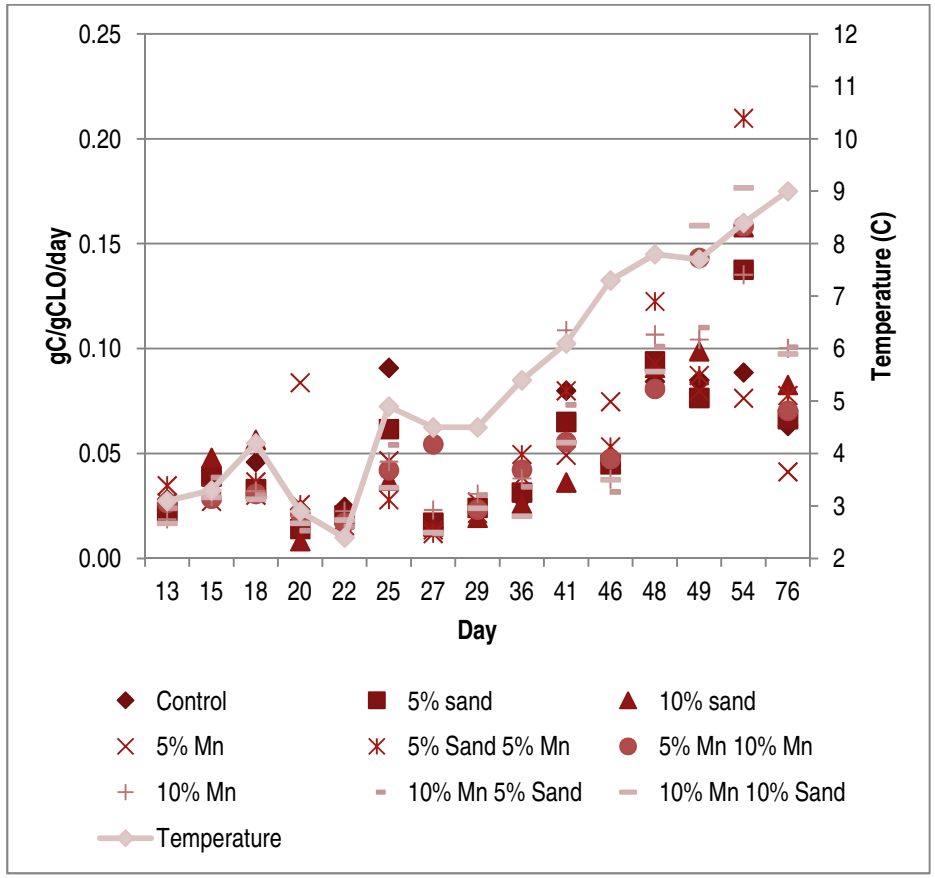


Figure 4.16: CO_2 flux against outdoor temperature on day of sampling over the 10 week trial period.

A good correlation is seen between the CO_2 flux and the soil moisture (r^2 0.70) and ambient temperature (r^2 0.88) as shown in Figures 4.14 and 4.25 respectively .This data largely reflects the results seen in the preliminary trials outlined in Chapter 3.8.1.

4.7.3 Humic acid analysis

Raw data are presented as the average of each sample at each week, plotted against the yield of humic acid extracted (Figure 4.17) and then against the E_4/E_6 ratio (Figure 4.18).

Again, control tests were carried out prior to this trial for both forms of analyses. Humic acid content of both sand and MnO_2 coated sand was measured using the 11-step alkali extraction method, detailed in Section 3.6.1.; no yield was given for either amendment. The absorbance for both sand and manganese at 465nm and 665nm were recorded using the method outlined in Section 3.6.2. The absorbance of both sand and Mn were below the lower limits of detection of the UV photospectrometer (absorbance <0.01nm). This should discount any potential of these amendments to skew the data.

For the alkali extraction the data were normalised, given that all the samples should have been the same at week one; any changes seen between samples over time will therefore be relative to week one. In Figure 4.17, the weeks are represented by the three series, as shown in the key.

Alkali extraction of humic acid

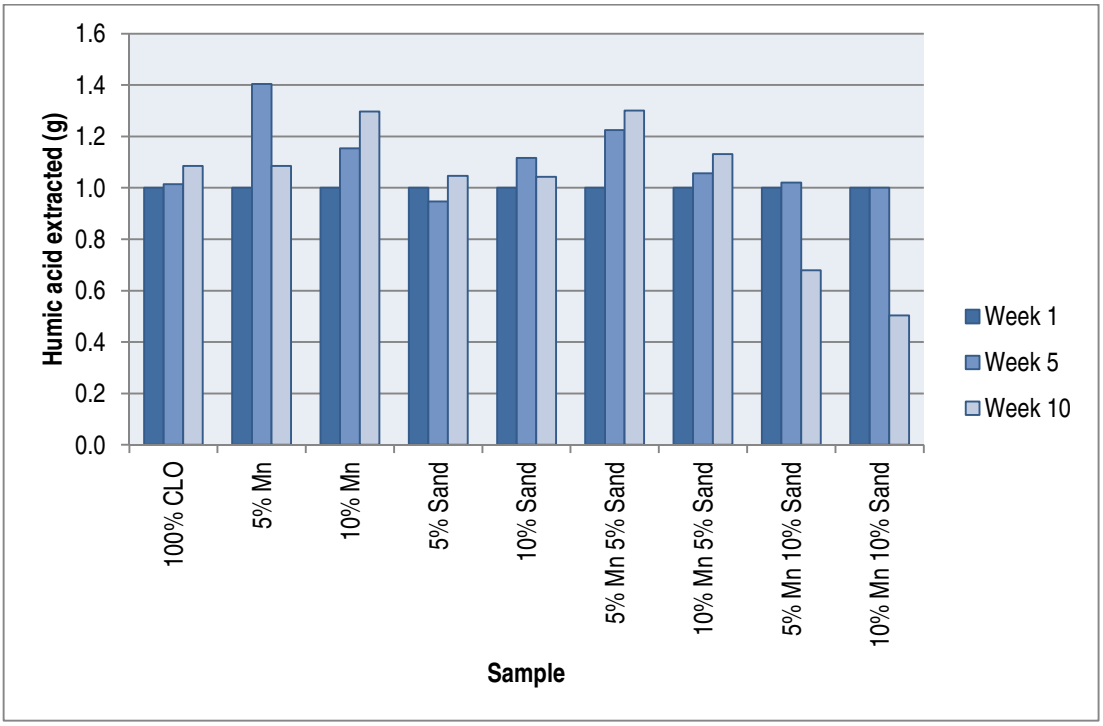


Figure 4.17 Normalised data showing the efficacy of each treatment over 10 weeks.

There are no significant differences shown between any of the samples at week one; this is to be expected, considering the control tests undertaken during trial one (i.e. no humic acid yield was seen for sand, and MnO_2-coated sand samples). Similarly to the CO_2 flux measurements, there were no significant differences seen between samples at week 1 and samples at week 5, suggesting that decomposition and humification were slow for the first 5 weeks possibly due to low temperatures and soil moisture.

The control sample shows a slight increase between weeks 1-5 (and a larger increase in yield between weeks 5-10 (0.4g and 2g respectively). The humic yield increased over the ten week period for all samples but those containing 5% Mn and 10 % sand; and 10% Mn and 10% sand which both decreased significantly. This could be due to an increase in aeration, leading to greater microbial activity and faster mineralisation of SOM; a corresponding increase of flux was observed for these samples. However, the same was not seen for the 5% sand with 10% Mn samples which would be expected when the same proportion of amendment has been added.

Absorbance

A decrease in absorbance ratio signifies an increase in humification and material which gives a ratio of <5 and is said to be humified. Figure 4.17 shows that all samples demonstrated an increase in humification over the ten week period. Only the samples with 5% sand; 10% sand; and 5% Mn with 10% sand had a ratio of below 5, indicating a humified sample after the 10 week aging period.

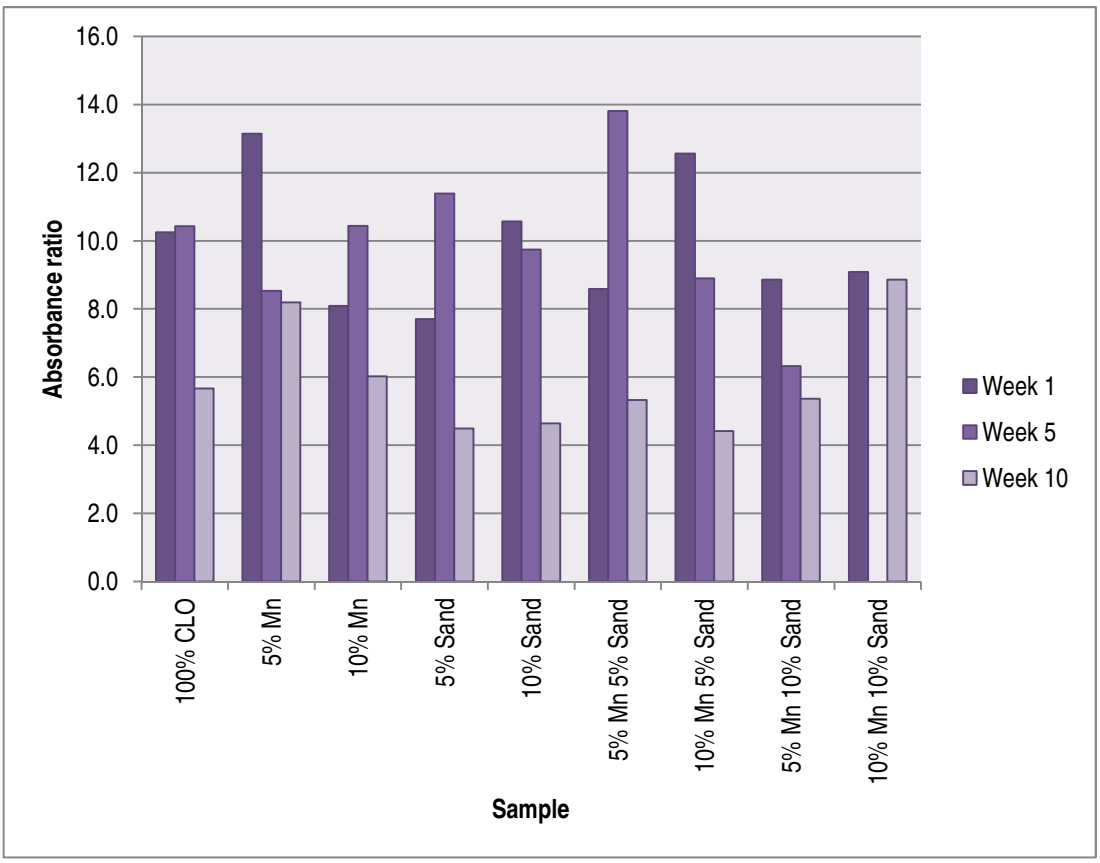


Figure 4.18: E_4/E_6 ratios for all samples as compared to the control sample at weeks 1, 5 and 10. Each series gives the mean of the three replicate samples.

Unlike for the carbon flux and alkali extraction data, differences can be seen between samples at week one and five, although they are only significant for the sample with 5% Mn (which decreases between weeks 5 and 10); 10% Mn (which decreases between weeks 1 and 5); and 5% Mn with 5% sand (which decreases between weeks 5 and 10).

For this data, the differences between replicate samples is again, pronounced. Figure 4.19 demonstrates the variation in absorbance between 3 replicates at week one.

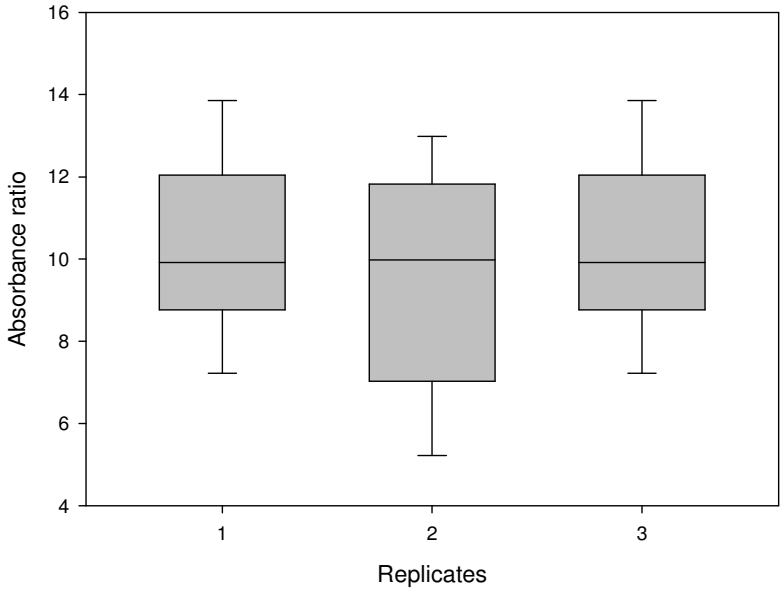


Figure 4.19: boxplot of three replicate control samples to show the spread of data at week one for E_4/E_6.

If at week one, such variation is seen when all samples should be roughly equal, it is unlikely that any differences will be observed between samples according to treatment or over time. So although there appear to be trends in the qualitative analysis given by the averaging of the triplicate results, ANOVA data reflects this, showing that no significant trends can be seen between the different treatments at each timeframe.

The only conclusion that can be drawn from the absorbance data is that the humic nature of all samples appeared to increase over the ten week period in the samples with added sand indicating the greatest degree of humification. This is not reflected in the results seen in both the alkali extracted humic and flux data.

In similar studies, the E4/E6 ratio of dissolved organic matter has been seen to decrease with an increase in pH which denotes a greater fraction of high MW being released into solution at higher pH values (You *et* al 1999*).* Because the pH values remained fairly consistent between samples over time, it was not possible to determine whether a similar correlation occurred in this trial.

4.8 Conclusions

4.8.1 Revisiting the objectives

1. *To find the ideal proportion of Mn to use during future trials.*

No consistent differences were observed between MnO_2 added at 5% and 10% in the three analytical methods used. Due to there being some evidence to suggest that samples with >10% added amendments might be experiencing increased mineralisation, 5% MnO_2 and 5% sand will be used in future trials.

2. *To test the metal species present in the manganese dioxide coated sand.*

The MnO_2 coated sand was found to include a number of toxic metal species, including cadmium, lead, copper and zinc. This problem would need to be addressed before this could be viewed as a viable source of MnO_2. However, much of the literature suggests that CLO's alkaline pH might have a heightened ability to bind these metals, making them less available/toxic. To test this, a Tessier extraction might be used or perhaps ICP analysis of leachate emanating from the lysimeters.

3. *To test the availability of the manganese within the given media.*

This was achieved by a Tessier extraction; the available manganese was initially thought to be within safe levels. However, it far exceeds the WHO guideline values for drinking water so should the MnO_2-coated sand prove to be successful in catalysing the humification process, this would have to be addressed and a safer concentration tested.

4. *To ensure that the manganese has no unexpected effect upon the analytical methods used*

For each analytical method, both the sand and the MnO_2- coated sand were tested as a control measure. All control tests showed that the amendments should not have any skewing effects on any data produced.

5. *To employ a sand control to exclude any physical effects.*

The sand was sieved to exclude any particle-size bias. As there were few significant differences shown between samples, it is difficult to determine whether any effects, physical or otherwise occurred. The samples with >10% of amendment added saw an increased flux rate and some decreases in HA extracted so a physical effect cannot be discounted.

6. *To assess study length, looking at humification in samples over time.*

Few differences were observed between the week 1 and week 5 samples for carbon flux and alkali extraction data. By week 10, it was possible to observe some differences between samples. Following trials could be longer in an attempt to establish further differences in humification over time and between samples. None of the samples saw an overall significant decrease in flux over the ten week period so a longer trail would be beneficial to ascertain whether this would happen in time.

7. *To test the catalytic properties of MnO_2 –coated sand in CLO.*

Results were largely inconclusive but some significant results were observed within the datasets.

The control samples showed neither an increase nor decrease in CO_2 flux over the trial period. No significant difference was seen in either of the humic data sets for these samples either.

When manganese was added at 5%, no significant increase or decrease in flux was seen. When the level was raised to 10%, and increase in flux was observed after five weeks. For the former, the absorbance data saw in increase in humification between weeks one and 10, but not within the fully humified range (<5). The latter saw an increase in the absorbance ratio

between week one and five and then a significant decrease in week ten; again, at week ten, the sample was still above the humic acid range.

In the parallel samples with 5% sand and 10% sand, both saw an increase in flux between weeks five and seven. Only with the 5% sand did this significantly decrease again by the end of the trial. Both sets of samples gave absorbance ratios of <5 at ten weeks, suggesting a high degree of humification.

The samples with 5% sand with 5% manganese also exhibited a significant increase in CO_2 flux between weeks five and seven. A significant increase in absorbance ratio was seen between weeks one and five followed by a significant decrease by the end of the trial.

A similar pattern in flux was seen in the samples with 10% sand with 10% manganese. A corresponding decrease in humic acid yield was also seen after week five. The absorbance data showed no significant increase in humification after ten weeks.

Although qualitative patterns were observed in the alkali extraction data, this method yielded few statistically significant results. On the whole, the difference between replicate samples was too great for any definitive conclusions to be drawn from the use of this method.

There appears to be some agreement between data sets but further trials will be needed to draw any decisive inferences. Not one treatment shows decisively a decrease in flux and a corresponding increase in humic acid when compared to the control sample. With a more consistent data set, perhaps comments can be made about the effect of pH and suppositions sketched on the catalytic abilities of Mn on the humification process.

4.9.2 Trial limitations

Having analysed the results from Trial 2, it is apparent that the CLO is too heavily contaminated with inorganic materials to see any trends in the organic carbon stability. The material is highly heterogeneous and thus the necessary subsampling did not prove representative of the material in its entirety. Within the composting pile, particles that are too large may have a shielding effect where humic material coats the particle, meaning that microbes cannot reach it. Particles that are too small may compact which could reduce porosity which in turn, could lead to anaerobicity. Conversely, if porosity exceeds 50%, the energy lost could exceed the heat produced.

The nutrient balance must be at a favourable level for microbial activity; if excess carbon exists, the microbes will not have enough nitrogen to metabolise the degradable compounds (Bernal *et al* 2009).

The results of this study did not identify any clear correlation between treatment and flux. Perhaps the manganese had no effect on the humification of CLO, or perhaps more sensitive methods are needed. The heterogeneity in the samples can account for a lack of significance between various samples but cannot be held accountable in every instance. For example, the alkali extraction and UV and fluorescence samples would be expect to show some correlation, being borne of the same sub-sample; the differences seen, therefore must be due to experimental error.

Only one source of MnO_2 was trialled; perhaps other sources could be used and compared to establish whether this would have any bearing on the results.

The subsampling of the trial pots is not ideal; when the CLO is laid to earth, it is unlikely that the soil will be turned regularly so results produced are not necessarily representative of what might pass *in situ.*

4.9.3 Implications for trial 3

A trial where the atmospheric conditions are controlled to some degree, particularly with respect to the ambient temperature and moisture content (thus limiting the Birch effect) of each sample, might provide some more definitive results. In this trial, the weather was so variable over the trial period that it may have overshadowed any subtle changes between samples, even though atmospheric temperature and soil moisture were measured and accounted for in flux measurements. Analysis might be a little more conclusive with tighter controls in place, meaning fewer factors in play to affect the data. If each test undertaken exhibits some experimental error, then small changes in flux and humic acid present within the different samples may easily be overshadowed.

The moisture content was also shown to be lower than 40% which may have a detrimental effect on the microbial community. Being able to control this may see more consistent patterns in flux. A higher temperature may also encourage microbial activity, thus increase the flux rate.

FACTOR	SPECIFICATION
Length of trial	12 months
Sacrificed sample time	10 weeks
Sampling frequency (flux)	twice a week then twice monthly after 10 weeks
Conditions controlled	Air temperature and moisture content
MnO_2	5%
Sand	5%

Table 4.3: proposed specifications for Trial 3.

4.9.4 Further comments

Since Trial 2, issues concerning the legality of the use of CLO as topsoil had some implications on the progression of the research. Due to electrical faults during the initial months of the Demonstrator phase, the temperature system was ineffective. This rendered the process non-compliant with the soil screening values (SVS) standard, thus the CLO produced had to be sent to landfill and the digesters halted until further notice. The process was once again deemed fit for purpose by the Environment Agency in June 2008. Trial 3 was suspended until the material had been permitted for use once again.

Chapter 5

Pseudo thermogravimetric analysis as a novel method for analysing soil organic matter

Given that the results obtained from trial 2 were contradictory and yielded few significant results, it was hoped that another form of analysis might provide elucidation of the effects of Mn on the humification of CLO.

Thermogravimetric analysis is a commonly used technique which can provide information on the organic and inorganic components present in a sample. Based on the premise that different carbon compounds decompose on heating at different temperatures, samples of CLO can be heated at various heat intervals and corresponding changes in weight measured. Resulting, weight loss curves can be produced and samples compared. Labile cellulosic material tends to combust at around 300-350ºC; refractory lignin at 400-650 ºC (Manning *et al.* 2005).

However, the heterogeneity and the particle size of the CLO render this method impossible to use. An adapted method was formulated where a larger, more representative sample could be studied in a similar manner.

5.1 Aim

To develop a pseudo-thermogravimetric (PTGA) analysis technique to allow the coarse, heterogeneous CLO to be studied with regards to its humic acid content.

5.2 Objectives

1. To determine appropriate sample weights for analysis.
2. To find a suitable temperature range and temperature intervals.
3. To select a comprehensive series of control samples.
4. To design a matrix of standard samples with which the CLO samples can be compared.
5. To employ the developed method against samples stored from previous and taken from subsequent trials.
6. To assess the efficacy of pseudo-TGA as a method for analysing humic material within the sample

5.3 Methodology

5.3.1 Experimental set up

In an experimental trial that is likely to be time consuming with many samples, it is paramount that it is undertaken as efficiently as possible. When deciding upon sample size, it was important to ensure that a representative sample was taken but of a size that would allow several samples to be processed concurrently.

A Carbolite furnace (CSF 1100) was sourced, being large enough to house several samples and also having a suitable temperature range. Two metal trays could fit comfortably into the furnace, each tray holding 18x 20ml ceramic crucibles. This allowed 36 samples to be run simultaneously (Figure 5.1). A sample size of 1g could comfortably fit neatly within each crucible (Objective 1).

When fulfilling trial objective 2, the components that comprise CLO had to be considered and the temperatures at which they combust. Water, having the lowest boiling point, meant that a minimum of 100°C was needed. Of the organic components, the lignin and humic acid would be expected to have the highest combustion temperature, therefore a maximum of 650°C was deemed appropriate.

Before each use, all crucibles were cleaned, rinsed with Milli-Q DI water and then dried over night at 105°C. They were then allowed to cool in a desiccator prior to being weighed to 4 decimal places on a Mettler AJ100 electronic balance. The appropriate weight of sample was then measured into each, labelled crucible. Trays of 2x 18 samples at a time were placed within the furnace (once the specified temperature had been reached and equilibrated) and then heated for a period of two hours (Heiri *et al.,* 2001).

Figure 5.1: Photograph of two of the metal trays, each containing 18 PTGA samples in labelled, ceramic crucibles.

After this period, the trays were removed from the furnace and transferred to a desiccator until cooled to room temperature. Meanwhile, the furnace temperature would then be increased by 50 °C and left to stabilise. Once cooled, the samples were each weighed and again recorded then returned to the oven once the defined temperature had been reached. This process was repeated until all samples had been heated for two hours at each temperature; the furnace was heated at 50°C increments, ranging from 105°C to 655°C

5.3.2 Materials used

Control samples

Duplicate 10g samples of glass, hard plastic, soft plastic, lignin, cellulose, lipid, protein, Aldrich humic acid, MnO_2, sand and char were taken and heated at each temperature for a period of two hours. After heating, each sample was cooled to room temperature and the weight recorded using a Mettler AJ100 electronic balance.

Standard sample matrix

A standard sample matrix was developed using known amounts of each major components of CLO. This allows for the comparison of subsequent CLO samples against this matrix in order to establish the likely composition and more importantly, the proportion of humic aid each contained.

humic acid	lignin	cellulose
0.0	0.9	0.1
0.1	0.9	0.0
0.2	0.8	0.0
0.3	0.7	0.0
0.4	0.6	0.0
0.5	0.5	0.0
0.6	0.4	0.0
0.7	0.3	0.0
0.8	0.2	0.0
0.9	0.1	0.0

Table 5.1: Sample of the standard sample matrix. The full sample matrix can be seen in the appendices.

Each sample weighed 1g in total, in a fully factorial matrix. The method outlined in 5.3.1 was followed; results entered into a Microsoft Excel 2007 worksheet and all data normalised to the 105°C measurement via:

Loss on ignition$_{T2}$ (LOI_{T2}) = ((DW_{T1} – DW_{T2})/DW_{105})*100

Where T1 represents the first temperature interval and T2 the second temperature interval; DW is the dry weight recorded for the sample after being heated to the specified temperature; and DW_{105} represents the dry weight of the sample at 105°C to which all samples are normalised, due to water loss that ought to have occurred. The weight loss should then be proportional to the OC contained within the samples and not affected by moisture content.

Trial 2 samples

Once the aforementioned materials had been analysed, the trial two samples were tested. Around 1 g of each sample was measured and its exact weight recorded. The method outlined in 5.3.1 was followed; results entered into a Microsoft Excel 2007 worksheet and all data normalised to the 105°C measurement.

5.3.3. Statistical analysis

All data were entered into a Microsoft Excel worksheet and then run in Minitab using both Analysis Of Variance (General Linear Model) as used in previous trials, and Principal Component Analysis in order to establish any patterns present in the data.

Principal Component Analysis

Where ANOVA deals with correlation between known variables within a data set, Principal Component Analysis (PCA) deals with uncorrelated variable. Using this method, trends in a multivariate data set can be isolated and a large quantity of data can be reduced, whilst still retaining as much as possible of the variation present. The principal components (PCs) are this smaller number of uncorrelated variables. The lack of correlation is important because it means that the PCs are measuring different dimensions within the data.

During this mathematical procedure, the dataset is transformed into a new coordinate system, in which new axes follow the direction of greatest variance in the data set. The appropriate number of variables is selected. In this case, the variables will be each temperature interval the samples are exposed to. Various combinations are analysed to produce these uncorrelated 'principal components' (PCs) or eigenvectors. PC-1 exhibits the greatest amount of variation, PC-2 the next and so on.

For this study Minitab 13 software was used to calculate the eigenvectors and eigenvalues within the correlation matrix via an iterative process known as the Monte Carlo method. Here, randomly generated data is simulated to assess the probability of the data occurring in the

order that it has by chance. This statistical technique that involves using a large number of repeated calculations is a methodical and formalised version of trial and error.

Once the PCs have been calculated, one must decide how many it is useful to retain; for this trial, all PCs with eigenvalues of ≥1 are retained, plus the first one in sequence that is <1. To ensure the correct number of PCs have been retained, a scree plot can be used; the eigenvalues are plotted against the PC numbers, and the components depicted on the curve of the graph are retained (Figure 5.3).

To make the study viable, it is important to have five times more data points than variables. In this study, there are twelve different temperatures/variables so at least sixty samples were needed. There are eleven standard samples, the sixty-four sample matrix described in section 5.3.2, plus the 3x9 samples from this trial. In total, this gives 101 samples so fulfils the minimum quota criteria.

5.4 Results

5.4.1 Control Sample matrix data

Table 5.2 shows the normalised values calculated for each of the samples and figure 5.3, the resulting temperature curves. Included are manganese dioxide coated sand and biochar ; the former which was used in trial 2 and the latter, to be used in trial 3, both as proposed catalysts; the sand was used as a control amendment during each.

Sample	N105°C	N155°C	N205°C	N255°C	N305°C	N355°C	N405°C	N455°C	N505°C	N555°C	N605°C	N655°C
Fat	1.000	1.000	1.000	0.995	0.940	0.418	0.164	0.000	0.000	0.000	0.000	0.000
Fat	1.000	1.000	0.995	0.974	0.929	0.454	0.189	0.000	0.000	0.000	0.000	0.000
Protein	1.000	0.995	0.995	0.836	0.639	0.546	0.448	0.049	0.049	0.049	0.044	0.033
Protein	1.000	1.000	1.000	0.817	0.624	0.538	0.452	0.065	0.054	0.054	0.054	0.054
Water	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Char	1.000	1.000	1.000	1.000	1.000	0.984	0.911	0.073	0.058	0.058	0.058	0.047
Char	1.000	0.995	0.995	0.995	0.995	0.974	0.885	0.068	0.047	0.047	0.047	0.037
Manganese	1.000	1.000	1.000	1.000	1.000	1.000	0.990	0.970	0.970	0.970	0.970	0.959
Manganese	1.000	1.000	1.000	1.000	1.005	1.005	0.995	0.949	0.949	0.949	0.949	0.944
Sand	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sand	1.000	1.000	1.000	1.000	1.000	1.010	1.010	1.005	1.005	1.005	1.005	1.005
Glass	1.000	0.994	0.994	1.000	0.988	0.987	0.987	0.987	0.987	0.987	0.986	0.987
Glass	1.000	0.985	0.982	0.985	0.986	0.986	0.986	0.986	0.986	0.986	0.986	0.986
Hard plastic	1.000	1.000	0.900	0.900	0.813	0.442	0.115	0.128	0.152	0.149	0.150	0.152
Hard plastic	1.000	1.001	0.943	0.914	0.859	0.483	0.207	0.075	0.121	0.119	0.121	0.121
Soft Plastic	1.000	0.813	0.813	0.751	0.739	0.664	0.538	0.049	0.039	0.038	0.035	0.032
Soft Plastic	1.000	0.892	0.783	0.783	0.723	0.647	0.549	0.039	0.037	0.038	0.032	0.031
Paper	1.000	0.870	0.844	0.741	0.399	0.333	0.282	0.168	0.158	0.156	0.151	0.148
Paper	1.000	0.859	0.841	0.764	0.392	0.306	0.259	0.146	0.135	0.130	0.122	0.117
Lignin	1.000	0.936	0.884	0.341	0.284	0.193	0.166	0.145	0.137	0.135	0.131	0.129
Lignin	1.000	0.930	0.889	0.361	0.301	0.198	0.176	0.152	0.143	0.142	0.138	0.137

Table 5.2: *Each control sample with its normalised gravimetric weight loss after having been heated to the corresponding temperature.*

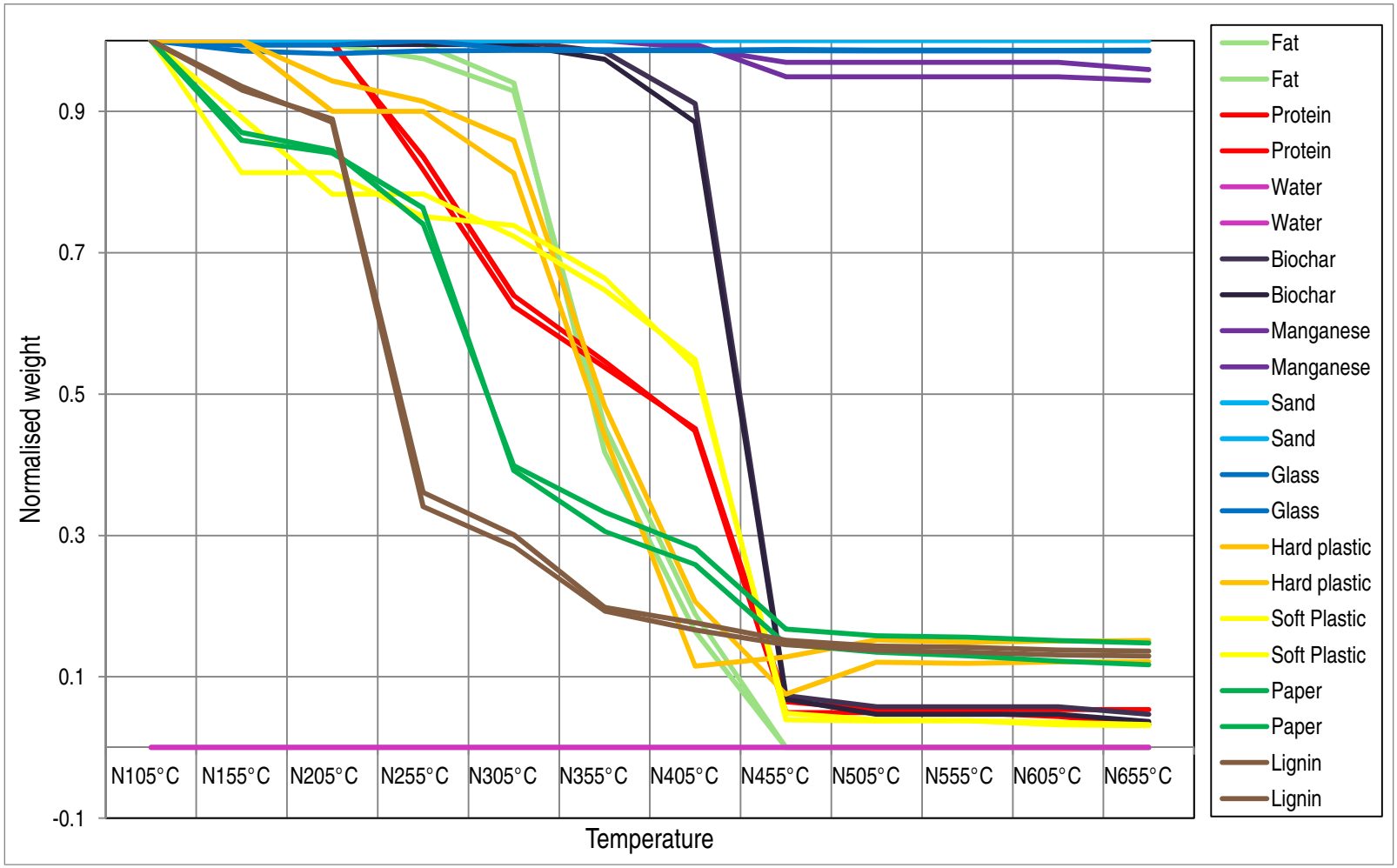


Figure 5.2: Temperature-weight graph showing the various gravimetric trends for the control samples.

It can be seen that water has been fully removed at 105°C. Glass, sand and manganese-coated sand remain more or less unchanged gravimetrically through to 655°C. Each of the other components appears to have characteristic temperature curves that should prove useful in the analysis of bulk CLO. Duplicate samples were consistent, and small anomalies that can be seen (for example, between the MnO_2 –coated sand at 455°C) could possibly be explained by OM on the surface of the material.

Principal Component Analysis

The 11 variables: temperature intervals (155°C-655°C) and their corresponding sample weights were entered into a Minitab 13 worksheet and the PCA calculation run. Two of the eigenvalues generated were greater than 1; a scree plot (Figure 5.3) was drawn to corroborate the number of eigenvalues retained. The first three PCs were retained and the scores for each are given in Table 5.3.

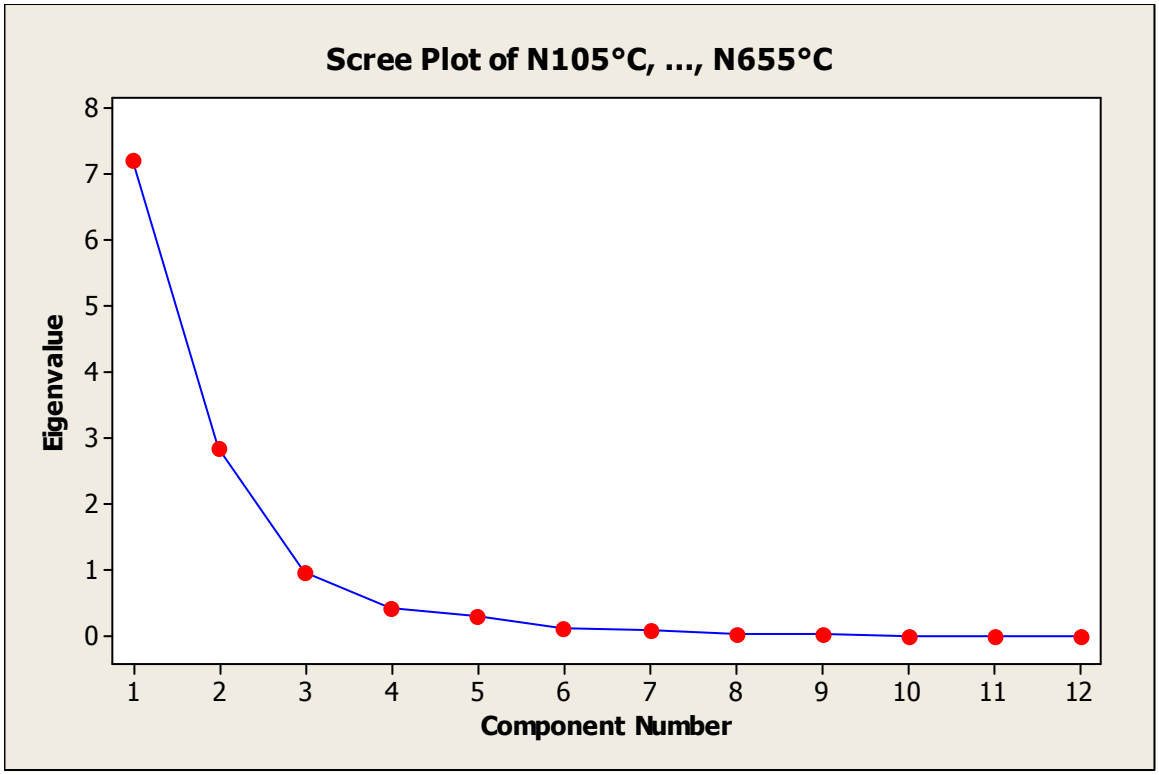


Figure 5.3: Scree Plot for PCA on PTGA data for control samples, used to indicate the number of principal components used.

Sample	PC-1	PC-2	PC-3
Lipid	-1.893	-2.437	-1.691
Lipid	-1.849	-2.346	-1.721
Protein	-1.512	-1.550	-1.076
Protein	-1.456	-1.517	-0.908
Char	0.656	-2.260	-3.070
Char	0.498	-2.205	-3.112
Manganese	8.003	0.853	0.643
Manganese	7.868	0.784	0.536
Sand	8.274	0.967	0.771
Sand	8.344	0.983	0.768
Glass	8.098	1.018	0.712
Glass	8.039	1.211	0.632
Hard plastic	-1.205	-1.089	-0.667
Hard plastic	-1.136	-1.488	-1.067
Soft Plastic	-1.714	1.348	-3.509
Soft Plastic	-1.667	0.638	-2.617
Lignin	-3.051	1.608	0.793
Lignin	-2.926	1.597	0.680
Humic acid	4.288	1.533	-0.141
Humic acid	4.233	1.598	-0.138
Humic acid	4.046	1.499	-0.180
Cellulose	-2.424	-1.231	0.463
Cellulose	-2.210	-1.127	0.406
Cellulose	-2.293	-1.142	0.424

Table 5.3: the principal component scores that explain the greatest variance within the control samples. Dark purple shows the samples with the highest positive score and dark pink, the highest negative score for each component.

PC-1 appears to be defined mainly by manganese, glass and humic acid; the samples with strong negative loadings are the lipid, lignin and cellulose. They seem to negatively correlate with combustibility: the higher the score, the less combustible the component.

For PC-2, the highest loadings are negative and for the char and lipid. Figure 5.4 better illustrates these patterns seen.

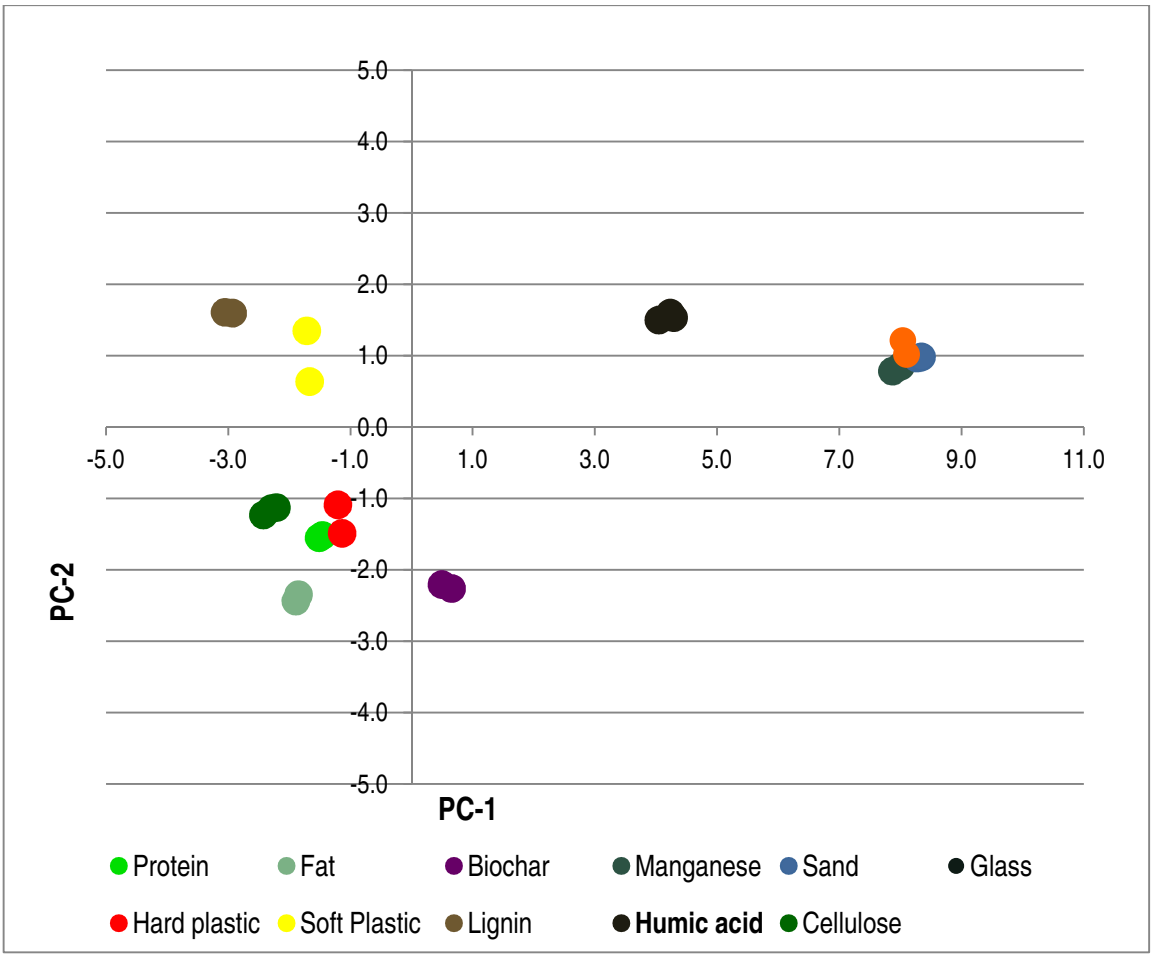


Figure 5.4: Score plot of PC-1 and PC-2 for the control samples.

Figure 5.4 presents clear divisions between the various control samples. The inorganic elements which remain non-combusted at 655°C (manganese-coated sand, glass and sand) each lie to the top right of the plot. The simple compounds (protein, cellulose and fat) occupy the bottom left area of the plot. Humic acid gives a high positive value for PC-1 where as are lignin, a high negative; both give the highest positive values for PC-2. Could this say something about aromaticity, and the difference in positive and negative PC-1 scores about molecular weight?

5.4.2 Standard sample matrix data

As described in section 5.3.2, humic acid, lignin and cellulose were added in discrete quantities in a fully factorial trial. Each of the 64 samples contained a total of 1g of combined materials, the matrix for which can be seen in the Appendix along with the resulting data.

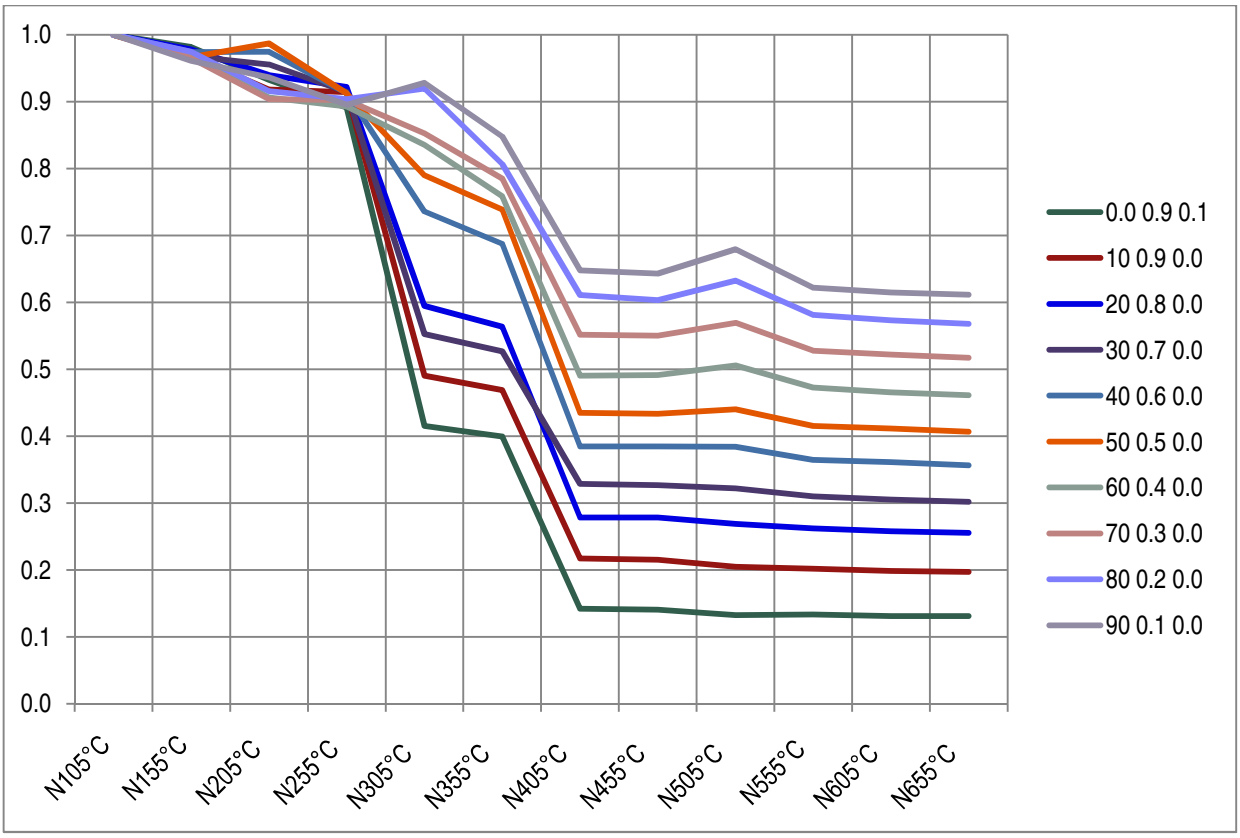


Figure 5.5: Temperature-weight graph, showing the first nine samples; the series show the relative proportions of humic acid (g), lignin (g) and cellulose (g) in this order.

The curves mimic the proportions of humic acid and lignin in each sample; as the humic acid increases and the lignin decreases, the weight measured after each temperature increases; this is illustrated in Figure 5.6.

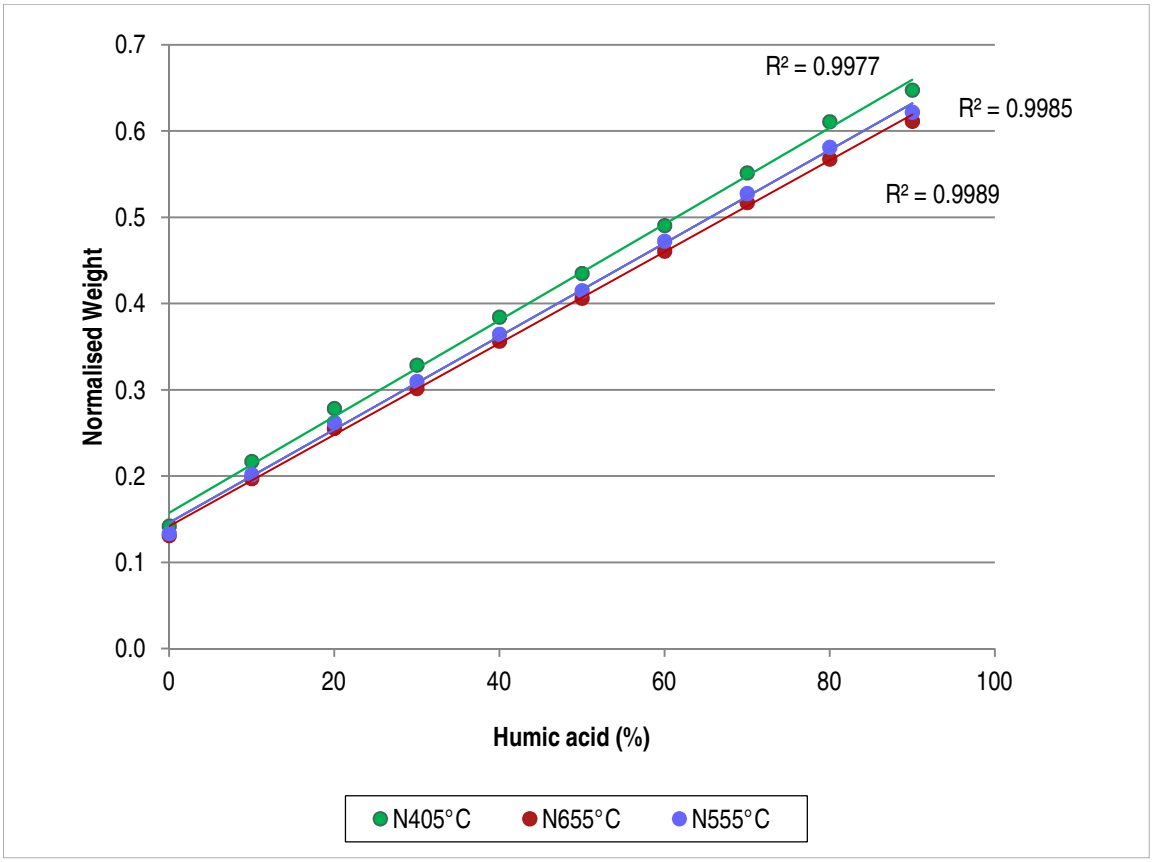


Figure 5.6: the relationship between the percentage of humic acid in the sample and the ensuing gravimetric result for three random temperatures.

Here the relationship between humic acid content of the sample and gravimetric weight loss is shown, with high R^2 values (0.9977-0.9989). Therefore, the samples that weigh the most at temperatures >405°C could contain more HA. However, any of the Trial 2 samples containing sand or MnO_2-coated sand could be misleading if tending to show this pattern.

Principal Component analysis

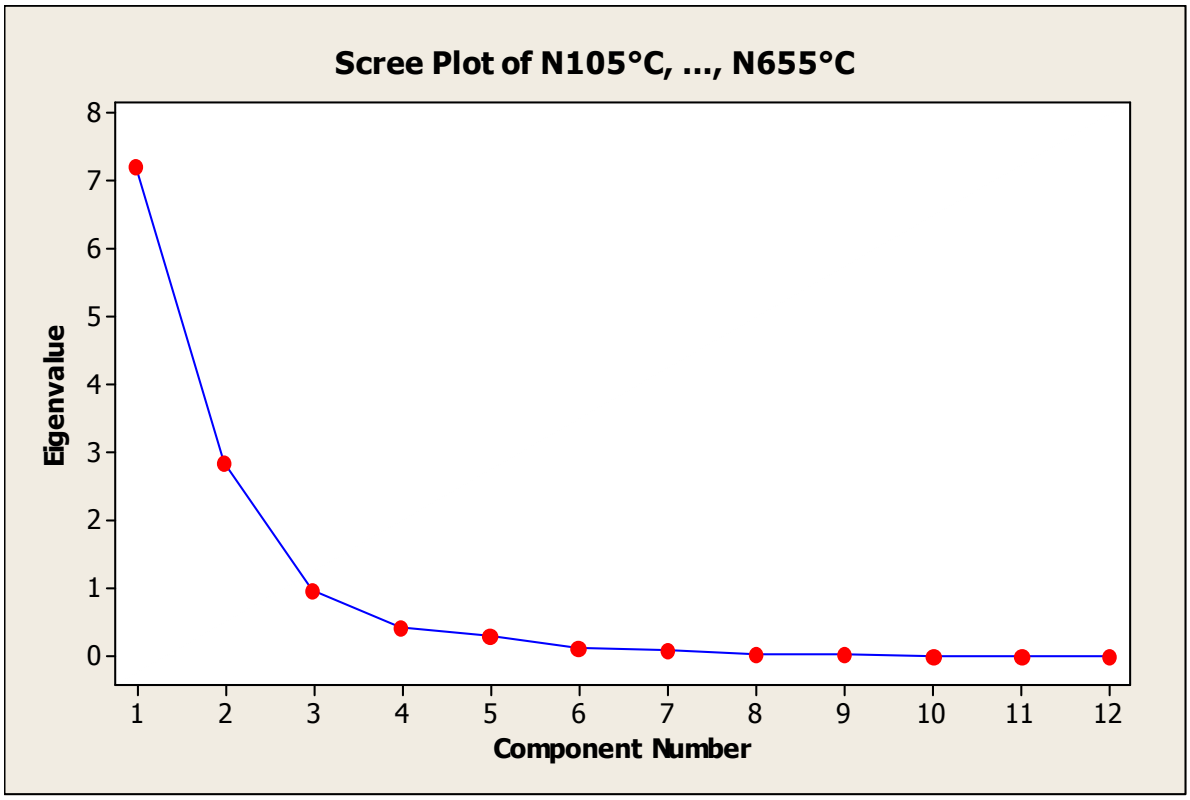


Figure 5.7: Scree Plot for PCA on PTGA data for standard samples.

The principal components were calculated as described in section 5.3.3 the first three retained, based upon the scree plot (Figure 5.7).The loadings for each PC are given in Table 5.4. the most significant positive loadings are indicated by deep violet, the secondary positive by a lighter violet. The most significant negative loadings are highlighted in deep red, the secondary negative loadings by a lighter red.

humic acid	lignin	cellulose	PC-1	PC-2	PC-3
0.0	0.9	0.1	-2.2014	-0.8236	0.1015
0.1	0.9	0.0	-1.1966	-0.4891	-0.0819
0.2	0.8	0.0	-0.1938	-0.3704	-0.3453
0.3	0.7	0.0	0.2877	-0.0759	0.0227
0.4	0.6	0.0	1.3992	0.0020	-0.6378
0.5	0.5	0.0	2.1348	0.2137	-0.7320
0.6	0.4	0.0	2.7912	0.7580	-0.8101
0.7	0.3	0.0	3.5417	1.0495	-0.7439
0.8	0.2	0.0	4.3102	1.2180	-0.7697
0.9	0.1	0.0	4.8721	1.4320	-0.7411
0.0	0.9	0.1	-3.5146	2.3284	-0.4493
0.1	0.8	0.1	-2.1316	1.5828	-0.7410
0.2	0.7	0.1	-1.3579	1.6645	-0.7794
0.3	0.6	0.1	-0.7727	2.1098	-0.5384
0.4	0.5	0.1	0.1589	1.8713	-0.6845
0.5	0.4	0.1	1.1606	1.8119	-0.6680
0.6	0.3	0.1	2.9014	0.3819	-1.1855
0.7	0.2	0.1	3.6348	0.6292	-1.0701
0.8	0.1	0.1	4.6941	0.5762	-0.8685
0.9	0.0	0.1	4.7422	1.3990	-0.8766
0.0	0.8	0.2	-3.8406	2.3224	-0.3043
0.1	0.7	0.2	-2.0230	0.6923	-0.4088
0.2	0.6	0.2	-0.5327	-0.4431	-0.3121
0.3	0.5	0.2	0.0677	-0.2593	-0.2583
0.4	0.4	0.2	0.9494	-0.1215	-0.3979
0.5	0.3	0.2	1.5733	0.1207	-0.3369
0.6	0.2	0.2	2.4440	0.2885	-0.4434
0.7	0.1	0.2	3.0594	0.5094	-0.5421
0.8	0.0	0.2	3.8232	0.6650	-0.4740
0.0	0.7	0.3	-2.6115	-0.9104	0.3484
0.1	0.6	0.3	-1.4489	-1.3255	0.3501
0.2	0.5	0.3	-0.5481	-1.0936	0.1871
0.3	0.4	0.3	0.0311	-0.9921	0.3347
0.4	0.3	0.3	1.0169	-0.7678	0.2628
0.5	0.2	0.3	1.7571	-0.6548	0.2918
0.6	0.1	0.3	2.4728	-0.4902	0.1610
0.7	0.0	0.3	3.5458	-0.2300	0.2596
0.0	0.6	0.4	-2.1480	-1.7167	0.2714

humic acid	lignin	cellulose	PC-1	PC-2	PC-3
0.1	0.5	0.4	-1.0067	-1.8622	-0.8344
0.2	0.4	0.4	-0.7722	-1.2766	0.8145
0.3	0.3	0.4	-0.0003	-0.9021	0.6970
0.4	0.2	0.4	1.0703	-0.7028	0.5193
0.5	0.1	0.4	1.7946	-0.5932	0.4257
0.6	0.0	0.4	2.1227	-0.5484	0.4446
0.0	0.5	0.5	-2.6788	-1.5733	0.9417
0.1	0.4	0.5	-1.6930	-1.3548	0.9038
0.2	0.3	0.5	-0.7702	-1.2402	0.6894
0.3	0.2	0.5	0.1234	-1.0524	0.5749
0.4	0.1	0.5	0.7862	-0.9235	0.5358
0.5	0.0	0.5	1.5026	-0.7180	0.6600
0.0	0.4	0.6	-3.1467	-1.5029	0.9052
0.1	0.3	0.6	-2.2158	-1.3829	0.9017
0.2	0.2	0.6	-1.5008	-1.2902	0.5351
0.3	0.1	0.6	-0.8094	-1.1130	0.6506
0.4	0.0	0.6	0.1290	-0.8931	0.8380
0.0	0.3	0.7	-3.4171	-1.7259	0.8488
0.1	0.2	0.7	-2.6037	-1.5048	0.9285
0.2	0.1	0.7	-1.8710	-1.3381	1.0059
0.3	0.0	0.7	-0.5697	-1.2862	0.0863
0.0	0.2	0.8	-3.3417	-1.8402	0.5433
0.1	0.1	0.8	-0.0918	-2.2007	-2.7316
0.2	0.0	0.8	0.7084	-1.8816	-2.6205
0.0	0.1	0.9	-0.9064	-2.3704	-2.6401
0.1	0.0	0.9	-1.0362	-2.4567	-2.7793

Table 5.4: The principal component scores that explain the greatest variance within the matrix samples.

For PC-1, the primary positive scores are highlighted in purple and the negative in red. One pattern that emerges is that most of the positive values seem to be dictated by high humic acid with low lignin contents whilst the high negative values seem to be driven by high lignin contents. The score plot shown in Figure 5.8 highlights the main patterns in data seen using pie charts of the relative proportions of humic acid, lignin and cellulose seen.

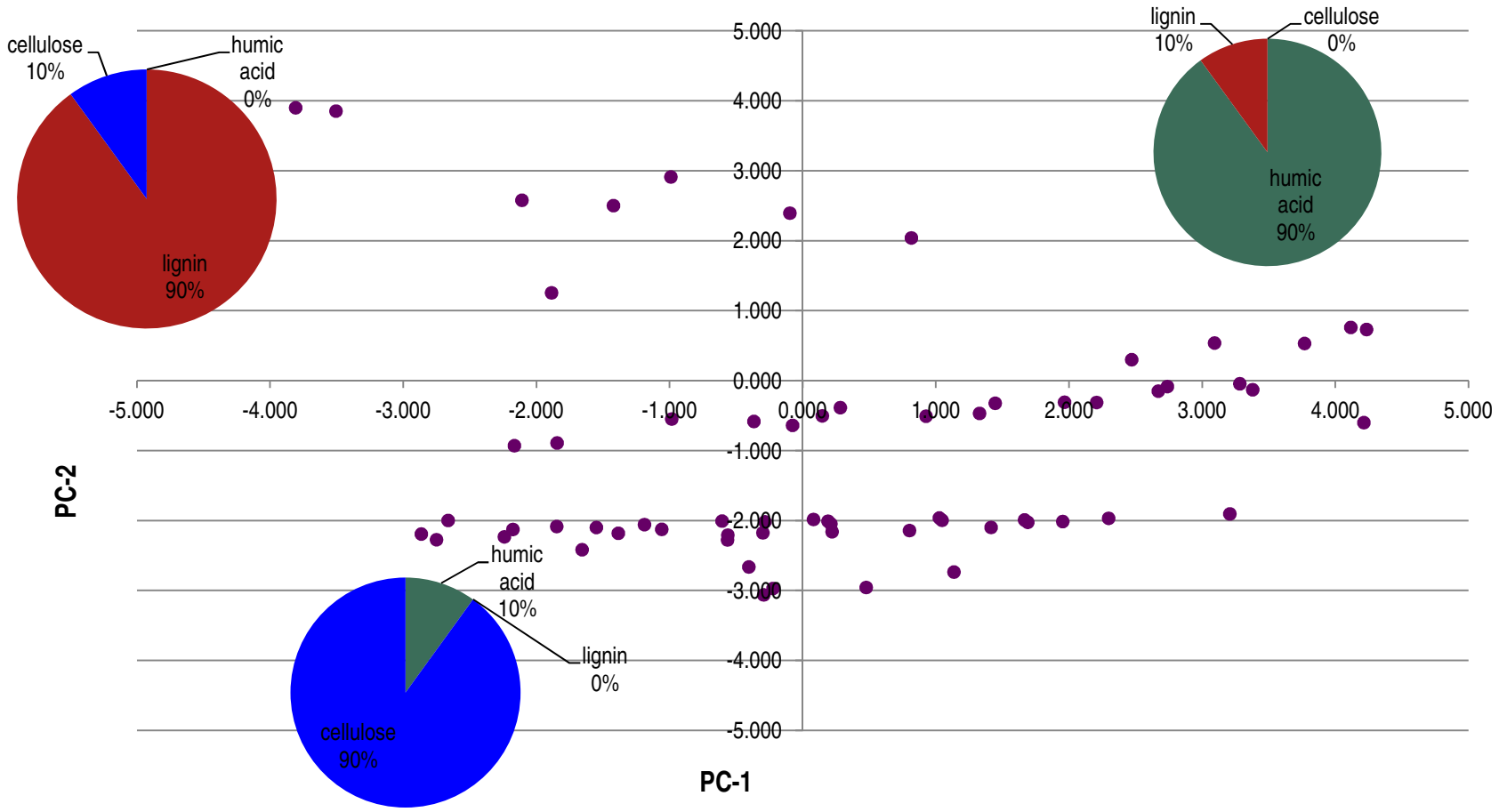


Figure 5.8: Score plot of PC-1/PC-2for the analysis of the standard samples

The patterns observed in Figure 5.4 for the control samples are reflected here; the 90 % humic acid samples occupy the top right of the plot (high positive scores for both PC-1 and PC-2); the 90% lignin sample has high positive scores for PC-2 but high negative scores for PC-1; and the 90% cellulose has high negative PC-1 and PC-2 scores.

Through PCA, it is anticipated that the samples with known components will characterise those samples of unknown components; both the control sample matrix and the standard sample matrix data should define the CLO sample data and help to establish the main constituents that have determined the gravimetric data. The matrices will be used for Trial 2 and Trial 3 CLO samples and analysed using this method to establish whether or not it is sufficiently effective in the quantification of humic acid present. The traditional TGA methodology relies on a certain degree of precision so it is imperative that a similar degree is observed using the adapted method if the results are to be reliable.

5.4.3 Trial 2 data

Sample	CLO %	Sand %	Mn %
1	100	0	0
2	95	0	5
3	90	0	10
4	95	5	0
5	90	5	5
6	85	5	10
7	90	10	0
8	85	10	5
9	80	10	10

Table 5.5: the trial matrix for trial 2, included here for ease of reference.

Figures 5.9-5.11 show the temperature-weight graphs for each sample at weeks 1, 5 and 10. Sample 9 was lost at 5 weeks so no data exists.

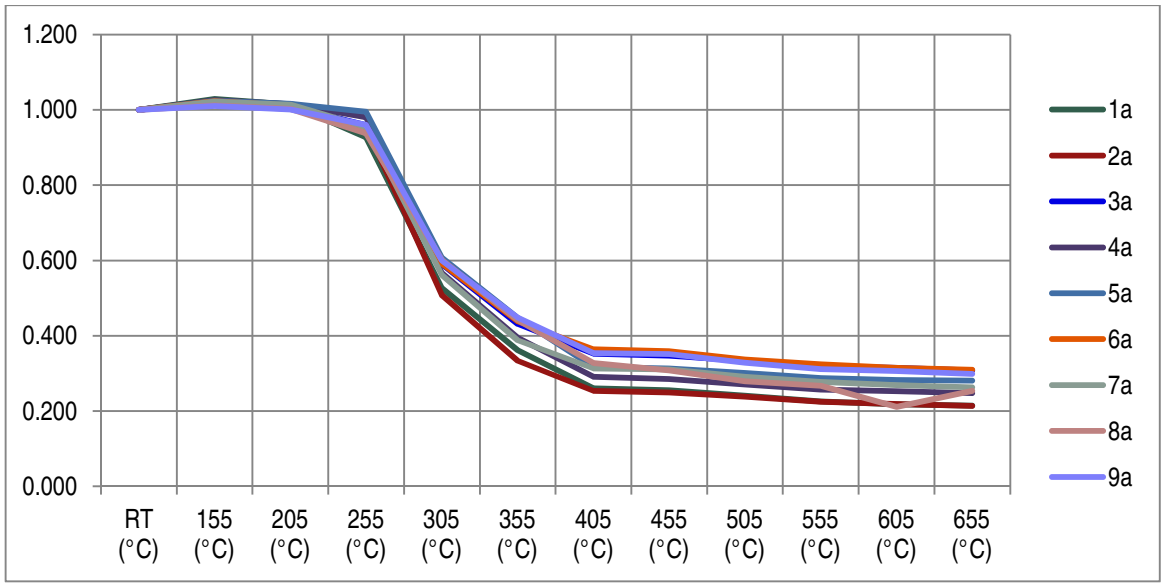


Figure 5.9: Temperature-weight graph for week 1 samples (each series is the mean of duplicate samples)

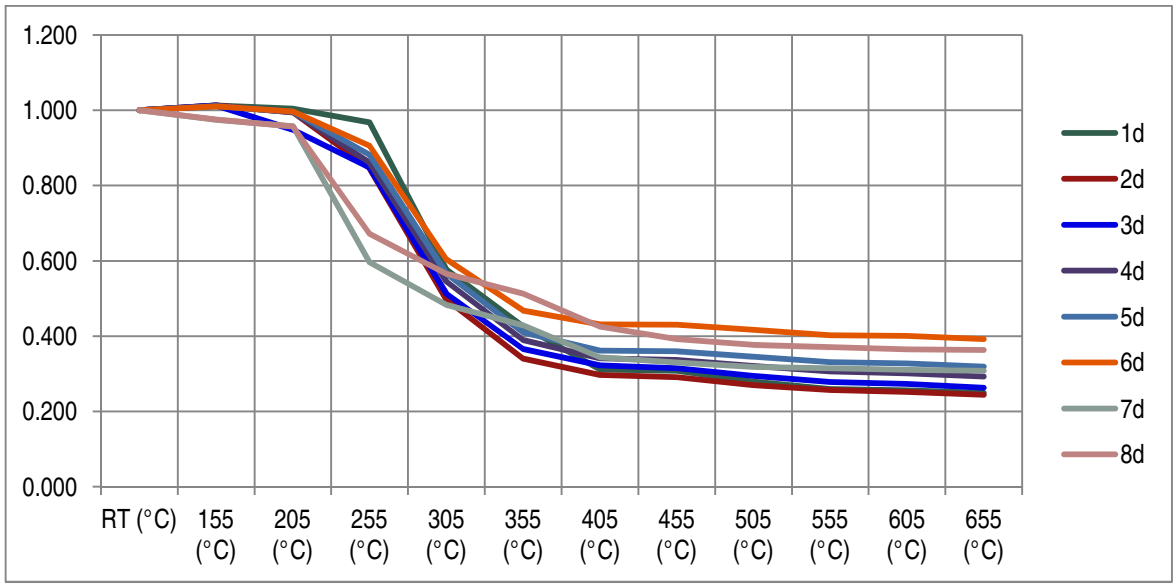


Figure 5.10: Temperature-weight graph for week 5 samples (each series is the mean of duplicate samples)

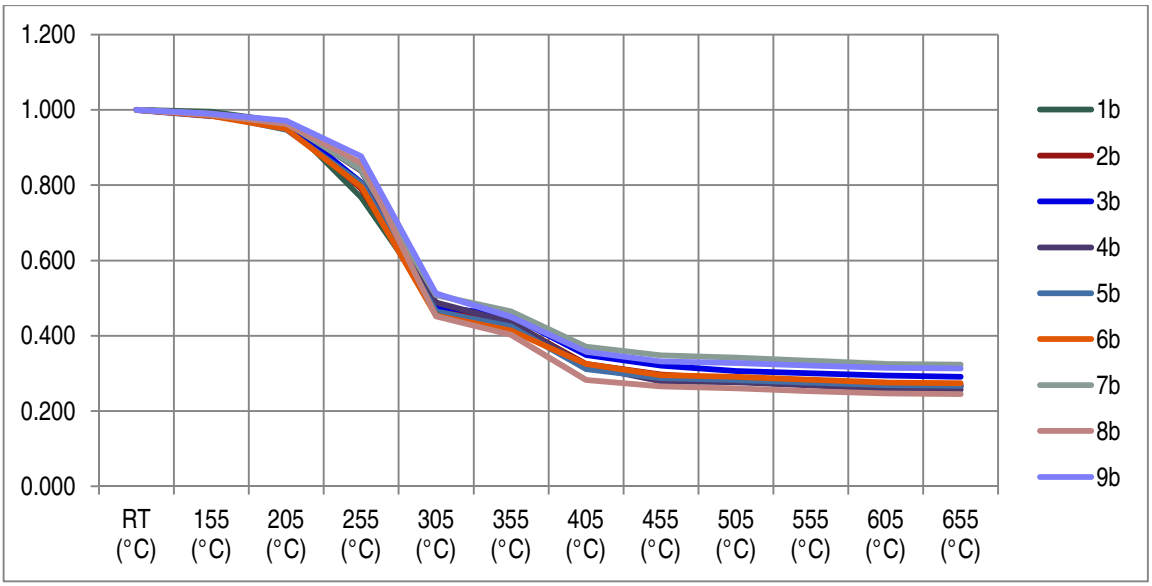


Figure 5.11: Temperature-weight graph for week 10 samples (each series is the mean of six replicate samples)

Differences in the curves between weeks 1 and 10 were can be observed between 205°C and 405°C which may indicate differences in the amount of labile material present. During week five the samples with 10% sand with 5% Mn, and 10% sand with 10% Mn slightly deviate from the other samples. At this stage, they show a significant loss on ignition at 205°C, much like the week 10 samples. Analysis of variance of the data sets should highlight any significant differences in samples over time and between samples, as compared to the control.

Analysis of Variance

There was a general trend for the samples to differ at each stage of the trial at both 155°C and 205°C. The weight dropped significantly between weeks 1 and 10 at 255°C. At 405°C, the samples weighed slightly more at week 5 than at week 1. At the higher temperatures, week 5 shows the greatest spread between samples. 6 replicate samples were tested at week 10, only 2 for weeks 5 and 10; this may have some bearing on the spread of data.

Few statistically significant differences were observed between samples. Samples 7 and 9, which both contain 10% sand, had a significantly higher weight than the control sample at higher temperature. This could simply be by virtue of the fact that a proportion of sand was present in the sample. When sub-sampling the lysimeters, it is difficult to know exactly how much sand or manganese-coated sand is present

The two samples which show significant changes over times are 6 and 8 which have 5% sand, 10% Mn and 10% sand, 5% Mn respectively. Both record a higher weight at week 5 than at week 1; at week 10 however, there is little difference seen between them and the week 1 samples. This trend occurred at temperatures >305°C. At temperatures ≥ 555°C, sample 7 was the sample that retained the most weight at the end of the ten week period. These samples are the only three samples that contain 10% sand. If it was the sand that remained, then perhaps the same should have been seen for samples containing 10% Mn coated sand also.

Principal Component Analysis

The data trial 2 data was analysed using PCA. Using the scree plot (figure 5.12), the first 3 components were retained.

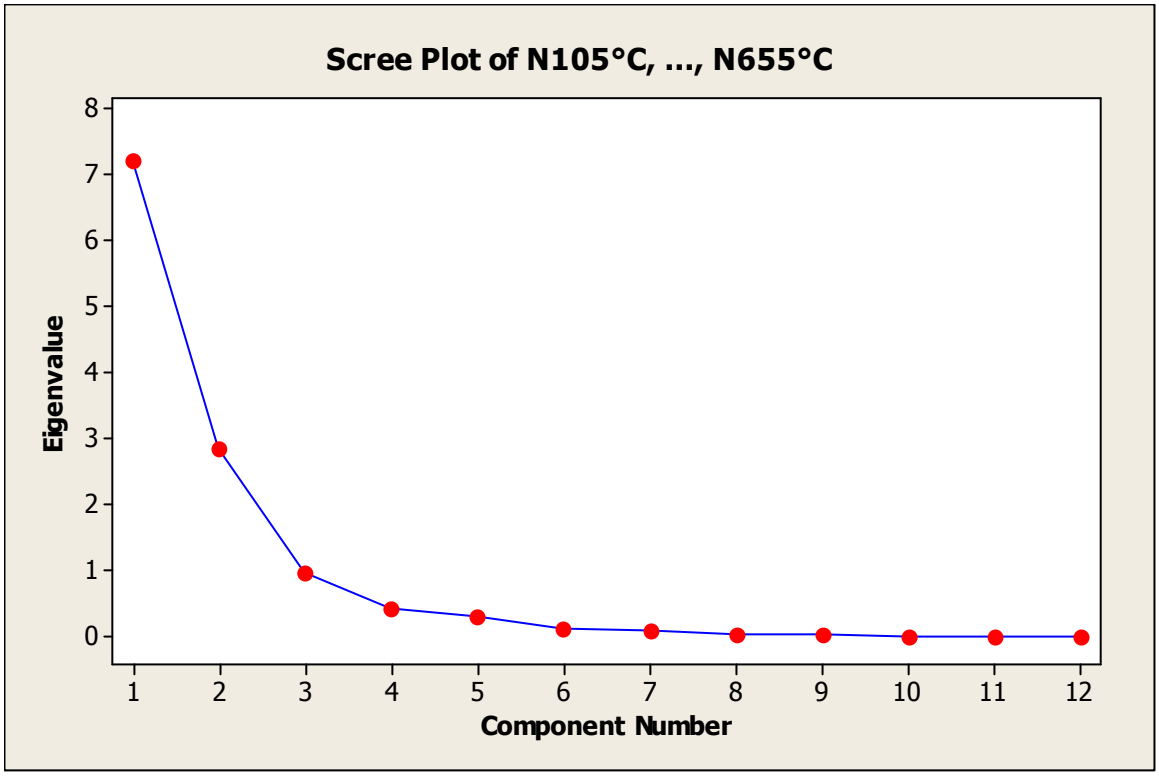


Figure 5.12: Scree Plot for PCA on trial 2 PTGA data.

The first 3 PCs were scored for the entire dataset and plotted against one another in Figure 5.13. In the overall distribution of these plots, at least one clear trend can be seen in the increasing PC-1 against increasing PC-2. PC-3 shows less clear trends against the other two components.

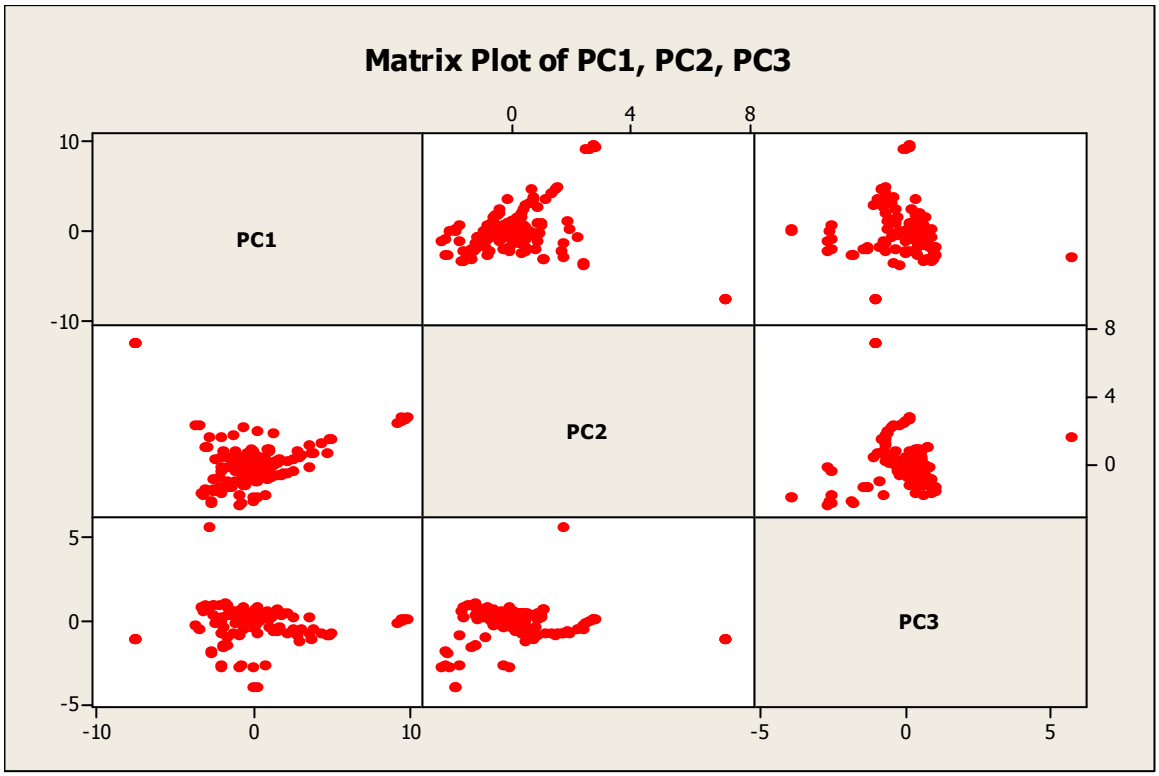


Figure 5.13: Principal component analysis plots showing the main trends in the PTGA data.

The score plots in Figure 5.14 and 5.15 show PC-1 plotted against PC-2. For Figure 5.14, no patterns can be seen between samples with different amendments. Figure 5.15, showing the difference between samples at each week does show a trend; when compared to the control and standards matrix, week one appears to have more labile material and week 10 more humic substances.

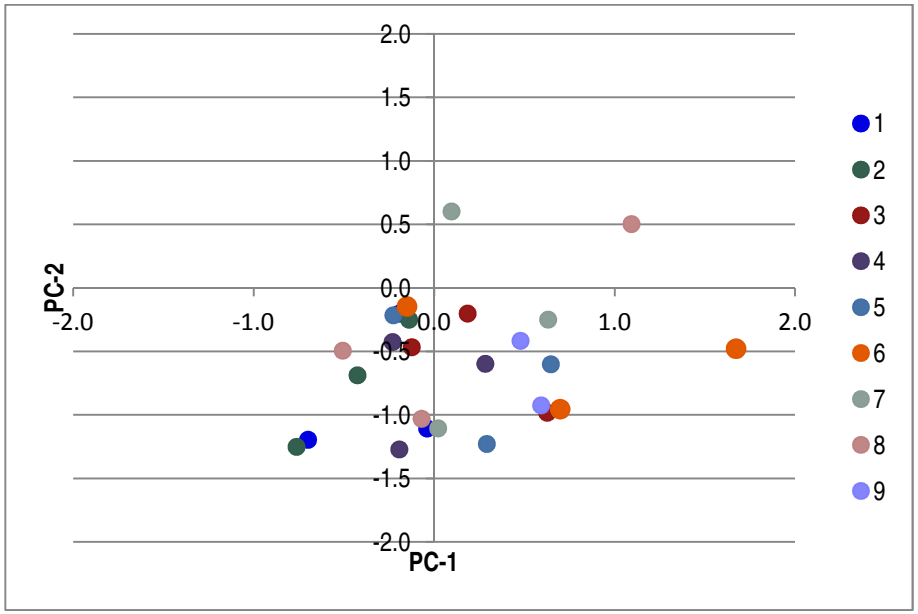


Figure 5.14: Score plot of PC-1 against PC-2 to illustrate differences between thetrial two samples.

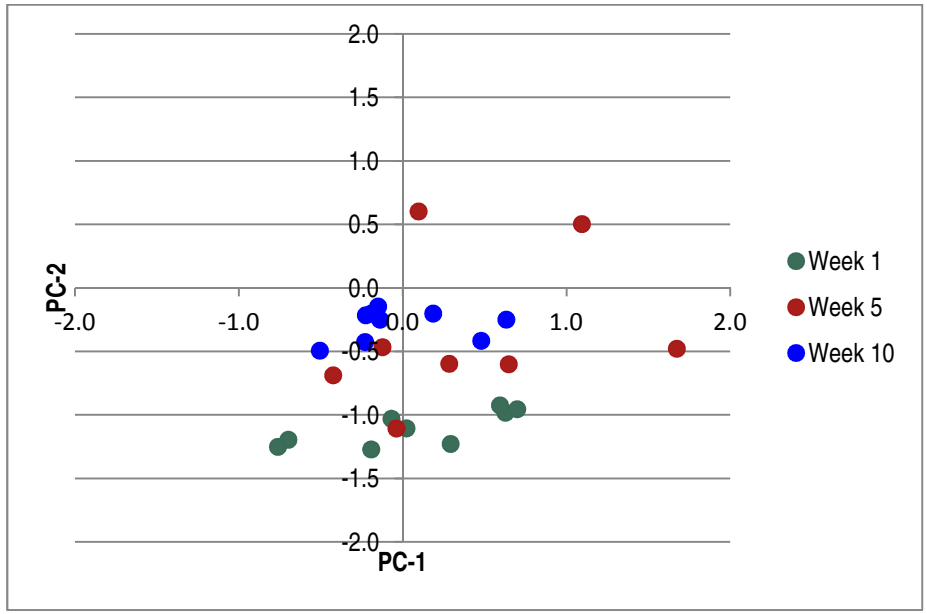


Figure 5.15: Score plot of PC- against PC2 to show the difference between the trial 2 samples at each week

5.5 Conclusions

5.5.1 Revisiting the objectives

1. *To determine the appropriate weight of sample for analysis.*

The sample weight chosen was a compromise between the numbers of samples that could be processed simultaneously and an adequate size that would account for the material's heterogeneity. An attempted was made to remove any large contaminating particles to ensure that the samples contained as much OM as possible. However, it was not possible in the time available to remove all contaminants so the OM in each sample will differ slightly. Any samples with hard plastics in, for example might show less of a loss on ignition at the higher temperatures than the sample ought to.

2. *To find suitable temperature range and temperature intervals.*

Again, it was necessary to cover the required span, with enough increments to capture suitably informative temperature curves with a consideration for the practicalities of analysis of a large number of samples. A range of 105°C - 655°C was chosen to analyse the organic components present. Differences can be seen between cellulosic and refractory components over the temperature ranges over the trial period. From the control samples, the only components that showed very little loss on ignition were the sand, manganese-coated sand and glass, where the weight of all organic material had significantly increased. The range and intervals chosen therefore were deemed appropriate.

3. *To select a comprehensive series of control samples*

The major contaminants were analysed, along with the three added materials (sand, MnO_2-coated sand and charcoal). When PC-1 was plotted against PC-2, some interesting patterns were seen with which to compare the standard matrix and trial samples. If this aspect of the trial were to be repeated, some ferrous and non-ferrous metal could also be tested as both are presents as contaminants in the CLO.

4. *To design a matrix of standard samples with which the CLO samples can be compared*

A matrix has been developed with which to compare samples in subsequent trials. Three of the main components of CLO were used in the construction of the control matrix (humic acid, lignin and cellulose). The trial with the major organic components, however, lacked protein and lipid. It was not until Trial 4 that it was decided to include these constituents. The matrix as it is produced some interesting patterns and correlated well with the controls matrix when the score plots in Figures 5.4 and 5.8 were compared. The protein and lipid in the control sample seem to occupy the same area of the chart as the cellulose so if these other labile components had been included in the standard matrix, it might have been difficult to differentiate between these components.

5. *To employ the method against the stored samples from previous trials and samples taken from subsequent trials.*

When this method was used with the stored samples from Trial 2, few trends were observed. The samples with 10% sand all appeared to differ in their temperature curves to the other samples tested. These samples all presented less of a loss on ignition than the other samples at the higher temperatures (>405°C). This was not true of the samples that contained 10% MnO_2-coated sand which would be expected if this was purely due to the physical effect caused by having a higher degree of non-cellulosic material present in the sample.

To test the matrix plot against the trial samples comprehensively, it would be necessary to run some complementary spectral analyses to prove that the samples that appear to have more lignin, humic acid or cellulose, do in fact do so and to rule out any skewing that could be caused by sand and MnO_2 –coated sand present in the samples.

Trial 3, discussed in the following chapter was a longer study. This PTGA method was repeated on samples from this trial and this objective revisited (see Section 6.8.1).

6. *To assess the efficacy of Pseudo TGA as a method for analysing humic material within the sample*

Precision was seen between replicate samples and the similarity between the control and standard matrix indicated a degree of accuracy. However, when manually weighing the sample between each combustion, spillages can happen which can effect this precision. The balance used must be sensitive enough to detect minor changes in weight. Further complementary spectral analysis would be needed to fully assess the robustness of this method, possibly in the form of a validation trial.

Ultimately, this method has the potential for effectively analysing trends seen in the samples temporally. Used in conjunction with PCA, trends seen in the humic acid content of samples over the trial period were seen. As in Chapter 4, very few significant trends were seen between control and amended samples. Perhaps the longer Trial 4 will highlight any noticeable changes in the humification process on the addition of manganese-coated sand.

5.5.2 Trial limitations

The standards matrix contained only humic, lignin, and cellulose when it perhaps ought to have included other groups such as lipids and proteins. However, there had to be limitations otherwise number of samples would have been too large, meaning that the analysis would not have been carried out in the time available.

The time factor also meant that the standards matrix was not analysed with replicate samples. The control samples and Trial 2 samples were each tested in duplicate and triplicate and a high degree of precision was seen. If the trial were to be repeated, some samples from the matrix ought to be duplicated to assess precision.

When taking samples from the lysimeters, one does not know how much sand/ Mn-coated sand is being removed. If the amount of CLO present in the sample is factored in to the normalising stage of the data analysis, there is no guarantee that 5 % or 10% of added material is necessarily going to be included in that particular sub-sample.

The furnace used may not have always given accurate temperatures. Often, the temperature was set and allowed to stabilise but when the door was opened to load the samples, the furnace overcompensated when re-equilibrating. This meant that some batches of samples may have been exposed to higher temperatures than intended for some temperature increments.

Chapter 6

The effect of char on the humification of CLO

This chapter details the design, implementation and results for the third experimental trial. Following trial 2, these experiments seek to build upon gained knowledge by specific adaptation to the experimental design. With certain modifications, it is hoped that the results from this trial might see less variation between samples and perhaps more agreement in the data derived from the various analytical methods. Samples with char will be added to the experimental matrix, as well as samples with sand, and manganese-coated sand, as before. It is anticipated that char will encourage the production of humic substances in the CLO as it has been proven to do in some natural soil systems.

6.1 Introduction

Following the legal action imposed by the Environment Agency on PWM in December 2007, CLO samples were unavailable analysis until September 2008. It was intended that this trial would run for twelve months; due to time constraints imposed by the lack of available of materials, the trial was designed to run for six months instead.

With an increase in kerb-side collection of recyclables, it was hoped that the waste might be a little better source-separated than with previous batches. Again, an attempt was made It was manually filter the CLO in order to obtain a more homogeneous material to analyse. The expectation was that this might give a clearer picture of the processes occurring within the organic fraction. The results would then be presented as what could potentially be achieved, should the CLO be better separated before it was applied to land. However, this proved to be impractical and so was abandoned once again and the whole material used.

6.2 The addition of char to soils

Black carbon (BC) or char is found in soils around the World as a result of wildfires and historic management practices. Slash and burn agriculture is practiced by around 300-500 million people globally, and affects around 1/3 of the planet (some 1500 million hectares of arable land) (Steiner *et al.*, 2007). Probably the most studied soil with BC is the Terra Preta or Amazonian Dark Earth (ADE), an anthropogenically carbon-enriched soil dating back some 9000 years which stabilises SOM, thereby increasing crop yield and fertility when compared to adjacent soils. (Steiner *et al.*, 2007; Solomon *et al.* 2007). Native infertile soils such as these can be transformed into fertile soils by addition of stable carbon in the form of char. This is known to reduce soil acidity and can also reduce the need for chemical fertilisers, meaning that the environmental benefits can be achieved at a relatively low capital costs. Such benefits are especially pertinent in developing countries.

The increased fertility is due to enrichment with phosphorous, magnesium, zinc and manganese; the increased water-holding capacity, cation exchange capacity (CEC), and pH. (Solomon *et al.* 2007; Fowles *et al.* 2007; Laird, 2008). ADE could decrease desertification, sequester atmospheric C, maintain biodiversity hotspots and decrease pressure on primary forests that are being extensively cleared for agricultural use with only limited fertility and sustainability (Glaser, 2007). Anthrosols rich in BC, when compared to BC-poor adjacent soils, assimilate added organic matter far better and have up to 125% more microbial biomass (Liang *et al.* 2010). ADE is thus an incredibly important carbon sink.

Having observed the beneficial effect that char can have on highly weathered, acid soils many studies have been conducted to examine char's potential as a viable tool for carbon sequestration in other part of the World. With its long-term chemical stability (compared to uncharred biomass), buried char could trap CO_2 within the pedosphere thus enhancing global carbon stores and reducing carbon emitted to the atmosphere.

6.2.1 Pyrolysis and biochar

The primary release mechanism for GHGs is the extraction and burning of fossil fuels from Earth's geological reservoir (Fowles, 2007). If fast-growing biomass or biomass waste could be used instead and burnt in a more sustainable way, this problem could be addressed.

Pyrolysis is thermochemical process where biomass is heated in the absence of oxygen producing heat, energy and char as a 'waste' product. Being based on a relatively mature technological premise, having a short processing chain (therefore inexpensive) and being fairly accessible to testing, it is subjected to increasing interest worldwide. The United States could potentially displace 1.91 billion barrels of fossil fuel a year (25% of current US consumption) using biomass to energy plus biochar (Laird, 2008).

Combining pyrolysis for bioenergy with the application of the char to soil may offer a strategy to reduce GHG emissions and deliver other environmental benefits. Char allows an ever-increasing C sink to be built up in soil, one which lifts agricultural production and limits leaching of nitrates into water (Winsley, 2007).

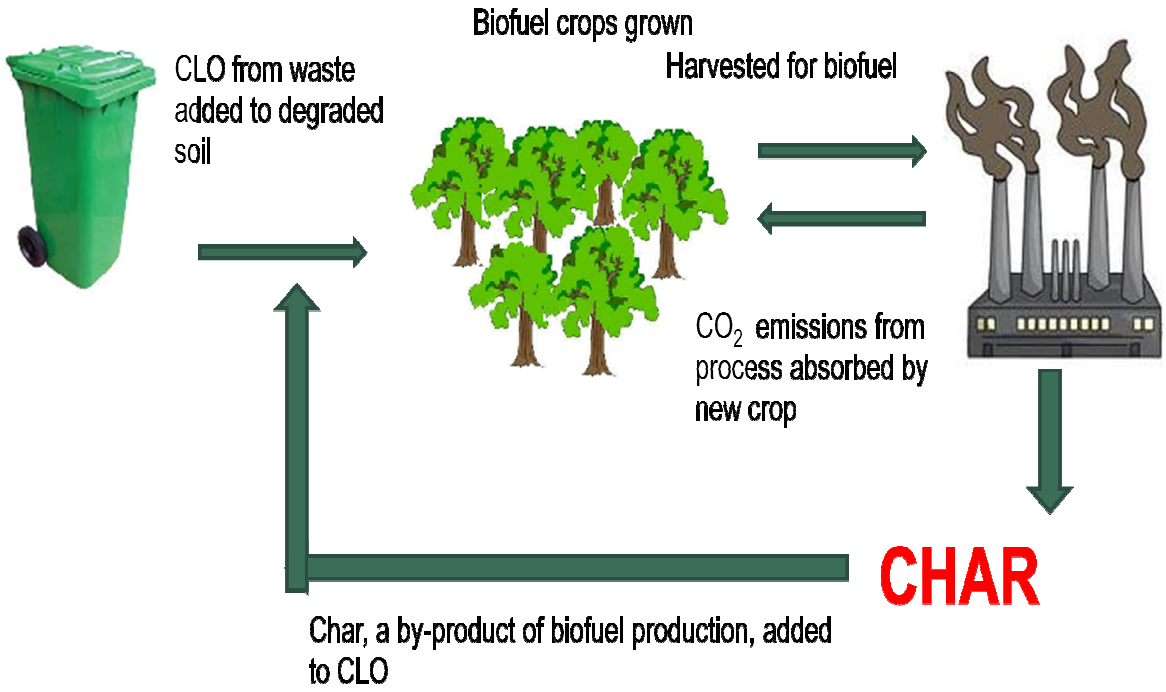


Figure 6.1: The 'waste to energy diagram re-visited with the addition of char.

Char produced via the pyrolysis process is now commonly called biochar. The UK Biochar Research Centre (UKBRC), based at the University of Edinburgh, give the following definition of this product:

> "Biochar is a black carbon material produced from the decomposition of plant-derived organic matter (biomass) in a low or zero-oxygen environment (i.e. pyrolysis or gasification) to release energy-rich gases which are then used for producing liquid fuels or directly for power and heat generation."

There are a broad range of materials that are currently defined as biochar. However, these chars vary depending on the type of feedstock (crop waste, energy crop, wood chip, municipal waste, and manure for example) and production process (mainly temperature, pressure and time). Such variables may determine the use or potential use of the product obtained.

6.2.2 Production process

The main technologies for producing biochar are fast, moderate and slow pyrolysis and gasification. Pyrolysis produces between 12 and 35% biochar with slow pyrolysis (at about 500 ^{o}C and with a very long vapour residence time of between 5 and 30 minutes) giving the best biochar yields. Gasification occurs at a higher temperature of at least 750^{o}C with a moderate vapour residence time of 10 to 20 seconds and generates approximately 10% biochar (UKBRC). Slow pyrolysis tends to produce more uniform chars where as fast pyrolysis gives more heterogeneous products (Smith *et al.* 2010).

Any carbon dioxide emitted from the process can be taken up again by biofuel crops making it a closed system. However, as Schalmadinger *et al.* (1995) state, the combustion of biomass and tree growth do not take place on the same timescale and in harvesting dedicated biofuel crops; part of the soil carbon pool size might decrease when part of plant litter is removed for bioenergy.

Problems occur in the developing world where earth pits/mounds; brick, concrete or metal kilns; or retorts are used in a batch process. This produces a low yield with no heat productions and significant environmental pollution. In the developed world, a closed system is generally used to avoid this.

Some authors have questioned whether or not this process is energetically favourable. Gaunt and Lehmann (2008) examined the energy balance and emissions associated with pyrolysis bioenergy production, combined with the sequestration of the biochar produced. They found that the land application of biochar reduced GHG emission to a greater extent than when the biochar was used purely as an alternative to fossil fuels.

6.2.3 Feedstocks

The feedstock used defines the biochar's complex structure, with some feedstocks being more productive than others. The wide variety of potential feedstocks includes:

- Dedicated biomass crop (e.g. Miscanthus, willow)
- Crop co-product/bi-product/residue (e.g. wood/timber wastes, leaves)
- Waste materials (e.g. agricultural, manure, paper mill sludge, food waste, green waste)

There are some concerns that dedicated biofuel crop like Miscanthus would remove carbon from the biosphere on harvesting; although this is a fast growing crop, perhaps waste residues are a better option, providing they can produce a suitable product.

6.2.4 Biochar product

Huge variability is seen in physical biochar structures, depending upon the parent material and formulation conditions, which leads to different turnover times in soils. Large charcoal particles from forest fires can stay in soils for thousands of years whereas smaller particles from grassland burning are barely detectable in Steppe ecosystems (Steinbeiss *et al.* 2009).

In a study on forest fire-derived BC in Western Kenya, Nguyen *et al.* (2008) found that BC degraded rapidly in soil over a 30 year period and then settled to a steady state. A mean residence time of only 8.3 years was calculated for BC, with losses via decomposition and transport processes; this is in general agreement with the results reported by Steinbeiss *et al.* (2009). However Kuzyoukov *et al.* (2009) found that char from a perennial Rye grass had a mean residence time of around 2000 years.

Regardless of feedstock source or synthesis method, some common benefits (improved water-holding capacity, CEC, soil fertility, soil aggregation and carbon content of amended soil) are observed (Smith *et al.* 2010). However, researchers at East Malling developed a biochar made from seaweed and found that has a vastly different mineral concentration to wood biochars; particularly high concentration of Cl was detrimental to plant growth. On the whole, addition of seaweed char severely reduced growth or killed maize plants when applied at rates greater than 20 gl^{-1}. When attempting to characterise chars and biochars this heterogeneity, and chemical complexity present many analytical challenges (Lopez-Capel, 2010).

The functional group chemistry of ADE is enriched with aromatic C structures, O-rich organic C moieties and a diverse group of refractory aliphatic compounds, possibly the key factor for its biochemical recalcitrance (Solomon *et al.* 2007). In forest fire-derived BC, surface molecular properties change more rapidly than the core with carbonyl groups increasing over the first 10-30 years (Nguyen *et al.* 2008). Unlike other components of SOM, char is largely made up of strongly bound, highly resistant moieties making it a potentially highly valuable carbon store. The structure of biochar is largely amorphous but contains highly conjugated aromatic compounds in a crystalline structure (Downie et al., 2009). There are voids in the biochar structure formed as micro and macropores and cracks from biomass origin (UKBRC, 2010).

There has been some suggestion that humic acids in soils are derived from BC rather than plant material. Haumaier and Zech (1995) discovered that oxidised BC showed “remarkable similarities” to the highly aromatic humic acids found in soils in their chemical composition and spectroscopic properties. Laird *et al.* (2008) poses the question that if BC in soil contributes substantially to the total aromatic carbon content of SOM then is the aromatic prevalence overstated in the various proposed models of HS structure?

6.2.5 Function/Reactivity in soils

Soil is independently variable and complex (chemically, biologically, and physically) in space and over time and can change with human intervention. Char is emerging as a similarly independent, diverse material which is complex both physically and chemically. The impact of char on soil is therefore unpredictable, especially when combined with random factor of plant interaction (Sohi, 2010).

The half-life of C in char in soil is greater than a thousand years, meaning a lasting contribution to soil quality and sequestered atmospheric carbon for millennia (Laird, 2008). Generally, the efflux of CO_2 from BC is so small that it cannot be compared to that of SOM (Kuzyoukov *et al.* 2009).

Many studies have shown that soils, on the addition of char, exhibit short-term increased mineralisation (usually >20 days) (Smith *et al.* 2010). Once this trend has passed, it is thought that a long-term storage effect can be achieved. Cheng *et al.* (2008) found that the organic carbon found in char-containing soil was more stable, with significantly less labile organic carbon and a longer half life in the recalcitrant OC fraction. Wardle *et al.* (2008) proposed an enhanced loss of SOC when char was added to a boreal forest humus layer. It was suggested that the char was responsible for promoting the growth of microbial communities and for enhancing the decomposition of labile C compounds, rather than stabilising them against degradation in soil. Sohi and Lehmann (2008) suggested that the study was flawed because the trial had omitted soil minerals, only carrying out the experiment in a litter bag.

Ordinarily, SOM turnover rates are usually higher in tropical climates (Zech *et al.* 1997) but there is evidence to suggest that after an initial rapid flux, soils with added char stabilise and flux is lower than that seen in adjacent native soils. Liang *et al.* (2008) studied CO_2 evolution from these anthrosols as compared to respective adjacent soils. The former had between 61-80% lower flux than the latter and furthermore, the age of the char in the soil (between 600-8700 years) gave no significant difference in carbon flux. The core regions remained the same regardless of age and no chemical/structural differences were observed between young and old char samples, providing further evidence for its recalcitrance. Liang *et al.* (2010) also found that the char-poor soils saw mineralisation to a significantly greater degree than the char-rich soils on addition of new organic matter.

The longevity of char itself under different climates is unclear, but in one study by Cheng *et al.,* (2008), its mineralisation tended to remain unchanged between cooler and warmer climates. However, as mentioned previously, not all chars are the same; chars burned at a lower temperature show a greater degree of mineralisation. Burnt biomass is largely labile and is mineralised within a matter of months or years; biochar is pyrolysed rather than burnt so is highly stable and resistant to degradation (Winsley, 2007). Although the positive effects of chars on crop yields in tropical soils are known, there is still relatively little known about the effects of char addition on soil microorganisms and soil carbon balance (Steinbeiss *et al.* 2009; Liang *et al.* 2010).

Soil microbial biomass has an important role in nutrient cycling. Biochar could potentially provide a habitat for these microbes, protecting them from predators and abiological stress. Durenkamp (2010) found that soil microbial biomass slightly increased on addition of biochar at permanent grassland but not at an arable site. Biochar did result in extra stimulation of microbial biomass upon ryegrass addition and did increase nitrification. After initial CO_2 flux, little increase in flux was seen. It was concluded that the impact of biochar on soil microbial biomass and activity mainly depends on the type of char but also on soil-type and experimental conditions. Soil microbial activity is increased by biochar especially that produced at lower temperature. The increase in soil microbial activity results from the direct microbial utilization of biochar as a nutrient source, and the secure environment provided by the physical structure of biochar (Luo, 2010).

When added to contaminated soils, biochar is found to adsorb DOC, increase soil pH (Beesley *et al.* 2010), increase key soil macro elements and immobilise water-soluble trace metals (Beesley and Marmiroli, 2010). However, this may not be true of all metals; in a 60 day study of an industrially polluted soil, Beesley *et al.* (2010) found that copper and arsenic concentrations increased in pore water by up to 30 times their original level. Char is also shown to have a high affinity for adsorbing and therefore reducing bioavailability of polycyclic aromatic hydrocarbons (PAHs) (Cornelissen *et al.* 2006), with up to a 50% decrease seen in more toxicologically relevant PAHs (Beesley *et al.* 2010). Oen *et al.* 2006 further suggest that it is the char, rather than the TOC that dictates the distribution of PAHs in the soil with the sorption to the former being far greater than to the latter. Cornelissen and Gustafsson (2006) studied the effect of adding humic acids and native PAHs on phenanthrene sorption to environmental char. The sorption was not decreased with the added humic acid, but was with other PAHS.

6.2.6 Problems associated with biochar

With any 'new' technology, a number of issues both scientific and social, need to be addressed. First the technology needed for safe and efficient local production of BC and other desirable by-products must be suitable for use on a national scale. The correct infrastructure has to be in place to support this and to make the process sustainable, the feedstock, production facility and location for end-use should be located in the same area.

Dr. Simon Shackley from UKBRC claims that from the C abatement perspective, traditional charcoal methods are damaging with unknown health impacts and not sustainable. This is due to the fact that during charcoal production, CH_4 (as well as organic vapours and nitrous oxide (N_2O)) are emitted. Charcoal making releases 1.9 tCO_2 eq. per tonne wood; burning charcoal releases a further 0.8 tCO_2. This is 60% more than C emission from straight combustion (Shackley, 2010). Any system in the UK would need to meet certain environmental criteria to ensure that it qualified as a green technology .It is also likely that the economics and organisation of reward scheme would be needed to encourage the utilisation of this technology.

There have been questions as to whether or not the energetics of the pyrolysis system are favourable when all of the input processes are taken into account. Gaunt and Lehmann (2008) explored this and found that even in systems where the conditions were optimised for the production of biochar (at the expense of energy production by around 30%), the energy produced per unit was still greater than comparable technologies. Furthermore, avoided emissions are between 2-5 times greater when biochar is applied to agricultural land than used solely for fossil energy off-sets.

There are also on-going issues for biomass feedstock. Effective land management is necessary to preserve global carbon stocks and land use change could be of potential concern. There is no clear understanding of what the demands will be for the crop co-products, by-products, residues or wastes as new technologies develop. If forest land is removed to grow short rotation coppice, an important carbon sink will be lost. The ideal solution is to use brownfield sites and contaminated land to grow dedicated feedstocks but whether this would have any deleterious effects on the end product remains to be seen.

The same potential problem exists when considering the use of certain waste materials as feedstock; although this eliminates the debate about biofuel crops competing for land with food crops and woodland, the 'waste' label could be a potential issue, leading to restrictions from waste regulation and prejudice from potential clients. This would not necessarily be wholly unjustified as there could be concerns relating to probable contamination from pollutants such as PCBs, toxic metals and PAHs which leads to the question:

When biochar incorporated is into soil, how safe is it to use?

To avoid PAH production, slow pyrolysis is the key. Chars studied from both ADE and Spanish wildfires were both below limits of detection for PAHs (Gaunt and Lehmann, 2008). However, more studies needed to assess levels of PAHs from biochars produced under different conditions.

Rather than an environmental pollutant, however, it could be argued that char added to soils follows a pathway that occurs in many natural soils ; many soils around the world contain high concentrations of char (some >20%) as a result of natural fires with no apparent ill effects (Fowles, 2007).

For all the addition of biochar to soils may improve crop fertility, it is also found to promote prolonged weed growth (Major *et al.* 2005). If the char is to be added to cropland to increase fertility, limiting the need for inorganic fertilisers, this could increase the need for herbicides.

6.2.7 Current/future research focus in UK

Although research in this area is active and growing, research has focused largely on the benefits to agriculture, rather than carbon sequestration (Fowles, 2007). Currently in the UK, research is chiefly focused on the characterisation of biochar as a product. To gain an empirical understanding, one needs available char, a willing land owner and a robust method for testing responses. But it can be difficult to ensure transferability and further, it is often very difficult to ensure that every study chooses a comparable range of measures (nutrient status, soil pH, soil texture...).

Classification of biochar would mean that it may become a readily available, commercial product; predictive capacity would enhance the market value and allow investment in active production. Parallels could be made between this PAS100 and PAS110 graded composts which have fulfilled these requirements and completed this process.

UKBRC are attempting to elucidate issues surrounding this relatively new science – classification system for government, industry, academia and the general public. They are in the process of developing rapid toolkit for screening short-listed products which emphasises five key functional attributes.

1. Carbon accounting purposes – how much biochar remains in soil over long-term
2. Fingerprint specific biochars so that different products are traceable
3. To assess reactions with and impact on soil
4. To establish viable methods for the analysis of biochar for rapid and inexpensive deployment
5. The ultimate aim is to create a database for a global 'charchive' which will be a web-based catalogue and physical library for a growing inventory of biochar samples.

Until this has been achieved, it remains difficult for academic studies using char to be wholly comparable.

6.2 Trial aims

To expand on trial 2 experiments by adding char and comparing it with manganese dioxide-coated sand in its ability to increase the humification of CLO.

6.3 Trial objectives

1. To control ambient conditions in order to better assess any significant differences between data sets.

2. To introduce peat as a soil standard.

3. To lengthen the trial period, assessing the degree of humification at 10 weeks and 30 weeks.

4. To avoid sub-sampling of lysimeters for flux measurements.

5. To re-examine the suitability of PTGA as a method of assessing humification.

6. To test biochar against Mn and sand-supplemented samples in terms of their individual capacities to stabilise organic matter.

7. To examine any difference in the carbon flux and humic acid content of free-draining and water-logged samples.

6.4 Materials

A 100kg batch of CLO was collected from the Thornley PARC recycling facility in October 2008. It was stored outdoors in sealed, breathable containers until use. On inspection, the material appeared no less contaminated than previous batches; a second attempt to manually remove macro-contaminants was once again aborted due to time constraints and volume of material.

Having encountered problems with heterogeneity during previous trials, sphagnum moss peat was introduced as a soil standard. Peat, like CLO, is high in organic carbon but being relatively homogeneous in character, it was thought to be a suitable standard.

As in Trial 2 (Chapter 4), the manganese–coated sand was collected from the Northumbrian Water Treatment Works in Castleside, County Durham; the sand was the same material as described in Chapter 4. Again, both were sieved prior to use to eliminate any particle size bias. For chemical composition of the manganese-coated sand, see Table 4.1.

Although the UK Biochar Research Centre (UKBRC) is currently working to produce a standard material with reliable properties, none exists as yet. Different feedstocks and different methods of production can produce a variety of chars with different properties. The char used in this trial was a lumpwood charcoal made from softwood, selected chiefly because of its commercially availability, known source and low cost.

Several trials have been undertaken to assess the effect of particle size on biochar's efficacy (UKBRC, 2010). Some state that it has no bearing on its soil-enhancing properties, others give the contrary view. To avoid bias and to ensure and reproducibility, the char in this trial was crushed and sieved to ensure that the particle size was comparable to that of the MnO_2-coated sand and sand.

6.5 Experimental set up

Based upon results from previous trials and new specifications from Premier Waste, some changes and additions were made to the experimental design for this trial. The Mn-coated sand, biochar and sand were all added at 5% (dry weight) based on results from trial two and on current literature from similar trials (Smidt *et al* 2008).

6.5.1 Ambient conditions

It was decided that trial three would be carried out under more controlled conditions so that any trends might be more easily seen.

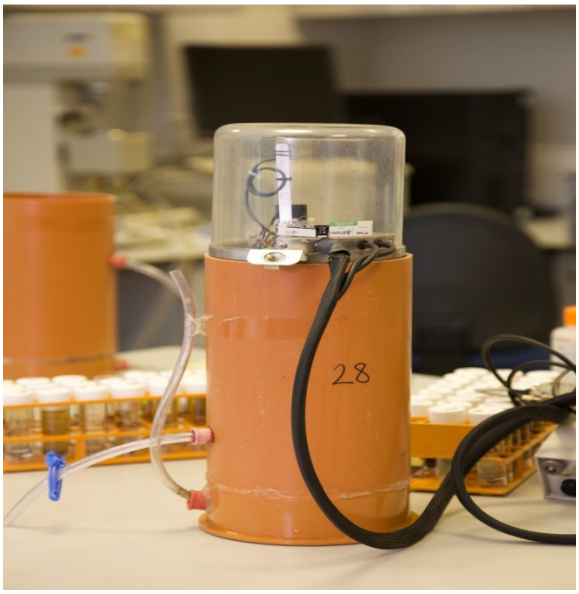


Figure 6.2: a lysimeter for a free-draining sample with the IRGA chamber attached.

Construction of lysimeters used 96 x 25cm lengths waste pipe and 96 x end caps. The lysimeters were made watertight with sealant. Drains and water-depth monitors were constructed using 20cm lengths of 6.5mm PVC tubing, rubber bungs and plastic ties/clips. Holes were drilled in the pipes for drainage tubing: one at base for water-depth measurements, one at 20cm for water-logged drainage. Lysimeters were laid out in trays in random order so that any sampling bias would be limited. Protective gauze frames were designed and built to cover the samples to minimise any spreading of dust within the laboratory.

6.5.2 Waterlogging

During this trial, a set of waterlogged samples were introduced alongside the free draining samples. As PWM have proposed the idea of creating a pseudo-peat bog made from CLO, it was considered important to see how the material behaved under waterlogged conditions.

As with the previous trials, dry weights were calculated and the relevant proportions added to each lysimeter, each in triplicate. A fourth replicate was made and sacrificed after 10 weeks; the other samples were measured for 26 so that any longer-term trends might be seen. Carbon flux is greatest when the rate of mineralisation is highest, as the labile components of the CLO decompose. Long-term behaviour is more likely to show any significant effects of the manganese addition after around two weeks.

Measurements of ambient temperature, soil temperature , pH and soil moisture were taken throughout the course of the experiment. The free draining samples were watered regularly to ensure that moisture content was sufficient for optimal microbial activity (Bernal *et al* 2009); and the waterlogged lysimeters were also watered so that they remained so. Milli-Q deionised water was used for this purpose. Small samples were taken regularly to monitor that the soil moisture content remained at least 40% (Huang and Hsu, 2008).

The leachate was collected at weeks 1, 10 and 26 in clean, labelled sterilins and stored below 4°C for further analysis

6.5.3 Flux measurement

Objective 4 for this trial was to avoid the sub-sampling of the lysimeters during flux measurements; In order for samples to remain undisturbed, the CLO was left to mature until the flux was within the IRGA's measurable limits. This facilitated the use of the integrated chamber that could be fitted directly over the lysimeter, and measurements to be taken directly (Figure 6.1). As the material had been stockpiled at the Thornley site for an indeterminate length of time, there was no knowing how old the CLO was at time of collection. That being the case, the material was already incomparable to previous batches so the further maturation period was of minor consequence.

6.6 Statistical analysis

6.6.1 Analysis of Variance

As described in Chapter 3.4, all data collected were entered into a Microsoft Excel spreadsheet, formatted and then ANOVA (General Linear Model) performed using Minitab 13. The results are given as p-values and all < 0.05 show a significant relationship between the two parameters compared. All results used from this point will be quoted as being statistically significant if they satisfy this requirement.

6.6.2 Principal Component Analysis

As in Chapter 5, PCA will be used to analyse the PTGA data. Again, Minitab 13 software was used to calculate the eigenvectors, eigenvalues and principal components (PCs) within the correlation matrix. Once the PCs have been calculated, all with eigenvalues of ≥1 are retained, plus the first one in sequence that is <1. To ensure the correct number of PCs have been retained, a scree plot is be used; the eigenvalues are plotted against the PC numbers, and the components depicted on the curve of the graph are retained (Figure 6.28).

The variables (temperature intervals 155-655ºC) were entered, giving eleven in total and the PCA run. Two eigenvalues were greater than 1; a scree plot (Figure 6.28) was drawn to corroborate the number of eigenvalues retained.

The control sample matrix and the standard sample matrix described in section 5.4.1 and 5.4.2 were used to compare the trial 3 samples against and inferences were then drawn.

To make the study viable, it's important to have five times more data points than variables. The 16 standard samples and the 64-sample matrix (described in 5.4.1-2) the 144 samples from this trial, gives a total of 224 samples which fulfils the minimum quota criteria (5x the number of variables).

6.7 Results

6.7.1 Physical analysis and observations

Over the thirty week trial period, apparent physical differences between the samples were observed. The waterlogged CLO samples tended to form a microbial, ocherous crust (figure 6.2) which appeared to impede carbon flux. This was not seen in the waterlogged peat samples.

Figure 6.3: Microbial/Fe film developed over waterlogged sample.

From the leachate collected at weeks 1, 10 and 26, the following data was collected.

pH

Sample	pH		
	W1	W10	W26
CLO	8.18	7.38	7.60
Waterlogged	7.71	7.67	7.68
CLO with sand	8.13	7.34	7.58
Waterlogged	8.11	7.71	8.17
CLO with MnO_2	7.29	7.26	7.36
Waterlogged	7.63	7.81	7.67
CLO with biochar	7.63	7.21	7.33
Waterlogged	8.16	7.84	7.54
Min	**7.23**	**7.12**	**7.09**
Max	**8.66**	**8.06**	**8.19**
Peat	4.46	4.07	4.41
Waterlogged	4.29	4.11	4.20
Peat with sand	4.23	4.01	4.23
Waterlogged	4.29	4.64	4.10
Peat with MnO_2	4.58	4.27	4.76
Waterlogged	4.95	4.61	5.02
Peat with biochar	4.30	5.28	4.91
Waterlogged	4.27	4.26	4.26
Min	**3.65**	**3.71**	**3.71**
Max	**5.49**	**5.63**	**5.44**

Table 6.1: leachate pH data from trial 3 (Average from three replicates; min and max values from individual sample readings).

The main differences in pH were seen between the CLO and the peat samples; the former having a significantly higher pH than the latter. No significant differences were seen between free-draining and waterlogged samples, between different treatments or over time.

The pH readings for all CLO samples were within the same range of those seen in Trial 2 (Chapter 4.7.1), measuring between 7.09 – 8.66 across the 26 week period. The peat samples measured between 3.65- 5.63, making them mildly acidic; these values are consistent with current literature (Gardea-Torresdey *et al. 1996; Andersen et al.,* 2010).

Moisture content

At the beginning of the trial, 50g extra material was added to each lysimeter for the purposes of moisture content analysis. At the end of week one, 50g were removed from each lysimeter; dried for 48 hours in a furnace at 105°C and then re-weighed. When the fourth set of samples was sacrificed for analysis at week 10, the same procedure was followed and each sample was analysed in duplicate. At week 26, the moisture content was measured again on the remaining triplicate samples. The results are shown in table 6.2.

Sample	Moisture content (%)		
	W1	W10	W26
Free draining CLO	42.4	45.7	48.2
Free draining CLO with Mn	45.7	40.0	46.6
Free draining CLO with Sand	42.7	37.4	31.4
Free draining CLO with char	40.9	32.4	51.4
Min	**36.1**	**27.2**	**28.9**
Max	**49.8**	**46.3**	**59.9**
Waterlogged CLO	73.6	72.9	73.6
Waterlogged CLO with Mn	72.5	73.8	74.0
Waterlogged CLO with Sand	73.1	74.7	73.5
Waterlogged CLO with char	74.0	75.9	75.3
Min	**69.1**	**70.1**	**69.6**
Max	**75.6**	**77.0**	**77.8**
Free draining Peat	42.8	37.6	36.5
Free draining Peat with Mn	34.7	34.8	38.1
Free draining Peat with Sand	35.7	40.1	38.5
Free draining Peat with char	40.5	40.3	44.4
Min	**31.4**	**31.6**	**29.1**
Max	**46.2**	**44.0**	**49.7**
Waterlogged Peat	80.0	81.1	**80.5**
Waterlogged Peat with Mn	80.1	78.5	80.3
Waterlogged Peat with Sand	77.9	79.5	80.3
Waterlogged Peat with char	78.1	79.8	81.2
Min	**77.2**	**77.4**	**77.9**
Max	**83.5**	**81.4**	**82.3**

Table 6.2: Moisture content of the CLO and peat samples at weeks 1, 10 and 26 (weeks 1 and 40 show the average of 6 replicates, week 10 is the average of 2 replicates).

As expected, the waterlogged samples have a higher water content than the free-draining samples. Peat holds slightly more moisture than CLO for the waterlogged samples but not for the free-draining samples. . The spread of data is much smaller for waterlogged samples than for free-draining samples with min-max values having a difference of ≤6 and ≤20 % respectively. The moisture content of the free-draining was ideally supposed to be maintained at above 40%. On the whole, the samples were lower than this; it was difficult to maintain this level of moisture without waterlogging the samples. This may have implications for microbial activity and water-bridge formation, as discussed in Chapter 4.7.1.

6.7.2 Degree of carbon flux

The carbon flux was collected in ppm with the IRGA as described in section 3.6.1 and then converted into gC/gCLO/day via the equation given in section 3.6.2; all data presented in this section are given in the converted units described.

CLO free-draining samples

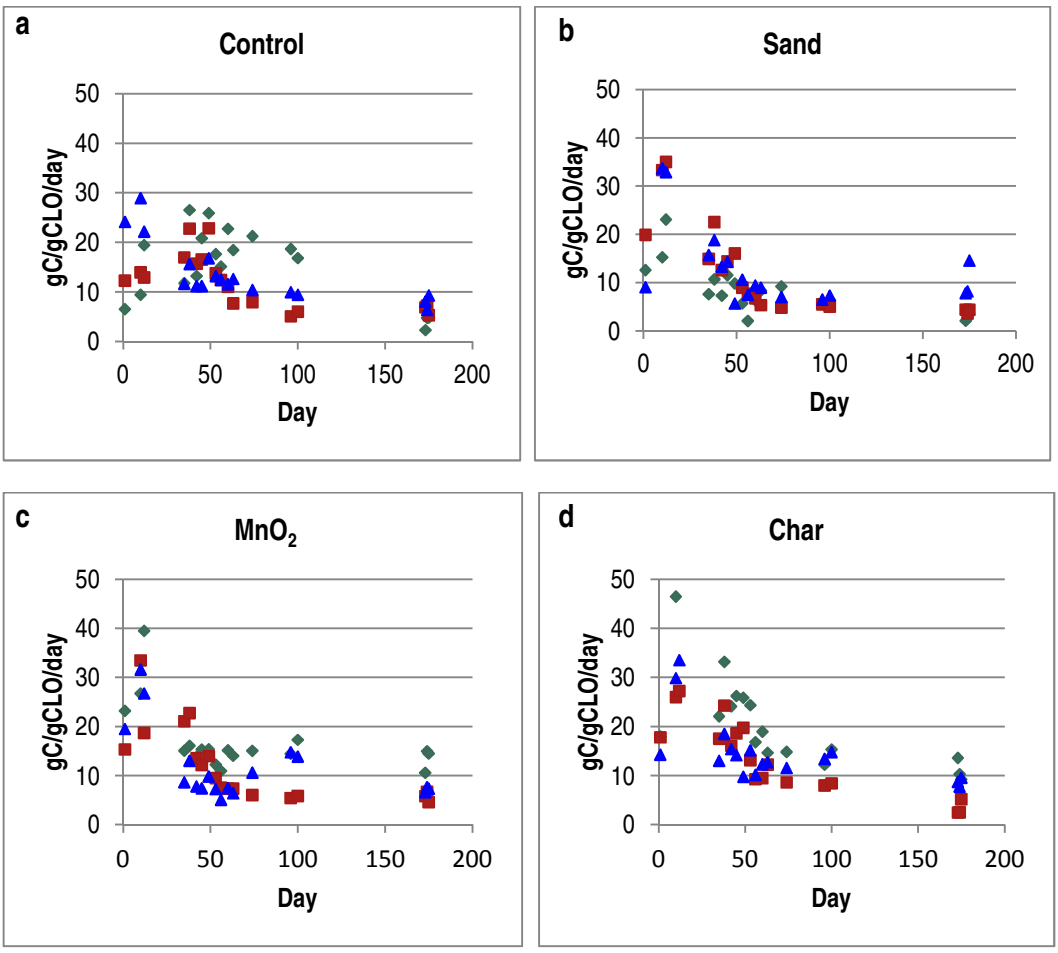


Figure 6.4a- d: Scatter graphs showing carbon flux of free-draining CLO over a 26 week period; each series represents the three triplicate samples at each data collection

There was a broad spread of data between replicate samples for all samples throughout the trial period with differences as large as 20gC/gCLO/day seen between some replicates. All samples showed the characteristic sharp increase in CO_2 flux over the first ten days and then steady decline over the 176 days.

CLO waterlogged samples

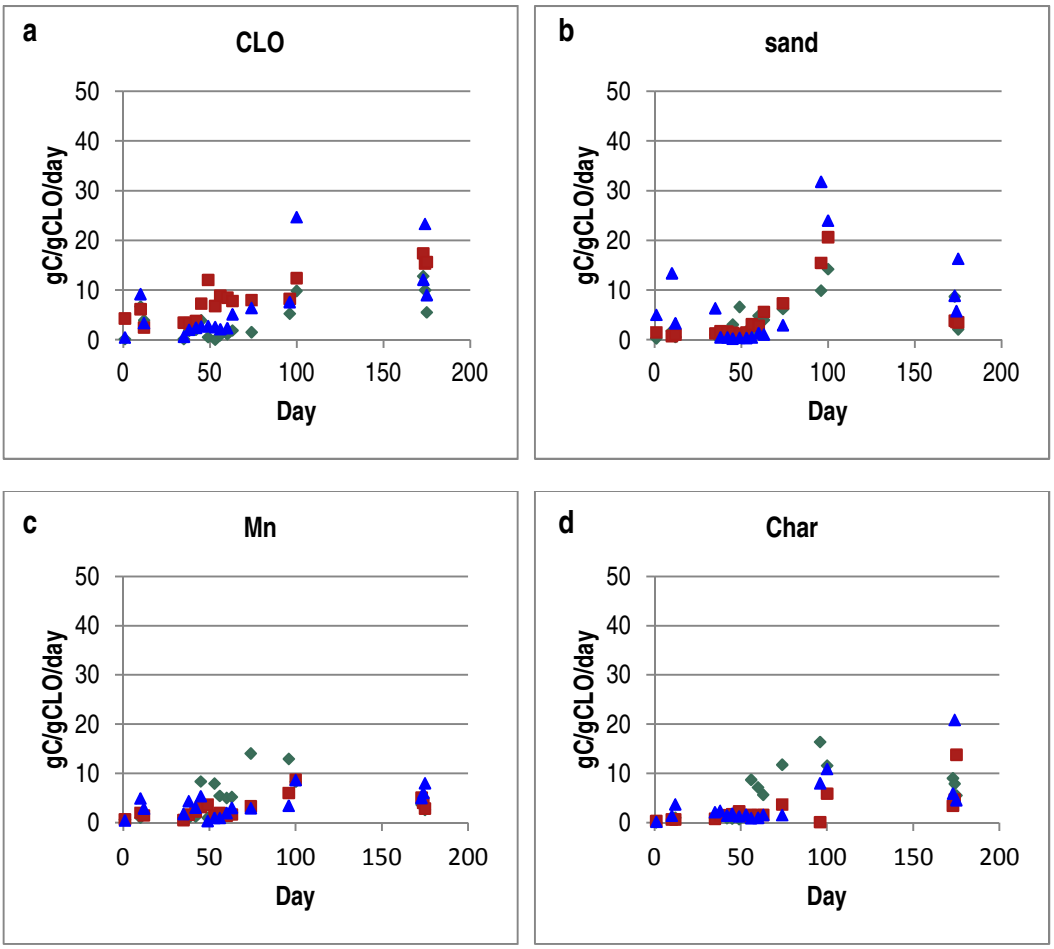


Figure 6.5a-d :Scatter graphs showing carbon flux of waterlogged CLO over a 26 week period; each series represents the three triplicate samples at each data collection

The spread of data and degree of flux is not as great in the waterlogged samples compared to the free-draining samples. There is also an apparent steady increase in CO_2 efflux over the trial period (from 0-≤32gC/gCLO/day), rather than the decrease seen in the previous charts in Figures 6.4a-d. Analysis of variance will give a quantitative account of this data.

Peat: Free-draining samples

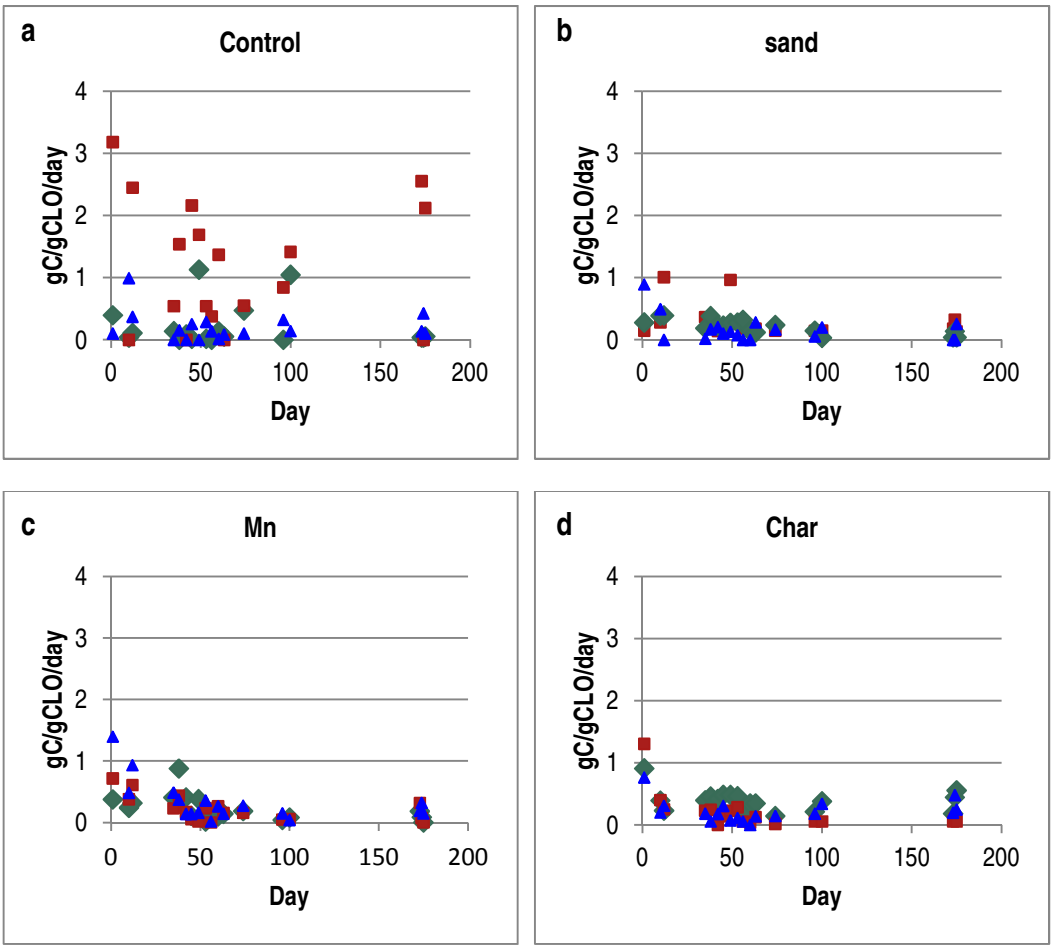


Figure 6.6a-d: Scatter graphs showing carbon flux off free-draining peat over a 26 week period; each series represents the three triplicate samples at each data collection

The peat samples have far less flux than the CLO samples, in the range of 0-4gC/gCLO/day as opposed to 0-40gC/g/CLO/day. The chart axes have been adjusted accordingly. Greater flux in CLO could be due to more labile material that may exist in CLO or a liming effect (Andersson and Nilsson, 2001) caused by a higher pH which is more conducive to the appropriate decomposer organisms.

The spread of data appears to be greatest for the control sample but still significant for other samples. There is no obvious increase or decrease of flux over time and no other apparent differences between samples.

Peat: Waterlogged samples

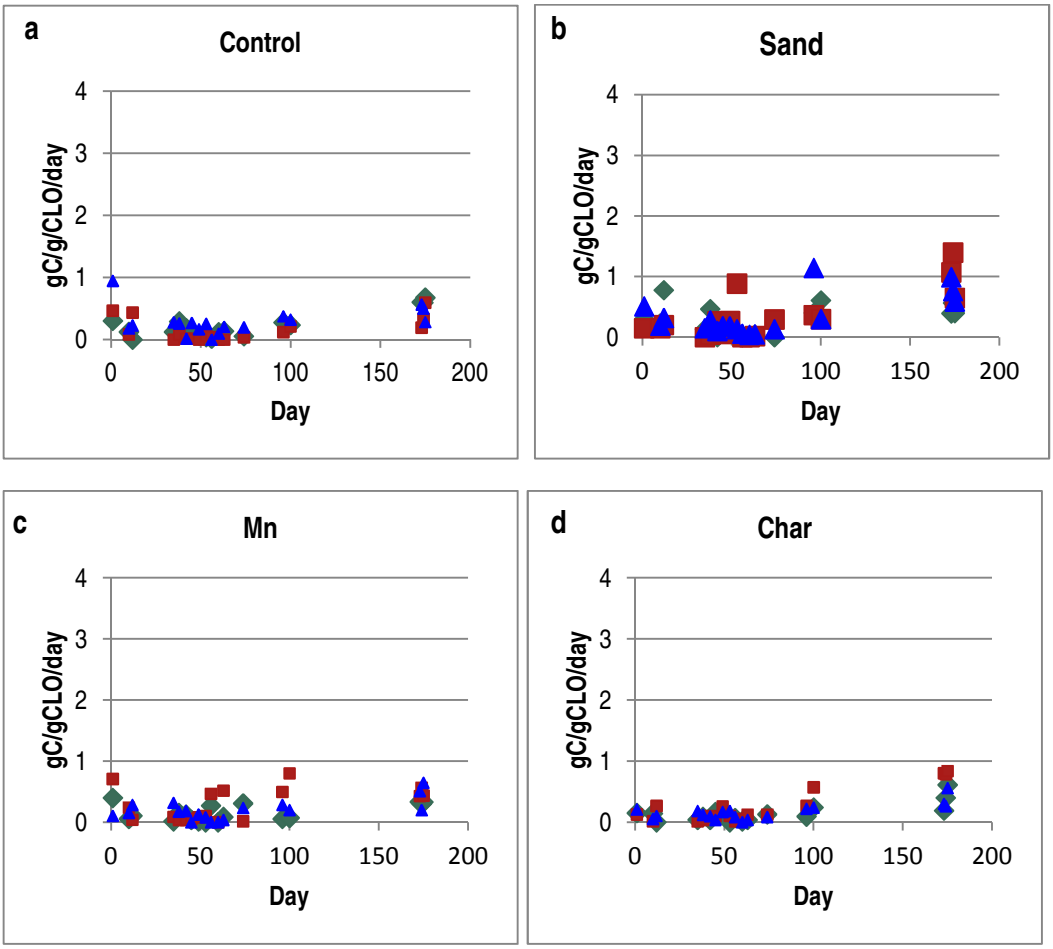


Figure 6.7a-d: Scatter graphs showing carbon flux of waterlogged peat over a 26 week period; each series represents the three triplicate samples at each data collection

The spread of data is not as great in the waterlogged peat control samples as it is in the free-draining peat control; for the other samples, the data-spread is similar between samples and to compared to their free-draining counterparts.

The free-draining CLO samples show the greatest flux which steadily decreases over time (from ≤ 3gC/gCLO/day - 0gC/gCLO/day); waterlogged CLO flux appears to increase slightly over the 26 week period. The peat samples give around ten times less flux that the CLO, possibly due to its relative stability.

General discussion for CO_2 flux data

Although samples were stored under the same conditions, some of the samples developed a microbial crust. This could account for some of the differences in flux between replicate samples. However, the range of data could be seen in the flux values of replicate samples in the previous trial when no microbial crust was seen so is not necessarily solely responsible for the lack of precision seen.

Significant differences were seen in CLO flux during weeks 1 and 2, possibly due to the water barrier, preventing respiration initially. Perhaps samples were analysed too soon after having been watered in the first week but by the second week, had drained a little, releasing CO_2 from the unobstructed soil pore spaces. This increase in flux rate observed over this short time period seen in the peat samples. Perhaps the microbial communities present in the peat sample had yet to become established in the CLO lysimeters, as was seen in the previous trial.

No statistically significant difference was observed between treatments for CLO samples in week one, as expected. By week two, the samples with added biochar exhibited a significantly higher flux than the control samples. This could be due to the trend often observed in soils with added char for short-term increased mineralisation within the first 20 days, as outlined by Smith *et al.* (2010).

At week 10, no difference in flux can be distinguished between the different treatments for any of the soil samples. The peat samples, whether free-draining or waterlogged, show no statistically significant difference between treatments at any point during the trial. Furthermore, waterlogging had no apparent effect on the flux of peat.

For the free-draining CLO samples, the decrease observed in the flux rate was significant between 1-10 and 1-26 weeks. The only significant difference for the waterlogged CLO samples was in the control samples with the increase in flux between weeks 1-26.

A significant difference can only be observed between treatments in the free-draining CLO samples; the CLO with char gives the greatest decrease in flux over time; the CLO with added manganese-coated sand also shows a slight reduction in flux when compared to the CLO control sample. All treatments in the waterlogged CLO samples show a significantly larger decrease in flux than the control samples over the trial period.

6.7.3 Humic analysis

Raw data are presented as the average of each the replicate at each week, plotted against the yield of humic acid extracted (Figures 6.8-6.11); against the E_4/E_6 ratio (Figures 6.12 -6.15); and finally, against the humification Index (HIX) (Figures 6.20 and 6.21).

For week 10, there are only two replicates as these were the dedicated sacrificial pots; the week 1 and week 26 samples comprise 3 replicates, analysed twice giving a total of 6.

ALKALI EXTRACTION: Free-draining CLO

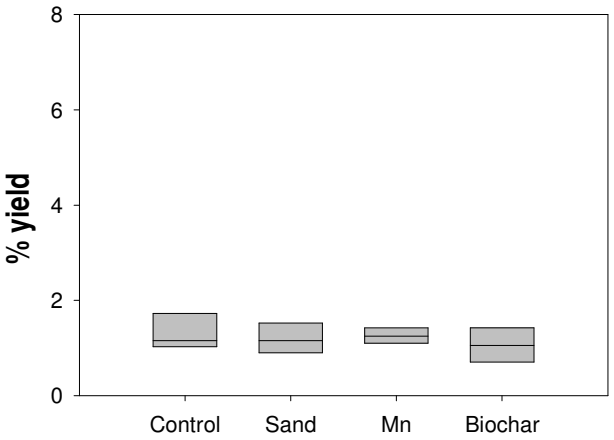


Figure 6.8a: Free-draining CLO samples at week 1 (six samples).

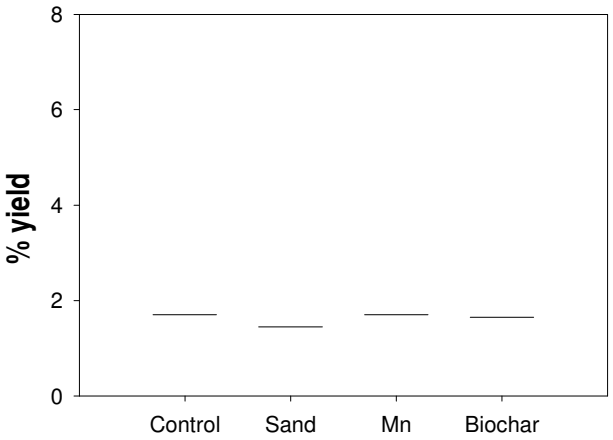


Figure 6.8b Free-draining CLO samples at week 10 (two samples).

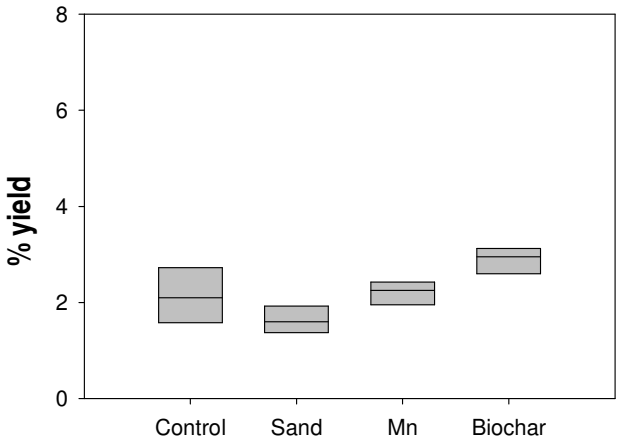


Figure 6.8c: Free-draining CLO samples at week 26 (six samples).

ALKALI EXTRACTION: Waterlogged CLO

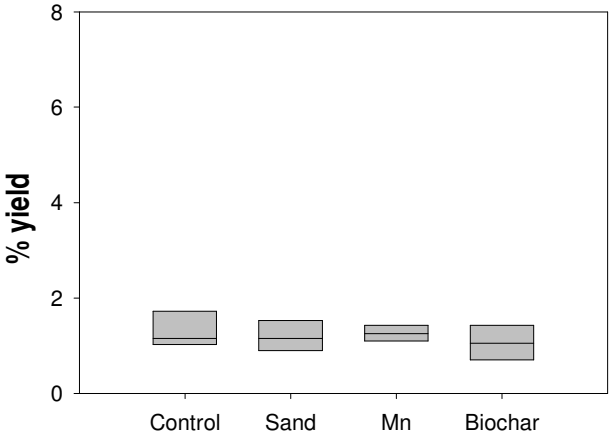


Figure 6.9a Waterlogged CLO samples at weeks 1 (six samples).

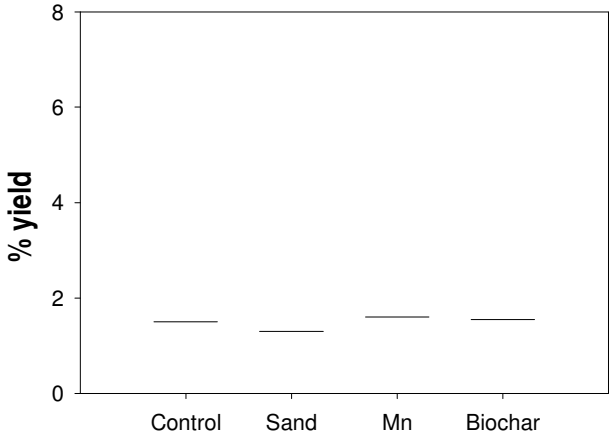


Figure 6.9b: Waterlogged CLO samples at 10 (two samples).

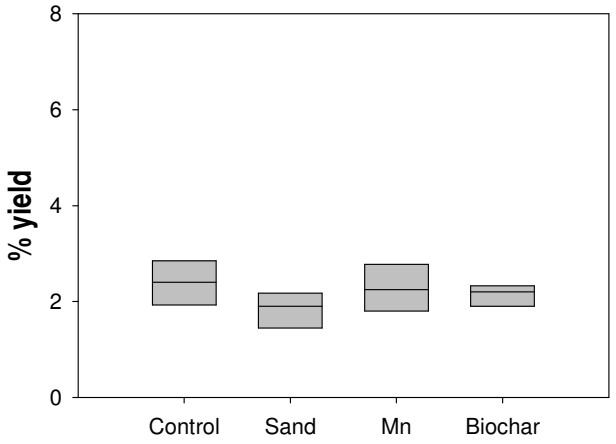


Figure 6.9c: Waterlogged CLO samples at 26 (six samples).

All free-draining samples appear to raise a similar yield of HA at week one, with little change seen at week 10. By week 26, the MnO_2-coated sand and char samples show a higher HA yield than the control sample with the char having the greatest. The samples with sand seem to have a slightly lower average yield than the control at week 26.

There appears to be very little difference between the samples over time and between each treatment in the waterlogged samples. The control samples show the largest data spread between replicates. Samples with char, followed by samples with MnO_2-coated sand give the greatest yield of humic acid; with sand samples present the lowest yield of HA.

For the control, manganese and char samples, little differences are seen in the average HA yield. The samples with sand appear to have the lowest yield. The MnO_2-coated sand and char-amended soils seem to be affected by waterlogging, giving lower yield than their free-draining counterparts. The spread of data is lowest in the char-amended samples.

Despite these apparent trends, when ANOVA was performed, no statistically significant differences were observed between waterlogging and free-draining samples, nor were any differences seen between the treatments, as compared to the control sample. The actual yield only varied from 0.9% for the sand-amended sample and 3% for the sample with added char so although the pattern looked apparent, the yields were so low that the difference between them was not statistically significant. The only statistically significant trend seen for alkali extraction procedure on CLO was the increased yield in humic acid in all samples over the trial period.

ALKALI EXTRACTION: Free-draining peat

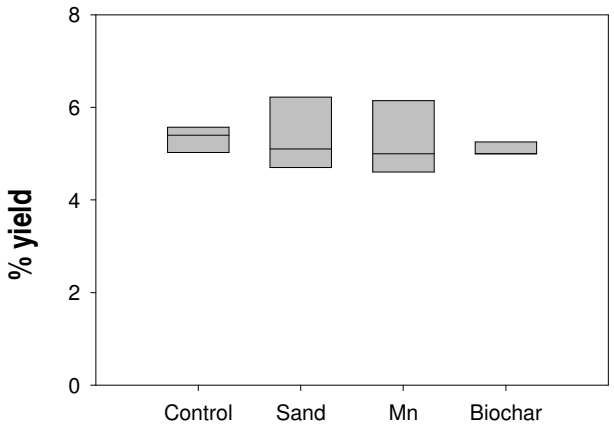


Figure 6.10a Waterlogged peat samples at week 1 (six samples).

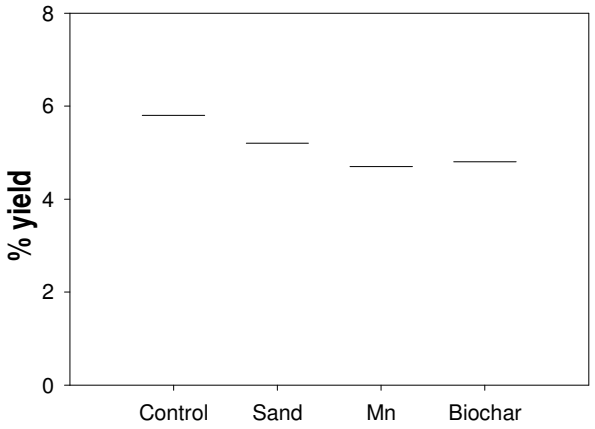


Figure 6.10b Waterlogged peat samples at week 10 (two samples).

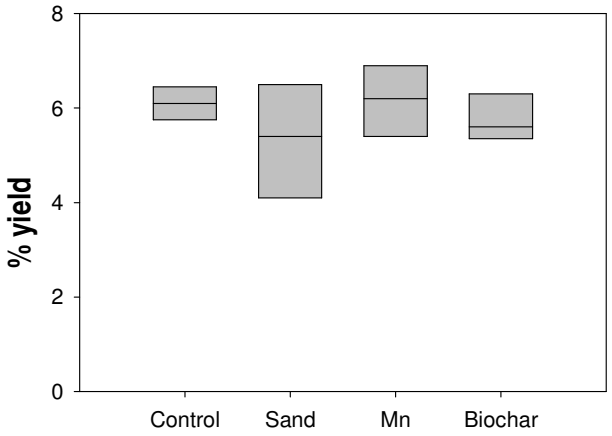


Figure 6.10c Waterlogged peat samples at week 26 (six samples).

ALKALI EXTRACTION: Waterlogged peat

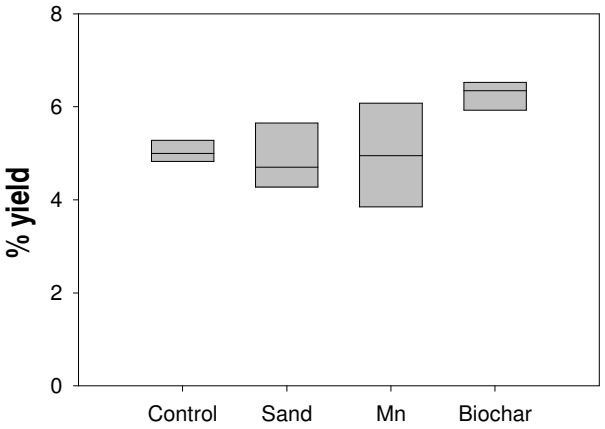


Figure 6.11a Waterlogged peat samples at weeks 1 (six samples).

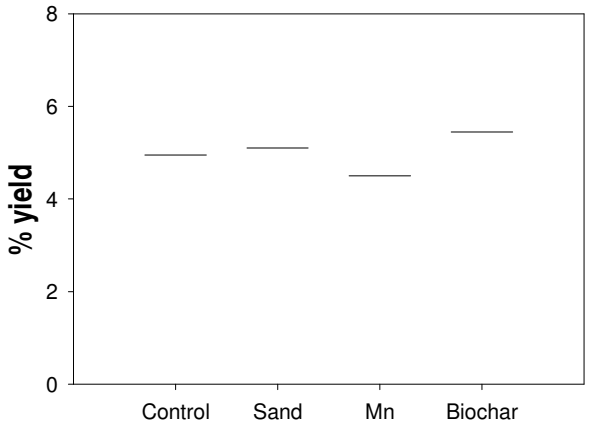


Figure 6.11b: Waterlogged peat samples at week 10 (two samples).

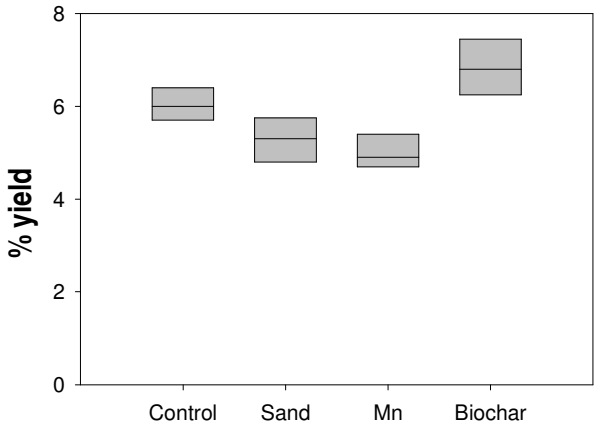


Figure 6.11c: Waterlogged peat samples at week 26 (six samples).

The peat samples have a higher average yield of humic acid than the CLO samples (4-7.5%) but again, show a spread of data between the replicates (as much as 2%). There appears to be little difference between the average yields seen in free-draining and waterlogged samples but the manganese MnO_2-coated sand amended samples appear to have the lowest average yield and the char samples the highest for waterlogged peat samples. This appears consistent over the 26 week period. Sand seems to have a lower average yield than the control for both free-draining and waterlogged samples.

Despite differences seen in Figures 6.10-6.11, no statistically significant differences were observed at 26 weeks between free-draining and waterlogged samples. The control peat sample is the only one to show any statistically significant difference between humic material in waterlogged and free-draining samples, with it having a slightly increased yield in the latter over 26 weeks but remaining the same in the former group.

The free-draining peat sample amended with sand and the sample amended with char each produced significantly less humic acid than the control sample at 10 weeks. At 26 weeks, the waterlogged peat samples with char yielded significantly more humic acid than the control sample.

UV DATA: CLO

The E_4/E_6 ratio decreased over the trial period for all control samples, more so with the waterlogged. For the amended free-draining samples, all three seemed to reduce their ratio more than the control over the 26 weeks with the manganese samples showing the lowest. The waterlogged samples are a little less clear but it appears the control samples look to reduce in ratio more than the other samples. For all samples, the error bars are large, especially at 26 weeks so the statistical analysis will show whether any of these relationships are significant.

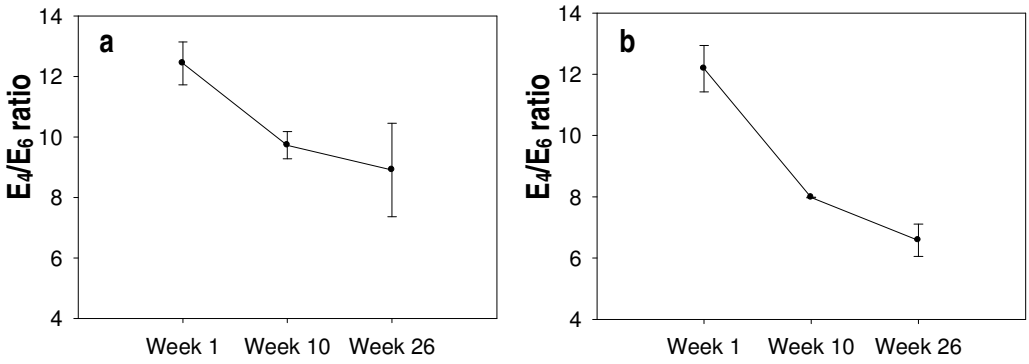


Figure 6.12 a and b: temporal changes in absorption ratio at 446nm and 665nm for free-draining and waterlogged CLO respectively.

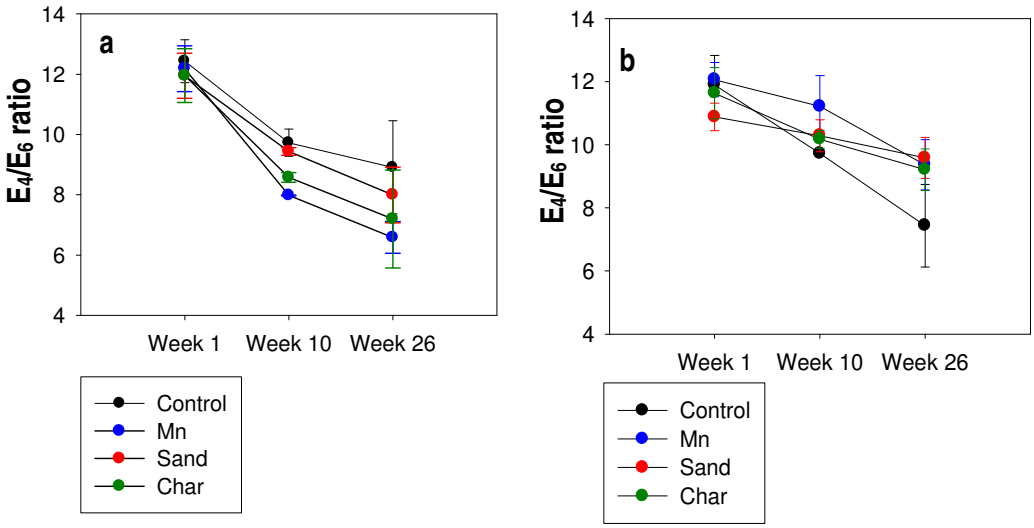


Figure 6.13 a and b: temporal changes in absorption ratio at 446nm and 665nm for different treatments for free-draining and waterlogged CLO respectively.

UV DATA: peat

The E_4/E_6 ratio is significantly lower for peat samples than for CLO, mostly being lower than 2 rather than between around 6-12. All free-draining and waterlogged samples appeared to decrease in E_4/E_6 ratio over the 26 weeks. Again, the error bars are large for all samples and so any apparent patterns in data could be deceptive.

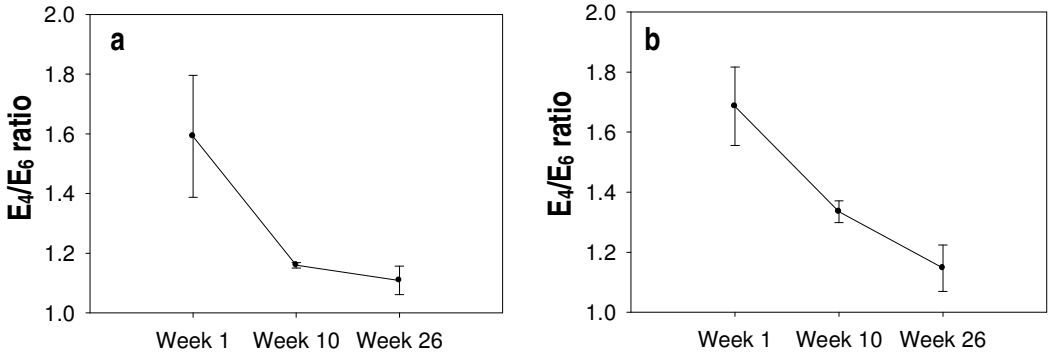


Figure 6.14 a and b temporal changes in absorption ratio at 446nm and 665nm for free-draining and waterlogged peat respectively.

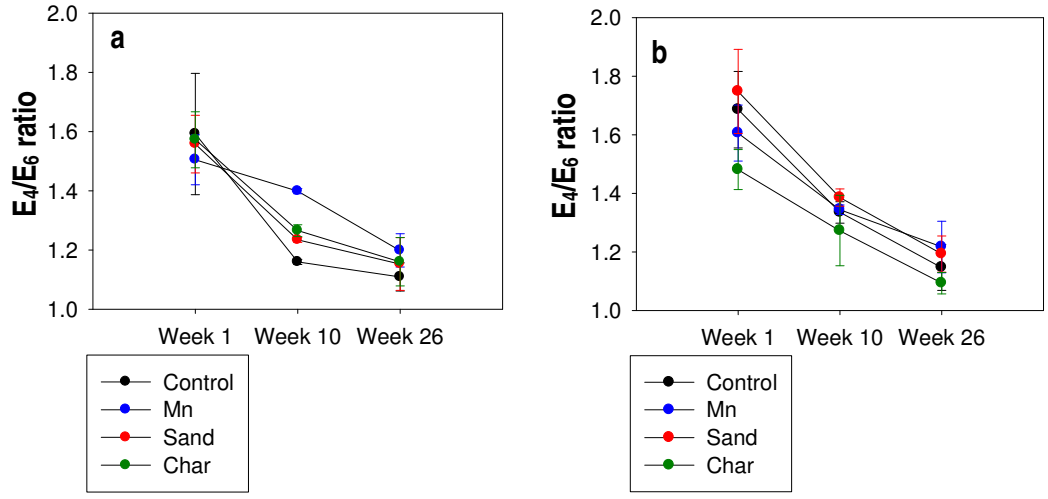


Figure 6.15 a and b: temporal changes in absorption ratio at for different treatments for different treatments for free-draining and waterlogged peat respectively.

There was a statistically significant difference between the CLO and the peat samples, the latter giving a lower ratio (in the order of 1-2 as compared to 6-13), thus denoting greater humification.

Within the peat samples, both free-draining and waterlogged samples decreased in ratio over the trial period. However, there was no difference between these samples, possibly because the small change seen in ratio which was in the order of 0.5, as compared to a change of around 6 for the CLO samples. No statistically significant changes were seen between different treatments either, possibly for this same reason.

No difference was seen in the CLO samples between treatments in week one, as to be expected. By week 10, the manganese-amended free-draining CLO sample had a significantly lower ratio than the control; this continued to be the case at week 26. By the end of the trial, the biochar-amended free-draining CLO also showed a significantly lower ratio than the control.

For the waterlogged and free-draining CLO, no significant difference was seen between control samples of each, possibly due to the large error bars seen. However, the MnO_2-amended and char-amended samples both show significant difference in humic material between their free-draining and waterlogged counterparts at weeks 10 and 26. At the end of the trial, the free-draining MnO_2-amended samples had a ratio of around 6.5 and the char-amended samples, around 7. For their waterlogged counterparts, the ratios were both around 9.5.

Within the waterlogged CLO samples, statistically significant differences in humic content were observed with all three treatments, as compared to the control at week 26. Unlike for free-draining samples, they appear to have a higher ratio which suggests less humic acid.

FLUORESCENCE DATA

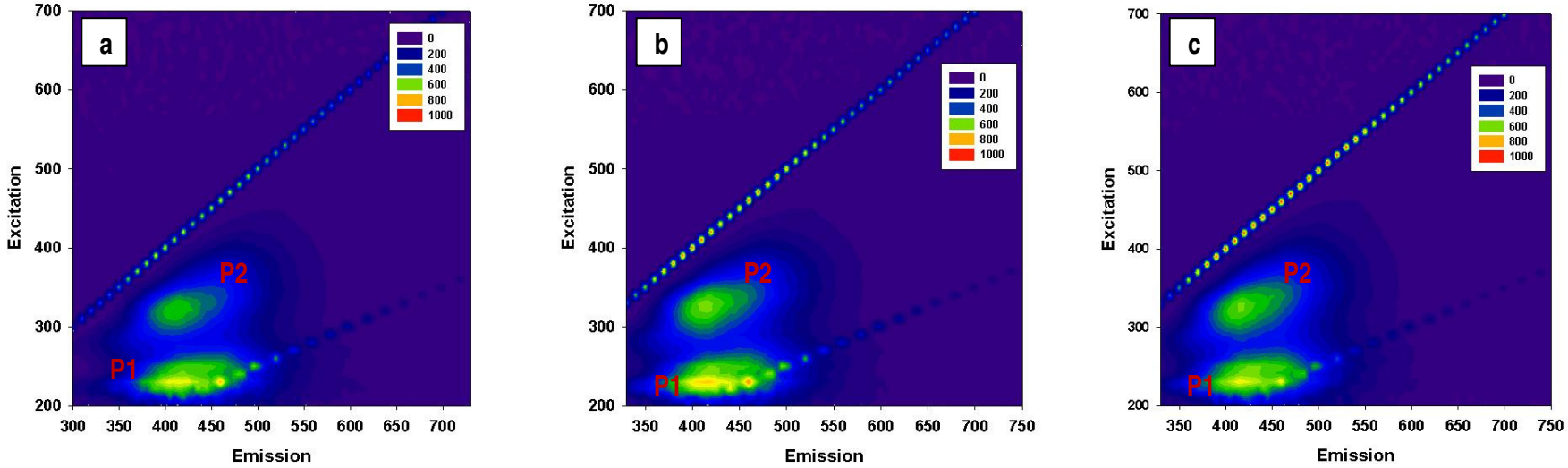
The fluorescence fingerprint charts illustrate the evolution of humic material over the 26 week period. The peak intensity (PI), with arbitrary units, is shown via the coloured key which ranges from 0 (indigo) to 1000 (red). For each sample's spectrum, two peaks exist at different excitation-emission wavelength pairs (EEWP): one at 220nm/440nm which for ease of reference will be referred to as peak 1(P1); and a second at 320nm/440nm which will be referred to as peak 2 (P2).

As fluorescent molecules become more condensed, their emission spectra tend to shift towards longer wavelength with more simple structures fluorescing at shorter wavelengths (Pedra *et al.,* 2008). For example, emission at 350nm tends to show relatively simple fluorophores, where emission at 480nm gives highly conjugated and condensed aromatic compounds (Fuentes *et al.,* 2006).

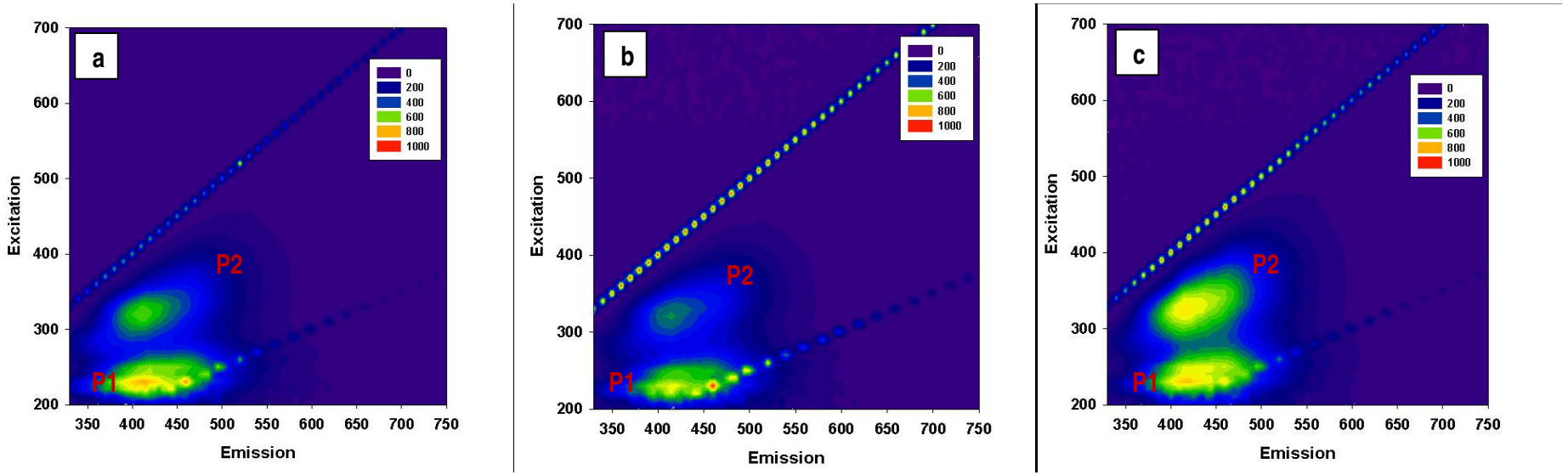
Vieyra *et al.* (2009) conducted a study on HA and FA formation in composting MSW over a period of 150 days. Samples were analysed using fluorescence spectroscopy and Excitation-Emission Matrices (EEM) produced. Two main peaks were identified, one at excitation-maximum <280nm and the second at >280. The position of these corresponded to fulvic acid-like and humic acid-like molecules respectively.

The two peaks seen in the Vieyra *et al.* (2009) spectra correspond almost exactly to the two peaks seen in Figures 6.16-6.18. It will therefore be assumed that P1 corresponds to FA-like compounds and P2, HA-like structures. Any shift in the spectra over time and between samples should indicate any changes or differences in their FA and HA content.

The week one samples all had an EEWP at 240nm/410nm (P1) and at 330nm/420nm (P2). The excitation wavelength remained the same in P1 for all samples. In P2, the excitation wavelength increased for all of the week 10 and 26 samples to 340nm. The emission wavelength remained the same for P1 samples also, with the exception of the CLO with char sample at week 26 where an increase in wavelength was observed (420nm to 430nm). For P2, the emission wavelengths exhibited an increase only in the week 26 samples for CLO with char and CLO with MnO_2-coated sand. The control samples saw no change in P2 position across the 26 weeks.



Figures 6.16a-c: fluorescence fingerprint charts for CLO control samples at weeks 1, 10 and 26 respectively. P1 corresponds to the FA-like fluorescence, P2 the HA-like fluorescence. Each sample was diluted x 100 using 0.2M NaOH.



Figures 6.17a-c: fluorescence fingerprint charts for CLO with char at weeks 1, 10 and 26 respectively. P1 corresponds to the FA-like fluorescence, P2 the HA-like fluorescence. Each sample was diluted x 100 using 0.2M NaOH.

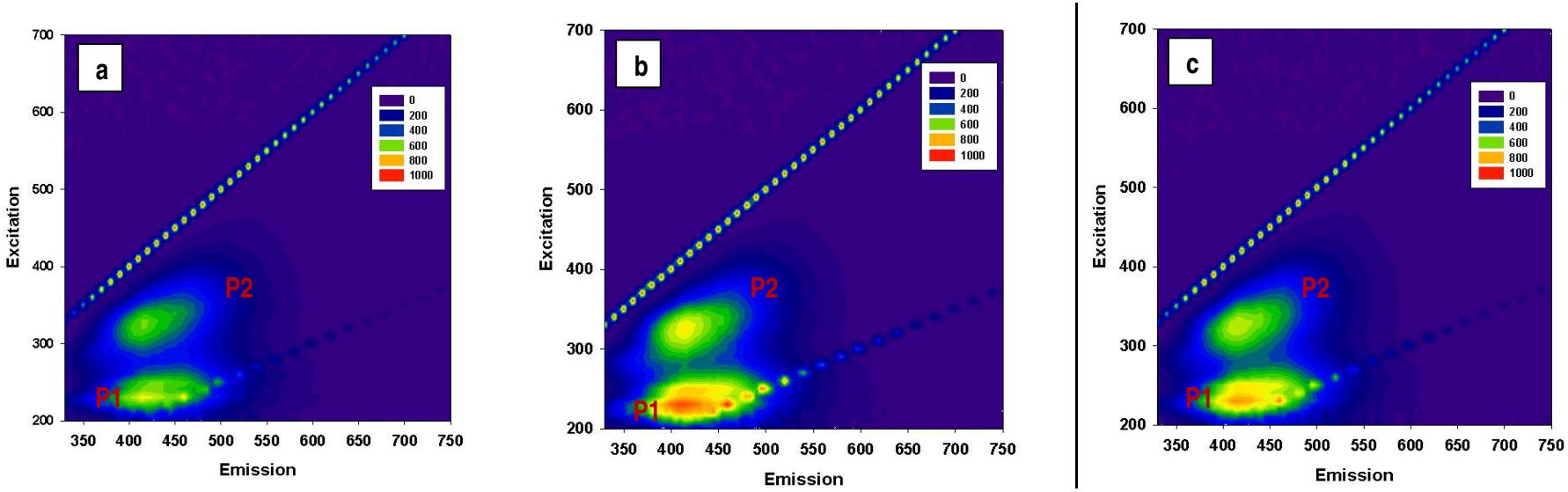


Figure 6.18a-c: fluorescence fingerprint charts for CLO with MnO_2 at weeks 1, 10 and 26. P1 corresponds to the FA-like fluorescence, P2 the HA-like fluorescence. Each sample was diluted x 100 using 0.2M NaOH.

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The peak intensities differ across the samples and over time. Low intensities tend to signify highly conjugated, high molecular weight molecules, whereas higher intensities are usually associated with fulvic acids and simple structural compounds with lower molecular weights. In Figures 6.16-6.18, the peak intensities of P2 are consistently lower than those in P1 with values being between 436 and 570 a.u., with the exception of the CLO with char at week 26 and the CLO with MnO_2-coated sand at week 10. Here, the peak intensities reached 706 and 689 a.u. respectively. This may suggest that the humic acid present contained more lower molecular weight moieties at that point than at the other stages of the trial.

The higher peak intensities seen in P1 suggest more FA-like material which is consistent with the data presented in the Vieyra *et al.* (2009) study. Here, intensities range from 684-933 a.u. Highest peak intensity values were seen in the CLO with MnO_2-coated sand samples, perhaps signifying a breaking down of HA into FA or higher molecular-weight HA molecules into smaller.

The fingerprint charts give a qualitative view of the humification occurring within the samples; for quantitative, more sensitive data that can be analysed statistically, the humification index (HIX) can be calculated (Ohno 2005).

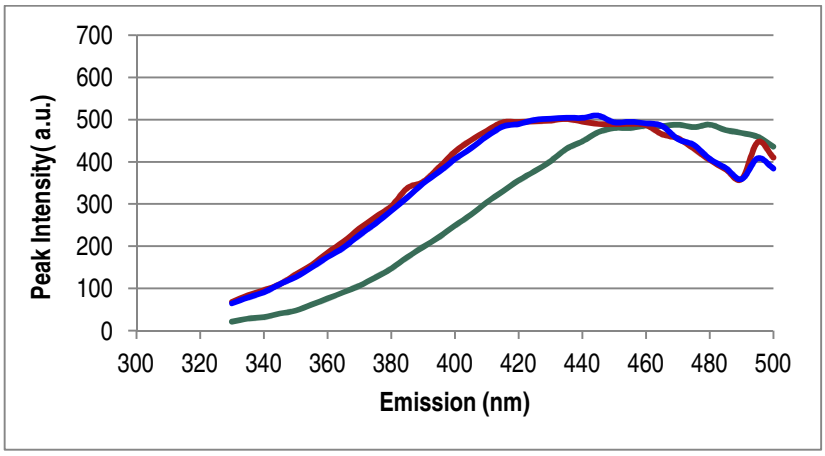


Figure 6.19: a graph to illustrate the peak intensity versus emission for the relevant area of the spectrum for calculating HIX. The three series shown are each of the triplicate samples.

As outlined in section 3.6.3, the HIX is calculated by comparing the emission seen at two different excitation ranges: 435-480 nm and 300-345 nm, calculated thus:

$$HIX = (\Sigma I_{435 \rightarrow 480})/(\Sigma I_{300 \rightarrow 345}) \qquad \text{(Ohno, 2002).}$$

The values calculated are between 0 -1 with the highest values signifying the greater degree of humification.

Humification Index

Samples	Weeks 0	Week 10	Week 26	ΔHIX[1]
CLO: Free-draining				
Control	**0.9744**	0.9290	0.9327	-0.0417
Sand	**0.9735**	0.9257	0.9429	-0.0306
Mn	0.9121	0.9296	**0.9389**	0.0268
Biochar	0.9111	0.9302	**0.9470**	0.0359
CLO: Waterlogged				
Control	0.9198	0.9196	**0.9373**	0.0175
Sand	0.9160	0.9283	**0.9328**	0.0168
Mn	0.9145	0.9232	**0.9322**	0.0177
Biochar	0.9079	0.9131	**0.9375**	0.0296
Peat: Free-draining				
Control	**0.9821**	0.9553	0.9715	-0.0106
Sand	**0.9699**	0.9596	0.9681	-0.0018
Mn	0.9545	0.9473	**0.9677**	0.0132
Biochar	0.9554	**0.9573**	0.9536	-0.0018
Peat: Waterlogged				
Control	0.9506	**0.9628**	0.9534	0.0028
Sand	0.9464	0.9617	**0.9766**	0.0302
Mn	0.9443	0.9579	**0.9658**	0.0214
Biochar	0.9539	0.9608	**0.9686**	0.0147

1 The change in humification index value over the trial period

Table 6.3: The humification index calculated for each sample at weeks 1, 10 and 26 (average of 2 replicates). The figures in bold represent the most humified sample across the trial period.

For the free-draining CLO samples, the control and samples with sand appeared more humified at the beginning of the trial, losing humic material over the first 10 weeks. Humification then appeared to increase again at 26 weeks. The free-draining CLO samples with MnO_2-coated sand and char both saw an increase at 26 weeks. This is in general agreement with the data presented in Figures 6.16-6.18.

The waterlogged CLO samples all increased in humic material over the trial period; the sample with added char showed the greatest increase.

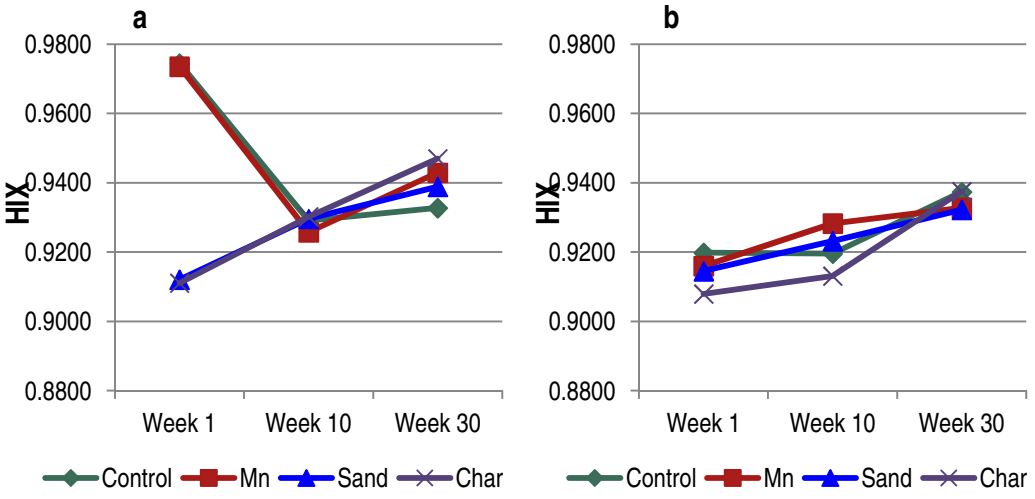


Figure 6.20 a and b: temporal changes in HIX (average of triplicate samples) of free-draining and waterlogged CLO samples respectively over 26 weeks

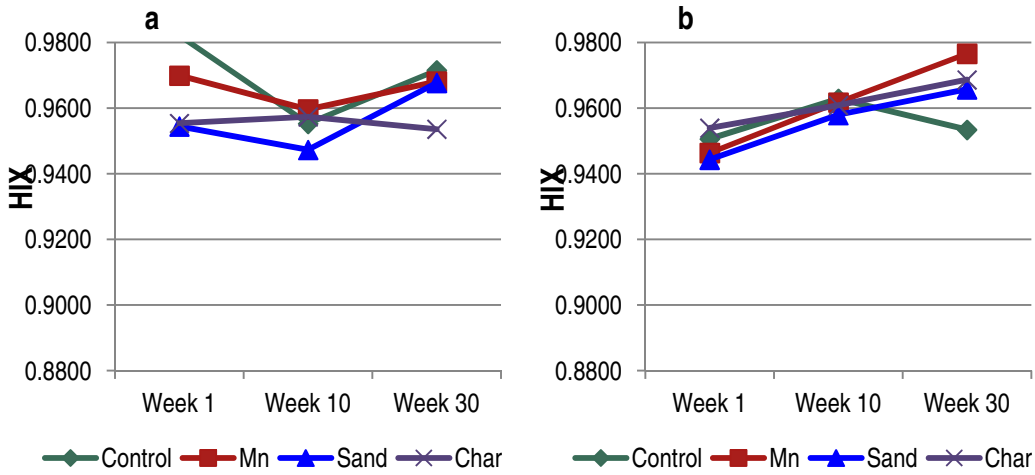


Figure 6.21a and b: temporal changes in HIX (average of triplicate samples) of free-draining and waterlogged peat samples respectively over 26 weeks

The HIX is high for all samples which suggest a high degree of humification in the humic substances present. All waterlogged samples saw an increase in HIX over the trial period which was a statistically significant trend for the control and Mn-coated sand amended CLO samples. The free-draining peat with Mn was had a significantly lower HIX than the control at each week.

There were no statistically significant temporal differences observed across all samples taken together. Perhaps more data would have elucidated this, especially for weeks 10-26 where p-value is close to 0.05. For individual samples, the free-draining CLO with added MnO_2-coated sand and char each indicated a significant increase in humification after 26 weeks.

Significant differences were seen between CLO and peat samples, which agree with the other forms of analysis. The waterlogged CLO samples all increased over the 26 week period, the only set of samples to do so. Both the free-draining CLO and peat showed a similar trend with the control and sand samples appearing less humified at the end of the trial than at the start.

The experiment would perhaps have been more sensitive if time had allowed for more replicate samples to be used.

Samples	Alkali extracted HA[a] (g)				E_4/E_6 A ratio[b]				HIX[c]			
	W1	W10	W26	Δg[d]	W1	W10	W26	ΔA	W1	W10	W26	ΔHIX
CLO: Free-draining												
Control	1.3	1.7	2.2	0.33	12.4	9.7	8.9	-3.52	0.9744	0.9290	0.9327	-0.0417
Mn	1.2	1.5	2.2	1.00	12.2	8.0	6.6	**-5.60**	0.9735	0.9257	0.9429	-0.0306
Sand	1.3	1.7	1.7	0.40	11.9	9.4	8.0	-3.95	0.9121	0.9296	0.9389	0.0268
Char	1.1	1.7	2.9	**1.83**	12.0	8.6	7.2	-4.76	0.9111	0.9302	0.9470	**0.0359**
CLO: Waterlogged												
Control	1.2	1.5	2.4	**1.25**	11.9	9.7	7.4	**-4.46**	0.9198	0.9196	0.9373	0.0175
Mn	1.6	1.3	2.3	0.73	12.1	11.2	9.4	-2.69	0.9160	0.9283	0.9328	0.0168
Sand	2.0	1.6	1.8	-0.20	10.9	10.3	9.6	-1.30	0.9145	0.9232	0.9322	0.0177
Char	1.5	1.6	2.2	0.65	11.6	10.2	9.2	-2.42	0.9079	0.9131	0.9375	**0.0296**
Peat: Free-draining												
Control	5.3	5.8	6.1	0.76	1.6	1.2	1.1	**-0.48**	0.9821	0.9553	0.9715	-0.0106
Mn	5.3	5.2	6.1	0.81	1.5	1.4	1.2	-0.31	0.9699	0.9596	0.9681	-0.0018
Sand	5.2	4.7	5.3	**1.00**	1.6	1.2	1.2	-0.41	0.9545	0.9473	0.9677	**0.0132**
Char	5.1	4.8	5.8	0.63	1.6	1.3	1.2	-0.41	0.9554	0.9573	0.9536	-0.0018
Peat: Waterlogged												
Control	5.0	5.0	6.0	**0.97**	1.7	1.3	1.1	-0.54	0.9506	0.9628	0.9534	0.0028
Mn	5.0	5.1	5.0	0.02	1.6	1.3	1.2	-0.39	0.9464	0.9617	0.9766	0.0302
Sand	4.9	4.5	5.3	0.33	1.7	1.4	1.2	**-0.56**	0.9443	0.9579	0.9658	0.0214
Char	6.3	5.5	6.8	0.55	1.5	1.3	1.1	-0.39	0.9539	0.9608	0.9686	0.0147

[a] *Weight of humic acid extracted (g; mean of three replicates).*
[b] *Absorbance ratio (465nm/665nm; mean of three replicates).*
[c] *Humification index using emission fluorescence spectra (ratio of areas: 435-480nm/400-345nm; mean of three replicates).*
[d] *Change between new and aged samples (weeks 1-26).*

Table 6.4: Comparison of the data from the three forms of humic acid analysis for CLO and peat at weeks 1, 10 and 26.

A summary of the humic analysis data

Table 6.4 gives an overview of the differences seen between each treatment over the trial period for all three humic analysis methods used. The numbers highlighted in bold print show the sample that has exhibited the greatest degree of humification within that particular sample set. For example, for the free-draining CLO samples, the sample with added char produced the greatest yield of HA after 26 weeks, 1.83g more than at week 1.

Although there is not absolute agreement between data sets in that the different methods will show a greater degree of humification in different samples, there is general agreement seen. On the whole, if the HA yield increases in the alkali extraction procedure, the E_4/E_6 ratio decreases and the HIX increases. An exception to this is the HIX values for the control and sand-amended free-draining CLO and peat samples; both show a decrease in HIX after 26 weeks. This could signify a reduction in molecular weight of HA components but not necessarily a reduction in HA. The fluorescence fingerprint charts in Figures 6.16-6.18 show an increase in excitation wavelength at week 26 for the HA-like peak (p2) which is indicative of a greater degree of humification.

For the alkali extraction procedure, no significant differ trend was seen between waterlogged and free-draining samples, both giving similar yields; the UV photospectrometry showed the same. For HIX, there were significant differences seen between waterlogged and free-draining samples with the former having higher HIX values by the end of the trial than at the beginning. This is more in line with the flux results seen where free-draining samples increased over time and waterlogged samples decreased. The HIX and CO_2 flux methods tend to be more sensitive than the alkali extraction procedure and the UV photospectroscopy where there tends to be more scope for experimental error. This may account for differences seen.

Both alkali extraction and UV photospectrometry showed significant differences between CLO and peat samples where the peat appeared to contain more humic material than the CLO; HIX showed no significant differences between the two materials where both were relatively high.

With regards to differences between treatments, the Mn-coated sand and char- amended free-draining CLO samples both had a significantly lower E_4/E_6 ratio than the control after 26 weeks, implying a greater degree of humification achieved. These samples also showed a greater decrease in CO_2 flux rate than the control sample. A corresponding increased HA yield in the alkali extraction was observed, along with higher HIX values.

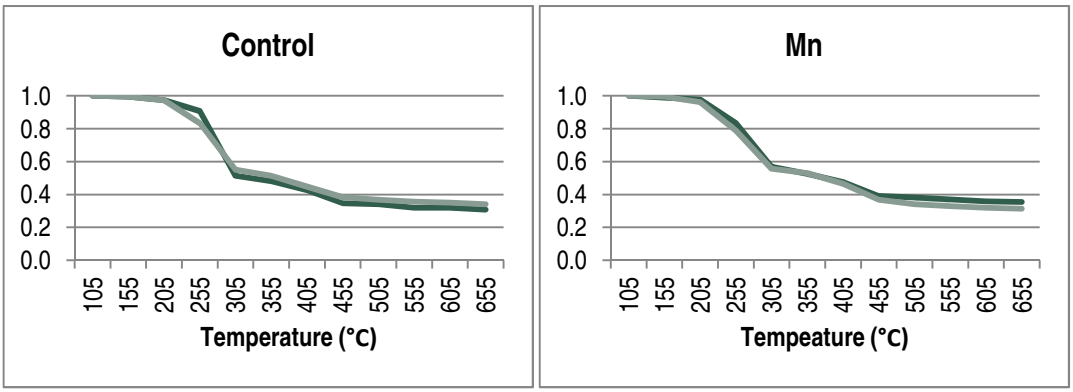
Some samples show an initial decrease in humic acid at week 10, only for it to rise again by 26 weeks. Veeken *et al* (2000) found that in composted biowaste, the humic acid content decreased during the initial stage but began to increase once again after 20 days. At the beginning of the process, the humic substances were found to mainly be of an aliphatic nature which were replaced by aromatic compounds during the composting process. Figures 6.16b and 6.18b both indicate that this might be the case in this study where an increase in the peak intensity is seen at week 10 in the FA-like peak.

6.7.4 Pseudo TGA data

This method provided some interesting data for the control and standards matrix with a temporal trend seen for the Trial 2 samples; therefore it was decided to repeat the analysis with the samples from this trial, in fulfilment of objective 5. The method outlined in Chapter 5.3.1 was followed and the results will presented in the same format, with comparisons made to the control sample matrix and the standard sample matrix (Chapter 5.4.2 and 5.4.3).

A selection of charts will be presented within the body of this section to highlight any relevant trends within the data series.

Precision between replicate samples



Figures 6.22 and 6.23: temperature curves for the control and Mn-amended free-draining CLO samples at week 1, both graphs showing the two replicates for that sample.

The reproducibility is high between the two replicate samples. Where two replicates were seen (weeks one and ten for all samples), this precision was moderately uniform across all free-draining and waterlogged CLO and peat samples. For week 26, where there were six replicates for each sample, more deviation between samples was seen (Figure 6.24 and 6.25).

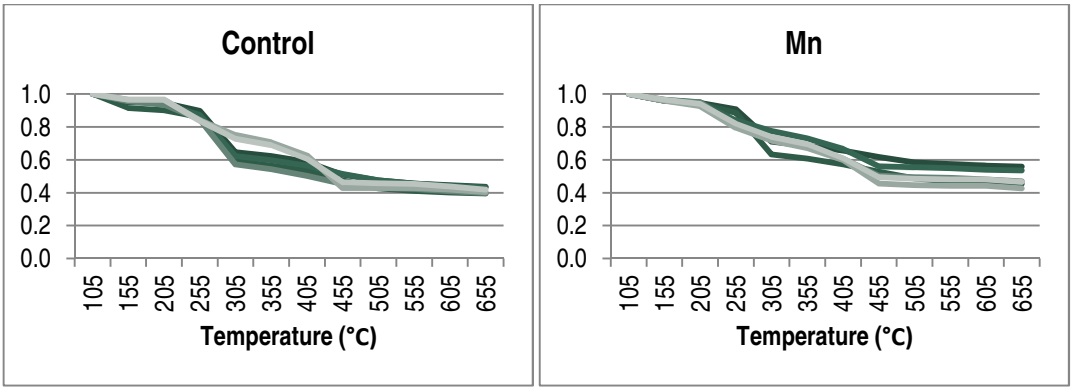
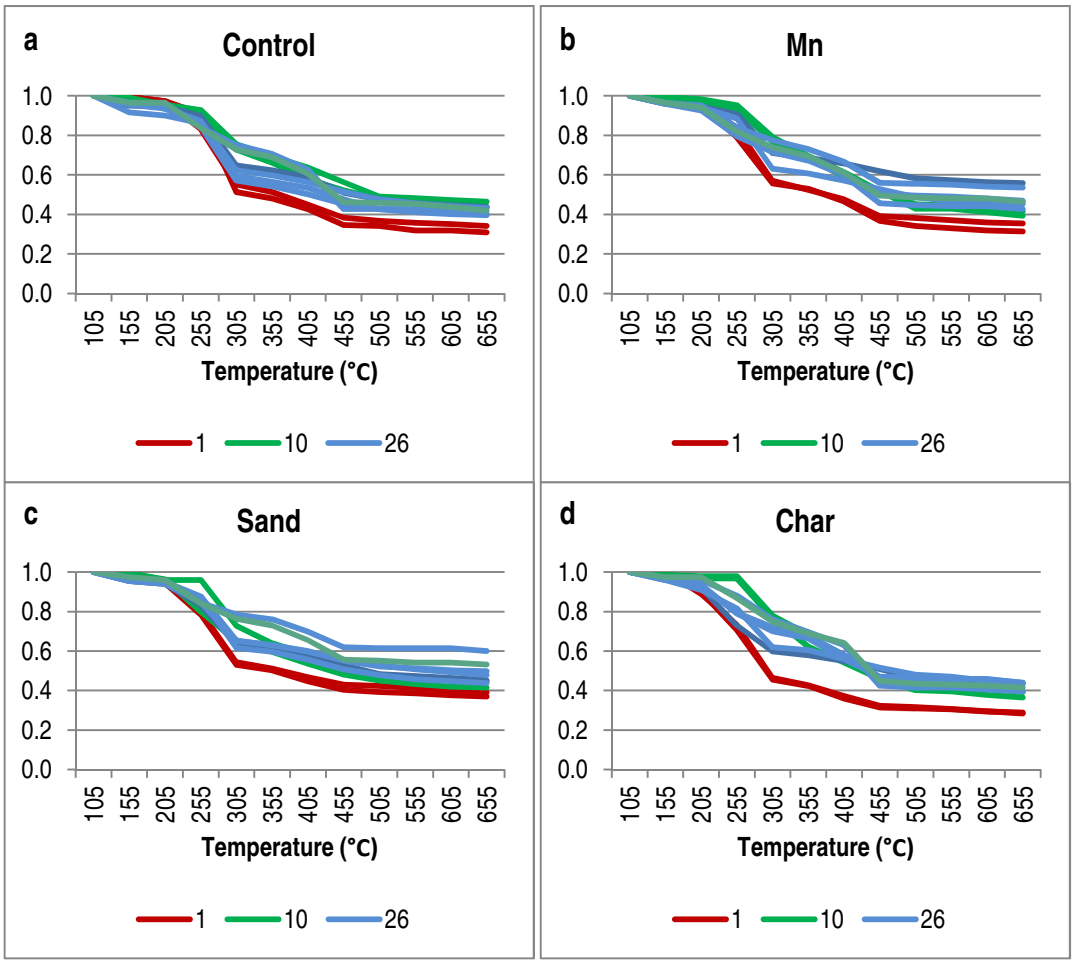


Figure 6.24 and 6.25: temperature curves for the control and Mn-amended free-draining CLO samples at week 26, both graphs showing the six replicates for that sample.

The heterogeneity of the CLO samples leads one to expect some deviation but the peat samples also showed a similar pattern, in some cases to a greater extent.

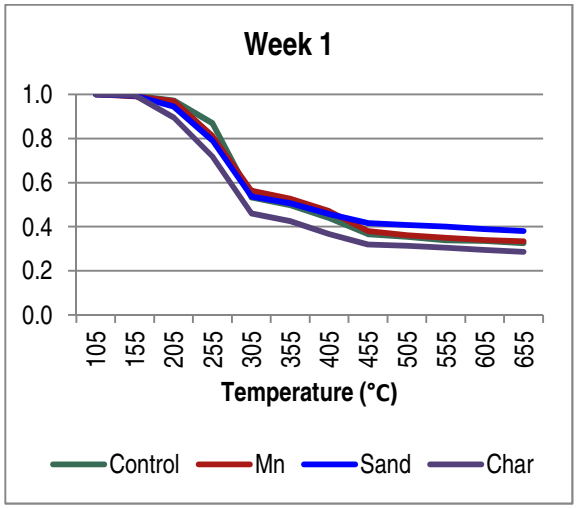
Temporal analysis



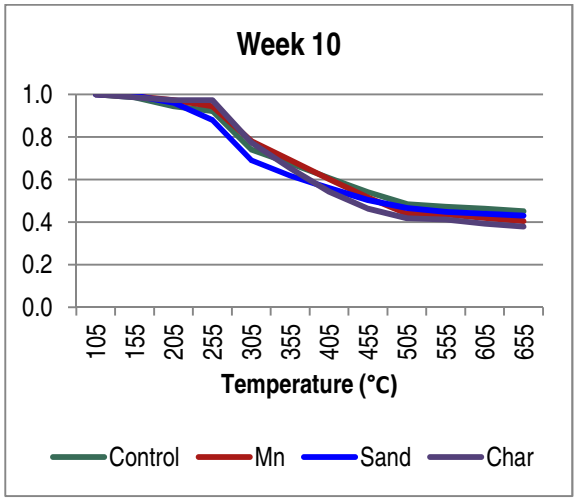
Figures 6.26 a-d: temperature curves for the free-draining CLO samples at weeks 1, 10 and 26.

The week 1 samples appear to decompose on heating to a greater degree than the week 10 and 26 samples; this is most apparent for char samples.

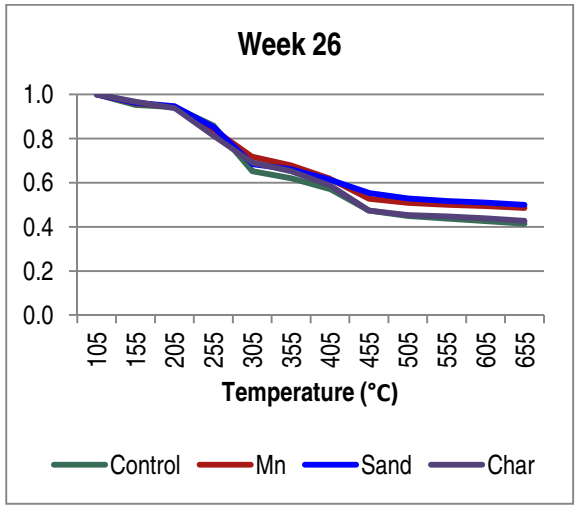
There were significant differences between all samples ≤ 455 °C between weeks 1 and 10, with the latter appearing to have less cellulosic material to burn. At week 26, this trend was only significant at ≤ 355 °C.



Figures 6.27 a: temperature curves for the free-draining CLO samples at week 1 showing the average readings for each of the treatment



Figures 6.27 b: temperature curves for the free-draining CLO samples at week 10 showing the average readings for each of the treatment



Figures 6.27 c: temperature curves for the free-draining CLO samples at week 26 showing the average readings for each of the treatment

No significant differences were seen between free-draining and waterlogged samples for either CLO or peat. However all CLO and peat samples were significantly different at each temperature interval > 255°C across the trial period. A difference in the shape of the curve can be observed between week 1 and 26 between 205°C and 305 °C where the more mature samples exhibit less of a loss on ignition.

The only significant difference seen between treatments was for the samples with added sand. In CLO, all the samples with sand weighed significantly more at ≥ 505 °C and for peat, ≥ 405 °C. Once again, the samples with Mn-coated sand would be expected to exhibit some differences also, when the control samples are considered.

Principal Component Analysis

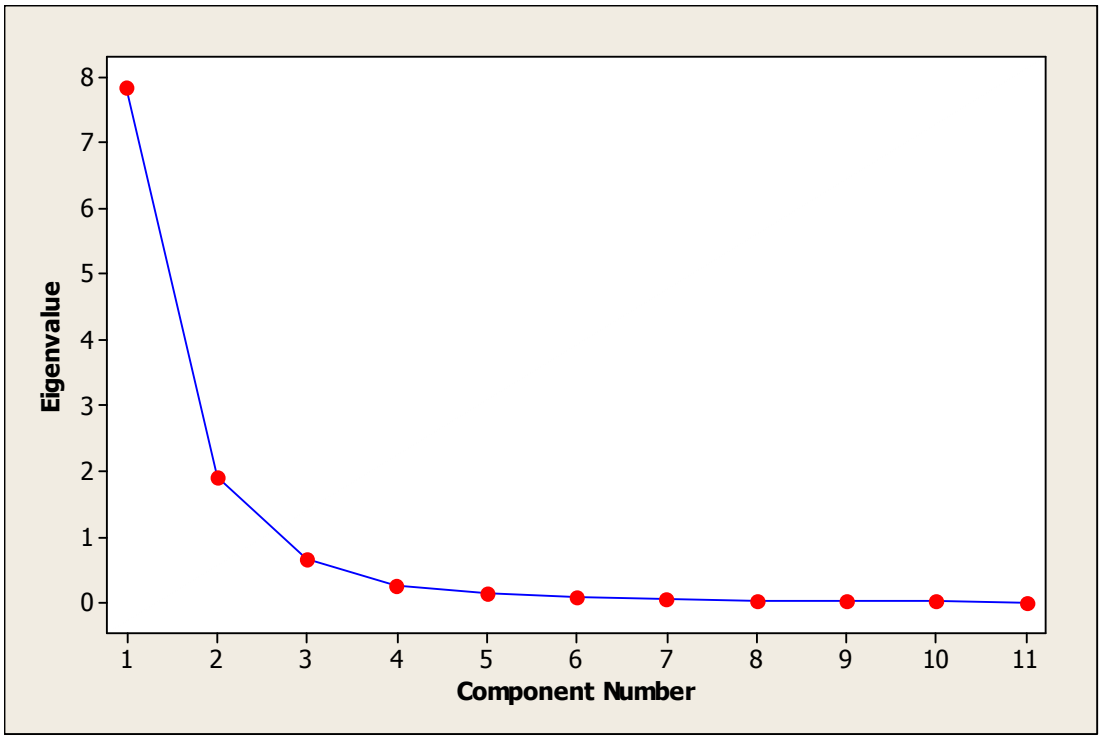


Figure 6.28: Scree plot to show the principal components retained.

As discussed in Chapter 5.33, the components with eigenvalues of ≥1 are retained, plus the first one in sequence that is <1. So in this case, the first 3 will be the principal components.

Temperature

Variable (°C)	PC-1	PC-2	PC-3
155	0.070	-0.523	0.587
205	0.153	-0.559	-0.016
255	0.210	-0.527	-0.158
305	0.289	-0.106	-0.537
355	0.324	-0.063	-0.383
405	0.346	0.053	-0.150
455	0.357	0.110	0.170
505	0.355	0.144	0.176
555	0.353	0.159	0.186
605	0.350	0.158	0.193
655	0.344	0.184	0.201

Table 6.5: the principal components for each temperature interval.

The first principal component, PC-1 shows the highest scores for 455 ºC, followed by 505 ºC, 555 ºC and 605 ºC. This ought to relate to the refractory components. The second principal component, PC-2 is dominated by the lowest temperatures, 155-255 ºC. The third principal component PC-3 has a high scores at 305 ºC and 355 ºC also but has the highest scores at the lowest temperature, 155 °C and so is perhaps dominated by the more labile materials.

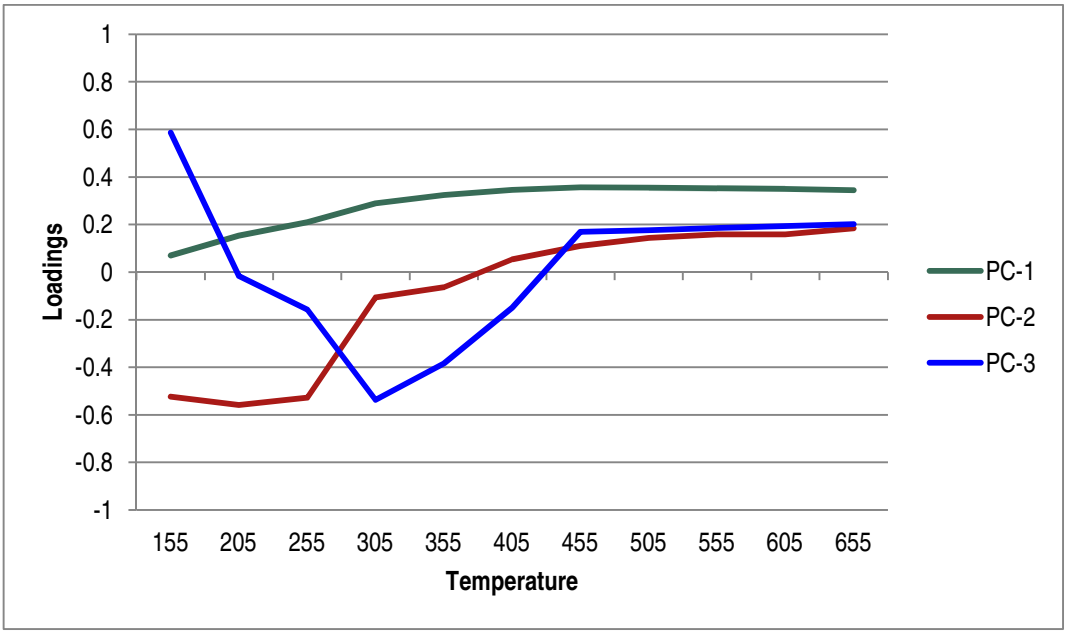


Figure 6.29: the loadings at each temperature for PC1, 2 and 3.

CLO: Control

Drainage	Week	PC1	PC2	PC3
Free-draining	1	0.4823	-0.6248	0.4711
Free-draining	1	0.7890	-0.3033	0.5173
Free-draining	10	2.353	0.034	-0.002
Free-draining	10	2.763	-0.114	0.012
Waterlogged	10	1.948	-0.817	-0.274
Waterlogged	10	1.807	-0.895	-0.388
Free-draining	26	2.104	0.377	-0.108
Free-draining	26	1.373	0.944	-0.364
Free-draining	26	1.968	0.385	0.138
Free-draining	26	1.342	0.446	0.191
Free-draining	26	2.056	0.108	-0.509
Free-draining	26	2.168	0.197	-0.295
Waterlogged	26	1.538	1.416	0.454
Waterlogged	26	1.715	2.037	0.808
Waterlogged	26	0.575	1.126	0.987
Waterlogged	26	1.490	0.827	0.414
Waterlogged	26	0.702	0.776	0.760
Waterlogged	26	1.040	0.706	0.554

Table 6.6 Principal component scores for all CLO control samples.

CLO: Mn

Drainage	Week	PC1	PC2	PC3
Free-draining	1	0.9588	-0.2437	0.4133
Free-draining	1	0.5740	-0.2103	0.3963
Free-draining	10	2.391	-0.588	-0.314
Free-draining	10	2.377	-0.330	-0.213
Waterlogged	10	4.944	0.326	0.303
Waterlogged	10	2.652	0.165	0.212
Free-draining	26	3.325	0.611	0.161
Free-draining	26	2.138	0.409	0.137
Free-draining	26	3.166	0.611	-0.103
Free-draining	26	1.637	0.229	-0.569
Free-draining	26	1.964	0.580	-0.253
Free-draining	26	1.754	5.411	-5.676
Waterlogged	26	1.680	0.694	-0.277
Waterlogged	26	1.729	0.818	0.386
Waterlogged	26	2.630	1.033	0.430
Waterlogged	26	2.088	1.196	0.391
Waterlogged	26	1.732	0.192	0.121
Waterlogged	26	0.558	2.166	-0.004

Table 6.7 Principal component scores for all CLO samples with manganese.

CLO: Sand

Drainage	Week	PC1	PC2	PC3
Free-draining	1	1.1402	0.0723	0.8341
Free-draining	1	0.8407	0.1434	0.6764
Free-draining	10	1.765	0.074	0.416
Free-draining	10	2.456	-0.321	0.121
Waterlogged	10	4.215	0.135	0.207
Waterlogged	10	4.929	0.166	0.392
Free-draining	26	2.102	0.604	0.120
Free-draining	26	2.450	0.665	0.166
Free-draining	26	2.560	0.442	0.259
Free-draining	26	1.948	0.464	0.125
Free-draining	26	3.798	0.778	0.133
Free-draining	26	3.141	0.443	0.027
Waterlogged	26	1.954	0.157	0.242
Waterlogged	26	1.741	0.172	0.507
Waterlogged	26	2.653	0.931	0.330
Waterlogged	26	2.646	1.056	0.287
Waterlogged	26	0.645	1.617	0.413
Waterlogged	26	1.616	1.302	0.224

Table 6.8 Principal component scores for all CLO samples with sand.

CLO: Char

Drainage	Week	PC1	PC2	PC3
Free-draining	1	-0.3280	0.3531	0.7816
Free-draining	1	-0.2370	0.1840	0.9172
Free-draining	10	1.765	0.074	0.416
Free-draining	10	2.456	-0.321	0.121
Waterlogged	10	2.060	-0.720	-0.178
Waterlogged	10	2.265	-0.609	-0.025
Free-draining	26	1.675	0.876	0.426
Free-draining	26	1.903	0.751	0.210
Free-draining	26	2.091	0.518	-0.091
Free-draining	26	2.010	0.509	-0.011
Free-draining	26	2.011	0.029	-0.647
Free-draining	26	2.194	-0.090	-0.366
Waterlogged	26	1.393	0.846	0.175
Waterlogged	26	1.163	0.600	0.213
Waterlogged	26	1.119	1.054	0.391
Waterlogged	26	1.178	1.018	0.301
Waterlogged	26	0.491	1.229	0.181
Waterlogged	26	0.846	1.378	0.305

Table 6.9 Principal component scores for all CLO samples with char.

Peat: Control

Drainage	Week	PC1	PC2	PC3
Free-draining	1	-3.7153	0.0200	0.6735
Free-draining	1	-4.0089	0.1829	0.9595
Free-draining	10	-2.004	-1.518	-1.087
Free-draining	10	-1.669	-1.427	-1.231
Waterlogged	10	-2.037	-1.432	-0.789
Waterlogged	10	-1.838	-0.980	-0.964
Free-draining	26	-2.930	-0.277	-0.247
Free-draining	26	-3.026	0.126	-0.001
Free-draining	26	-3.253	-0.188	0.021
Free-draining	26	-3.976	0.935	0.817
Free-draining	26	-2.967	0.229	-0.848
Free-draining	26	-2.530	0.347	-1.191
Waterlogged	26	-3.698	1.054	-0.294
Waterlogged	26	-3.580	0.421	-0.001
Waterlogged	26	-3.540	0.542	-0.180
Waterlogged	26	-4.355	1.900	-0.091
Waterlogged	26	-4.117	1.359	0.528
Waterlogged	26	-4.152	1.475	0.684

Table 6.10 Principal component scores for all Peat control samples.

Peat: Mn

Drainage	Week	PC1	PC2	PC3
Free-draining	1	-3.6293	0.3093	0.9826
Free-draining	1	-3.5948	0.3304	0.8962
Free-draining	10	-0.844	-0.911	-0.820
Free-draining	10	0.702	-1.044	-0.376
Waterlogged	10	-2.645	-0.479	-0.623
Waterlogged	10	-2.386	-0.903	-0.006
Free-draining	26	-4.237	1.412	0.759
Free-draining	26	-3.908	0.943	0.607
Free-draining	26	-3.394	1.169	0.741
Free-draining	26	-2.642	1.628	1.026
Free-draining	26	-3.179	0.617	-0.683
Free-draining	26	-0.078	0.695	-0.643
Waterlogged	26	-3.434	0.300	-0.176
Waterlogged	26	-2.823	0.689	-0.181
Waterlogged	26	-3.420	1.523	0.222
Waterlogged	26	-3.552	1.214	0.026
Waterlogged	26	-3.441	1.699	-0.167
Waterlogged	26	-3.168	2.770	0.513

Table 6.11 Principal component scores for all Peat samples with manganese-coated sand

Peat: Sand

Drainage	Week	PC1	PC2	PC3
Free-draining	1	-3.2057	0.3178	0.8573
Free-draining	1	-3.1525	0.4329	0.9596
Free-draining	10	-3.733	1.157	0.394
Free-draining	10	-2.754	0.081	-0.713
Waterlogged	10	-1.740	0.696	0.093
Waterlogged	10	-2.491	1.201	0.009
Free-draining	26	-3.361	1.434	0.669
Free-draining	26	-3.733	1.157	0.394
Free-draining	26	-1.432	-0.122	-0.885
Free-draining	26	-2.404	0.540	-0.192
Free-draining	26	-0.656	0.445	-0.713
Free-draining	26	-1.089	0.352	-0.853
Waterlogged	26	-1.740	0.696	0.093
Waterlogged	26	-2.491	1.201	0.009
Waterlogged	26	-1.976	0.759	0.308
Waterlogged	26	-1.546	1.272	0.761
Waterlogged	26	-2.688	1.777	-0.024
Waterlogged	26	-2.233	1.356	-0.080

Table 6.12 Principal component scores for all Peat samples with sand.

Peat: Char

Drainage	Week	PC1	PC2	PC3
Free-draining	1	-3.9617	0.4082	0.8959
Free-draining	1	-3.9627	0.1180	0.8277
Free-draining	10	-1.023	-1.611	-1.511
Free-draining	10	-1.190	-1.728	-1.235
Waterlogged	10	-2.657	-0.667	-0.634
Waterlogged	10	-2.645	-0.479	-0.623
Free-draining	26	-2.707	0.863	-0.036
Free-draining	26	-2.671	0.081	-0.577
Free-draining	26	-2.542	-0.151	-1.233
Free-draining	26	-2.350	-0.411	-1.388
Free-draining	26	-2.035	-0.710	-1.565
Free-draining	26	-1.956	-0.692	-1.562
Waterlogged	26	-3.549	0.842	-0.218
Waterlogged	26	-3.938	1.082	0.063
Waterlogged	26	-2.754	0.081	-0.713
Waterlogged	26	-3.506	0.758	0.223
Waterlogged	26	-4.185	1.126	0.759
Waterlogged	26	-4.050	1.244	0.544

Table 6.13 Principal component scores for all Peat samples with char.

The highest positive scores for PC-1 in the CLO samples (as highlighted in purple) are across weeks 10 and 26. There seems to be little difference between waterlogged and free draining samples. The highest positive scores seen for PC-2 are almost exclusively seen in the waterlogged samples at 26 weeks. The loadings for PC-3 are relatively low, perhaps suggesting a lower proportion of labile material present in the samples.

All of the highest scores for peat were negative for PC-1. Overall, no difference was seen between weeks or between waterlogged and free-draining samples. The peat with sand saw the highest PC-1 scores for free-draining samples; the peat with char, were highest at week 1 and all of the waterlogged samples at week 26.

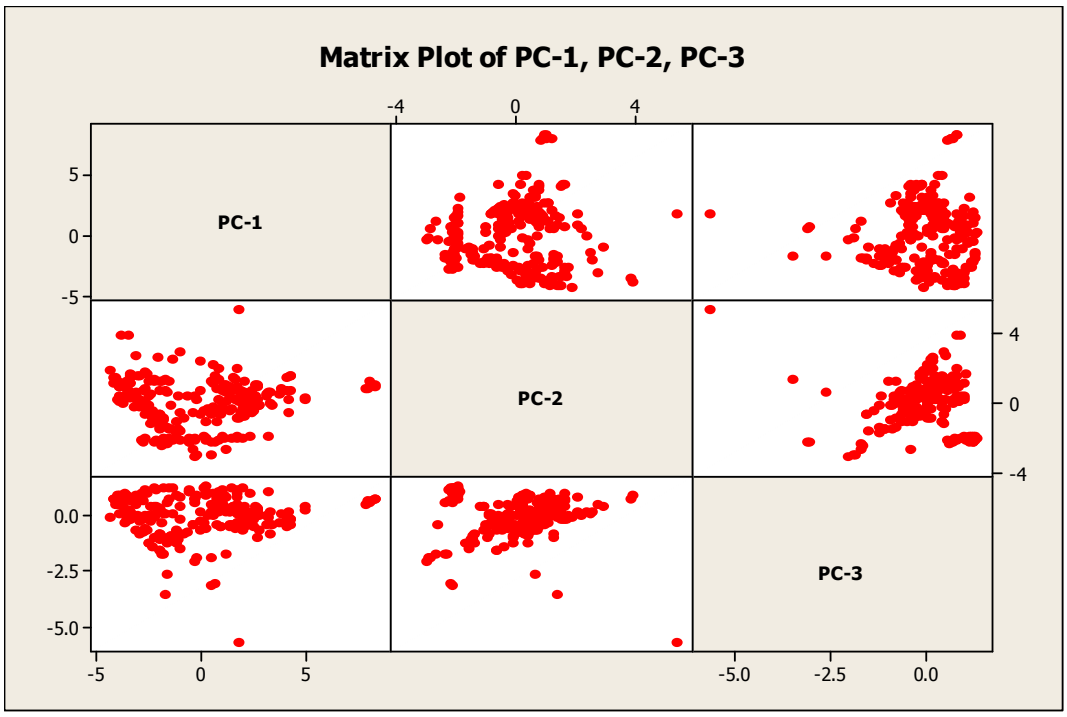


Figure 6.30: A matrix plot of PC-1, PC-2 and PC-3 scores from all trial 3 data.

The distribution of data show several possible trends. Plotting the scores from specific samples against one another should make this clearer. The score plots in Figures 6.31 - 6.33 separate the CLO and the peat samples into different plots (a and b). Firstly, the relationship between the different treatments will be examined; secondly, any temporal differences between samples; and lastly, any distinctions between waterlogged and free-draining samples.

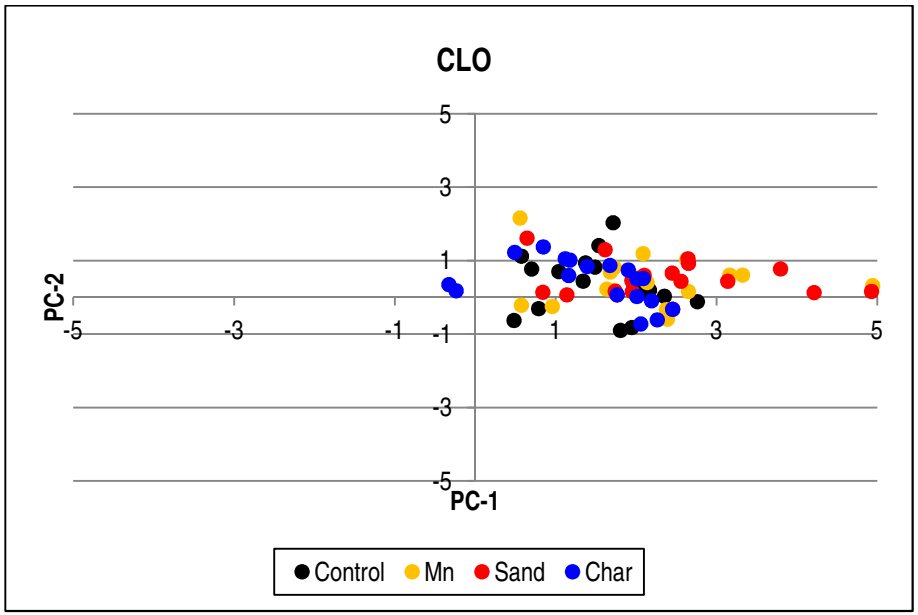


Figure 6.31a: Score plot of PC-1/PC -2 for all CLO samples

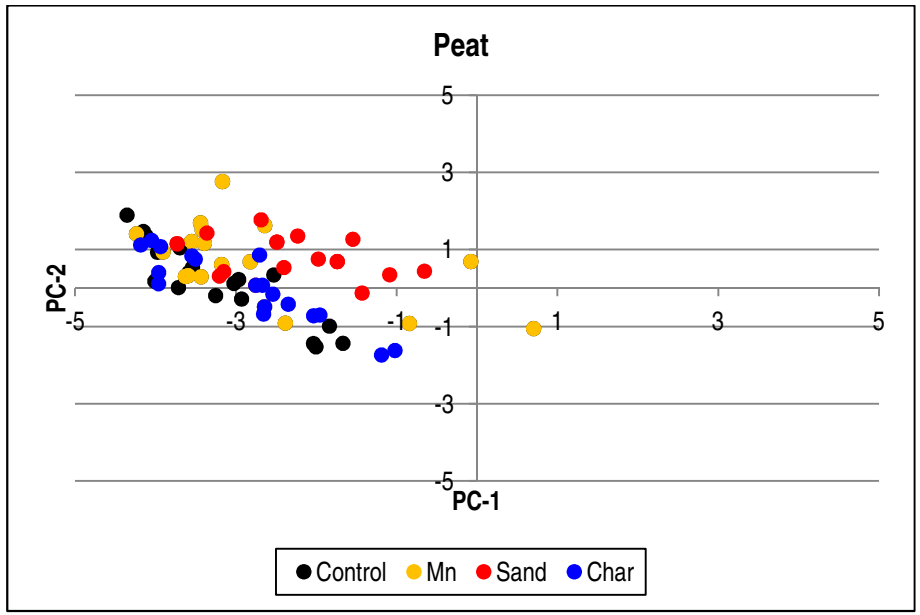


Figure 6.31 b: Score plot of PC-1/PC-2 for all Peat samples.

The CLO samples largely have positive values for both PC-1 and PC-2 whereas the peat samples have mainly negative PC-1 and positive PC-2. In Chapter 5, Figures 5.4 and 5.8 showed the score plots for PC-1 and PC-2 for the control and standard samples respectively. When comparing Figures 6.29 and b, some trends can be seen.

This matrix data showed that the top left quarter of the chart was dominated by lignin-rich samples; the top right quarter by humic acid-rich samples and the bottom left quarter by cellulose-rich samples. Comparing the data from this trial with the matrix data, it seems that the peat samples may be more lignin (and possibly cellulose) rich whereas the CLO samples could be more humic-acid rich.

As for differences between samples, the samples with Mn-coated sand, sand and char from this trial seem to reflect the 'pure' Mn-coated sand, sand and char samples from the matrix.

When assessing temporal differences for all samples (Figures 6.32a and b), patterns can be seen for both CLO and peat. Week 1, when compared to the standards matrix, appears to have an average composition of 30% HA: 60% lignin: 10% cellulose (or other labile components, if the control matrix when taken into account). At week 10, the data points are more spread across the positive PC-2 axis and are seen both above and below 0 on the PC-1 axis. These data points seem to move away from a higher lignin and cellulosic area to a higher humic area of the plot. At week 26, a spread of data is once again seen but there is a strong positive trend towards high humic acid contents. The peat samples appear to trend towards a greater proportion of lignin-like material towards 26 weeks.

In comparing the waterlogged and free-draining samples, any trends that there might be in the data are less clear (Figures 6.33a and b). The waterlogged samples for both peat and CLO, however, seem to have slightly higher lignin and HA contents respectively than their free-draining counterparts.

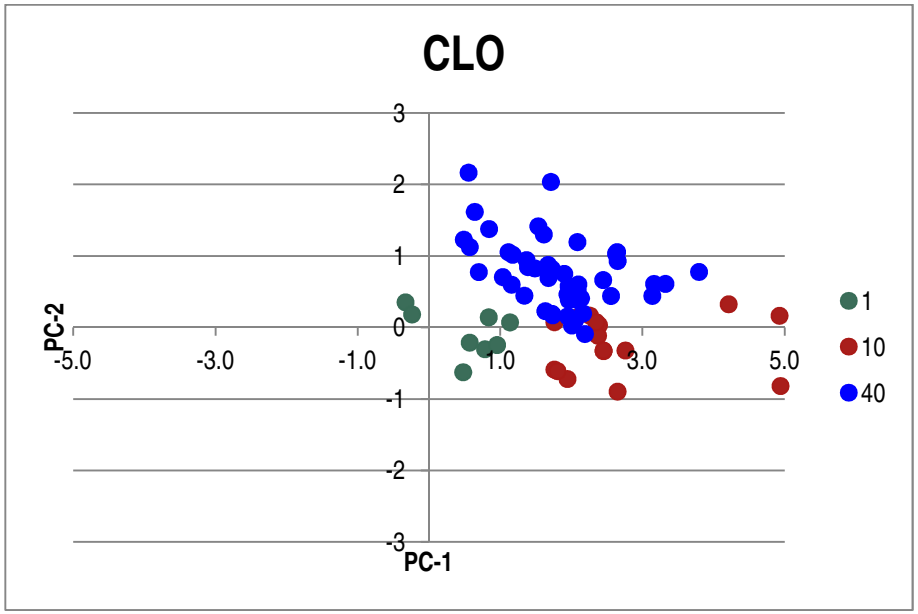


Figure 6.32 a: Score plot of PC-1/PC -2 for all CLO samples at weeks 1, 10 and 26

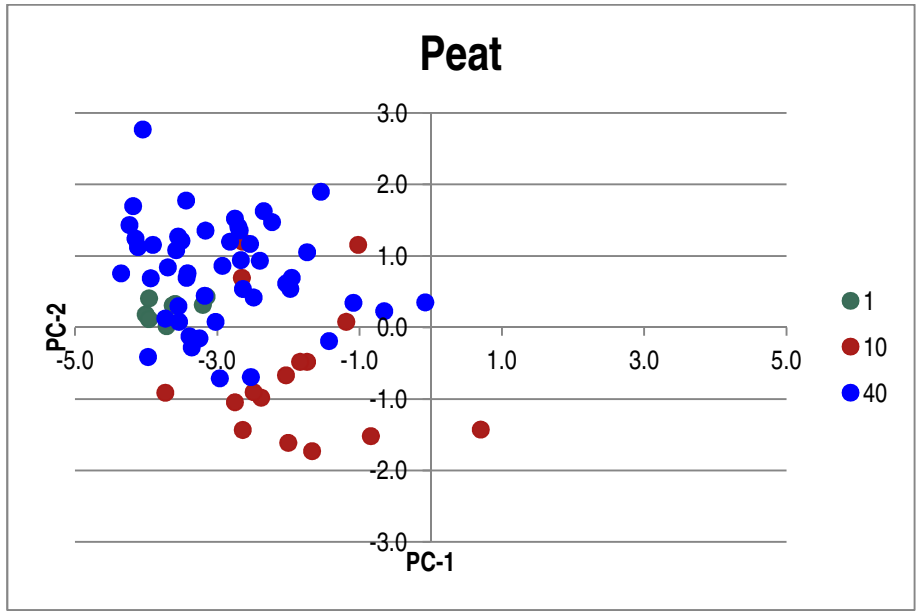


Figure 6.32 b: Score plot of PC-1/PC -2 for all Peat samples at weeks 1, 10 and 26

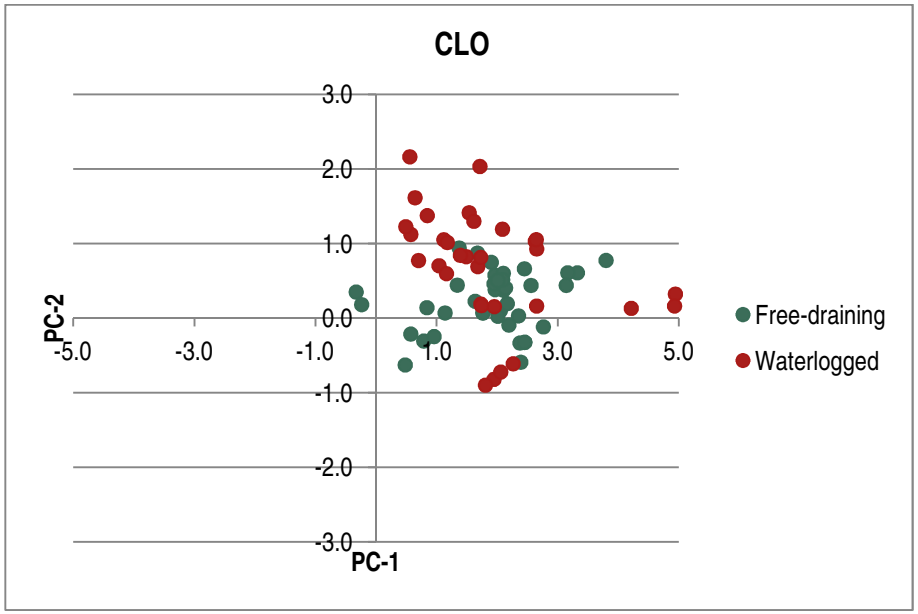


Figure 6.33 a: Score plot of PC-1/PC -2 for all Free-draining and waterlogged CLO samples across all weeks

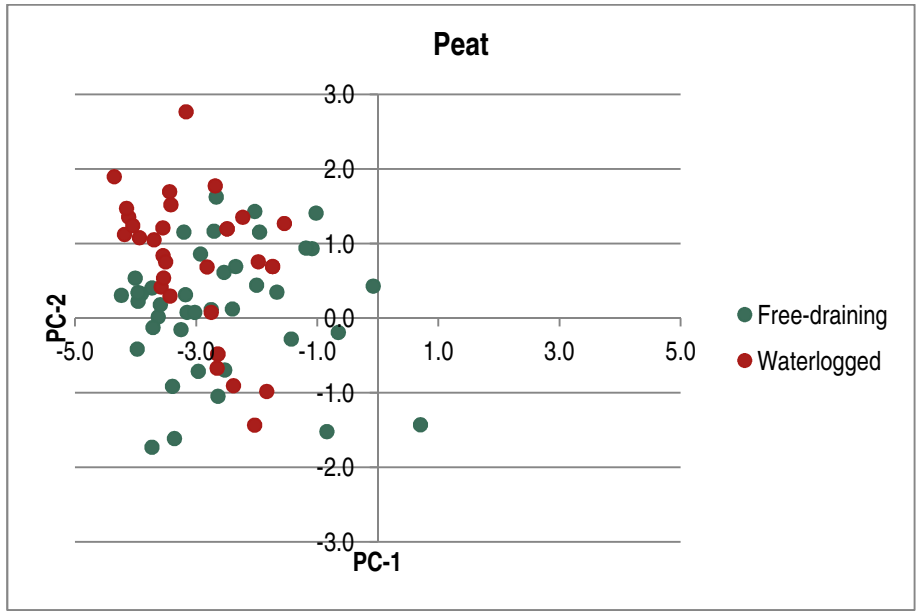


Figure 6.33 b: Score plot of PC-1/PC -2 for all Free-draining and waterlogged Peat samples across all weeks.

6.8 Conclusions

6.8.1. Revisiting the objectives

1. *To control ambient conditions in order to better assess any significant differences between data sets.*

The soil moisture was monitored and although an attempt was made to ensure that it was kept above 40%, this was not always achieved. The temperature in the laboratory however was kept relatively constant.

Temporal trends were seen for most samples with every form of analysis, along with some differences seen between CLO and peat samples, and waterlogged and free-draining samples. The differences between control and amended samples were seen more clearly than in the previous trial where the ambient conditions were not controlled.

On the whole, this objective was met successfully although the trends seen in this trial could be attributed to the increased trial length, as well as the controlled laboratory conditions.

2. *To introduce peat as a soil standard.*

Peat was introduced to assess the effect of CLO's heterogeneity. It was expected that the peat, being a more homogenous material, would show better precision between replicate samples and therefore highlight trends between different sample sets. However, this was not the case with a spread of data seen between replicate samples in this data set too. When inspecting the peat, it was clear that although it contained no contaminating species, the particle size of the material still varied with pieces of non-degraded wood present. This may have affected the precision.

Although peat has similarities to CLO in its high organic carbon content, it is a far more mature and stabile soil and as such, already much more humified. This being the case, it is possible that peat may not be susceptible to catalysis if the humification process has largely occurred for the material present. The E_4/E_6 ratios were very low, some close to the lower limits of detection for the photospectrometer (absorbance <0.01), indicating a high level of humification. This was significantly lower than the CLO E_4/E_6 ratios, a trend also seen in the flux and alkali extraction data.

3. *To lengthen the trial period, assessing the degree of humification at 10 weeks and 26 weeks.*

Temporal trends were seen across all forms of analyses. Current literature provides contrasting time periods under which humification of composted material takes place (Eklind and Kirchmann, 2000; Paredes *et al.,* 2002; Smars *et al.,* 2002; Cayuela *et al.,* 2008; Chang and Hsu, 2008). This, combined with the lack of knowledge on the age and maturity of the CLO batch sampled, it is difficult to know whether a longer trial period still might be yet more informative.

4. *To avoid sub-sampling of lysimeters for flux measurements.*

Sampling straight from the lysimeters was much less time consuming than sub-sampling so the method used was successful. More significant trends where seen in the flux data in this trial than in Trial 2 (Chapter 4).

If the age of the material tested had been known, the method followed may not have been suitable. If the study was repeated with a better characterised batch of CLO, another method may have to be sought.

5. *To re-examine the suitability of PTGA as a method of assessing humification.*

This method proved effective in finding trends between the different data sets. Patterns in possible labile and refractory content within samples were seen across the trial period. the more mature samples saw less of a loss on ignition between 205-305°C for example.

This method, along with the other forms of analyses established temporal trends, with the more mature samples appearing more humified; between CLO and peat: the former showing HA-like characteristics and the latter, lignin-like characteristics; waterlogged and free-draining samples with the former appearing slightly more humified/lignified. It was, however less effective at distinguishing between different treatments. Its limitations, some of which are discussed in section 5.5.2.

6. *To test biochar against Mn-coated sand and sand-supplemented samples in terms of their individual capacities to stabilise organic matter.*

For the flux data, no difference was observed between treatments for CLO samples in week one, as expected. By week two, the samples with added biochar exhibited a significantly higher flux than the control samples, possibly due to the trend often observed in soils with added char for short-term increased mineralisation within the first 20 days, as outlined by Smith *et al.* (2010). This pattern is mimicked in the humic data also with some samples show an initial decrease in humic acid at week 10, only for it to rise again by 26 weeks (see Table 6.4). The fluorescence fingerprint charts in Figures 6.16b and 6.18b both show an increase in the peak intensity at week 10 in the FA-like peak which reduces again by week 26.

By the end of the trial, a significant difference in flux between treatments can only be observed in the free-draining CLO samples; the CLO with char gives the greatest decrease in flux over time; the CLO with added manganese-coated sand also shows a slight reduction in flux when compared to the CLO control sample.

With regards to differences between treatments in the humic data, the Mn-coated sand and char- amended free-draining CLO samples both had a significantly lower E_4/E_6 ratio than the control after 26 weeks, implying a greater degree of humification achieved. A corresponding increased HA yield in the alkali extraction was observed, along with higher HIX values. The HA-like peak (P2) in the fluorescence fingerprint charts (Figures 6.17 and 6.18) also exhibited an increase in emission wavelengths in the week 26 samples for CLO with char and CLO with MnO_2-coated sand, indicating an increase in humification. The control samples saw no change in P2 position across the 26 weeks.

In conclusion, there is a strong case in the data produced to suggest that both MnO_2 –coated sand and char amendments both have a positive impact on the humification of CLO. The both appear to reduce CO_2 flux rate and to increase humic acid content with evidence from several forms of analysis.

7. *To examine any difference in the carbon flux and humic acid content of free-draining and water-logged samples.*

Differences between these two samples were more apparent with some forms of analyses than others. The greatest differences were observed in carbon flux where the free-draining CLO samples saw a decrease over the 26 weeks where as the waterlogged CLO samples increased. The former had a greater flux rate than the latter also. In terms of humic acid, these differences were not quite so pronounced. HIX values suggested a slightly greater degree of humification in the waterlogged samples than in the free-draining samples but no significant differences were observed between data sets for alkali extraction or E_4/E_6 data.

In the PTGA data, the waterlogged samples appeared to be more lignified and humified for peat and CLO respectively. Spectral analysis such as IR spectroscopy or NMR would need to be carried out to confirm this, however.

6.8.2 Trial limitations

Leaving the batch of CLO to mature in order that it might be within the IRGA's detection range was not ideal; this was only an option because of the lack of information available about the age of the material collected.

The moisture content ought to have been controlled more carefully to ensure that a minimum of 40% was achieved at all times.

The temperature of the laboratory was at a constant 19-20 °C. This would only perhaps be representative of temperatures in the UK during the summer months. If a longer trial was to be carried out under the same conditions, the temperature would have to be adjusted accordingly.

The importance of using replicate samples and statistically analysing the data have been highlighted; when assessed qualitatively using charts produced from the raw data, a trend was often apparent but quantitative analysis showed that the spread of data between replicate samples to be too great for that trend to be statistically significant.

The electrical conductivity of the leachate emanating from the lysimeters was not tested. This could have provided useful information on the amount of dissolved ions in the different samples, particularly between the samples with amendments. It was intended that during this trial, inductively coupled plasma-optical emission spectrometry (ICP-OES) would be used to monitor any differences in cations present in the leachates of each samples. This method of analysis was not available, however, at the time of the trial.

Once again, the CLO material was tested 'neat' without any inorganic carbon present. Laboratory trials cannot always fully predict what might happen in the field.

Chapter 7

A microstudy

In the previous chapters, CLO sourced directly from PWM's PARC recycling facility has been used for all trials. It was hoped that this final study would be field-based, so that results in previous trials could be tested *in situ*. Although laboratory studies can provide us with valuable information, there is no guarantee that what happens in these controlled, simplified systems will translate to a complex natural system. Throughout all trials, CLO has been used 'neat' so no information exists about its interactions with inorganic soil carbon which is known to have an important stabilising effect on relatively fresh OM (Huang, 2002; Sohi and Lehmann, 2008).

No plants have been grown on the soil so primary productivity has not been assessed, nor has the effect of a continuous source of fresh organic matter (Bear, 1964; Huang *et al.,* 2002). Macro fauna such as earthworms have an important influence on soil carbon turnover (Huang *et al.,* 2004) but as all trials so far have more or less been undertaken in a closed system effects such as these are yet to be seen.

With materials that have been classified as waste, it can be difficult to obtain permits to carry out land-based trials. BSI PAS 100 and BSI PAS 110 cover composts and source-separated anaerobically digested wastes respectively. As yet, co-mingled wastes are unpredictable; their feedstocks can differ widely and can be highly contaminated. The MnO_2-coated sand and char may require separate consideration if they are to be mixed with the CLO and laid to land also**.** When the time came to plan this trial, the Environment Agency had imposed restrictions on Premier's CLO, resulting in it temporarily having to be sent to landfill. It became clear that a field-trial was not going to be possible at this time. Rather than this macro-field study, it was decided to carry out a micro-study instead, exploring interactions between some of the major component of the CLO against the previously tested catalysts in order to gain a greater understanding of possible reaction pathways of the humification process.

7.1 Introduction

This study will take four major components of CLO. Cellulose comprises around 60% of MSW CLO and is the principal contributor to the degradable fraction of BMW, followed by lignin at around 20%. Proteins and fat contribute around 4 and 8% respectively. Each of these components will be reacted against one another in a fully, factorial study. The amendments from the previous trials (sand, MnO_2-coated sand and char) will also be incorporated into some of the samples.

As PWM also produce PAS 100 certified compost, this study could be useful in determining the lowest proportion of green waste they can feasibly set aside for their MSW feedstock for aerobic digestion. It is expected that a certain volume will be needed to balance the high proportions of animal fats and proteins found in kitchen wastes.

The large proportion of fat from food waste is one of the key factors that set CLO apart from natural soils. Initially, it can have an inhibitory effect on decomposition being difficult to decompose and having a shielding effect on other molecules from microbial attack. The acidity of food waste due to the presence of short-chain organic acids may also pose problems. These may be present in initial materials but may also be generated during the initial stages of the composting process (Yu and Huang, 2009). This can further reduce the pH which eventually inhibits microbial activity (Beck-Friis *et al.,* 2003)**.** This can be controlled, however, by the addition of an alkali amendment. Yu and Huang (2009) advocate the use of sodium acetate as a buffer salt which combines with the acetic acid to form a buffer solution, having positive effects on degradation. Proteins have been found to be a major factor that control the rate of composting, due to the fact that the bacteria require it to gain nutrients for their cell structures.

During the biooxidative stage of composting, the rapid degradation of simple compounds is seen and the temperature increases rapidly. During the following thermophilic phase, fats, cellulose and lignin are degraded (Senesi, 1989). In a composting pile of around 2000 kg, this stage is considered finished at around 90 days (Paredes *et al.,* (2002). The maturation phase which follows this is associated with the stabilisation and humification of the remaining organic matter and requires several weeks to months to give a stable end product (Veeken *et al.,* 2000).

In Section 2.2.2., a number of feasible routes via which humification might occur was proposed: the degradation of lignin; the polymerisation of quinones (derived from cellulose or lignin); and the condensation of sugars and proteins via the Maillard reaction. These routes involve the four components that will be tested in this trial.

In natural systems, both MnO_2 ((Senesi and Calderoni 1998, Bryan et al., 2001) and char (Cheng *et al.* 2008, Smith *et al.* 2010) have been found to stabilise SOM and. Conversely, Sunda and Kieber (1994) propose that manganese oxides can actually split humic substances to form lower molecular weight compounds, thus making them accessible to soil microbes. Similarly, Wardle *et al.* (2008) proposed that an enhanced loss of SOC occurred when char was added to a boreal forest humus layer. It was suggested that the char was responsible for promoting the growth of microbial communities and for enhancing the decomposition of labile C compounds, rather than stabilising them against degradation in soil. These opposing arguments were explored in Chapters 4, 5 and 6 where the catalytic potential of each was assessed when added to CLO. These chapters presented the data from trials 2 and 3 where humification was assessed using three different techniques: alkali extraction procedure, E4/E6 ratio given by UV photospectrometry and HIX as determined by fluorescence spectroscopy. The spectroscopic methods each indicated the degree of aromaticity of the sample and thus, the degree of humification. This micro-study will see the use of the same catalysts and humification will be assessed using the same analytical methods as mentioned above.

Similar micro-studies that have assessed the individual components present in biowaste composts (Eklind and Kirchmann, 2000; Paredes *et al.,* 2002; Smars *et al.,* 2002; Cayuela *et al.,* 2008; Chang and Hsu, 2008) have employed similar experimental controls. They have all maintained a minimum moisture content of 40%; ensured an ambient temperature (19-30°C); added bulking agents to ensure the correct C/N ratio and to achieve aerobic conditions; and used sample sizes of >10 kg . Finally, all biowaste samples were added to a natural soil, rather than being assessed in their neat forms.

Variation was seen, however in their trial length which ranged from 8 to 600 days. Cayuela *et al.,* (2008) found that the bio-oxidative phase was complete after 92 days in a 200kg pile, but their compost was then allowed to mature over a two month period. Moral *et al.,* (2009) suggest that the mineralisation of C and N in manure composts can take between 70 and 364 days. During the composting process of these biowastes, Veeken *et al.,* (2000) saw the HA content decrease between days 0-10 (the mesophilic phase) with the degradation of fatty

acids and polysaccharides but increased once again after 20 days. Smars *et al.,* (2002) witnessed low activity or a lag phase during mesophilic stage, possibly due to low pH which persists until neutralised by microbial activity.

7.2 Trial aims

To explore the interactions between four of the major components of CLO (lignin, cellulose, a lipid and a protein); both with and without the three amendments used in previous trials (sand, manganese-coated sand and char) in order to gain a greater understanding of possible reaction pathways of the humification process that occurs in CLO.

7.3 Trial objectives

1. To conduct a fully factorial study, assessing humification occurring between four major components of CLO: cellulose, lignin, a lipid and a protein.
2. To gauge the effect of char and Mn-coated sand and sand on these reactions.
3. To ascertain whether or not the introduction of an established microbial community via seeding of the samples will enhance humification.
4. To examine any differences seen in samples stored aerobically and anaerobically.
5. To use alkali extraction, UV photospectrometry and fluorescence spectroscopy to analyse the extent of humification achieved by each sample.

7.4 Materials

7.4.1 Substrates

Based upon the literature reviewed in Chapter 2, four substrates were chosen to represent an artificial soil. Cellulose is the major component in most CLO feedstocks, being abundant in food, garden and paper sourced wastes. Lignin is thought to be instrumental in two of the four humification pathways and is also abundant in most CLO feedstocks. Proteins comprise around 15% of food waste and one of the necessary substrates for the Maillard reaction and are used rather than amino acids as they are metabolised more slowly (Hayes *et al, 1999*). Finally, animal fat is one of the major components that differentiate CLO from natural soil.

Aldrich lignin and Aldrich egg albumin protein; ash-free paper was used to represent the cellulose component and lard, the lipid fraction. Table 7.1 gives an example of the matrix used to calculate the proportion of each substrate and catalyst.

7.4.2 Catalysts

The char was 100% lump-wood charcoal, as used in Trial 3. As before, it was crushed and sieved through a 4mm mesh to ensure that the surface area was comparable to the Mn-coated sand, and sand. The Mn-coated sand was again collected from Northumbrian Water and the sand was builders' sand from a local hardware shop. These were also sieved through the same size mesh to ensure control on the size and surface area.

7.4.3 Seeding

During industrial composting/anaerobic process, each new batch is seeded to ensure that an effective microbial community is established. The breakdown of most organic C in soils due to decomposer microorganisms which are affected by pH, redox, temperature, soil texture, quality of OM – C/N ratio, phenols, lignin and fat content: energy and nutrients for microbes (Cayuela *et al.,* (2008). The inoculum used to seed the appropriate samples was taken from the previous trial, given that the conditions in the CLO ought to be similar to that of its constituent parts, therefore fulfilling the above criteria. The leachate from a CLO control sample was used (pH 7.4); it was important not to use a sample that had been catalysed so as not to contaminate the Trial 4 samples with any traces of these.

7.4.4 Oxygen

As the sample matrix in Table 7.1 shows, some samples were aerobic, whilst others were anaerobic. A difference was observed between the free-draining and waterlogged samples in the previous trial which is why this parameter has been incorporated into this experiment. However, Eklind and Kirchmann, (2000) state that a litter addition is needed to achieve aerobic conditions in the composting of household waste. This trial will not be including a bulking agent so there may be no difference seen between samples. The albumin and lignin substrates are powders so together with the lard, may suffer compaction, also promoting anaerobic conditions.

7.5 Experimental set up

Sample	lignin	cellulose	lipid	protein	manganese	black carbon	sand	no catalyst	unseeded	seeded	aerobic	anaerobic
1a	x	x	x	x	x				x		x	
2a	x	x	x	x		x			x		x	
3a	x	x	x	x			x		x		x	
4a	x	x	x	x				x	x		x	
225a	x		x		x				x		x	
226a	x		x			x			x		x	
227a	x		x				x		x		x	
228a	x		x					x	x		x	

Table 7.1: A sample of the experimental matrix; the full version of which can be seen in the Appendices.

For each sample, 1 g of each component was used. This meant that the 512 samples could be constructed in a cost and time effective manner. Larger sample sizes would have been difficult to accommodate in the laboratory space available.

Each sample was weighed and transferred into a clean, labelled, sterile 30ml polypropylene screw cap container. The inoculated samples were injected with 1ml of leachate from Trial 3, the unseeded samples were injected with 1ml of Milli-Q DI water. The inoculated and sterile samples were stored separately to minimise the risk of cross-contamination. The anaerobic

samples were sealed and the aerobic samples loosely covered with foil to prevent cross-sample contamination. The samples were loaded in to trays and placed in the dark under controlled laboratory temperature of 22°C for a period of 10 weeks.

Eight control samples, which consisted of 1ml Milli-Q DI water, were prepared alongside the 512 samples and stored under the same conditions for the trial period. They were analysed with the other samples using the methods described in section 7.6 in order to establish lower limits of detection (LLD) for each method.

Figure 7.1: some of the 512 samples in labelled 30ml polypropylene screw cap containers.

Once again, the length of the trial was dictated by time constraints; the 10 week period chosen in order to make it comparable to Trials 2 and 3. In the other studies outlined in Chapter 7.1, some trials were as short as 8 days and all detailed significant changes seen in the maturation and stability of OM in the first 70 days. This, therefore, should be a suitable timeframe in which to witness any differences seen in humification between samples.

7.6 Methods

To make this study comparable to the previous studies, some of the same analytical techniques used were employed. This also allowed the many samples to be tested quickly via well understood, robust and inexpensive methods. The samples sizes were too small to measure flux with the IRGA and too numerous to measure using PGTA in the time available.

After 10 weeks, the samples were transferred into 100ml polyethylene centrifuge bottles with 0.2M NaOH. Samples were processed in batches to ensure that they didn't degrade before they were analysed.

7.6.1 Alkali extraction

The alkaline extraction procedure as described in Section 3.7.1 was carried out for each sample. In summary, a version of the IHSS method was employed whereby 100ml of 0.2M NaOH was used with two extraction stages. HCl was used during the HA/FA separation step.

7.6.2 UV analysis

The same method was followed as detailed in Section 3.7.2. This methods were successfully integrated with the first stage of the alkali extraction (pre-acidification), meaning that a great deal of time and materials were saved. Efficiency is imperative when many samples must to be analysed in a short period of time. The results are given as E_4E_6 ratio.

7.6.3 Fluorescence

The same method was followed as detailed in Section 3.7.3 as in Trial 3, some of the samples needed to be diluted to avoid IFE. Absorbance of each sample was tested and if below 0.3 at 337 nm excitation, was diluted accordingly with 0.2M NaOH. Results are presented as HIX values.

7.6.4 Statistical analysis

As described in Chapter 3.4, all data collected were entered into a Microsoft Excel spreadsheet, formatted and then ANOVA (General Linear Model) performed using Minitab 13. The results are given as p-values and all < 0.05 show a significant relationship between the two parameters compared. All results used from this point will be quoted as being statistically significant if they satisfy this requirement.

7.7 Results

7.7.1 Substrate

This section reviews the data with respect to the substrates, assessing whether there are any key differences or relationships between any of the different combinations. Table 7.2 summarises the data given for each of the control (no amendment added), aerobic and seeded samples.

Sample	E_4/E_6	HIX	Alkali extracted humic acid (g)
(ln, c, l, p)	3.752	0.8802	0.45
(ln, c, l)	No data	0.9161	0.19
(ln, c)	No data	0.9248	0.34
(ln)	4.329	0.8801	0.77
(c, l, p)	No data	0.6135	0.00
(c, l)	No data	0.7301	0.00
(c)	No data	0.7203	0.00
(l, p)	No data	0.4528	0.00
(l)	No data	0.7965	0.13
(p)	No data	0.5278	0.00
(c, p)	No data	0.7784	0.00
(ln, c, p)	2.459	0.8463	0.28
(ln, p)	2.479	0.8596	0.58
(ln, l, p)	No data	0.8546	0.37
(ln, l)	No data	0.9228	0.47
(none)	No data	No data	0.00

Table 7.2: The measurements taken after 10 weeks for all control, aerobic, seeded samples; where ln is lignin, c is cellulose, l is lipid and p is protein.

Many of the samples produced no data via UV analysis with the absorbance values reading below the lower limits of detection of the UV photospectrometer (absorbance <0.01nm) as calculated with the blank samples. When an attempt was made to extract humic acid from the samples, many yielded none. Furthermore, only one sample containing no lignin yielded HA, giving the lowest positive yield of 0.13g; this was the sample containing the lipid solely. This was only for one of eight the lipid-only sample and was only marginally above the lower limits of detection (0.1g). However, Its HIX value was also larger than the other samples containing no lignin, suggesting that it was not a case of the lipid adhering to the sample pot, giving a false reading.

Overall, data for alkali extracted humic acid showed the highest yields existed in those samples containing lignin. The relative yields of the samples containing all four substrates with each amendment and under each of the four experimental conditions can be seen in Figure 7.2. The sample with lignin alone, which produced the greatest amount of HA is shown in figure 7.3.

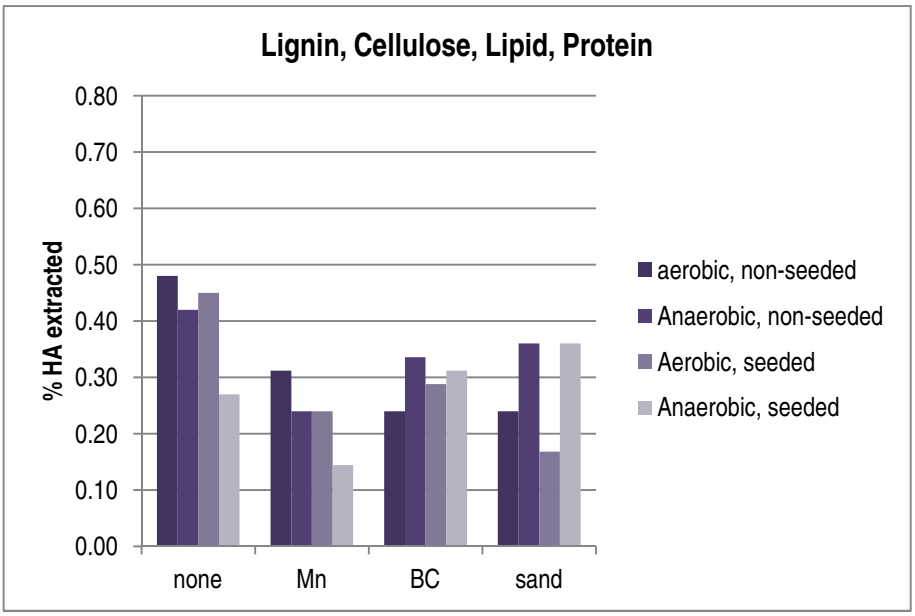


Figure 7.2: Percentage humic acid extracted for the sample containing all four substrates (average of two samples).

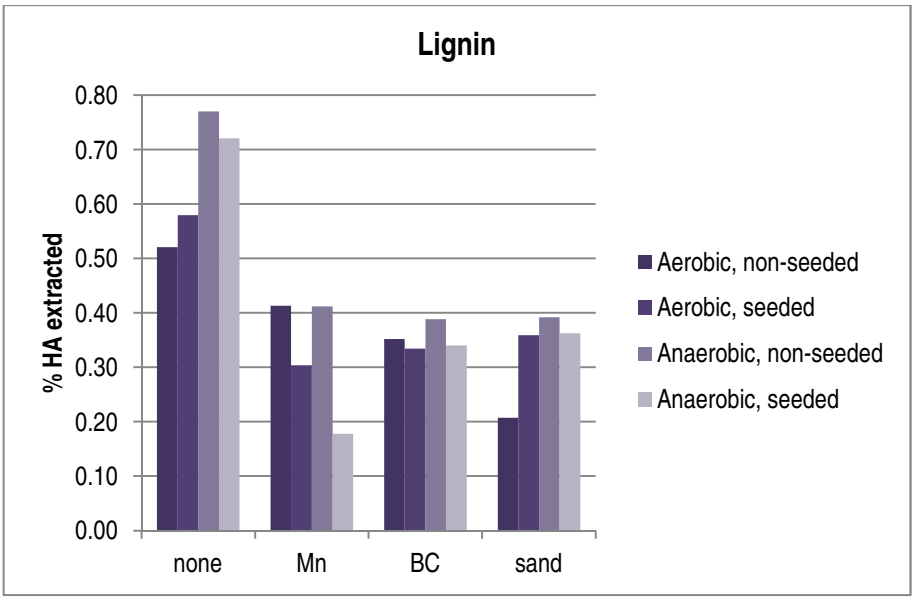


Figure 7.3: Percentage humic acid extracted for lignin only sample (average of two samples).

The sample containing lignin alone yielded the most humic acid (Figure 7.3), followed by the samples containing all four substrates (Figure 7.2). The cellulose samples yielded no humic acid; though both the protein and the lipid samples appeared to produce a small amount of humic acid these were discounted, as they were below lower limits of detection for this method (0.10g). In a similar trial, initial lignin content strongly correlated with residual amount of organic carbon seen at the end of the trial (Eklind and Kirchmann, 2000).

The corresponding HIX values for the samples with lignin were all significantly higher than all of the other samples. For the E_4/E_6 data, the same pattern was observed; the only samples that produced data were those which contained lignin. However, not all samples that contained lignin produced data.

As both methods are concerned with the degree of aromaticity of the substance, it is perhaps unsurprising that the lignin, being the most aromatic of the substrates, gives high readings for each. Could this, perhaps, have any bearing on the results from previous trials? Samples with high yields during the alkali extraction and high HIX values might in fact be rich in lignin rather than HA.

Current literature suggests that the maximum fat content should be kept below 40% as the other substrates may become covered with lard which can lead to a significant slowing down of composting process (Chang and Hsu, 2008; and references therein). This could offer an explanation as to why the sample with all substrates returned a lower yield of HA than the lignin alone (Figures 7.2 and 7.3).

7.6.2 Catalysis

As illustrated briefly in Table 7.1 and fully in the Appendices (Trial 4), 16 samples were prepared for each combination of substrate. Of these 16, 4 had no amendments added (control), 4 had MnO_2-coated sand added, 4 had char added and 4 had sand added. Each of this sub-set of four was then subjected to the various experimental conditions (Aerobic, non-seeded; anaerobic, non-seeded; aerobic, seeded and anaerobic, seeded). The following data will highlight any differences observed between samples with the different amendments (None, MnO_2-coated sand, char and sand). Figures 7.2 and 7.3 show that the amended samples appear to yield less HA than the control in most cases. Despite this, no statistically significant trends were seen with regards to this, perhaps due to the spread of data.

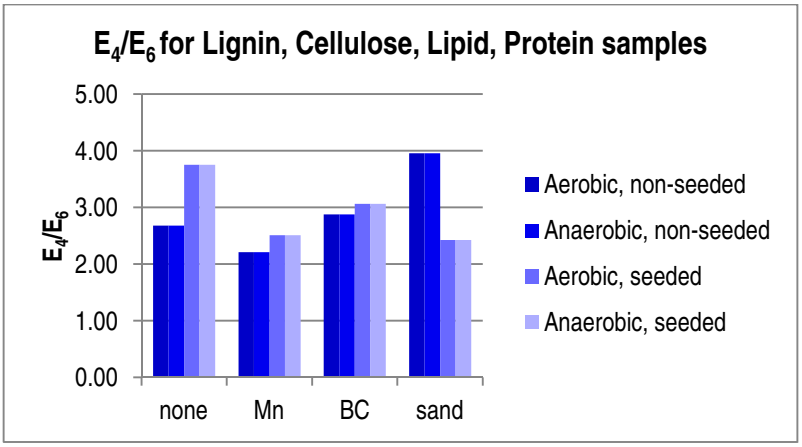


Figure 7.5: Absorbance ratio for samples 1-16 (average of two samples).

For the absorbance data illustrated in Figure 7.5, the samples with manganese appeared to show the greatest degree of humification overall. However, ANOVA showed no significant differences for any of the treatments compared to the control samples. Figure 7.6a illustrates the lack of uniformity between HIX data for the different samples under the same catalyst. If the Aerobic, seeded sample is considered on its own (Figure 7.6b), again, sample with added Mn appears to give the highest HIX value (0.8925) and thus the greatest degree of humification but once more, ANOVA found no statistically significant differences between any of the treatments.

Because of the volume of samples, the conditions in this case were treated as the replicate samples. As Figure 7.6a shows, there is very little agreement between samples when subjected to the different experimental conditions which could account for this lack of statistical significance. The HIX values are also only vary by a maximum of 0.06 which is possibly too small a difference to be statistically significant.

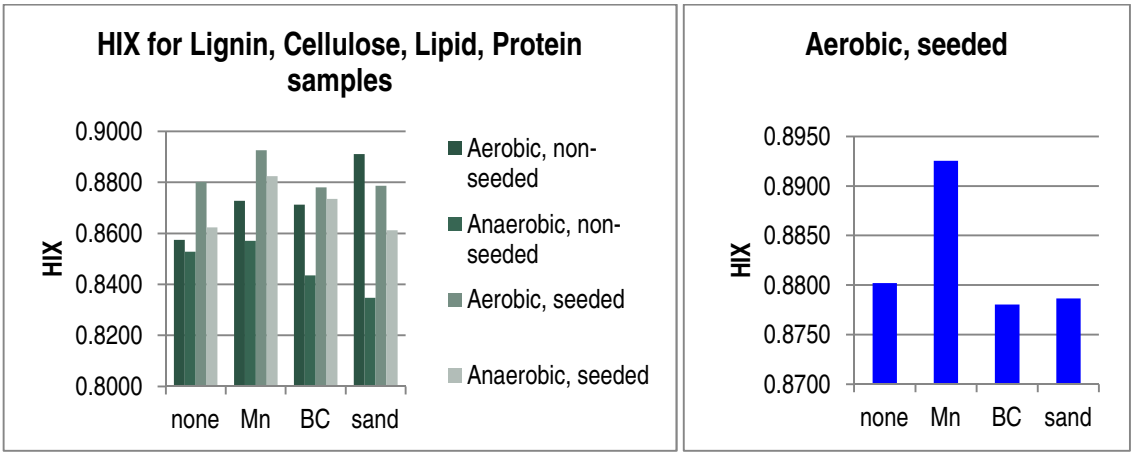


Figure 7.6a: HIX for samples 1-16 with all conditions and 7.6b samples 1-16 for aerobic and seeded only.

7.7.3 Humification conditions

This section will identify any significant differences between samples that were humified under aerobic conditions and samples which were humified under anaerobic conditions; and seeded and non-seeded samples. As the total number of samples was numerous, the first sixteen samples were again chosen to examine this relationship. These samples comprised lignin, cellulose, lipid and protein and under each different experimental condition, a control sample, and a sample with each of sand, manganese-coated sand and char were tested. Each sample was made in duplicate but not all duplicates were tested to ensure that all samples could be analysed within the time available.

Sample	HA extracted	Absorbance ratio	HIX	Catalyst	Aeration	Seeding
1	0.48	2.680	0.8575	Control	Aerobic	Non-seeded
2	0.31	2.210	0.8728	Mn	Aerobic	Non-seeded
3	0.24	2.877	0.8712	Char	Aerobic	Non-seeded
4	0.24	3.957	0.8910	Sand	Aerobic	Non-seeded
5	0.42	2.680	0.8528	Control	Anaerobic	Non-seeded
6	0.24	2.210	0.8571	**Mn**	Anaerobic	Non-seeded
7	0.34	2.877	0.8436	Char	Anaerobic	Non-seeded
8	0.36	3.957	0.8347	**Sand**	Anaerobic	Non-seeded
9	0.45	3.752	0.8802	Control	Aerobic	Seeded
10	0.24	2.510	0.8926	Mn	Aerobic	Seeded
11	0.29	3.066	0.8780	Char	Aerobic	Seeded
12	0.17	2.428	0.8787	Sand	Aerobic	Seeded
13	0.27	3.752	0.8624	Control	Anaerobic	Seeded
14	0.14	2.510	0.8825	Mn	Anaerobic	Seeded
15	0.31	3.066	0.8735	Char	Anaerobic	Seeded
16	0.36	2.428	0.8612	Sand	Anaerobic	Seeded

Table 7.3: samples 1-16, each treatment containing 1g of all four substrates. The red text signifies the most humified; the blue text signifies the least.

Table 7.3 provides the results from each form of analyses for these first 16 samples. Very little agreement is seen in this data for example, sample 16 gave the highest HA yield and most humified result as tested by E_4/E_6 (2.428) but the lowest HIX value (0.8612). Furthermore, no statistically significant differences were seen between the samples for any of the analyses. Figure 7.7 shows the percentage of alkali extracted HA in each of the samples (1-16).

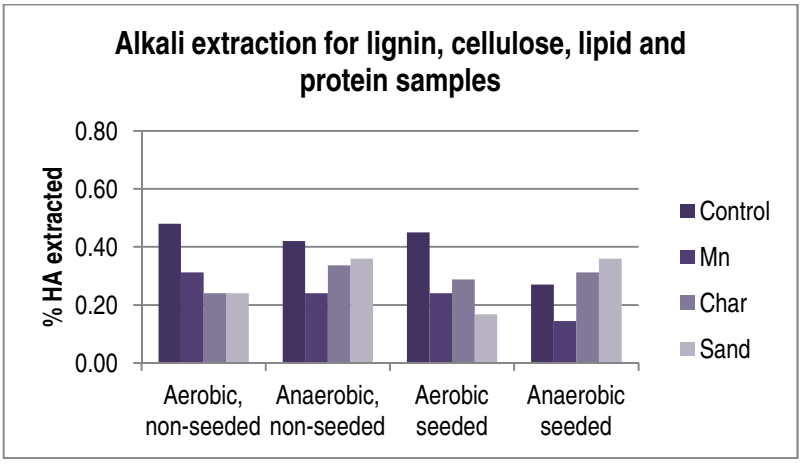


Figure 7.7: Alkali extracted HA for samples 1-16 (average of two samples).

Values range from 0.12% and 0.48% and little agreement is shown between the various samples. The yield was particularly low when compared to alkali extracted HA from CLO and peat (around 1-3% and 4-7% respectively). Of the organic material extracted, the samples tended to give high HIX values, which correspond with a higher level of humification (Figure 7.8)

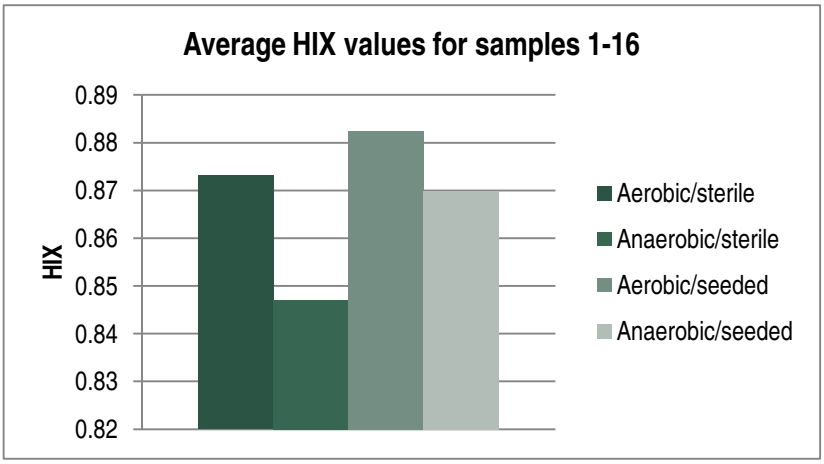


Figure 7.8: Average values for each group, samples 1-16.

In Figure 7.8, the aerobic samples appear to have higher humification index than the anaerobic samples and the seeded samples give greater values than the non-seeded samples. However, ANOVA gave no statistical significance to these results, possibly due to the lack of agreement between replicate samples or the closeness of the HIX values (between 0.846-0.882).

7.9 Conclusions

The trial produced very little statistically significant data. UV photospectrometry proved to be an unsuitable method of analysis with most of the data being below the lower limits of detection for the instrument. Perhaps this was simply because no humic acid was produced. The alkali extraction procedure only yielded humic acid when lignin was present in the sample. This could have some implications on the results from previous trials, if lignin is in fact extracted as well as humic acid during this procedure.

None of the added amendments had any statistically significant affect on the substrate samples. Again, no statistically significant differences were seen between seeded and non-seeded samples, or differences between aerobic and anaerobic samples.

Section 7.9.2 will present some of the possible reasons why it is likely that no statistically significant trends were seen, and only a small amount of humic acid produced during this trial.

7.9.2 Trial limitations

The key limitation to this trial was the fact that too large a sample set with too many variables was attempted to be analysed at once, meaning that precision was sacrificed. If this experiment was to be repeated, several changes would be made.

Firstly, as with all previous trials, a preliminary trial would be undertaken to test the suitability of the experimental methods. Psuedo-TGA, for example, provided some interesting results with the previous trials' samples so could be assessed for its suitability here.

Once the methods have been chosen, several smaller trials could be designed to run consecutively. The various conditions that were tested concurrently (seeded/non-seeded, aerobic/anaerobic, catalyst/non-catalyst) would each be analysed in a different trial. This way, more replicates could have been used and possibly larger volumes of sample tested.

As well as these fundamental changes, several other improvements could be made to ensure a more robust study in the future. No time zero samples were taken during this trial so how humification progressed over 10 weeks is not known. The same can be said for pH and conductivity due to the samples' extraction with NaOH. This was largely due to the time constraint posed by such a large sample matrix.

Moisture content would have been different for each sample; 1ml of either water or inoculum was added for each, the samples ranged from 1-5g in weight, depending upon their composition so this variable was not controlled. The samples were kept in the dark; perhaps photodegradation is necessary process in the formation of radicals which then polymerise – something which ought to be tested.

Bulking agents are often used in food-waste composting to improve structure, enhance aeration, to absorb excess liquids and to provide microorganisms with an extra energy source to balance the normally high N content. Composting without litter can lead to anaerobic conditions. This could be a potential problem in this study. Chang and Hsu (2008), for example, effectively used rice husks as a bulking agent in a similar study on food waste composting. If some of the samples had particularly high C/N ratios, they may not have humified.

Rather than the same proportions of each, perhaps should have been more representative of actual food waste composition. Chang and Hsu (2008) used 89.5% carbohydrate, 9.4% protein, and 1% fat. However, vast differences are seen in compositions of MSW CLOs so perhaps there is not necessarily such a thing as a 'typical' composition.

For ideal microbial conditions during the composting process, temperatures should be in the range of 52-60 °C and optimum O_2 concentration is 15-20 %; excess moisture lead to anaerobic conditions where as too little will lead to slow degradation as microbial action diminishes (Bernal *et al.,* 2009). Neither the temperature nor the moisture was monitored throughout the trial.

Particles that are too small may compact, reducing porosity and thus having implications for microbial action and potential to lead to anaerobicity (Bernal *et al.,* 2009). The protein and lignin were in powder form and together with the lard, made a compacted sample. The addition of a bulking agent may provide a solution for this issue.

No carbon dioxide, methane, nitrous oxide fluxes were measured meaning that microbial activity was left unmonitored. There was not enough material to measure CO_2 flux via IRGA and methods not in place to measure any other gas fluxes.

Based upon the results for this trial, it would be useful to investigate the major functional groups present in both the CLO and the control peat. In future work it would be useful to make use of solid state NMR, FT-IR and GC-MS to gauge the change in functional groups present in newly produced CLO and after a maturation period. This should provide valuable information about the stable compounds present.

Ultimately, too much was undertaken in too short a time period. This trial, if repeated should take all of the above in to consideration and split into a series of smaller trials.

Chapter 8

Conclusions and recommendations

8.1 General Conclusions

This chapter will draw together the major conclusions from each trial in a comprehensive summary. The second part of the chapter will offer recommendations based on the main findings whilst the third and final part will propose suggestions for further work within this area.

Chapter 2 explored the composition of SOM; the structure, possible formation pathways and the conditions under which HS degrade. As many other reagents all co-exist in soils, it is probable that there are several pathways that occur closely and interact to form HS.

Despite the high levels of CO_2 released during mineralisation of composted wastes, the humification stage contributes to carbon sequestration. The chemical properties of HA will differ depending upon the composition of the organic waste source, which varies temporally and geographically.

Once the humic acid is formed, susceptibility to biodegradation depends upon its structural characteristics. The depth of the material is also an important factor with decomposition in surface leaf-litter being much more rapid than in deeper, mineral soils. Molecules adsorbed onto clay minerals decrease the rate of biodegradability rendering them unavailable for microbial attack. When the CLO is added to natural soils, it ought to be well mixed to prevent rapid mineralisation of a potentially immature material.

Stability of the CLO was assessed by measuring CO_2 flux using an IRGA; maturity by alkali extraction, UV photospectroscopy and fluorescence. A Pseudo TGA method was developed as a third suite of analyses with some success. The measurement of CO_2 flux using an IRGA is documented to offer a high degree of accuracy and ease of use, however, it was not ideal for use on the relatively high-emitting CLO. It was necessary to employ methods that would be cost-effective, plus quick and simple to run due to the vast number of samples. The methods, on the whole, fulfilled these criteria. Had circumstances allowed, it would have been useful to obtain some ICP-OES data from the leachate to study the flux of metals from each sample.

The alkali extraction procedure lacked sensitivity so experimental error and differences between replicate tended to mask trends between samples on the whole. Perhaps more success would have been realised with a less contaminated product. However, this method is subject to much criticism and it is often advised that it be used in conjunction with several other methods. The microstudy detailed in Chapter 7 suggested that perhaps lignin was extracted during this process. When the PTGA results are considered, this could mean that peat's relatively high humic acid contents might in part be lignin.

The results from the absorption and fluorescence data were less reliant on the amount of organic matter being uniform in replicate samples. It was still necessary, however, for samples to be homogenous enough that the humic matter extracted was representative of the whole lysimeter. For example, if one subsample had a large proportion of plastics, plus some paper-derived material and lignin-typed components from green waste, they would offer different results from a sample that had and equal proportion of non-extractable plastic plus some digested food waste. For some of the samples tested (particularly in trial 4), data generated were below the limits of detection for these methods.

Chapter 5 saw the development of a new technique: PTGA. Ultimately, this method highlighted some interesting patterns and has the potential for effectively analysing trends seen in the samples, particularly temporally. As in Chapter 4, very few significant trends were seen between control and amended samples, however. Although it was useful for highlighting trends in data sets, it is not yet significantly robust to use as a standalone method and would require validation testing before it could be used reliably.

In the 10 week Trial 2 (Chapter 4), there appeared to be some agreement between data set. When manganese was added at 5%, no significant increase or decrease in flux was seen. When the level was raised to 10%, and increase in flux was observed after five weeks. The latter saw an increase in the absorbance ratio between week one and five and then a significant decrease in week ten. In the parallel samples with 5% sand and 10% sand, both saw an increase in flux between weeks five and seven. Only with the 5% sand did this significantly decrease again by the end of the trial. Both sets of samples gave absorbance ratios of <5 at ten weeks, suggesting a high degree of humification. Although qualitative patterns were observed in the alkali extraction data, this method yielded few statistically significant results. On the whole, the difference between replicate samples was too great for any definitive conclusions to be drawn from the use of this method. Further trials were needed

to attempt to draw any decisive inferences. None of the treatments show decisively a decrease in flux and increase in humic acid when compared to the control sample.

Trial three (Chapter 6) showed some interesting temporal results over the 26 week period, with more agreement between analyses than seen the previous trial. Although this was initially intended to be one year long, the results after six months showed significant differences in many cases than the 'new' and ten week old materials. It would still be of interest to see what differences, if any, would be observed over a longer timescale. Differences between free-draining and waterlogged samples were more apparent with some forms of analyses than others. The flux, PTGA and HIX data all suggested that the waterlogged samples may be slightly more humified after 26 weeks; the alkali extraction procedure and the E_4/E_6 ratios showed no difference between the data sets. There is a strong case in the data produced to suggest that both MnO_2 –coated sand and char amendments both have a positive impact on the humification of CLO. The both appear to reduce CO_2 flux rate and to increase humic acid content with evidence from several forms of analysis.

The microstudy designed for trial 4 (Chapter 7) to attempted to observe interactions between four of the major components of CLO (lignin, cellulose, a lipid and a protein); both with and without the three amendments used in previous trials (sand, manganese-coated sand and char) in order to gain a greater understanding of possible reaction pathways of the humification process that occurs in CLO. A fully factorial study was conducted over a 10 week period but the trial produced very little significant data. UV photospectrometry proved to be an unsuitable method of analysis with most of the data being below the lower limits of detection for the instrument. Perhaps this was simply because no humic acid was produced. The alkali extraction procedure only yielded 'humic acid' when lignin was present in the sample. This could have some implications on the results from previous trials, if lignin is in fact extracted as well as humic acid during this procedure. This trial had many limitations which would need to be considered if a similar trial was to be repeated.

8.2 Future work and recommendations for Premier Waste

For most of the samples across most of the trials, some significant results were produced. The material does appear to see significant stabilisation and increasing maturity over a relatively short period. With less contaminated and more heterogeneous product, perhaps more significant data could be produced and the product better characterised and understood. It was not possible to make comparisons between different trials due to the batches not being uniform or regulated. Intra-trial observations could be compared but not inter-trial between samples.

Firstly, the interaction of CLO with fresh soil inorganic carbon would be necessary in order to establish a unified system, as seen in natural soils. Any humic acid produced needs the protection of inorganic carbon particulates if it is to avoid degradation. No information exists from this research about interaction with soil fauna and flora, another important factor present in natural systems. The latter is of great importance when it comes to having a continuous fresh input of OM to the system. If materials were to pass the relevant Governmental standards, a field trial would be the best way to achieve this. Here, the sample would be subjected to all of the conditions that it would be when laid to land so its behaviour would be better predicted. A longer time-scale would be useful too, in order to assess whether the Mn-coated sand or char had any beneficial catalytic effects, or whether waterlogged would aid humification.

In terms of laboratory analyses, leachate experiments using ICP-OES would be useful to see what effect the added Mn and biochar has on the release of metals from the CLO. This may have some bearing on their suitability as a soil amendment.

The PTGA method showed some potential in its ability to characterise the organic fraction of CLO. However, the method would need to be validated using techniques such as solid state NMR, FT-IR and GC-MS to gauge the change in functional groups present in newly produced CLO and after a maturation period.

In terms of carbon flux, only CO_2 was measured during the trials; it would be useful to monitor CH_4 flux also, particularly from the waterlogged samples. If a significant volume is produced, then waterlogging becomes a far less attractive prospect.

Different sources of MnO_2 and char could be trialled to see whether this had any effect on the humification of the CLO. It is known that different feedstocks that produce the char can give a different product with different properties. Manganese oxide could be sourced from mining waste and its catalytic ability tested, for example. CLO could be added to acidic soils to see whether a liming effect will be seen, caused by its alkaline pH.

If CLO is to become a marketable product, many of the above will need to be addressed before it can be fully characterised. This is a potentially useful product which not only diverts waste from landfill, but has the potential to store carbon and to improve degraded soils. Further research in this area is paramount in order to maximise the benefits of this potentially valuable resource. If PWM intend to use CLO as a viable carbon store/soil improver, the separation techniques used must be improved for co-mingled wastes. Only then can useful experimental trials be carried out. Section 8.5.1 outlines some ideas for future trials that PWM could undertake to better characterise and understand their CLO produced. Perhaps if some of these are undertaken, a more stable and mature material could be produced.

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