Use of Recycled Organic Wastes as nutrient sources to aid the growth of energy crops on brownfield sites

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By

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Abstract

This thesis presents the results of research into the use of Recycled Organic Wastes (ROWs) as soil amendments to aid the growth of energy crops on brownfield sites in the UK. In order to divert biodegradable waste from landfills, food waste is source segregated by households or is mechanically separated from mixed Municipal Solid Waste (MSW) before being biologically decomposed to form stable ROWs. The source of waste stream influences the future market and use of ROWs. Three different ROWs were characterised to assess the effect of the initial waste stream and the influence of aerobic or anaerobic biodegradation processes on the final organic products.

Growth trials were conducted to assess the use of ROWs as nutrient sources for three fast growing tree species, *S.viminalis*, *E. nitens* and *P. tomentosa*. Preliminary growth trials found that high total dissolved solids were associated with some ROWs were detrimental to plant health and survival rates.

Further growth trials identified the optimum total nitrogen application rate of ROWs when mixed with Kettering Loam, a clay loam. Analysis of leachate collected from the pot trials identified changes in quantities of heavy metal and nitrogen leaching during the growth trials and allowed comparison to water quality limits for aquatic life to assess the risk to the wider environment.

Column growth trials assessed the effect of saturated and unsaturated conditions on a specific ROW known as Compost- Like Output derived from MSW, the impact on plant growth and the effect on leachate composition. Saturated conditions were detrimental to *E. nitens*, whereas *S. viminalis* adapted to saturated conditions with greater biomass production compared to the unsaturated columns. A reducing environment resulted in differences in heavy metal leaching and nitrogen forms compared to the unsaturated columns.

The use of ROWs on brownfield sites can improve the overall soil quality while providing sufficient nutrients to improve biomass yields of energy crops. However the source of ROWs and biological treatment methods can influence the characteristics and future use of the ROWs. CLO derived from MSW had significantly higher heavy metal concentrations than source segregated material.

Higher concentrations of heavy metal, particularly of copper, zinc and lead were identified within the fines (< 1 mm) of MSW derived CLO. Leachate analysis from the optimum and column growth trials identified changes within the pore water chemistry, which also influenced the mobility of heavy metals. The application of ROWs to brownfield sites has the potential to release soluble nitrogen forms and heavy metals into the wider environment; however application to contained sites or lower applications will reduce the risk.

Glossary

AD	Anaerobic Digestion
BD	Bulk Density
BMW	Biodegradable Municipal Waste
CE	Cation Exchange
CEC	Cation Exchange Capacity
CLO	Compost-Like Output
C:N	Carbon: Nitrogen
DOM	Dissolved Organic Matter
EC	Electrical Conductivity
IC	Ion Chromatography
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LAS	Landfill Allowance Scheme (NI, Scotland and Wales)
LATS	Landfill Allowance Trading Scheme (England)
MBT	Mechanical Biological Treatment
MC	Moisture Content
MSW	Municipal Solid Waste
PAS	Publically Available Specifications
PPS	2- phenylphenol
ROL	Radial Oxygen Loss
ROW	Recycled Organic Waste
SRC	Short Rotation Coppice
SRF	Short Rotation Forestry
SOMs	Separated Organic Materials (Organic Recycling Group)
WDA	Waste Disposal Authority

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Chapter 1 INTRODUCTION

Historically waste disposal comprised of large volumes of Municipal Solid Waste (MSW) and industrial waste being disposed of in landfills, commonly large voids created by open cast mining in the UK. The anthropogenic impact of landfills was not fully considered until the 1980s when engineered landfills were introduced to reduce the environmental impact. Landfilling has remained the most common means of waste disposal in the UK, however due to global and national legislative pressure on environmental impacts the reliance upon landfilling, has decreased over the last 10 years.

Under the European Landfill Directive (99/31/EC) the UK must reduce the amount of biodegradable material reaching landfills to 35% of the 1995 levels by 2020 (European Council, 1999). In order to help meet these targets the waste hierarchy (Figure 1.1) was introduced to ensure the generation of waste was firstly prevented, followed by being reused and recycled before final recovery methods. Recovery methods can include energy from waste facilities, with any remaining material that cannot be processed further being disposed of using landfills (Defra, 2011).
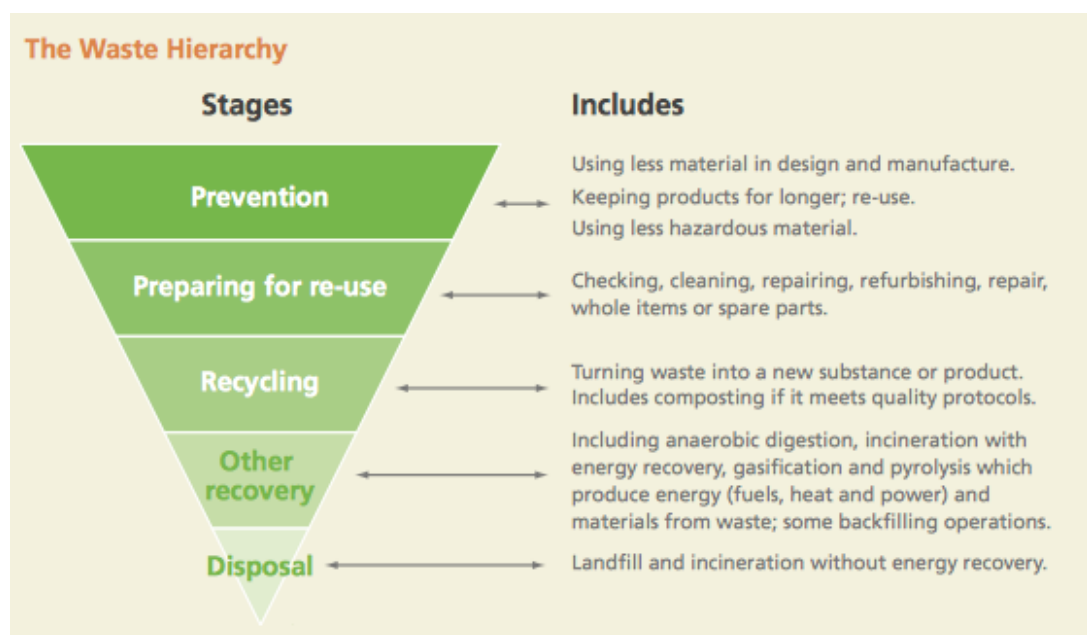
Figure 1.1 Waste Hierarchy (Defra, 2011).

As well as the reduction of recyclable material such as plastics and paper reaching landfills, the amount of biodegradable waste such as food and garden waste reaching landfill must also be limited to reduce the production and release of greenhouse gases into the atmosphere. To divert biodegradable material from landfills household food and garden waste can be segregated by households or separated from mixed MSW before being biologically decomposed to produce Recycled Organic Wastes (ROWs). Decomposition of organic waste by either aerobic composting or anaerobic digestion ensures organic material is broken down in controlled environments to produce a biological stable material, which is free from hazardous pathogens.

Where source segregation at households is not possible, biodegradable materials are mixed with MSW, which must be pre-treated before disposal by facilities such as Mechanical Biological Treatment (MBT) plants. MBT plants are designed to sort and segregate recyclable and organic material from non-recyclable residue waste. The organic fraction separated from MSW is aerobically composted to produce Compost-Like Output (CLO). Due to the methods used to separate out the waste CLO has the potential to contain plastics, glass and metals resulting from its mixed waste source. From an agricultural perspective CLO is a valuable source of slow release organic matter and nutrients, however the use and application of CLO is restricted to brownfield sites due to the potential to contain contaminants and the relatively unknown risk to human health and the wider environment.

The use of CLO on brownfield sites is managed by the Environment Agency (England) and requires bespoke permits for land application to sites where 'an ecological benefit' can be shown. CLO application is assessed on site-specific conditions and CLO characteristics due to the risk of contaminants entering the food chain and water by the Environment Agency (England) (Environment Agency, 2009a). Brownfield sites such as closed landfills can benefit from the application of CLO as a soil amendment by improving soil structure and nutrient content in order to support vegetation as part of the restoration of landfills (Bardos *et al*., 2007; Merrington *et al*., 2010).

Closed landfill sites represent substantial areas of brownfield land that have a limited range of potential uses once restored, however one use that has attracted

interest is that of energy crop production. In such approaches biomass is produced from energy crops and used to generate electricity and heat. As well as being a renewable energy source, energy crops also improve industrial landscapes, prevent erosion and can increase biodiversity. Furthermore, increased biomass production supports the UK Bioenergy Strategy that aims to meet European Renewable energy and carbon emission targets for 2020 and 2050 (Defra, 2012).

Numerous studies have investigated the heavy metal content and long-term application of mixed MSW compost to land to assess the risk to the wider environment and potential for contaminants to reach the food chain and affect human health. However there is limited work on the behavior of CLO when used to grow Short Rotation Coppice (SRC) or Short Rotation Forestry (SRF) crops in the UK. This study assesses the future use of CLO on brownfield sites to support the growth of energy crops and the potential risk of heavy metals and nitrogen to the wider environment.

This chapter presents the background and driving forces in the production of ROWs from different waste source streams, as well as an overview of current standards and characteristics of ROWs and identifies the current economic markets for ROWs.

1.1 BACKGROUND

Organic wastes including food and garden waste from residential, commercial and industrial processes were previously deposited in landfills before the introduction of the European Landfill Directive (99/31/EC) (European Council, 1999). Natural degradation processes occur to waste material deposed in landfills with limited oxygen supply resulting in anaerobic conditions. The microbial degradation of waste in anaerobic conditions releases methane and carbon dioxide gases, which can be slowly released from landfills into the atmosphere. To prevent the release of landfill gases to the atmosphere, landfill gas is extracted from engineered landfill sites to power electricity turbines due to the high calorific value of methane and carbon dioxide. Figure 1.2 shows the typical composition of landfill gas from an engineered landfill site (Environment Agency, 2002). Landfills are the largest anthropogenic source of methane emissions; however through effective landfill design and gas extraction systems landfill gas released into the atmosphere has decreased by 59% between 1990 and 2007 (Environment Agency, 2010). Limiting

the release of methane to the atmosphere is of particular importance as the global warming potential of methane is between 21 and 62 times greater than carbon dioxide (Environment Agency, 2002).
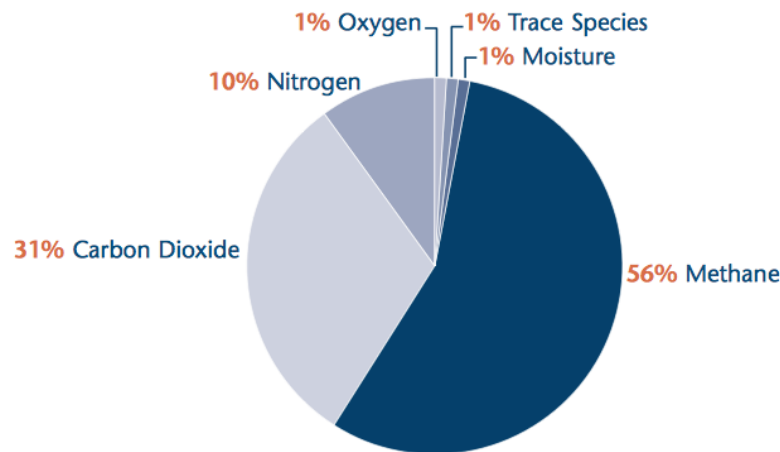
Figure 1.2 Average Composition of an engineered landfill gas (Environment Agency, 2002).

1.1.1 *LEGISLATION*

To restrict the global release of greenhouse gases from anthropogenic sources such as methane gas from landfills, international commitments and national legislation have been developed. Under the Kyoto Protocol 1997 signatory countries are committed to reducing the release of anthropogenic greenhouse gases. This has been transposed into UK legislation via the Climate Change Act 2008, which commits the UK to reducing emissions by at least 80% in 2050 of the 1990 levels (Environment Agency, 2010).

Alongside the Climate Change Act 2008, the European Landfill Directive (99/31/EC) (European Council, 1999) was introduced to reduce the reliance on waste disposal in landfills and to drive waste up the waste hierarchy by reducing, reusing and recycling resources. The overall aim of the Directive is:-

> *'to prevent or reduce as far as possible negative effect on the environment, in particular the pollution of surface water, groundwater, soil and air, and on the global environment,*

including greenhouse effect, as well as any resulting risk to human health, from the landfilling of waste, during the whole life-cycle of the landfill'

(Defra, 2009a, p 1)

The European Landfill Directive (99/31/EC) was implemented to limit the environmental impact of landfills, reduce risk to human health and ensure a minimum standard in landfill design across the EU. The Directive:-

- Set minimum standards for the location, design, construction and operation of landfills.
- Set targets for the diversion of Biodegradable Municipal Waste (BMW) from landfill.
- Controls the nature of waste accepted for landfills.
- Defined the different categories of waste as municipal waste, hazardous waste, non-hazardous waste and inert waste.

The European Landfill Directive is transposed into UK law by the Environmental Permitting Regulations 2010 (England and Wastes). The diversion of BMW from landfill aims to reduce the generation and release of greenhouse gases from landfills. By 2020, the UK must reduce the amount of BMW reaching landfills to 35% of the 1995 levels (Defra, 2009a).

The diversion of BMW from landfills is the responsibility of Waste Disposal Authorities (WDAs). Each WDA has been set targets for reducing tonnage of waste reaching landfill with specified allowance allocations for the amount of BMW reaching landfills. In England waste targets and allowances can be traded between WDAs under the Landfill Allowance Trading Scheme (LATS) in order to meet individual WDAs targets. In Wales, Scotland and Northern Ireland no such trading is permitted and the system is known as the Landfill Allowance Scheme (LAS). WDAs record their LATS and LAS data using WasteDataflow, an online recording system that allows accurate local and national recycling and landfill figures. The Environment Agency (England) is responsible for monitoring LATS in England; Natural Resources Wales is responsible for monitoring LAS in Wales, the Scottish

Environment Protection Agency monitors Scotland's LAS and the Department of Environment and Heritage Service monitor Northern Ireland LAS (CIWM, 2013).

Once landfills have reached their capacity and life span, the landfill operators must restore landfills with engineered caps with continued management and aftercare. The Environment Agency is responsible for regulating over 2000 landfills, of which over 800 sites are closed in accordance with the Landfill Directive (Environment Agency, 2014). Restored landfill sites represent substantial areas of land, which can be used to establish areas of woodland or potentially provide land for the growth of energy crops. This latter option has a number of advantages including, improvement of the wider landscape, contributing towards the generation of renewable biomass and reducing the use of agricultural land used for biomass production (Edwards *et al*., 2012).

Increased biomass production from energy crops is an important part of the UKs Renewable Energy Strategy to ensure 15 % of energy is from renewable resources by 2020 under the European Renewable Energy Directive (2009/28/EC) (European Council, 2009). In 2012, 11.3 % of electricity generation was from renewable energy sources, with total renewable energy accounting for 4.1 % of energy consumption in the UK (Defra, 2013). In order for the UK to meet renewable energy targets there has been growing interest in the growth of energy crops on restored landfill sites with field trials taking place across the UK using perennial grasses such as Miscanthus and rye grass or SRC and SRF (WRAP, 2013).

1.2   RECYCLED ORGANIC WASTE

The majority of BMW reaching landfills is food and garden waste. To meet BMW targets new waste infrastructure and processes have been developed. MBT plants have been designed to sort, segregate and pre-treat mixed MSW. Biological treatment of organic waste includes aerobic composting and anaerobic digestion (AD) in which the primary purpose is to produce energy from the methane production.

Aerobic composting is the decomposition of organic material in the presence of oxygen. Organic material is piled into rows known as windrows, this maybe outdoors or indoors. Rapid decomposition causes self-heating within the piles with

temperatures exceeding 65 °C within 7 days (British Standards Institution, 2011). Specialist thermophile microorganisms decompose the organic material into water vapour, carbon dioxide gases and solid organic residue. The piles are turned regularly to ensure consistency in the material decomposition and the moisture content can be maintained to ensure optimum conditions are maintained. Sanitization of the organic material occurs during the thermophilic stage where high temperatures destroy human and animal pathogens such as *E. coli* and *Salmonella* (British Standards Institution, 2011). As degradation rates decrease, temperatures reduce and mesophilic microorganisms continue to degrade and stabilise the organic material. As the rate of biological activity and decomposition decreases the organic material becomes stable and very low biodegradation activity indicates maturity (British Standards Institution, 2011).

Organic waste can also be decomposed in anaerobic conditions in the absence of oxygen. The degradation of organic waste in anaerobic conditions generates methane and carbon dioxide and when conducted in a contained system the gases can be collected and used to generate power. AD of organic material requires optimum conditions to be maintained to allow thermophilic or mesophilic microorganisms (depending on AD process) to biodegrade the organic material into biogas and whole digestate. The biogas contains approximately 60 % methane and 40 % carbon dioxide and traces of other contaminant gases (Anaerobic Digestion, 2013). Biogases are combustible and are used to generate energy, which can be used to locally run the AD system, or produce electricity for the national grid or biofuel for vehicles. As with aerobic composting, a pasteurisation or sanitisation process of heating occurs to reduce the levels of pathogens to acceptable levels. The rate of biodegradation reduces as the organic digestate stabilises. After the digestion process and biogas extraction, a final solid residue and liquor are produced as by-products (British Standards Institution, 2010).

The final organic residue of any these methods can be described as Recycled Organic Waste (ROW). The source and treatment of organic material can determine the future use and characteristics of the ROWs.

1.1.1 PAS 100/110

Food and garden waste source segregated by households can be classified under the British Standards Public Available Specification (PAS) criteria (British Standards Institution, 2010; British Standards Institution, 2011). As part of the PAS certification producers must implement a quality management system to ensure consistency in the final material produced onsite, which sets a quality standard with traceability.

Organic material aerobically composted is classified as PAS 100 and the solid digestate from AD is certified as PAS 110. However both materials have the same standards and must be from a source segregated waste stream (British Standards Institution, 2010; British Standards Institution, 2011). The PAS specification ensures suitable methods for composting/digestion are met; the selection of input materials, the minimum quality of final product materials and the storage, labelling and traceability of products are fulfilled.

Table 1.1 shows the maximum allowable values for a number of potential toxic elements and pathogens that source segregated material must not exceed in order to achieve the PAS 100 or PAS 110 certification (British Standards Institution, 2010; British Standards Institution, 2011).

Table 1.1 PAS certification quality limits (British Standards Institution, 2010; British Standards Institution, 2011).

Parameter	PAS limit
Cadmium (mg/ kg dry matter)	1.5
Chromium (mg/ kg dry matter)	100
Copper (mg/ kg dry matter)	200
Lead (mg/ kg dry matter)	200
Mercury (mg/ kg dry matter)	1.0
Nickel (mg/ kg dry matter)	50
Zinc (mg/ kg dry matter)	400
Escherichia coli (CFU/ g fresh mass)	1000
Salmonella spp (25 g fresh mass)	Absent

If material does not reach the required minimum standards after processing, the material can be disposed of, reprocessed or dispatched as non-PAS certified material (British Standards Institution, 2010; British Standards Institution, 2011).

PAS 100 certified materials are permitted for use as a soil improver, mulches or as substrate for growing media and as a constituent of topsoil manufacturing. These products are then available for agricultural, horticultural, land restoration, soft landscaping and sport recreation purposes (British Standards Institution, 2011). Digestate certified under the PAS 110 standard can be used as a fertiliser and soil improver for agriculture, forestry, land restoration, land reclamation and land remediation purposes (British Standards Institution, 2010).

1.2.2 CLO

Organic waste within MSW is pre-treated using MBT plants. This involves the mechanical sorting and removal of the biodegradable fraction as well as other recyclable materials such as plastic, paper and metals. The biodegradable fraction is then biologically treated using aerobic processes to reduce the dry mass and stabilise the material. The final organic residue of the biological process from a MSW source is known as Compost-Like Output (CLO). The composition of MSW derived CLO can vary with household type, sorting and pre-treatment methods as well as with regional and seasonal changes (Veeken and Hamalers, 2002). Due to the mixed waste source, CLO has the potential to contain contaminants such as plastic, glass and metals (Merrington *et al.*, 2010), which influence the application of CLO to land. The Organics Recycling Group (2013) has developed a standard for Separated Organic Materials (SOMs) such as CLO for use in land restoration projects to provide guidance to producers and users in order to enable restoration projects. The heavy metal limits identified in the standard are derived from the Sludge (Use in agriculture) Regulation (1989) shown in Table 1.2 (Organics Recycling Group, 2013). The standard ensures CLO is not applied to land where grazing animals and crops are to be grown due to the potential presence of contaminants. The application of CLO to any land requires a bespoke environmental permit, which assesses the site-specific risk to humans and environmental health while ensuring an 'ecological benefit' to the site can be demonstrated (Environment Agency, 2009a).

Table 1.2 Separated Organic Materials Land Restoration End-Use Standard heavy metal quality limits (Organics Recycling Group, 2013).

Parameter	SOMs Limits	PAS limit
Cadmium (mg/ kg dry matter)	20	1.5
Chromium (mg/ kg dry matter)	1000	100
Copper (mg/ kg dry matter)	1000	200
Lead (mg/ kg dry matter)	750	200
Mercury (mg/ kg dry matter)	16	1.0
Nickel (mg/ kg dry matter)	300	50
Zinc (mg/ kg dry matter)	2500	400
Escherichia coli (CFU/ g fresh mass)	1000	1000
Salmonella spp (25 g fresh mass)	Absent	Absent

Both source segregated and mixed MSW organic residues are valuable sources of slow release organic matter and nutrients with the potential to improve soil structure and quality (Edwards *et al*., 2010; Merrington *et al*., 2010). Source segregated material certified under the PAS standard can used as a nutrient source for agricultural land. However CLO from a mixed MSW cannot be certified under the PAS standard and its application to land is limited (Environment Agency, 2009a).

1.3 UK ROW MARKETS

The UK is committed to reducing climate change and improving the environmental impact of landfills through legislation and targets. To meet targets, steps have been taken to reduce methane production from landfills by limiting BMW, this has in turn produced ROWs and new markets must be found for these materials.

Horne *et al*. (2013) conducted a survey in 2012 to quantify the recycling of organic wastes by composting, AD and MBT facilities in the UK. It was estimated that 7.54 million tonnes of organic waste was treated by composting and AD facilities, with a further 2.51 million tonnes processed by MBT plants.

The UK has an estimated annual composting capacity of 7.48 million tonnes from 323 operational sites in 2012. The majority of input material is sourced from local authorities (88 %) with the remaining sourced from agricultural, retail, hospitality,

food manufacturing and other treatment facilities. An average composting period including sanitisation, stabilisation and maturation is 11.4 weeks across all composting processes. An estimated annual total output from the composting sites is 3.47 million tonnes with the dominant of the end market (68 %) being agriculture and field horticulture. The market share of PAS 100 compost being used for landfill restoration decreased to 3 % in 2012 compared to 8 % in 2010 which has been attributed to the use of CLO for landfill restoration instead of PAS 100 (Horne *et al*., 2013).

Of the composting facilities surveyed over half were producing PAS 100 certified compost. There were 3.2 million tonnes of input organic waste treated in 2012 and certified as PAS 100 compost; certification had a positive impact on market price compared to uncertified materials (Horne *et al*., 2013).

AD in UK can be classified as commercial, demonstration, industrial and on-farm sites. There is a total annual capacity of 2.07 million tonnes in the UK, with a total input of 1.68 million tonnes in 2012. It was identified that the majority of AD facilities in the UK used mesophilic processes (85 %) compared to thermophilic processes. The feedstock input for AD plants is mainly separated solid food (41.4 %), purpose grown energy crops (21.1 %), liquids and manures (both 16.6 %) with the remaining inputs from mixed food, green waste and other sources. Of the sites surveyed it is estimated 430,000 tonnes of whole digestate, 40,000 tonnes of fibre and 2.34 million tonnes of liquor were generated by UK AD plants in 2012. End uses of whole digestate varied between types of AD plants. On-site farm facilities used digestate onsite; commercial sites either used digestate within the business either on site or off site whereas industrial sites paid for the digestate to be removed from site. At the time of the survey 10 operators were certified as PAS 110, processing 270,000 tonnes of input in 2012 to produce 260,000 tonnes of wet digestate (pre-separation) (Horne *et al*., 2013).

There were 30 active MBT plants in the UK in 2012, treating an estimated 2.52 million tonnes of mixed MSW; of which 390,000 tonnes of organic fraction outputs were generated. Other MBT outputs include dry recyclables; refuse derived fuel and inert aggregate material. Of the CLO produced, 17 % (by weight) was landfilled at a cost to the operator however 80 % of CLO (by weight) was used for restoration and

remediation projects however this was at a cost to the operator or given away free of charge (Horne *et al*., 2013).

PAS 100 composts have an established market with average sale prices varying between £0.63 and £12.64 per tonne depending on the end use. The PAS 110 market is still developing with limited figures disclosed within the study, however CLO costs the operator (Horne *et al*., 2013) and does not have a profitable market at the moment.

1.3.1 OTHER EUROPEAN COUNTRIES

Biodegradable material is also diverted from landfills by other European countries by similar pre-treatment methods such as MBT plants, however there are differences in the final quality and use of the segregated organic material. There is no European standard for compost quality; however there are some voluntary standards and classes to differentiate compost quality although they vary between countries. As with the PAS standard in the UK, there are metal limits for cadmium, chromium, copper, mercury, nickel, lead and zinc. Germany, Denmark and Sweden have also included limits for organic pollutants such as dioxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and nonylphenols. Compost class and waste source influence the end use of material, with most countries applying source-segregated material to agricultural land. The CLO produced by MBT plants is only a pre-treatment in Germany before stabilised compost is landfilled or incinerated as refused derived fuel, compared to France, Spain and Italy, which apply CLO to land. There is evidence of variability between quality standards, treatment methods and end uses across Europe leading to a lack of public confidence and limited markets (Stretton-Maycock and Merrington, 2009).

1.4 AIMS AND OBJECTIVES

The overall research question driving this project is to assess the viability of CLO as a nutrient source and evaluate the risk to the wider environment from the use of ROWs as soil amendments to aid the growth of energy crops on brownfield sites in order to access future markets for CLO.

To address this research question a number of objectives were established to:-

- Investigate different ROWs and the effect of waste source and biological pretreatment on the characteristics of the ROWs.

- Identify the ability of different tree species to grow in soils amended with ROWs at different concentrations and the effect on biomass production.

- Assess the heavy metal uptake and potential phytoremediation benefits by different tree species on the ROWs.

- Determine the optimum total nitrogen application rate of ROWs for various candidate energy crop species.

- Identify the soluble concentrations of different nitrogen forms and heavy metals present in ROWs leachate and the risk to the wider environment in growth trials using high total nitrogen application rates.

- Explore the effect of different redox conditions on biomass production and assess the potential risk from changes in nitrogen forms and heavy metals solubility from ROWs.

The scope of this study focuses upon the heavy metal and nitrogen content and risk to the wider environment from ROWs. The tree species investigated include a standard UK species (*Salix,* common name Willow) as well as two novel tree species (*Eucalytpus* and *Paulownia*), which are relatively new to the UK for biomass production.

1.5 THESIS OVERVIEW

This thesis investigates the use of ROWs as nutrient sources for the growth of energy crops on brownfield sites. This included the characterisation of three different ROWs from source segregated (PAS 100 and PAS 110) waste streams and mixed MSW streams (CLO) and the influence of aerobic or anaerobic biodegradation processes on the final organic products. Growth trials were conducted to identify the influence of ROWs on different tree species, at different application rates and within different environmental conditions. The potential risk to the wider environment from the use of ROWs focused on the heavy metal and nitrogen forms leached from the materials and phytoremediation of heavy metals within the biomass material.

This thesis is presented in 8 chapters addressing the previous research in the field, the methods and results collected and the findings and conclusions of the research.

Chapter 2- Literature Review: This chapter reviews the environmental risks from the use of ROWs focusing upon heavy metal and nitrogen forms and the development of energy crops to restore and remediate brownfield sites.

Chapter 3- Materials and Methods: This chapter discusses the materials collected, the analytical equipment and methods used to characterise the ROWs, the procedures for three separate growth trials and the development of a plant image analysis program to estimate plant growth.

Chapter 4- ROWs Characterisation and Preliminary Growth Trials: This chapter presents the results of the characterisation of each ROW and the biomass production from the preliminary growth trials. The calibration results of a plant image analysis program are also presented in this chapter.

Chapter 5- Optimum Nitrogen Growth Trials: This chapter presents the results from the optimum nitrogen application rate growth trials including biomass production and weekly leachate analysis.

Chapter 6- Column Growth Trials: This chapter presents the results from the column growth trials comparing the effect of unsaturated and saturated conditions on biomass production and leachate composition over time.

Chapter 7- Discussion: The results from ROWs characterisation are discussed before the results from the all the growth trials are discussed based on biomass production, leachate composition and phytoremediation of heavy metals.

Chapter 8- Conclusions and Further work: The final chapter summaries the main findings of this thesis and suggests areas of additional investigations to further understand the uses and risks of different ROWs.

Work conducted as part of this thesis has been presented at two conferences; 16th European Biosolid and Organic Resources conference in 2011, and the International Solid Waste Congress in 2012. An article has been published by the Science of the Total Environment. All papers are presented in Appendix 1.

Chapter 2 LITERATURE REVIEW

This chapter reviews current literature available on the characteristics and use of ROWs as soil amendments. This is a focused literature review evaluating the heavy metal and nitrogen forms in ROW and the application of ROW to land; there are other related areas that have not been addressed as part of this study. The beneficial value and the potential environmental risks associated with the use of ROWs as soil amendments are considered as well as the potential to support the growth of energy crops. Three energy crop species are reviewed for use in the UK as well as their ability to uptake and accumulate heavy metals.

2.1 ROW AS SOIL AMENDMENTS

The fallowing of land and the addition of nutrients or fertilisers to land is not a new concept; the Romans would rotate crops, add liming acids and manure to boost soil fertility. The development of inorganic fertilisers and available nutrients increased crop yields, however the excess mobile nutrients also led to environmental pollution (Hillel, 2008). Organic fertilisers are an important source of organic matter and nutrients that are released slowly by microorganisms. The addition of organic matter improves soil structure, moisture holding capacity, soil pH and redox, enhances plant growth, soil biological activity and is a source of plant nutrients (Merrington *et al*., 2010; Smith, 2009).

The application of organic amendments is not restricted to agricultural land they can also be applied to brownfield sites, which commonly have poor quality soils with low nutrient content as a result of previous commercial or industrial use. The addition of organic materials such as ROWs have the potential to improve the physical structure as well as the chemical and biological quality of soil on brownfield sites in order to aid the restoration and reuse of valuable land. Increased organic content of soils can decrease the bulk density (BD) of compacted soils, which increases the soil porosity and improves the available water holding capacity of a soil (Kilbride, 2006).

The application of ROWs to land can improve the chemical composition of soil by increasing the soil pH. This is attributed to carbon mineralisation and the production

of OH$^-$ ions by ligand exchange and the introduction of basic cations such as calcium and magnesium (Mkhabela and Warman, 2005). Garcia- Gil *et al*. (2004) showed that the addition of organic matter increased the buffering capacity of bulk soils as a result of the high humus and the ability to neutralise H$^+$ ions. The stability of soil pH is important in controlling the mobility of nutrients and heavy metals as acidic conditions can increase metal mobility and the risk of leaching to the wider environment (Walker *et al*., 2004).

The electrical conductivity (EC) of ROWs can be a limiting factor for land application, particularly for mixed MSW sourced materials. Materials such as CLO have greater concentrations of soluble salts as a result of the original mixed waste stream; although source segregated material can also have high EC values. Cameron *et al*. (2009) identified EC values of between 4- 8 mS/ cm from waste-derived organic materials that included MBT derived CLO and source segregated green waste, PAS 100 compliant. Similar values were evident in PAS 110 certified digestate (5.5-7.5 mS/ cm), which may increase soil salinity when used as a soil amendment (Rigby and Smith, 2011). Critically EC values as low as 1.4 mS/ cm have been known to be detrimental to root development and can affect plant growth and yields (Cameron *et al*., 2009).

As well as organic matter, soil amendments are a source of valuable nutrients essential for plant growth. Nitrogen is one of three major macronutrients required along with potassium and phosphorus that are required in large quantities.

Nitrogen is present in organic forms such as protein molecules and humus or inorganic forms such as nitrate and ammonium. Organic nitrogen is mineralised by microorganisms to form ammonium, which is then nitrified into nitrite followed by nitrate. Nitrate and ammonium are the dominant forms of nitrogen utilised by plants, although nitrate is the preferred source. Nitrate is very soluble and is readily leached whereas ammonium is generally bound to exchange sites but is leachable in excessive concentrations and low exchange sites (Di and Cameron, 2002).

PAS 100 aerobically composted material contains on average between 7.5 and 11 kg/ tonne of total nitrogen of which up to 0.6 kg N/ tonne is present in available forms (Defra, 2010). Anaerobically digestate contains approximately 5 kg/ tonne of total nitrogen of which 4 kg N/ tonne are present in available forms (WRAP, 2011).

Anaerobically digested material is a valuable source of readily available nitrogen forms whereas aerobically composted material represents long term, slower release of nitrogen and nutrients (WRAP, 2011).

The ratio of carbon and nitrogen (C:N ratio) during initial biodegradation processes (aerobic and anaerobic) is important for the microorganisms populations and rate of decomposition. During the degradation process the C:N ratio decreases and is used to assess the maturity of degraded material before application to land. A maximum ratio of 20:1 is recommended for PAS 100 certified material, as higher ratios may lead to nitrogen immobilisation where available nitrogen is consumed by microorganism limiting the available nitrogen for plants. Merrington *et al*. (2010) identified CLO samples to range between 9.5 and 11.5 suggesting suitable maturity for application.

As well as macronutrients, plants require secondary macronutrients and micro-nutrients (Hillel, 2008). Magnesium, calcium and sulfur are secondary macronutrients; and boron, chlorine, copper, iron, manganese, molybdenum and zinc are micro-nutrients required in trace levels measured in the parts per million. Deficiencies in any of the essential nutrients can have a detrimental effect on the health of plants (Hillel, 2008). Hargreaves *et al*. (2008) reviewed the use of MSW compost materials in agriculture identifying the physical, biological and chemical properties from different studies. Hargreaves *et al*. (2008) found that MSW compost has the potential to contain all the macronutrients, secondary nutrients and micronutrients required by plants. However the bioavailability of nutrients varied between studies with feedstock and maturity being reported to affect their availability and mobility.

Nutrients can be present in soil in organic or inorganic forms; microorganisms within soil mineralise organic material into inorganic forms, which are available for plant uptake. Plant roots uptake soluble inorganic nutrients from the soil solution by three main transport mechanisms: diffusion, mass flow and active uptake. Diffusion, the movement of nutrients down a concentration gradient from high to low concentrations occurs across root membranes. Mass flow is a result of the transpiration stream, a catenary transport process in which water is evaporated from leaves into the atmosphere, drawing up water via the vascular systems from the

roots. Active uptake however is the selective absorption of ions required by the plant from the soil solution against a concentration gradients using cellular energy (Hillel, 2008).

The application of inorganic fertilisers provides soluble and readily available nutrients for plants, however they do not supply any organic material or long term forms of nutrients. In contrast ROWs are valuable sources of plant nutrients in readily available and slow release organic forms. A collaborative project conducted by Defra, WRAP, WRAP Cymru and Zero Waste Scotland investigated the nutrient value and potential of anaerobic digestates and compost for agricultural use over four years using field experiments. Anaerobic digestate had approximately 80 % of total nitrogen in available nitrogen forms such as nitrate and ammonium. As a result of the high available nitrogen content, it was found that anaerobic digestate could directly replace inorganic fertilisers. Composted material however has less than 5 % available nitrogen with the majority of nitrogen present in organic forms, which will be slowly released over time. Total phosphate and potash (potassium) levels were greater within the composted materials compared to the digestate, although the availability of phosphate and potash in composted and digested materials were consistent; 50 % (phosphate) and 80 % (potash). It was reported that the use of ROWs could reduce the amount of inorganic fertilisers required for plant growth or even completely replace the need of inorganic fertilisers (WRAP, 2011).

2.2   ENVIRONMENTAL RISK

There are many benefits associated with the application of organic wastes to land in order to improve soil quality and nutrient content. The type of ROW affects the final use of a material and type of land application; however there are still environmental risks from the application of any ROWs.

2.2.1   HEAVY METAL RISK

The application of ROWs to agricultural land is restricted to source segregated material certified under the PAS standard as discussed in section 1.2.1. The PAS standard assesses soil amendment quality by the total heavy metal content of the material. However it has been argued that although total heavy metal content gives a general insight into the contamination of a material, it provides little information

of the bioavailability, mobility and toxicity of metals from soil amendments (Alvarenga *et al*., 2007). The majority of studies focus on the concentrations of those heavy metals monitored as part of the PAS certification; cadmium, chromium, copper, lead and zinc.

2.2.1.1 HEAVY METALS IN ROWS

The origin of heavy metals and contaminants in organic composts is a result of the original waste streams. Household waste can contain batteries, paints, inks, plastics and metal products which contribute to the source of heavy metals in MSW and the separated organic fraction (Smith, 2009).

Veeken and Hamelers (2002) investigated the source of cadmium, copper, lead and zinc in biowastes from the organic fraction of MSW. The heavy metal content of different size fractions of biowaste was compared to the natural heavy metal content of the constituents of biowaste. Biowaste was collected from different sources representing both rural and urban collection areas. It was found the type of household affected the organic matter content, with urban apartment buildings disposing of greater quantities of food waste and flowers, compared to rural households that disposed of greater volume of outdoor garden waste. Particle size distribution analysis identified greater masses of heavy metals were associated with particles of less than 0.05 mm in rural biowaste, as a result of the presence of organic topsoil compared to urban households who had an average particle size of greater than 1 mm. The heavy metal content of the different particle size fractions of biowastes was consistent with background levels indicating biowastes are not contaminated by other sources such as atmospheric deposition. However the heavy metal concentrations exceeded the Netherlands legal limits restricting its application to land which are stricter than the PAS limits used in the UK (Veeken and Hamalers, 2002). The composition and total heavy metal concentration in ROWs can be influenced by household type, sorting and pre-treatment methods, regional and seasonal variations (Veeken and Hamalers, 2002).

Disregarding the source of heavy metals, the biodegradation process of either source segregated or mixed MSW is the same. During decomposition the physical and chemical composition of organic material changes, organic material is broken down to stable humus, which can readily adsorb organic and inorganic constituents

due to the high cation exchange capacity (CEC) and are a source of slow release nutrients (Hillel, 2008). During the decomposition of organic matter heavy metals are not degraded; concentrations of heavy metals actually increase as the mass of material decreases due to carbon and water loss from microbial respiration (Smith, 2009). The mobility of heavy metals can change during the decomposition process as a result of heavy metals forming carbonate and hydroxide complexes with organic matter. The maturity and humus content of an organic material can influence the concentration of soluble organic matter, well matured material forms immobile stable complexes, whereas immature material has a relatively higher concentration of soluble organic matter which can increase the mobility of metals (Madrid *et al*., 2007). Heavy metal binding during the decomposition stage influences the availability of heavy meals as either readily available metals weakly bound at exchange sites or immobile stable complexes strongly bound to humic material.

Farrell and Jones (2009) investigated heavy metal speciation during the composting process of two wastes; one a waste consisting of only MSW and the other being MSW mixed with source segregated green waste. Samples of compost were analysed using sequential extraction to identify heavy metal content within the exchangeable, reducible, oxidisable and residue fractions after day 0, 18, 83 and 145 of composting. There was an overall increase in heavy metal content during the composting, with a notable difference in total heavy metals between the two wastes, due to the natural dilution of heavy metals from MSW when mixed with green waste. Sequential extraction identified zinc to have the greatest exchangeable fraction, 46 % of the total zinc compared to less than 5 % of the total copper, nickel and lead being exchangeable. Copper and lead were mainly present in the oxidisable fraction and nickel was present in the residue fraction. Copper was the only metal to change its speciation over time from the reducible to the oxidisable fraction.

Paradelo *et al*. (2011) investigated the distribution and availability of trace elements in MSW compost by comparing different extraction techniques including sequential extraction. Particle size distribution identified higher heavy metal concentrations within the fines of less than 1 mm compared to the coarser material except for nickel where concentration did not vary with particle size. The sequential extraction

found copper was associated with the oxidisable fraction and chromium and nickel were present in the residue fractions also reported by Farrell and Jones (2009). Lead was equally distributed between fractions and zinc was present in the reducible fractions whereas Farrell and Jones (2009) found lead and zinc present in the oxidisable and exchangeable fractions respectively. Paradelo *et al*. (2011) also conducted water soluble, Toxicity Characterisatic Leaching Procedure (TCLP) and calcium chloride/ DTPA extractions to investigate the water soluble, slightly acidic medium leaching and plant available trace elements respectively. In all extraction methods copper, zinc and lead were the most available with greater concentrations leached from all material and extractants. Water extractions from MSW composts showed concentrations were in the order, copper > zinc > lead, whereas when MSW was mixed with green waste, concentrations were lead > zinc > copper indicating a change in the binding of copper particularly. TCLP extractions showed concentrations were zinc > copper > lead and calcium chloride/DTPA concentrations were zinc > lead > copper. The difference in binding and availability of metals within different fractions and materials was evident from the changes in the concentrations of each metal. From the different extraction methods applied by Paradelo *et al*. (2011) it was concluded copper and zinc pose the most potential risk for the use of MSW compost in agriculture and impact upon environmental health as a result of the high total concentrations present in MSW compost and the greater extractability.

Farrell and Jones (2009) and Paradelo *et al.* (2011) both used the modified BCR sequential extraction method, which varies to the original BCR method by the extraction solution being used to identify the reducible fraction. Mossop and Davidson (2003) concluded the modified method using an increased concentration of hydroxylammonium chloride at pH 1.5 provided greater extraction from iron and manganese oxyhydroxides. Although both methods were designed for soils and sediments; Paradelo *et al*. (2011) identifies potential errors when used on compost due to the high organic content and suggests the oxidisable fraction may be under estimated as the previous extraction steps can influence organic matter. As both Farrell and Jones (2009) and Paradelo *et al*. (2011) used the same method, any potential errors due to the organic matter are consistent and variation in the

speciation of metals is likely to be a result of the variability between the MSW composts.

2.2.1.2 ROWs LAND APPLICATION

The application of ROWs to land has many potential benefits as discussed in section 2.1, however the loadings of heavy metals to soil, subsequent leaching to groundwater and accumulation in food crops pose environmental and health risks. The availability and mobility of heavy metals vary between metals, soil type, humification of organic matter, other heavy metals and salts, soil pH and CEC of a soil (Walker *et al*., 2004). The application of MSW composts increases the heavy metals concentrations compared to background levels; however the risk posed by the heavy metals varies (Madrid *et al*., 2007; Ayari *et al*., 2010; Businelli *et al*., 2009).

Madrid *et al*. (2007) investigated the application of MSW compost for three consecutive years to food crops to assess the heavy metal accumulation, availability and displacement within a sandy soil in Spain. There was an increase in total heavy metals after two MSW compost applications, with lead and zinc displacement to lower layers after the third application and growing season. There were also increases in the available heavy metal concentrations over time, with increments of available concentrations exceeding the total concentrations suggesting the availability of heavy metals within the MSW compost to be greater than native metals within the soil (Madrid *et al*., 2007). This is a result of organic material containing fairly resistant metal complexes; however, when applied to land, the organic metal complexes are more labile than the background metal complexes which originate from underlying geological minerals (Smith, 2009).

Similar agricultural trials in Tunisia were conducted by Ayari *et al*. (2010). The accumulation and uptake of heavy metals into wheat from MSW compost amendments were investigated. MSW compost amended soils accumulated greater concentrations of cadmium, copper, nickel, lead and zinc after 4 years of application compared to control soils. The wheat plants grown on the MSW compost amended plots showed evidence of uptake and translocation of all metals, however zinc and copper were translocated to the edible grains (Ayari *et al*., 2010).

This indicates the risk to agricultural land and the potential to reach the human food chain.

The use of ROWs from mixed MSW streams is restricted to brownfield sites in the UK to prevent heavy metals reaching the human food chain. The application of organic wastes still has the potential to pose a risk to the wider environment as a result of the mobility of heavy metals. Businelli *et al*. (2009) investigated the long term distribution, mobility and plant availability of heavy metals from a single application of MSW compost used as landfill cover over 10 years. The most abundant metals present and leached were copper and zinc as suggested by Paradelo *et al*. (2011). Heavy metals associated with dissolved organic matter maybe responsible for the mobilisation of organo-metal complexes from amended topsoils to subsequent soil horizons. Naturally occurring plants on site were sampled over the 10 years to identify heavy metal availability and uptake within plant material. Heavy metal accumulation was generally associated with copper, lead and zinc, with highest concentrations present in the root material. There was evidence of zinc translocation into the above ground tissue; however there was variability between species (Businelli *et al*., 2009).

Many brownfield sites contaminated with heavy metals can be remediated by the application of some organic wastes. The ability for organic waste compost to be used to immobilise heavy metals on brownfield sites is dependent upon the heavy metals, soil types and amendment type. It was found by van Herwijnen *et al*. (2007) that plant-based composts such as the green waste PAS 100 compost reduced leaching compared to manure based composts such as the sewage sludge which increased leaching. It was suggested that this was a result of the different composition in organic matter and the formation of complexes between metals and organic material (van Herwijnen *et al*., 2007).

Farrell *et al*. (2010) investigated the effect of MSW derived compost and a green waste derived compost on the chemistry and heavy metal mobility within highly acidic and heavy metal contaminated soil from North Wales. Column growth trials using *Agrostis capillaris* (common grass) and soil solution sampling through the profile found pH increased after amendments. The metal leaching through the profile that did occur was attributed to leached salts competing for exchange sites

through the soil profile as there was no correlation between metal leaching and dissolved organic content as seen by Businelli *et al.* (2009). There was an improved yield in grass growth for both applications; however there was still potential risk from the mobility and leaching of heavy metals (Farrell *et al.*, 2010). Businelli *et al.* (2009) and Farrell *et al.* (2010) showed the ability of non-specific plants to establish, tolerate and thrive in MSW compost and contaminated brownfield sites while naturally remediating the soil and immobilising bioavailable heavy metals.

A number of studies have identified the ability of MSW compost amendments to reduce the mobility of contaminants when used on contaminated sites even though MSW compost can have high heavy metal content. Paradelo and Barral (2012) investigated the biosorbent capacity of different concentrations of copper, lead and zinc of two MSW composts using isotherms. Batch leaching experiments using a green waste compost with relatively low initial copper, lead and zinc concentrations and a MSW compost with higher initial heavy metal concentrations were mixed with solutions containing different concentrations of copper, lead or zinc (nitrate salts). The adsorption isotherms of all three elements were similar in both composts even though the initial heavy metals concentrations were different. Metal adsorption increased with metal concentrations until a plateau was reached. Although the green compost had lowest heavy metal concentrations, the organic matter content and the CEC are factors which influence the sorption of contaminants further supporting the use of MSW composts for the remediation of contaminated sites (Paradelo and Barral, 2012).

The binding and release of heavy metals can also be affected by changes within environmental conditions, with one chemical reaction influencing further reactions causing changes in the mobility of ions. For example changes in redox conditions can influence metal mobility due to changes in soil pH and metal binding with iron and manganese oxides as well interactions with sulfides. Oxidising conditions release H^+ ions, decreasing solution pH compared to reducing conditions, which consume H^+ ions, increasing pH values (Frohne *et al.*, 2011). Table 2.1 shows important acid producing and acid buffering reactions common in oxidising and reducing conditions (Flyhammer and Håkansson, 1999; Bozkurts *et al.*, 2000).

Table 2.1 Common acid producing and consuming reactions (Flyhammer and Håkansson, 1999; Bozkurts *et al.*, 2000).

Acid Producing	Reactions
Sulfur	$H_2S + 2\,O_2 = SO_4^{2-} + 2H^+$
	$S^- + 3/2\,O_2 + H_2O = SO_4^{2-} + 2H^+$
Sulfur and iron	$FeS + 9/4\,O_2 + 3/2/\,H_2O = FeOOH + SO_4^{2-} + 2H^+$
	$FeS_2 + 15/4\,O_2 + 5/2\,H_2O = FeOOH + 2SO_4^{2-} + 4H^+$
Iron	$Fe^{2+} + ¼\,O_2 + 5/2\,H_2O = Fe(OH)_3 + 2H^+$
Nitrogen	$NH_4^+ + 2O_2 = NO_3^- + H_2O + 2H^+$
	$NO_x + ¼(5\text{-}2x)\,O_2 + ½\,H_2O = NO_3^- + H^+$
Acid Consuming	
Carbonate minerals	$CaCO_3 + 2H^+ = Ca^{2+} + H_2O + CO_2$
Silicate minerals	Primary rock mineral $+ 2H^+ + H_2O =$ cations $+ H_4SiO_4$ + secondary minerals
Sulfur	$SO: Me + H^+ = SO: H_2 + Me^{2+}$
Iron	$Fe_2O_3 + 6H^+ = 2Fe^{3+} + 3H_2O$
Aluminium	$Al2O_3 + 6H^+ = 2Al^{3+} + 3H_2O$
	$Al(OH)_3 + 3H^+ = Al^{3+} + 3H_2O$

In aerobic conditions metals bound to sulfides can be oxidised releasing metal oxides, sulfate ions and H^+ ions. In well-buffered material such as those with a high organic content, carbonates react with the available H^+ ions releasing carbon dioxide, water and calcium ions as shown in Table 2.1 (Bozkurts *et al.*, 2000). In anaerobic reducing conditions when there is limited oxygen; nitrate, iron and manganese oxides and sulfate become terminal electron acceptors. By accepting electrons the valency and form of an ion alters, for example in anaerobic respiration, iron oxide III (insoluble) is reduced to iron II (soluble) releasing previously bound metal ions into solution. As seen in the sequential extraction studies evaluated in section 2.2.1.1 heavy metals are commonly bound with iron and manganese oxides as stable metal complexes and therefore reducing conditions pose a potential risk in terms of heavy metal release from iron and manganese oxides. However when sulfate is reduced to sulfide, insoluble metal sulfide precipitates form, reducing the mobility of heavy metals (Bozkurts *et al.*, 2000). Metal mobility is not restricted to these redox reactions only.

The ability to precipitate heavy metals, by reducing sulfate has been used in the treatment of contaminated land, particularly acid mine drainage which poses significant risk to the environment. In-situ treatment methods induce sulfate reduction using sulfate-reducing bacteria by the oxidation of organic carbon, which in turn increases pH and precipitates metal sulfides limiting the risk to the environment. Gibert *et al*. (2003) investigated the use of municipal compost as a carbon source with limestone to treat metal rich acidic water in permeable reactive barriers. The presence of limestone and municipal compost reduced metal solubility mainly as a result of an increase in pH, metal hydroxide precipitation and sorption to organic matter rather than metal sulfide precipitation.

Where reducing conditions can limit metal mobility, oxidising conditions have the potential to release metals. Flyhammer and Håkansson (1999) investigated the mobility of heavy metals in partly anaerobically stabilised MSW when exposed to air. Previously saturated and stabilised MSW was exposed to oxygen for 7 and 15 days. Within a 1 day, pH decreased from approximately 9 to 8, with increased concentrations of calcium, magnesium, manganese and sulfate. This suggested rapid oxidation of metal sulfides, increasing the metal and sulfate ions soluble concentrations and releasing protons, which were then neutralised by the dissolution of carbonate ions causing the release of calcium ions.

Changes in redox conditions can have a significant effect on metal mobility as a result of variations in H^+ concentrations and pH which in turn influence binding and release of metal ions from complexes. There is limited work on the potential release of heavy metals from ROWs in different redox conditions and as shown by Flyhammer and Håkansson (1999) significant changes in metal mobility can occur in short periods of time posing a risk to the wider environment.

The application of organic waste to land has been shown to improve soil quality and provide nutrients for plant growth (Merrington *et al*., 2010; Smith, 2009; Hargreaves *et al*., 2008). Although previous studies have also identified potential risks from the heavy metal loading to soils, the potential mobility of heavy metals through soil profiles and the uptake and accumulation in plants (Madrid *et al*., 2007; Ayari *et al*., 2010; Businelli *et al*., 2009). Short-term changes in environmental conditions also have the potential to influence metal mobility although ROWs

behavior is relatively unknown as well as the long-term release of heavy metals from the stable residue fraction of organic material. It has been hypothesised that over time the residue fraction will be slowly mineralised by micro-organisms releasing heavy metals although there is little evidence to support this (Silveira *et al*., 2003; Smith, 2009). A review of the current literature concerning heavy metal risk from ROW shows potential in the application of ROWs to land, although there are gaps in the research in which this thesis intends to address.

2.2.2 NITROGEN RISKS

As discussed in section 2.1, nitrogen is an essential nutrient for plant growth and ensuring sufficient nitrogen within soils is one of the main driving forces for the application of any inorganic fertilisers or organic amendments.

Nutrient applications to land from any source are restricted by the total nitrogen content because of the solubility of some nitrogen forms and the risk to the wider environment. Nitrate and ammonium are soluble forms of nitrogen, which are particularly detrimental to water bodies due to eutrophication, as nitrogen promotes excess plant growth restricting the available oxygen to aquatic organisms. Nitrate in drinking water is also harmful to human health as it can cause methemoglobinenia in infants, which affects the oxygen carrying capacity of haemoglobin and stomach cancer in adults (Di and Cameron, 2002).

In the UK total nitrogen to be applied to agricultural land is restricted to 250 kg/ Ha/ year. In environmentally sensitive areas such as near watercourses, nitrate vulnerable zones have been designated and nitrogen application is restricted to 170 kg/ Ha/ year (Defra, 2012). The maximum nitrogen loading values includes the total nitrogen present in the soil before additional application. Nitrogen leaching and water contamination is a result of excess soluble nitrate in soils with high drainage volume. The production of crops and ploughed pastures represent land uses with the greatest risk of nitrate leaching with the lowest risk associated with forests (Di and Cameron, 2002). Effective application and land management is required to ensure nitrate leaching is limited by understanding the transformations in the environment the nitrogen uptake rates of different species.

It is during decomposition of organic material that microorganisms mineralise organic nitrogen to soluble inorganic forms such as nitrate and ammonium. Figure 2.1 represents the transformations of nitrogen during decomposition (Guardia *et al.*, 2010). Temperature, moisture content (MC), waste particle size and C:N ratio can affect the rate of decomposition. Hamoda *et al.* (1998) identified the optimum composting conditions for the organic fraction of MSW based on the total organic carbon content. An optimum temperature of 40 °C with a MC of 60 % and particle size of 40 mm were identified as optimum variables during decomposition. The C:N ratio of initial organic material is an important factor that can affect the rate of decomposition by organisms. High C:N ratios increase microorganism demand for nitrogen, immobilising the available nitrogen for plant uptake. Hamoda *et al.* (1998) identified an optimum C:N ratio of 30:1. The C:N ratio reduces during decomposition to ratios of less than 20:1 before application to land (Edwards *et al*, 2010). Anaerobic digestion requires similar C:N ratios to ensure optimum conditions for microorganism without the presence of oxygen.
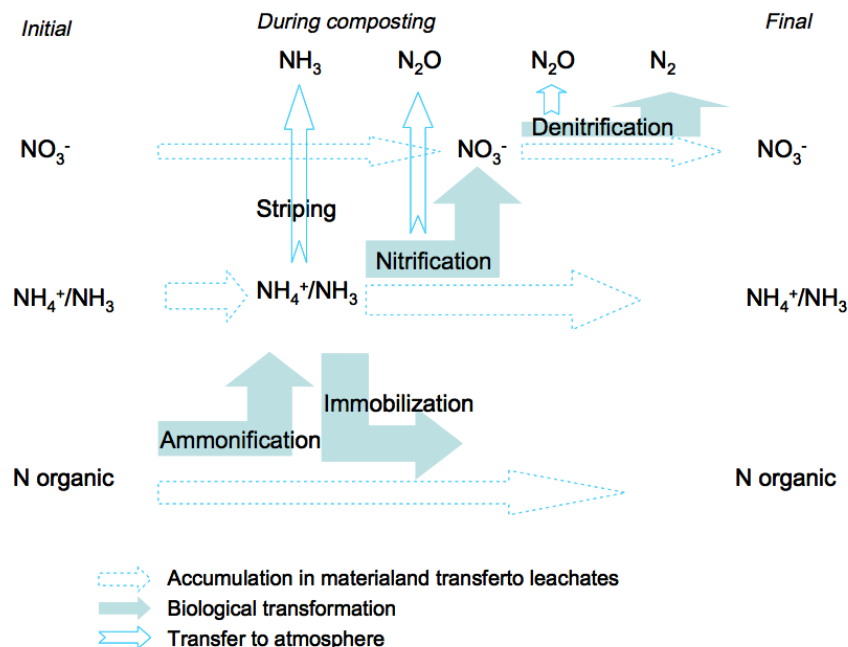
Figure 2.1 Nitrogen transformations during composting (Guardia *et al.*, 2010).

Farrell and Jones (2009) investigated the changes in the speciation of heavy metals and the extractable nitrate and ammonium concentrations during the composting process (2.2.1.1). Ammonium concentrations gradually decreased during in-vessel composting; it would be expected that the nitrate levels would increase as ammonium was oxidised, however nitrate levels remained fairly constant and less than 2 mg/ kg. Once the compost was removed from the in-vessel treatment and allowed to mature in static piles, ammonium levels stabilised while nitrate levels rapidly increased at day 145. Although the results are presented, Farrell and Jones (2009) do not discuss the nitrogen changes during the composting process. As a result of the sampling regime at day 83 (end of composting) and day 145 (end of maturing), there is limited information of changes in the ammonium, nitrate or heavy metal concentrations during this time. Rapid changes in the nitrogen forms from ammonium to nitrate have the potential to pose greater risk to the environment as nitrate (anion) is highly soluble, whereas ammonium (cation) has greater binding potential to negative exchange sites (Di and Cameron, 2002).

Mineralisation of organic material is continuous even after application to land the rate of mineralisation is affected by the organic matter characteristics, the soil properties and environmental factors. As previously discussed the temperature, moisture content and C:N ratio can affect the rate of mineralisation during the aerobic decomposition and once applied to land. Burgos *et al*. (2006) investigated the nitrogen dynamics and leaching from organic amended soils using incubation and column trials. The incubation trials identified nitrogen immobilisation within the first week before ammonium concentrations decreased and nitrate concentrations increased indicating nitrogen mineralisation. Leachate collected from column trials showed similar patterns with higher nitrate leaching from MSW compost amendments compared to a paper sludge and agroforestry compost amendments due to the higher rate of nitrogen mineralisation. Nitrate concentrations in leachate from the soil columns amended with MSW compost exceeded the maximum 50 mg / l under the Drinking Water Directive (98/83/CE) indicating some risk to water courses and human health.

The available forms of nitrogen vary between materials and treatment methods as mentioned in section 2.1, with greater amounts of available forms present within anaerobically digested material (WRAP, 2011). Hargreaves *et al*. (2008) reported

that the low available concentrations of nitrogen associated with MSW compost could limit the growth of crops within the first year of application. This has resulted in large application rates to ensure sufficient available nitrogen for growth; however this has lead to the excess loading of other nutrients and trace elements to sites (Hargreaves *et al*., 2008).

The available nitrogen forms are also dependent upon environmental conditions such as moisture content and redox conditions. In aerobic conditions, ammonium can be oxidised to nitrate by nitrification (Figure 2.1), however in anaerobic conditions this process is limited due to low oxygen concentrations and restricted microorganism activity (Cronk and Fennessy, 2001). When soils become anaerobic any nitrate present may be reduced to gaseous nitrogen (nitric oxide, nitrous oxide and dinitrogen) by denitrification and released to the atmosphere. This is a natural cycling of nitrogen and removal of nitrogen from land however denitrification is also a significant source of atmospheric greenhouse gas nitrous oxide (Butterbach-Bahl and Dannenmann, 2011). Ammonium cannot be oxidized to nitrate in anaerobic conditions and although ammonium has a lower solubility than nitrate, ammonium can still be leached when there are excess concentrations and limited binding (Cameron *et al*., 2009).

Changes in environmental conditions and forms of nitrogen can influence nitrogen uptake by plants. In saturated conditions oxygen is present near the surface and within the root zone of plants as a result of Radial Oxygen Loss (ROL) from plant roots. The development of aerenchyma tissue within plants allow the transfer of oxygen from above ground material to roots in order to oxygenate the rhizosphere. This allows the oxidation of essential nutrients such as nitrate from ammonium in saturated conditions (Cronk and Fennessy, 2001).

Although nitrate is the preferred nitrogen form for many plants some species have shown a greater affinity for ammonium (Garnett and Smethurst, 1999). It has been suggested that the nitrogen form utilised by plants may be related to soil pH and temperature as these can influence the nitrogen forms and uptake rates (Garnett and Smethurst, 1999). Garnett and Smethurst (1999) showed ammonium uptake by *E. nitens* was 200 % greater at pH 4 than pH 6, whereas pH had no effect on nitrate uptake and temperature changes from 20 °C to 10 °C reduced the uptake of both

nitrate and ammonium. The preferred uptake of ammonium by *E. nitens* may be a result of the natural habitat of *E. nitens* in which ammonium was the dominate form due to cold temperatures and acidic conditions. An understanding of species-specific preferred nitrogen forms could allow optimum growing conditions and nutrient management particularly in environmentally sensitive areas to reduce the risk from soil amendments.

2.3   ENERGY CROPS

The UK is committed to generating 15 % of energy from renewable resources by 2020 under the European Renewable Energy Directive (2009/28/EC) (European Council, 2009). Biomass production is a key component in the UK Energy Strategy with particular interest in the growth of energy crops on restored landfill sites (WRAP, 2013). Energy crops can include perennial crops such as grasses (Mischanthus, and Rye) and SRC/SRF (Willow and Poplar trees), as well as agricultural crops such as sugar, starch or oil crops and aquatic crops grown hydroponically such as algae and weeds (Biomass Energy Centre, 2014).

SRC is a traditional method of cropping fast growing tree species such as *Salix, Populus* spp. to a low stump every 3-5 years, whereas SRF is the cropping of trees every 8-10 years depending on the tree species (*Eucalyptus, Sycamore*) (Biomass Energy Centre, 2014). Available energy crop data suggests over 10,000 hectares of energy crops (*Miscanthus* and SRC) were planted under a government funded grant scheme (Energy Crop Scheme) and approximately 45,000 tonnes of energy crops were used in biomass power stations between 2010 and 2011 (Aylott and McDermott, 2012).

Species selection is an important factor to ensure successful biomass yields. SRC/SRF species are generally fast growing with the ability to tolerate poor soil conditions, adverse climate conditions and be resistant to pests and disease (Nixon *et al*., 2001). *Salix* spp. and *Populus* spp. are the most successful SRC species used in the UK and Northern Europe with a number of clone variations available. Weih and Nordh (2005) investigated the use of pot trials to predict the field performance of different hybrid *Salix* spp. over three years. Shoot biomass production in the pots trials showed good correlation with the shoot production in the field for the first

year only, as there was little correlation in the second and third year as a result of growth pattern changes. The pot trials plant leaf area and field shoot biomass however showed better correlation after the first year reflecting the potential photosynthetic and biomass production. The biomass production over the three years showed clone specific relationships when comparing pot trials and field trial production, resulting in clone specific pot trials in the future to predict field performance.

Assessing plant growth from pot and field trials is time consuming and can include measuring plant height, stem thickness, shoot numer, leaf number and leaf area or involve destructive techniques to identify biomass production. A number of studies have investigated the use of photos to develop rapid and non destructive methods. Leaf area is linked to plant growth and can be used to compare different plant treatments. Rico-García *et al*. (2009) developed a Matlab processing code to estimate leaf area from digital photos and found a correlation of greater than 99 %. Lindsey and Bassuk (1992) investigated the use of a tree silhouette area to estimate the leaf area with significant correlation for a number of different species. It is evident that the ability to use photos and a processing program allows quick and simple estimation of plant growth parameters.

New fast growing SRC/SRF species such as *Eucalyptus* spp. and *Paulownia* are gaining interest for use in the UK. *Eucalyptus* spp. are a fast growing species suitable for SRF with coppicing rotation every 10 years. A small number of *Eucalyptus* spp. can tolerate cooler UK temperatures and produce straight stems, high-density wood with low moisture content. However even tolerant *Eucalyptus* spp. can be damaged by extreme cold temperatures experienced in the UK as well as moisture deficiencies in some regions during summer months (Leslie *et al*., 2012). Paulownia was originally introduced to the US and Europe as an ornamental tree, however its rapid growth, high tolerance levels and its biomass production have been identified as a suitable SRF crop (Dourmett *et al*., 2008). There are concerns however over the invasive nature of *Paulownia* in warmer climates (Woods, 2008; USDA, 2013).

The development of SRC/SRF on brownfield sites is not a new concept and has been a priority for England Forestry Strategy (Forestry Commission, 1999 cited in

Hutchings, 2002). Forest Research (2008) presented the results of a 10 year study into the establishment of woodland on landfills. The study showed the ability of some tree species to survive in the poor soil conditions and the gradual increase in height and yields. Drought and low nitrogen contents were identified as the main limiting factors to tree growth (Forest Research, 2008). Energy crops require maximum yields to be successful and economically viable. Drought, soil compaction, poor nutrients and the influence of landfill gas can all affect the biomass production on landfill sites (Nixon *et al*., 2001). The drought, soil compaction and poor nutrient content on landfills are a result of the engineered clay cap required to reduce the environmental impact and risks. The compacted clay cap is designed to limit infiltration and is a significant barrier in the development of SRC/SRF on landfills. Engineered clay caps impede plant growth and yields as a result of poor root development and poor soil structure, which can lead to seasonal droughts and water logging. Organic amendments such as sewage sludge were used on landfill caps to improve soil structure, moisture holding capacity and nutrient content to ensure biomass yields were not limited (Nixon *et al*., 2001).

It has been suggested that MSW derived ROWs can be used a soil amendment to landfill caps to aid the growth of SRC/ SRF energy crops. Bardos *et al*. (2007, p 1) identified this as: -

> *'an important opportunity to combine materials recycling with land restoration, renewable energy and providing a "bankable", i.e. economically self-sustaining means of managing degraded land'*

The quality of CLO and perceived potential detrimental effects to land from its application were identified as a barrier with land managers and site owners (Bardos *et al*., 2007). There is limited academic research into the use of ROWs as a nutrient source for SRC/SRF specifically, although numbers of growth trials are in progress in the UK at the use of AD digestate (WRAP, 2013).

2.3.1 PHYTOREMEDIATION

In recent years SRC/SRF species have been used on brownfields as previously discussed, however brownfield sites are commonly contaminated from historical

use. The use of SRC/SRF restores brownfield sites, by stabilising the soil and reducing soil erosion. They have also been shown to naturally remediate sites by stabilising or removing bioavailable contaminants from soil and accumulating them in plant biomass (Hutchings, 2002).

Phytoremediation is a fairly low cost, in-situ treatment of contaminants. The ability of plants to tolerate, uptake and accumulate heavy metal varies widely. Natural hyperaccumulating plants are typically near areas of natural mineralisation and mine spoils and are capable of accumulating potentially phytotoxic elements in shoots at concentrations that are significantly greater than non-hyperaccumualtors (Pulford and Watson, 2003). It is common for heavy metals to accumulate within the root material, to immobilise contaminants and prevent their movement to other plant organs, whereas some species have the ability to translocate metals from the roots to shoots, to compartmentalise metals within leaf material before defoliation (Pulford and Watson, 2003).

Salix, Eucalyptus and *Paulownia* spp. have all shown evidence as metal tolerant species with the ability to remediate contaminated soils (Pulford and Watson, 2003, Rockwood *et al.,* 2004; Kim and Owens, 2010).

Several studies have investigated the ability of *Salix* spp. to adapt to poor soil conditions and tolerate contaminated soils as well as their ability to accumulate heavy metals (Pulford and Watson, 2003). Zacchini *et al*. (2009) compared the tolerance, accumulation and translocation of cadmium when *Poplar* and *Salix* clones were grown hydroponically. *Salix* clones showed greater consistency between clones in the accumulation and distribution of cadmium compared to *Poplar* clones. To assess the tolerance and phytoremediation ability of species the tolerance factor, translocation factor and bio-concentration factors were calculated based on equations (Equation 1), (Equation 2) and (Equation 3). Zacchinni *et al*. (2009) showed *Salix* clones had greater tolerance index values and translocation factors however *Poplar* clones had greater bio-concentration factors and accumulation with the root material.

$Tolerance\ Index$

$$= \frac{Dry\ weight\ of\ the\ plant\ grown\ in\ contaminated\ soil}{Dry\ weight\ of\ the\ plant\ grown\ in\ control\ soil}\ x\ 100$$

(Equation 1)

$Bio - Concentration\ Factor$

$$= \frac{Metal\ concentration\ in\ the\ harvest\ plant\ material}{Metal\ concentration\ in\ the\ solution}\ x\ 100$$

(Equation 2)

$Translocation\ factor$

$$= \frac{Concentration\ of\ metal\ in\ aerial\ material}{Concentration\ of\ metal\ in\ the\ root\ material}\ x\ 100$$

(Equation 3)

The heavy metal accumulation of 13 different *Eucalyptus* clones were investigated by Mughini *et al*. (2013). The heavy metal contaminated soil only affected the biomass production of 1 clone when compared to the control *Eucalyptus*. All *Eucalyptus* clones accumulated heavy metals within the leaf and stem material suggesting translocation of contaminants. There was evidence of some clone specific uptake of metal pairs, suggesting clone selection of one metal may also improve the uptake of another metal for specific site conditions. Mughini *et al*. (2013) highlighted the use of *Eucalyptus* for both biomass and phytoremediation, however their study was for Mediterranean sites specifically and a similar study would be required in the UK to investigate climate suitable clones.

Doumett *et al*. (2008) investigated the influence of complexing agents on the metal mobility and accumulation in *Paulownia tomentosa*. The accumulation of heavy metals in the root and shoot material followed the concentration pattern of zinc > copper > lead > cadmium for controls and pots containing complexing agents, however bioavailable concentrations of heavy metals showed zinc > lead > copper > cadmium. It was concluded that concentration gradients between pore water and root was not the dominant uptake mechanism for *Paulownia* and the use of

Paulownia for remediation is driven by its rapid biomass production rather than metal accumulation capability (Doumett *et al*., 2008).

Salix, *Eucalyptus* and *Paulownia* all have potential to produce high biomass yields as well as phytoremediate heavy metals from soils, however the tree type and biomass incineration are important issues to consider. The tree type determines the loss of leaf material annually. The potential for *Salix* and *Paulownia* trees to accumulate heavy metals within leaf material requires harvesting of the aboveground mass to take place before leaf loss to ensure heavy metals are not returned to the soil. *Eucalyptus* trees are generally evergreen and therefore biomass collection is not controlled by leaf loss (Mughini *et al*., 2013). The use of SRC/SRF to phytoremediate soils results in heavy metal accumulation within the harvested biomass, which in turn is incinerated for energy, resulting in heavy metals potentially occurring in flue gas and accumulating within incineration ash which can lead to other environmental issues (Keller *et al*., 2005; Wu *et al*., 2013).

Although the ability of SRC/SRF species to accumulate heavy metals is beneficial to remediation soils, it is important to ensure the metal accumulation does not affect the biomass production, especially long term after repeated coppicing and accumulation with root material.

2.4   SUMMARY

Due to global and national policy commitments the diversion of biodegradable organic waste from landfills has resulted in the creation of ROWs as by-products. ROWs are valuable sources of slow release organic matter and nutrients. The value and use of the ROWs is in part dependent upon the risk posed to humans and the environment. Source segregated materials have lower concentrations of contaminants and are permitted to be used on agricultural land, whereas mixed MSW composted material such as CLO contains glass, plastics and heavy metals and is only permitted on brownfield sites providing it will provide 'an ecological benefit' (Environment Agency, 2009a).

Many studies have identified the benefits of the application of ROWs to land, such as improved soil structure and moisture holding capacity, as well as the environmental risks such as the potential heavy metal loading and nitrate leaching.

This chapter discussed the heavy metal fractions and identified copper, lead and zinc as the heavy metals that posed greatest risk due to the higher concentrations and mobile fractions (Farrell and Jones, 2009; Paradelo *et al*, 2011). Long term studies also identified the potential mobility and uptake of heavy metals by plants (Ayari *et al*., 2010; Businelli *et al*., 2009). Previous literature also assessed the use of *Salix*, *Eucalyptus* and *Paulownia* as energy crops with the ability to tolerate and accumulate heavy metals. There is however a lack of research into the effect of ROWs on the growth of different SRC/SRF and risk of heavy metal leaching and nitrogen forms to the wider environment, which this thesis intends to address.

Chapter 3 MATERIALS AND METHODS

3.1 OVERVIEW OF PROJECT STRUCTURE

This chapter describes the materials and methods used in the collection and analysis of results presented in this thesis. The ROWs under investigation were initially characterised using a number of standard methods, before three different growth trials were conducted. Figure 3.1 shows the experimental overview and aims of each stage of this investigation.

Three different ROWs were initially investigated and characterised, PAS 100, PAS 110 and CLO. PAS 100 and PAS 110 are source-segregated materials, which are aerobically composted and anaerobically digested respectively in order to degrade and stabilise the organic material. CLO however is derived from a mixed MSW source after separation at MBT plants and is aerobically composted. The source of the waste stream and the type of biological treatment can influence the characteristics of the materials. Basic experimental analysis was conducted to identify the pH, EC, BD, MC and particle size distribution of the ROWs. A number of different leaching and extraction methods were conducted to identify the readily available nutrients and the binding fractions of heavy metals within the ROWs. All cation metal analysis was conducted using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), soluble anions were measured using ion chromatography (IC) and ammonium was colourmetrically analysed via the modified Berthelot method. The total nitrogen and carbon content of each ROW was investigated to understand the nutrient content and maturity of the composts.

PAS accredited ROWs can be used as a fertiliser replacement on agricultural land, however the use of CLO is restricted to brownfield sites as a soil amendment. It has been proposed that CLO can be used as a nutrient source to aid the growth of SRC/SRF energy crops on brownfield sites, particulary restored landfills. Growth trials were conducted to identify the effect of ROWs on the growth and yield of SRC/SRF energy species. Three fast growing species were investigated; a traditional UK species, *Salix viminalis* and two relatively new species to the UK *Eucalyptus nitens* and *Paulownia tomentosa*. Three growth trials were conducted;

preliminary growth trials, optimum application rate growth trials and column growth trials.

Preliminary growth trials investigated the sole influence of ROWs on the growth of *S. viminalis, E. nitens and P. tomentosa*. ROWs were mixed with perlite, an inert mixing medium at application rates equivalent to 1500 and 3000 kg N / Ha. Plant growth was assessed weekly, with photos taken for the development of a plant image analysis program. Plant material was analysed after the growth trials to assess the uptake of heavy metals into the root, stem and leaf material.

Conclusions from the preliminary growth trials influenced the optimum application rate growth trials. The optimum application rate growth trials aimed to identify the optimum total nitrogen application rate of CLO and PAS 110 based on the yield of *S. viminalis*, and *E. nitens* after 12 weeks. Perlite was replaced with Kettering Loam to replicate mixing material that is not inert, while having relatively uniform characteristics and repeatability. Excess leachate was collected from pot saucers under each pot and analysed to identify the composition of the leachate. The pH, EC, soluble heavy metals (ICP-OES), soluble anions (IC) and ammonium concentrations were analysed.

The optimum application rate based on the total nitrogen content was identified and used as an application rate in the column growth trial. The column growth trials were designed to investigate the effect of saturated conditions on CLO and Kettering Loam mixes compared to unsaturated conditions. The column trials assessed the influence of different environmental conditions on soil pore water chemistry, SRC/SRF growth rate and yields of *S. viminalis*, and *E. nitens.* An additional experiment was designed to identify if root development before saturation impacted the survival rate of *E. nitens*. Columns containing CLO and Kettering Loam were planted with *E. nitens* and saturated immediately after planting, after 3 weeks and after 6 weeks of unsaturated growth. Plant growth and leachate were analysed to identify changes over time and the impact on plant growth.

Experimental Work

Overall Aim: Assess the use of CLO and evaluate the risk to the wider environment from the use of ROWs as soil amendments to aid the growth of energy crops on landfill sites.

Characterisation of ROW

Aim: To understand the properties and potential contaminants present in ROWs and assess the difference between material sources and biological treatment.

- pH, electrical conductivity
- Bulk density, moisture content.
- Total heavy metal (*aqua regia* Digestion)
- Available and water soluble metals
- Total nitrogen/carbon
- Mercury analysis
- Batch leaching
- Sequential extraction
- Particle size distribution

Preliminary Growth Trials

Aim: Investigate the effect of ROWs from source segregated and mixed MSW streams on the growth of 3 different energy crop tree species and the potential heavy metal phytoremediation.

- CLO, PAS 100 and PAS 110
- Equivalent to 1500 and 3000 kg N/Ha
- Inert perlite
- *S. viminalis, E. nitens, P. tomentosa*
- Weekly plant growth measurements
- Weekly photos
- Plant image analysis
- 22 weeks
- Heavy metal uptake and accumulation in root, stem and leaf material

Optimum Application Rate Growth Trials

Aim: Identify the tolerance of energy crop tree species to toxicity arising from the use of ROWs at different application rates, assess the effect on leachate composition and diffuse pollution risk and understand the influence on biomass yields.

- CLO and PAS 110
- Kettering Loam
- Application rates equivalent to 0, 250, 1000, 3000, 6000, 10000 kg N/Ha
- *S. viminalis, E. nitens*
- Leachate analysis
- Weekly plant growth measurements
- 12 weeks
- Heavy metal uptake and accumulation in root, stem and leaf material

Column Growth Trials

Aim: Examine the effect of different redox conditions on contaminant release from CLO in leachate and potential risk to the wider environment as well as the impact on SRC biomass production.

- CLO equivalent to 3000 kg N/Ha
- Kettering Loam
- Saturated and unsaturated
- *S. viminalis, E. nitens*
- Rhizon and base soil pore water sampling points
- Leachate analysis
- Weekly plant growth measurements
- 12 weeks
- Heavy metal uptake and accumulation in root, stem and leaf material

E. nitens Point of Saturation

Aim: Identify the time required for *E. nitens* roots to develop before column saturation.

- CLO equivalent to 3000 kg N/Ha
- Kettering Loam
- Saturated on day 0, after 3 weeks and 6 weeks
- Rhizon and base soil pore water sampling points
- Leachate analysis
- Weekly plant growth measurements
- 12 weeks
- Heavy metal uptake and accumulation in root, stem and leaf material

Figure 3.1 Experimental Design.

3.2 MATERIAL COLLECTION AND STORAGE

ROW samples were collected from 3 different processing sites where the ROW materials were stored in different storage piles. Samples were collected from different storage piles before material was mixed together on large plastic sheeting and re-bagged. Kettering Loam a clay loam and perlite and inert material were used as controled mixing mediums within the growth trials.

3.2.1 COMPOST-LIKE OUTPUT

The CLO was sourced from a MBT plant in the south of England (UK) from a mixed MSW stream. At this facility the biodegradable fraction of MSW is separated from the mixed waste and composted in bio-stabilisation halls for 6 weeks with repeated aeration and irrigation to maintain optimum composting conditions. The organic material is finally sanitised in order to destroy hazardous pathogens. CLO was obtained in June 2011 (approximately 20 kg) and March 2012 (approximately 40 kg).

3.2.2 PAS 100 COMPOST

Organic compost was collected from a recycling centre in South Wales (UK) which is PAS 100 accredited by the Composting Association (British Standards Institution, 2011). The compost was derived from segregated food wastes, which had been in-vessel composted for between 7 and 21 days before being further matured for up to 10 weeks. Within this thesis aerobically composted material certified under the British Standards PAS will be referred to as PAS 100. PAS 100 was obtained in January 2011 (approximately 20 kg). PAS 100 was only investigated during the characterisation and preliminary growth trials as a result of extensive previous research into the material.

3.2.3 PAS 110 ANAEROBIC DIGESTATE

Solid anaerobic digestate was collected from an AD plant in South Wales, which treats source segregated food waste only. Three different feedstocks, effluent sludge, potato sludge and food waste are blended at different ratios and fed into the AD system. The site is working towards British Standards PAS 110 accreditation and currently applies the solid digestate to local fields to replace chemical fertilisers

and disposes of the liquid fraction to waste water drains. Within this thesis anaerobically digested solid residue will be referred to as PAS 110. PAS 110 was obtained in July 2011 (approximately 20 kg) and March 2012 (approximately 40 kg).

3.2.4 KETTERING LOAM AND PERLITE

Kettering Loam and Perlite were sourced from appropriate suppliers and stored in cool dry conditions before analysis and use within the growth trials. Kettering Loam and perlite were control materials and were used rather than brownfield material to identify the sole influence of the ROWs. Kettering Loam and perlite are consistent and allow repeatability of the investigation. Kettering loam and perlite were characterised using the same techniques as the ROWs to understand the inputs within the growth trials.

3.2.5 MATERIAL STORAGE

All materials were stored at < 4 °C in airtight containers before analysis and use in the growth trials. Material was retested before each growth trial. Perlite and Kettering Loam were used as mixing mediums within the growth trials and were stored in dry cool conditions.

3.3 CHARACTERISATION OF ROW MATERIALS

To characterise the ROWs and analyse soil and leachate samples a number of standard analytical methods were conducted. Perlite and Kettering Loam that were used in the growth trials as mixing mediums with the ROWs were also analysed with all ROWs samples to ensure all inputs were known.

3.3.1 SAMPLE PREPARATION AND PRESERVATION

For all analyses, ROWs were scoop-sampled (approximately 3 kg) from different storage bags and mixed together. Riffle boxes were used to ensure representative samples of each ROW were available for analysis. Unless otherwise stated, all ROW samples required for analysis were dried at 75 °C to prevent excessive drying and loss of volatiles in accordance with BS 13040 (British Standards Institution, 2007a). Dried ROW samples were stored in cool and dry conditions in sealed bags.

All plant material before and after plant growth trials were separated from pot material and the roots washed with deionised water. Plants were divided into root, stem and leaf material and dried at 75 °C to prevent excessive drying and loss of volatiles in accordance with BS 13040 (2007a). All dried plant material was stored in cool and dry in sealed bags.

Soil and plant material was prepared for analysis by grinding dried material in a Labtech Essa grinder to homogenise sample material.

Soil and compost samples were stored at less than 4 °C in sealed containers to preserved their 'as received' state. Compost samples were stored for approximately 12 months in a cold environment. To ensure there were no substantial changes in the composition of the compost samples before the final column growth trials, material was reanalysed. Dried soil and compost samples were stored in sealed bags, in dry and cool environments. Liquid samples for ICP-OES analysis were preserved with 10 % nitric acid (100 µl per 40 ml of sample). Liquid samples to be analysed for IC or ammonium were analysed as soon as possible or frozen < -10 °C and analysed within 3 weeks (British Standards Institution, 2003).

3.3.2 DEIONISED WATER

Deionised water was used to rinse glassware, dilute samples and make up analytical solutions and standards. All deionised water was approximately 18.2 MΩ/ cm (Millipore, Synergy 185).

3.3.3 PH AND ELECTRICAL CONDUCTIVITY

A Mettler Toledo SevenMulti dual meter with specific pH and EC electrodes were used to measure pH and EC of liquid samples. The electrodes were calibrated using pH 4 and pH 7 and 1413 µS/ cm standard solutions for pH and EC respectively. After calibration electrodes were tested with deionised water blanks before sample analysis was conducted to ensure calibration solutions were removed from electrodes. The equipment was calibrated for every batch of samples analysed with automatic endpoint and automatic temperature compensation.

To determine the pH and EC a sample of ROW equivalent to 60 ml of dry mass was mixed with 300 ml deionised water a 1: 5 (*V:V*) ratio and shaken for 1 hour on a

shaking table at room temperature (British Standards Institution, 2000a and British Standards Institution, 2000b). Each liquid sample was then filtered using 8-µm filter membrane (Whatman Grade 40) before pH and EC probes were submerged and pH and EC values were recorded.

3.3.4 MOISTURE CONTENT

The MC of each ROW was determined by drying triplicate samples of approximately 100 g at 105 °C for 16 hours or until, there was no further change in weight. The MC (%) and dry matter content (%) for each replicate sample was calculated and averaged for each ROW (British Standards Institution, 2007a).

3.3.5 BULK DENSITY

The BD of each ROW was determined using two methods; the British Standard laboratory compaction method (British Standards Institution, 2007a) and the BD (loose) of fine-grained fertilisers (British Standards Institution, 2001a).

The compacted BD was identified by filling a 1 litre cylinder with a representative sample of each ROW in its 'as received state' (wet sample) and compacted using a plunger (650 g) for 3 minutes. The mass of the ROW was weighed and the compacted BD identified for the known volume (British Standards Institution, 2007a).

The loose BD was identified by filling a 1 litre cylinder with a representative sample of each ROW (dried sample) and left to stand for 2 minutes with no compaction. The mass of the ROW was weighed and the loose BD identified for the known volume (British Standards Institution, 2001a).

The compacted BD method was conducted to characterise the wet material however, the dry loose BD method had a greater representation of the compaction required for plant growth. When preparing the pots trials and planting the saplings/ willow rods the ROW/Kettering Loam or ROW/perlite mixes were not compacted to ensure the pot were suitable for plant root growth. Each method was repeated three times with different material before average bulk densities were calculated based on the mass and volume.

3.3.6 TOTAL CARBON AND NITROGEN

For the ROWs characterisation, preliminary growth trials and optimum nitrogen application rate growth trials the total carbon and nitrogen content was analysed using a Carbon Hydrogen Nitrogen Sulfur (CHNS) elemental analyser (Perkin Elmer 2400). Approximately 0.01 g of ground sample was prepared in 5 mm by 9 mm tin capsules, before the capsule was folded and sealed in preparation for auto injection into the combustion chamber of the CHNS analyser. Three calibration materials were used to standardise the equipment; acetanilide (71% carbon, 10% nitrogen), nicotinamide (60% carbon, 23% nitrogen) and marine sand standard (4% carbon, 0.1% nitrogen). All standards were repeated with standard errors of less than 5%.

The CHNS elemental analyser was unavailable for use for the column growth trials sample analysis. Samples were analysed using Thermo Scientific FlashEA1112 at Forest Research laboratories (Alice Holt, Farnham, UK). Approximately 0.025g of ground sample was prepared in 5 mm by 9 mm tin capsules; capsules were folded and sealed before auto injection into the FlashEA1112. Aspartic acid (36% carbon, 10.5% nitrogen), LGC-euro 2 (11% carbon, 0.26% nitrogen) and thermo11 (2.2% carbon, 0.21% nitrogen) were used as standard calibration materials. ROWs previously analysed using the CHNS elemental analyser were also tested using the Thermo Scientific Flash EA 112 analyser to ensure consistency between analytical equipment.

All results for total carbon and nitrogen analysis were presented as a percentage of the total mass.

3.3.7 TOTAL HEAVY METAL ANALYSIS

Total metal analysis on solid and liquid samples was performed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Perkin Elmer Optima 2100 DV, UK). Solid samples were first microwave digested using an Anton Paar Multiwave 3000 with an *aqua regia* solution (1: 3 32% hydrochloric acid and 70% nitric acid) (British Standards Institution, 2001b). Approximately 0.1 g of each ROW sample (analysed in triplicate) was digested using *aqua regia* (6 ml) solution. The samples were microwave digested by heating samples to 200 °C for 30 minutes

before samples were cooled to room temperature. Samples were filtered using Whatman cellulose 0.45 μm filter membranes and made up to volume (50 ml) using deionised water. The ICP-OES was calibrated using standard solutions (0, 0.1, 1.0, 10, and 100 mg / l) dependent upon elements to be analysed (Fisher Scientific, UK). Deionised water blanks were run with every batch of leachate samples analysed. Liquid samples were also analysed using the ICP-OES without the microwave digestion step.

All leachate samples were analysed for Mg, Al, Ca, Cd, Cr, Cu, Ni, Pb, and Zn. Column growth trials also analysed Fe concentrations in leachate samples.

Solid ROW and plant material were also analysed using an Innov-X X-ray Fluorescence (XRF-X 5000) system. The XRF was calibrated using a stainless steel calibration disc provided by the manufacturer. The 3-beam soil program was used to analyse all samples tested. Approximately 20 g of ground material (analysed in triplicate) was prepared in an XRF sample pot and analysed. Individual readings for each sample were averaged to determine final values to the nearest mg/ kg. The complete list of elements detected by the XRF 3-beam soil program and detection limits can be found in Appendix 2. Cadmium, chromium, copper, nickel, iron, lead, and zinc were investigated and presented in this study.

3.3.8 MERCURY ANALYSIS

The total mercury content of liquid samples were analysed using AMA254 LECO Mercury Analyser. The mercury content of solid samples was anlaysed after aqua regia digestion as described in section 3.3.7. The mercury analyser was internally calibrated which is compared to known standards. Fisher Scientific standard solution 100 mg / l was diluted using deionised water to standards of 0.1, 0.05 and 0.01 mg / l including deionised water blank controls.

3.3.9 ANION ANLAYSIS

A Dionex ICS- 2000 Ion Chromatography system with an AS40 auto sampler and an IonPac AS11 analytical column was used to identify the soluble anions present in soil leachate samples. Standard calibration solutions were used to produce analysis standards of 1, 10 and 100 mg / l for chloride, nitrate, nitrite and sulfate (Fisher Scientific, UK). Deionised water blanks were run before every calibration

and before every batch of leachate samples to ensure any previous sample residue was removed from the analytical column.

3.3.10 AMMONIUM ANALYSIS

The concentration of ammonia/ ammonium in liquid samples was analysed using the modified Berthelot method (Rhine *et al*., 1998). Ammonium concentrations were measured colourmetrically using spectrophometic analysis.

The principle of the Berthelot reaction involves the reaction of ammonia/ ammonium with hypochlorite and phenol under alkaline conditions to produce a blue-green colour (indophenol). The intensity of the colour change indicates the ammonium concentration. A modified method replaced phenol with sodium salt of 2-phenylphenol (PPS) and allowed colour development at lower pH levels.

Three solutions were prepared for the ammonium analysis; citrate reagent, 2-phenylphenol-nitroprusside reagent, and buffered hypochlorite reagent as described in Table 3.1.

Table 3.1 Berthelot Reagent methodology (Rhine *et al*., 1998).

Reagent	Preparation
Citrate	Trisodium citrate (11.36 g) was dissolved in approximately 180 ml of deionised water in a 250 ml volumetric flask. The pH was adjusted to 7.0 by the addition of 0.1 M hydrochloric acid and the solution was made to volume with deionised water.
PPS- nitroprusside	In a 250 ml volume metric flask, 7.32 g of PPS and 0.034 g of Na nitroprusside were dissolved in deionised water and brought to volume.
Buffered hypochlorite	Trisodium phosphate (2.27 g) was dissolved in approximately 180 ml of deionised water. 22.5 ml of sodium hypochlorite (0.7 M NaOCl) was added and the pH was adjusted to 13.0 using sodium hydroxide (2.0 M), and the solution was made up to volume (250 ml).

A 0.3 ml aliquot of filtered leachate was added to a 5 ml vial. Citrate reagent (0.3 ml) was dispensed into the vial and swirled briefly with the leachate sample. After 1 minute, 0.3 ml of PPS- nitroprusside reagent and 1.8 ml of deionised water was added. Colour formation was initiated by the addition of 0.18 ml of the buffered hypochlorite reagent. 0.12 ml of deionised water was added and the colour allowed to develop for 45 minutes at room temperature.

Of each batch of samples analysed, 10% were repeated to ensure reliable results. Colour intensity was analysed using spectrophotometric analysis and compared to the known standards.

The spectrophotometer (Hitachi U-1900) was calibrated using known ammonium solutions diluted from Fisher Scientific (UK) 1000 mg / l ammonium standard. Standard calibration solutions of 0, 0.5, 1, 5, 10, 25, 50, 75 and 100 mg / l were prepared and calibration curves were generated. A linear relationship between absorbance values and standard concentrations of $R^2 = 0.9939$ and $R^2 = 0.9943$ were generated for optimum application rate and column growth trials respectively (Appendix 3).

Deionised water blanks were run with every batch of leachate samples. All samples were analysed at 660 nm wavelength. Absorbance values of greater than 1.0 (> 10 mg / l ammonium) were discarded due to concentrations exceeding the calibration curve. Samples exceeding 10 mg / l were diluted with deionised water to ensure absorbance values were within calibration.

3.3.11 WATER-SOLUBLE AND AVAILABLE NUTRIENT EXTRACTION

The water soluble and available nutrient extractions were conducted on all ROW following BS 13652 (British Standards Institution, 2001c) and BS 13651 (British Standards Institution, 2001d). Control samples containing only extraction solutions were also included.

Water-soluble nutrient extraction: Samples of ROW equivalent to a volume of 60 ml were placed in 500 ml conical flasks (2 replicates per material) and 300 ml of deionised water extracting solution. Samples were shaken for 1 hour at room temperature. Liquid samples were centrifuged at 3000 rpm for 20 minutes before the supernatant was decanted and filtered (0.45 μm Whatman cellulose filter

membranes). The filtrate was analysed for pH, EC (section 3.3.3), total heavy metals (section 3.3.7), total mercury (section 3.3.8), soluble anions (section 3.3.9) and ammonium (section 3.3.10).

Samples of ROW equivalent to a volume of 60 ml were placed in 500 ml conical flasks (2 replicates per material) and 300 ml extracting solution (0.01 M calcium chloride, 0.02 M diethylenetriaminepentaacetic acid). All samples were shaken for 1 hour at room temperature. Samples were centrifuged at 3000 rpm for 20 minutes before the supernatant was decanted and filtered (0.45 µm Whatman cellulose filter membranes). The filtrate was analysed for pH, EC (section 3.3.3) and total heavy metals (section 3.3.7). Soluble anion and ammonium analysis were unsuitable for available nutrient extractions analysis due to the interference of the extractant with analytical methods.

3.3.12 PARTICLE SIZE DISTRIBUTION

Approximately 150 g of each ROW (triplicate samples) was placed in a sieving tower to assess the particle size distribution of materials. Sieves of mesh sizes 19, 10, 6.3, 4, 2, 1.18 mm and a reception tray (> 1.18 mm) were placed on a vibrating shaker with the ROW samples for 7 minutes. The weight of each fraction was recorded and fraction distribution of the sample was calculated based on the total mass of the sample (British Standards Institution, 2007b). Large plant materials such as twigs were included in the samples, however large pebbles and stones were removed from the sample. Plastic strips and cigarette filters identified in sample fractions (particularly CLO samples) were included in the samples, however on two occasions metal screws were found and discarded.

The size fractions were ground to < 2 mm and analysed for total heavy metal content using XRF (section 3.3.7).

3.4 SEQUENTIAL METAL EXTRACTION

To understand the chemical distribution of heavy metals within ROWs the Bureau Communautaire de Référence (BCR) sequential extraction procedure was followed (Paradelo *et al.*, 2011). Table 3.2 shows the 4-step extraction technique and the associated extractants. The modified method used an increased concentration of hydroxyl ammonium chloride and a lower pH. This method is fully described in

Paradelo *et al*. (2011) and was used by Farrell and Jones (2009) to assess the metal speciation and fate during the composting of MSW.

Table 3.2 Modified BCR sequential extraction. Extractants and associated fractions (Paradelo *et al*., 2011).

Step	Fraction	Target phases	Modified BCR method
1	Exchangeable, water and acid soluble	Soluble species, carbonates, CE sites.	0.11 M acetic acid
2	Reducible	Iron and manganese oxyhydroxides	0.5 M hydroxyl ammonium chloride at pH 1.5
3	Oxidisable	Organic matter and sulfide	Hydrogen peroxide followed by 1.0 M ammonium acetate at pH 2
4	Residual		Aqua regia

Step 1- Exchangeable, water and acid soluble

1 g ($\pm$ 0.01g) of each dried ROW (< 2 mm) was added to 100 ml polypropylene centrifuge tubes. In a fume cupboard, 40 ml of 0.11 M acetic acid was added to each tube and placed on a shaking table for 16 hours at room temperature.

All samples were centrifuged for 20 minutes at 3000 rpm to separate solid residue and the supernatant liquid was decanted. All liquid samples were filtered using Whatman cellulose 0.45 µm filter membranes and stored at 4 °C prior to analysis. Solid residue remaining in the centrifuge tubes was washed by adding 20 ml of deionised water and shaken for 15 minutes and centrifuged for 20 minutes at 3000 rpm. The supernatant was decanted and discarded; none of the solid residues were discarded.

Step 2- Reducible

Hydroxyl ammonium chloride solution (0.5 M) containing 25 ml of nitric acid (2 M) was prepared on the day of extraction. 40 ml of 0.5 M hydroxyl ammonium chloride (1.5 pH) was added to the washed residue from step 1 and the solid material re-suspended. The tubes were placed on a shaking table for 16 hours at room temperature. All samples were centrifuged, filtered and the remaining residue washed as described in step 1.

Step 3- Oxidisable

10 ml of hydrogen peroxide (8.8 M) was carefully added in small aliquots to the residue in the tubes from step 2 in a fume cupboard. The tubes were covered loosely with a plastic sheet and left for 1 hour at room temperature, with occasional manual shaking. Digestion was continued at 85 °C in a water bath with occasional manual shaking for 30 minutes. The remaining solution was reduced to 3 ml by further heating. The tubes were allowed to cool before 10 ml of 1 M ammonium acetate adjusted to pH 2 with nitric acid (2 M). All tubes were then placed on a shaking table for 16 hours at room temperature. All samples were centrifuged, filtered and the remaining residue washed as described in step 1.

Step 4- Residue

The solid residues in each tube after step 3 were allowed to air dry at room temperature until there was no change in mass. Solid residue was then digested using the *aqua regia* method described in section 3.3.7.

All supernatant samples were analysed using ICP-OES (section 3.3.7).

3.5   BATCH LEACHING

To characterise the ROW, regulatory compliance batching leaching with liquid to solid ratio of 1:10 was conducted. The BS 12457-2 (British Standards Institution, 2002) method for granular waste materials and sludges with a particle size of less 4 mm (with or without size reduction) allows comparison with other waste materials to identify the leaching properties. Batching leaching analysis was unsuccessful with the PAS 110 samples due to the high adsorbency of the digestate and limited effluent collection.

A representative sample of each ROW was sorted to ensure materials exceeding 4 mm were cut or broken to reduce the sample size. Stones, pebbles and nails of greater than 4 mm were removed and weight recorded. The known MC (section 3.3.4) was used to prepare an as received 'wet' test sample containing 0.090 kg (± 0.005 kg) of dry mass and placed in a conical flask (500 ml).

To calculate the volume of deionised water as the liquid leaching solution, the known moisture content ratio of each test sample was subtracted from liquid ratio

(10) and multiplied by the mass of dry matter in each test sample (section 3.3.4). The volume of deionised water was added to the corresponding conical flask and agitated for 24 hours at room temperature ensuring sediment did not settle in the base. Each material had 2 replicates with an additional two blank controls containing deionised water only.

After 24 hours the flasks were allowed to settle for 15 minutes before the leachate was filtered using a Whatman cellulose 0.45-µm filter membrane. The filtrate was analysed for pH, EC (section 3.3.3), total heavy metals (section 3.3.7) and soluble anions (section 3.3.9).

3.6   STATISTICAL ANALYSIS

SPSS v. 20 (IBM Corporation, 2011) was used to assess the statistical significance of results in chapter 4, 5 and 6. Data sets were imported in SPSS v 20 from excels and data variables were classified as nominal, ordinal, interval or ratio. Nominal data included the plant type, the ROW or the application rate. Scale interval data included percentage change in dry biomass or zinc concentration with the leaf material. Univariate general linear model procedures were conducted (two way ANOVA) on all data sets. This test examines the influence between two different independent variables on one dependent variable as well as the joint effect of the two-independent variables on the one dependent. This test was chosen to allow comparison of heavy metal uptake by different plant species grown in different ROWs as well the effect of both species and ROW on heavy metal uptake. Where required the data files were split by tree species or material type to assess the impact of environmental conditions on plant biomass for species grown in ROW and control pots (Brace *et al*., 2006.). All available statistical output files are available in Appendix 5.

3.7   GROWTH TRIALS

Growth trials were conducted to identify the effect of the ROWs from mixed and source-separated waste streams on different biomass tree species.

3.7.1   APPARATUS

All growth trials were conducted under Sylvania Activa fluorescent tubes (172/ 58W) with daylight spectrum and a colour temperature of 6500 Kelvin. The lights were approximately 1 metre above the surface of the growth pots or columns. The fluorescent lights were set on a timer for 16 hours photoperiod and 8 hours of dark for the duration of all the growth trials.

All growth trials were conducted at room temperature. Temperatures were recorded manually for the preliminary growth trials; an Easysense Q5+ data logger was used during the optimum and column growth trials. The logger was placed in the centre of the table within the plant pots or columns. Three different probes measured the temperature; an internal thermometer in the logging device and two additional probes at different locations across the table. A light sensor recorded the light intensity from the fluorescent tubes, surrounding room lights and natural light. Humidity and air pressure were also logged throughout the growth trials. The data logger took readings every hour. All data logging information can be found in Appendix 6.

3.7.2   PLANT SPECIES

Three tree species were investigated during this study; *Salix viminalis* (Willow), *Eucalyptus nitens* (Shining gum), *Paulownia Tomentosa* (Princess tree).

S. viminalis cuttings were sourced from a nursery in Buckinghamshire (Water Willows, UK) for all experimental growth trials. *S. viminalis* were supplied as rod cuttings of approximately 300 mm with no roots or buds (Figure 3.2). The cuttings were stored at < 4 °C wrapped in black bags to prevent shoots developing. Before growth trials the *S. viminalis* rods were cut to approximately 150 mm (± 20 mm) in length and hydrated in tap water for 24 hours prior to planting.

Figure 3.2 *S. viminalis* rods before planting.

E. nitens were sourced from a nursery in Scotland (UK) (Sourced by Forest Research) for the preliminary growth trials. All plants were grown in commercial compost and were approximately 300 mm (± 80 mm) in height with well-developed roots, stem and leaves. *E. nitens* sourced for the optimum growth trials appeared to have small bumps over all the leaves. Figure 3.3 shows the potentially infected leaves of *E. nitens*. The plants appeared to be infected with Eucalyptus Gall wasp (*Ophelimus maskelli)* from the Hymenoptera family and originates from Australia. Females lay eggs on new foliage in early summer and larvae develop within the leaves, the visible bumps on the surface of leaves or galls may not be visible until the following spring when larvae pupate within the galls (Macleod, 2007; Bowdrey, 2010).

Replacement *E. nitens* were sourced from a Forestry Commission (Delamere) nursery in Cheshire (UK). To ensure these were not infected and could not affect plant growth during the growth trials, all plants were treated with Provado Ultimate Bug Killer a systemic insecticide. *E. nitens* leaves were sprayed liberally and left for 3 weeks before growth trials were conducted. Spare trees were prepared and analysed before and after insecticide application. There were no visible signs of Eucalyptus Gall wasp developing on any of the plants. For the column growth trials, *E. nitens* were supplied by a Forest Commission (Delamere) nursery in Cheshire (UK). There was no evidence of Eucalyptus Gall wasp on any leaves.

Figure 3.3 *E. nitens* with Euc Gall and healthy plant.

P. tomentosa was investigated for the preliminary growth trials due to its rapid growth characteristics and potential as a new UK biomass crop. Saplings of approximately 150 mm (± 30 mm) in height with 4-6 leaves (Figure 3.4) were supplied by Forest Commission (Delamere) nursery in Cheshire (UK). *P. tomentosa* was not investigated beyond the preliminary growth trials due to the invasive characteristics of the species seen in America, limiting its potential market in the UK due to restricted availability of growth data (Woods, 2008; USDA, 2013).

Figure 3.4 *P. tomentosa* sapling.

3.7.3   PLANT ANALYSIS

Before each growth trial, spare trees of each species were analysed to assess initial MC and background concentrations of heavy metals. Plant material was prepared as previously discussed in section 3.3.1 and analysed for total heavy metal content in plant material using XRF (section 3.3.7). All plant material heavy metal content is expressed in mg/kg dry material.

At the end of each growth trial, trees were prepared as described in section 3.3.1 and analysed using XRF for total heavy metal content (section 3.3.7).

To assess plant growth and the biomass production of each species the MC of each plant was identified at the end of each growth trial and was assumed constant throughout the test period. This was used to estimate the initial dry biomass to calculate the percentage change in dry plant biomass over the growth trial and the effect of different variables on species growth. All plant percentage change in dry biomass was calculated using the same method therefore any errors associated in assuming the constant MC would be similar in each species.

3.7.4   PLANT IMAGE ANALYSIS

Matlab (Mathworks, 2011) was used to create a plant analysis program to provide a quick, simple and non-intrusive method of measuring plant growth over time. The Matlab plant image analysis code was written by an undergraduate engineering student as part of a Cardiff Undergraduate Research Opportunities Programme (CUROP) placement (Morgan, 2011). The Matlab code and documents can be found in Appendix 4. The plant image analysis was designed and tested during the preliminary growth trials.

The aim of the program was to estimate plant height as well as estimate the plant biomass by assessing the plant volume. Three photographs were taken from fixed horizontal points at 0°, 45° and 90° of each plant once a week during the preliminary growth trials. A graphical user interface was developed to allow plant images to be uploaded, cropped, analysed and output values to be exported to excel.

Images were uploaded into the program and the plant height was calculated from a datum established at the top of the plant pot from each cropped image. The camera

was moved to ensure all plant material was included in the photo and the program was calibrated weekly using a known point on a ruler as shown in Figure 3.5. A scaling factor within the Matlab coding used the known height (mm) divided by the image height in pixels. Photos of each plant were converted into black and white pixels. The scaling factor was then used for each photo to calculate the plant height based on the number of white pixels. The plant volume was calculated from the 2-dimensional areas of pixels from three images. The average width of the plant was halved to identify the radius and an idealised cylindrical shape assumed.

An 'envelope volume' was calculated based on the solid space taken up by the plant by filling gaps in the binary images of each plant. To assess leaf density of each plant and the difference between species and treatments, density factors were calculated from the percentage difference between the plant volumes and envelop volumes. The output values could then be exported to an excel file for each plant weekly to be compared to manual height measurements and program calibration.

Figure 3.6 shows the processed images used to analyse the plant height, plant volume and envelope volume. The density factor is a percentage comparison of the plant volume value and plant envelope volume value to give an indication of leaf density.
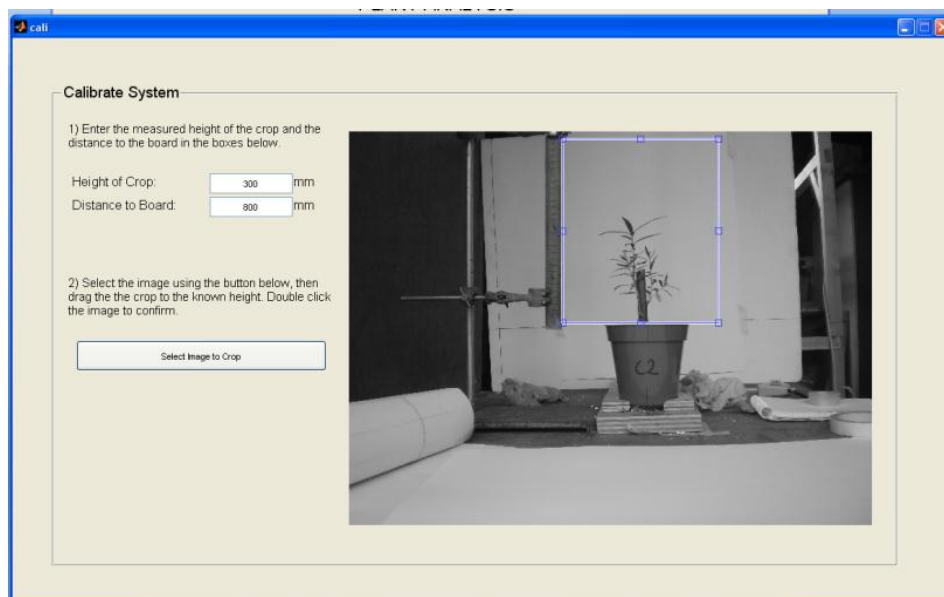
Figure 3.5 Matlab Graphical user interface Plant Image Analysis.

Figure 3.6 Plant image manipulation for plant height, volume and envelope volume.

The Matlab weekly heights were compared to the manual height measurements taken to calibrate the program. The volumes of the plants were assessed at the end of the preliminary growth trial by measuring the displacement of water when the above ground plant material was submerged into water. This was compared to the Matlab calculated volumes.

3.7.5   TOTAL NITROGEN APPLICATION RATES

Nutrient management of fertiliser application ensures excess nutrients do not cause pollution. Fertiliser application to agricultural land is based on the total nitrogen per hectare and is limited to 250 kg of total N/ Ha/ yr (Defra, 2009b). The Environment Agency (England) regulates the application of CLO to land through bespoke environmental permits, which ensure there is no risk to the environment or human health. The maximum application rate of CLO is dependent upon site-specific conditions, CLO characteristics and contamination limits (Environment Agency, 2009b).

Forest Research field trials were conducted on a brownfield site in the south of England between 2011 and 2013. A bespoke permit was issued to apply CLO at rates greater than the 250 kg N / Ha. Forest Research used application rates ranging between 250-10,000 kg N/ Ha as seen in this research.

To calculate ROW application rates per 1 litre pots or columns equivalent to different total nitrogen per hectare application rates, the total nitrogen content (section 3.3.6), mixing depth and MC of each material was identified (section

3.3.4). A mixing depth of 150 mm was employed based on field trials and Good Practice Guide for PAS 100 landscaping projects (Edwards *et al*., 2012). Preliminary and optimum growth trials were conducted in 1 litre pots (0.001 m^3); the column growth trials had a volume of 0.002602 m^3.

The application rate (i.e. 250 kg N / Ha, column A) was divided by the total volume the nitrogen is to be spread over (10,000 m^2 x 0.15 m = 1500 m^3) to identify the total nitrogen application per m^3 (column B). The volume of the pot (0.001 m^3) was multiplied by the total nitrogen application per m^3 value to give the nitrogen mass per pot (column C). The nitrogen mass per pot was divivded by the total nitrogen content (%) of the ROW (i.e. CLO = 2.07 %, PAS 110 = 2.37 %). This gave the dry weight of the ROWs applied to each pot to correspond to the application rate required as seen in Table 3.3. The MC was used to correct the dry mass to the 'wet' or as received ROWs.

Table 3.3 Example of CLO optimum total nitrogen application rate calculations.

Application Rate (kg N / Ha)	N application rate by volume (kg N / m^3)	N mass per pot (kg)	Dry mass sample (kg)	Dry mass content (100-MC) (%)	Wet sample per pot (kg)
ChaptA	B	C	D	E	F
CLO	(A/1500m^3)	(0.001 X B)	(B/2.07%)	CLO	(D/E) X 100
250	0.167	0.000167	0.008	58.97	0.014
1000	0.667	0.000667	0.032	58.97	0.055
3000	2.00	0.0020	0.097	58.97	0.164
6000	4.00	0.0040	0.193	58.97	0.328
10000	6.67	0.0067	0.322	58.97	0.546

Control pots, not supplied with ROW were watered with Hoagland nutrient solution. A nutrient solution was applied to control pots during the preliminary and optimum application rate growth trials to ensure sufficient nutrients were available for plant growth, while controlling the nitrogen forms and heavy metal inputs. Hoagland solution provides all required nutrients for plant growth and is commonly used in diluted form (25 %) as seen in previous studies by Jenson *et al.* (2009) and Punshon *et al.* (1995).

To make up 25 % strength Hoagland solution, 0.4 g of Hoagland basal salt mixture was added to a 1 litre volumetric flask and made up to volume with deionised water.

The 25 % Hoagland solution was stored in the dark at less than 4 °C. Table 3.4 shows the salt mixtures in 25 % Hoagland Solution in mg/ l. The nitrogen content of 25 % Hoagland solution is 49.02 mg/ l, with an average pH of 5.822 and EC of 0.598 mS / cm. Hoagland solution was applied weekly, rather than a single dose in both growth trials.

Table 3.4 Basal salt 25 % Hoagland solution, mg/ l (Sigma Aldrich, 2013).

Ammonium Phosphate	28.76	Magnesium Sulfate	60.19
Boric Acid	0.715	Manganese Chloride	0.4525
Calcium Nitrate	164.1	Molybdenum Trioxide	0.004
Cupric Sulfate	0.02	Potassium Nitrate	151.65
Ferric Tartrate	1.33	Zinc Sulfate	0.055

3.7.6 PRELIMINARY GROWTH TRIALS

The aim of the preliminary growth trials was to investigate the use of three differently sourced/ treated ROWs as nutrient sources for three different tree species and the effect on their growth. *S. viminalis*, *E. nitens* and *P. tomentosa* were grown in CLO (mixed MSW source), PAS 100 (source segregated, aerobic composting), and PAS 110 (source segregated, anaerobic digestion) at total nitrogen application rates equivalent to 1500 and 3000 kg N / Ha. An inert material (perlite) was used as a mixing medium with the ROWs at different application rates. Species control pots containing perlite only and water with 25 % Hoagland solution were included in the experimental design. Table 3.5 shows the experimental matrix of variables.

Table 3.5 Preliminary growth trial experimental matrix representing the total number of plants for each species and conditions.

Species	Control [c]	CLO [a]	CLO [b]	PAS 110 [a]	PAS 110 [b]	PAS 100 [b]
S. viminalis	3	3	3	3	3	3
P. tomentosa	3	3	3	3	3	3
E. nitens	3	3	3	3	3	3

[a] *Application rate equivalent to 1500 kg N/Ha (CLO: Perlite, 1.25; PAS 110: Perlite 0.498 wet masses)*
[b] *Application rate equivalent to 3000 kg N/Ha (CLO: Perlite, 3.21; PAS 110: Perlite 1.09; PAS 100: Perlite, 3.6 wet masses)*
[c] *control pots containing perlite only and watered with 25% Hoagland solution equivalent to 160kg N/Ha over 22 weeks.*

S. viminalis cuttings approximately 165 mm in length were planted in commercial compost for 4 weeks to allow roots and shoots to develop. The *P. tomentosa* saplings were approximately 155 mm with well-established roots around commercial compost with 4 - 6 green healthy leaves. *E. nitens* saplings were approximately 300 mm with well-established root systems around commercial compost. For replanting with ROWs, the root systems were washed with deionised water to remove the majority of compost material around the roots. The plant weight, plant length, and root length were recorded.

Control pots containing perlite only were watered weekly with 100 ml of 25% strength Hoagland solution (section 3.7.5) as a nutrient supply (equivalent to 160 kg N / Ha applied over 22 weeks, section 3.7.5), and additional 100 ml deionised water. ROWs pots were watered twice weekly with a total of 200 ml of deionised water to control the nutrient and ion input. The irrigation regime was determined by observing the plants and moisture content of the soil based on the environmental conditions and growth of the plants. All water input was recored.

Pot trials were grown under daylight spectrum fluorescent bulbs for 16: 8 hours of light: dark, at temperatures ranging between 11 - 28 °C on a diurnal temperature cycle, for 22 weeks between September 2011 and January 2012. Manual height measurements, leaf counts and observational notes monitored the growth rates of all species weekly. Weekly photographs were taken of each plant from 3 fixed points as described in section 3.7.4. Figure 3.7 shows the preliminary growth trials experiment set up. At the end of the 22 weeks plant material was prepared (section 3.3.1) and analysed as described in section 3.3.7.

Figure 3.7 Preliminary growth trials set up.

3.7.7   *OPTIMUM APPLICATION RATE GROWTH TRIALS*

The preliminary growth trials findings were used to influence and develop the secondary growth trials. Two variables previously investigated were not continued after the preliminary growth trials; PAS 100 (source segregated, aerobically composted) and *P. tomentosa*. For the optimum application rate growth trials, perlite was replaced with Kettering Loam as perlite did not simulate the natural environment due to the limited binding sites and low cation exchange capacity associated with the inert material. Kettering Loam contains approximately 24 % clay and is commonly used for cricket pitches. The loam has a consistent composition required for wicket construction and therefore has good repeatability as a control mixing medium for the use in these growth trials. Perlite and Kettering Loam were subjected to the same characterisation analysis as the ROWs as described in section 3.3.

The aim of the secondary experiments was to identify the tolerance of *S. viminalis* and *E. nitens* to toxicity arising from the use of ROWs at different total nitrogen application rates while assessing leachate composition and the impact on biomass production.

Pot trials were conducted with application rates equivalent to 250, 1000, 3000, 6000 and 10,000 kg N / Ha. The average ratios of CLO to Kettering Loam were 0.015, 0.07, 0.2, 0.7 and 2.7 for application rates equivalent to 250, 1000, 3000, 6000 and 10,000 kg N / Ha respectively. The average ratio of PAS 110 to Kettering Loam were 0.008, 0.03, 0.12, 0.5 and 1.3 for application rates equivalent to 250, 1000,

3000, 6000 and 10,000 kg N / Ha respectively. Each condition had 3 replicates with additional control plants grown in Kettering Loam only. See Table 3.6 for the experimental matrix.

S. viminalis cuttings of approximately 145 mm in length were grown in Kettering Loam only for 3 weeks to allow root and shoot development. *E. nitens* were supplied as developed sapling of approximately 220 mm in length, in commercial compost. To reduce replanting death and risk of inconsistent results all pot conditions were initially double-planted for the first 3 weeks of the growth trial. Figure 3.8 shows the experimental set up.

Table 3.6 Optimum application rate growth trials experimental matrix representing the total number of plants for each species and conditions.

Application rate kg N/Ha	0*	250	1000	3000	6000	10,000
PAS 110 + Kettering Loam						
E. nitens		3	3	3	3	3
S. viminalis		3	3	3	3	3
CLO + Kettering Loam						
E. nitens		3	3	3	3	3
S. viminalis		3	3	3	3	3
Kettering Loam						
E. nitens	3					
S. viminalis	3					

*Kettering Loam only, watered with 25 % Hoagland solution, equivalent to 87 kg N / Ha over 12 weeks.

For experimental planting excess soil material was washed off roots using deionised water. The total weight, root length, above ground length and number of shoots/leaves of each plant was recorded.

Each ROW pot was watered with 200 ml of deionised water each week to control external nutrient input. The irrigation regime was determined based on observational results and previous experience. A basic moisture meter was used however the high organic content and high moisture holding capacity of the higher application rates exceeded the moisture meter values although the pots did not appear to be over watered. Control pots containing Kettering Loam only were watered with 100 ml deionised water and 100 ml of 25 % strength Hoagland solution (section 3.7.5), equivalent to a total nitrogen application rate 87 kg N / Ha

over 12 weeks. Excess water within pot saucers were collected once a week and filtered for pH, EC (section 3.3.3) and total heavy metals (section 3.3.7) analysis. The soluble anions (section 3.3.9) and ammonium concentrations were analysed (section 3.3.10) fortnightly.

After 3 weeks, one plant from each pot was removed ensuring the healthiest plant remained within the pot. The healthiest plant remaining in the pot was based on the number of shoots/ number of leaves rather than height only. Photos were taken to show evidence of distress in the leaves and shoot colour and differences. The removed plants were measured, the number of leaves/shoots and observational notes of why chosen to be removed were recorded. The total weight, above ground length and root length were recorded for each plant that was removed. The plant material was prepared and analysed as described in section 3.7.3.

Figure 3.8 Optimum application rate growth trials set up.

The pot trials continued for the remaining 9 weeks assessing plant growth with height, observational and visual records. Weekly analysis of the pot leachate continued for pH, EC (section 3.3.3) and total heavy metal content (section 3.3.7), as well as fortnightly soluble anions (section 3.3.9) and ammonium analysis (section 3.3.10).

After a total of 12 weeks of growth, the plant mass, above ground length and root length were recorded. Individual plants were dried and prepared as described in section **Error! Reference source not found.**3.7.3 for total heavy metal content using XRF analysis (section 3.3.7).

3.7.8 COLUMN GROWTH TRIALS

The findings from the optimum nitrogen growth trials were used to develop the column growth trials.

Column growth trials were conducted to investigate the effect of different environmental redox conditions on the leachate chemistry of CLO (mixed MSW source, aerobically composted) and the effect on growth on *S. viminalis* and *E. nitens*.

Columns of 93 mm by 400 mm with diameter: length ratio of greater than 1: 4 (Lewis and Sjöstrom, 2010) and volume of 0.002602 m^3 were used in replacement of 1 litre pots. The optimum nitrogen application for CLO (3000 kg N / Ha equivalent to CLO: Kettering Loam ratio of 0.2, wet masses) identified in the optimum application rate experiments (section 3.7.7) were mixed with Kettering Loam with 3 replicates per condition.

Control columns containing equivalent volumes of perlite in replacement of CLO were mixed with same volume of Kettering Loam (Perlite to Kettering Loam ratio of 45, wet masses). Plant-free controls with CLO or perlite were also included to identify the influence of plant species on the leachate chemistry. Table 3.7 shows the experimental matrix.

Table 3.7 Column growth trial experimental matrix representing the total number of plants for each species and conditions.

Plant	CLO unsaturated	CLO saturated	Perlite unsaturated	Perlite saturated
E. nitens	3	3	3	3
S. viminalis	3	3	3	3
Plant free	3	3	3	3

In total 36 columns and 24 plants were grown in either unsaturated conditions or saturated condition. Unsaturated columns had both air and water in the pore spaces, which reproduced conditions present in a soil vadose zone. The irrigation regime was determined based on observation results and the volume of excess leachate collected at the base of the columns. All water input and output was recored. The saturated columns had pore spaces filled with water to mimic flooded zones that may occur on brownfield sites as a result of poor soil structure, flooding and perched water tables. To maintain saturated condtions of the saturated columns, water was added on a daily basis to ensure the surface of the soil mixtures were submerged by at least 1cm of water. All water input and output was recored.

Figure 3.9 shows a diagram of the column design and packing. Columns made of polyvinyl chloride were cut to approximately 650 mm and fitted with an end cap at the base of the column with a prepared drainage point. To limit material loss through the base drainage point, 2 mm gauze and 80 µm mesh was placed at the base of the column. A layer of inert perlite and another 80 µm mesh separated the CLO/ Kettering Loam and perlite/ Kettering Loam mixes with the column drainage system.

93 mm Diameter

20 mm from the top of the column left clear

400 mm CLO/ Perlite and Kettering Loam mixed and evenly compacted

Rhizon Sampler inserted 200 mm into column to collect pore water

140 mm Filter layer containing 80 μm nylon mesh, perlite and 2 mm gauze

Base leachate sampling point
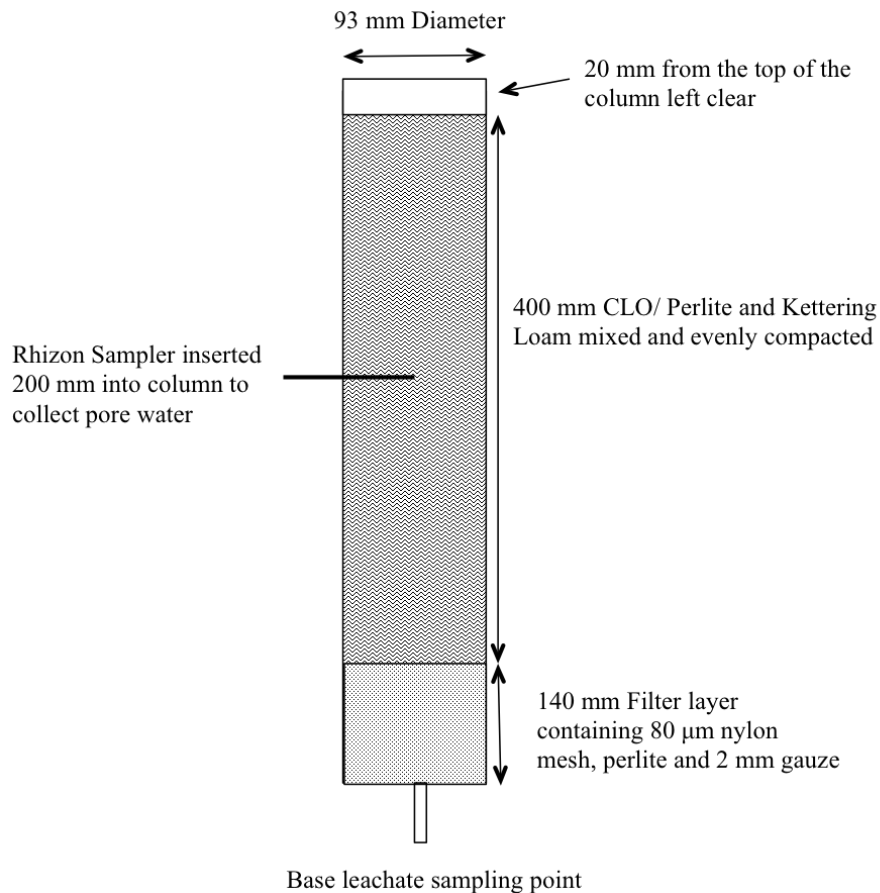
Figure 3.9 Column design and Packing.

The CLO and Kettering Loam were hand mixed in buckets at a total nitrogen application rate equivalent to 3000 kg N / Ha. The perlite and Kettering Loam were hand mixed in buckets at a total nitrogen application rate equivalent to 0 kg N / Ha. Columns were damp packed with CLO/ Kettering Loam or perlite / Kettering Loam mixes in 100 mm lifts with average bulk densities of 1191 and 1042 kg / m^3 respectively. Between each lift the material was lightly scarified to ensure hydraulic connectivity between layers (Lewis & Sjöstrom, 2010). Columns were allowed to equilibrate for 2 days before saturation and planting.

To assess the change in leachate through the profile of the column two sampling points were incorporated into the column design; 200 mm from the soil surface and the base of the column (400mm). To collect the soil solution 200 mm down the column profile, Rhizons (Figure 3.10) were inserted into the soil column. Rhizons are specially designed soil samplers with a 2.5 mm diameter hydrophilic porous material to sample pore water. A vacuum is applied to the Rhizon by a syringe, which samples soil pore water from the soil profile in unsaturated and saturated

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conditions. The pore water is filtered by the 50 mm hydrophilic porous material (0.15 µm) and collected in the syringe for analysis.

All columns were saturated with tap water to approximately 10 mm above the soil surface. After 6 hours, half of the columns were drained under gravity to be the unsaturated test columns. Leachate collected from the unsaturated and saturated columns after 6 hours were analysed for pH and EC (section 3.3.3) and total heavy metal content (section 3.3.7), soluble anions (section 3.3.9**Error! Reference source not found.**) and ammonium (section 3.3.10).

The soil solution in each column was collected from the Rhizons and at the base of the columns as shown in Figure 3.11. Soil leachate samples were collected from all columns rhizon and base collection points after 24 hours, 96 hours and 1 week to assess the change in redox conditions between the saturated and unsaturated columns.

Saturated columns were topped up when required with tap water to ensure saturated conditions were maintained. Unsaturated columns were watered with 200 ml of tap water weekly and were free draining at all times. Tap water samples were collected and analysed weekly to understand inputs into the column. Control columns containing perlite / Kettering Loam were also watered with tap water; no nutrient solution was used in these experiments.

After 1 week of saturation *S. viminalis* and *E. nitens* were planted in the columns and monitored weekly for 12 weeks biomass growth. Leachate samples collected from the Rhizons and base samplers were analysed for pH and EC (section 3.3.3). Lechate samples from the Rhizons and base samplers were anlaysed for heavy metal (section 3.3.7), soluble ions (section 3.3.9) and ammonium (section 3.3.10) fortnightly (approximately 40 ml from each sampling point). The plant height and number of leaves were recorded weekly.
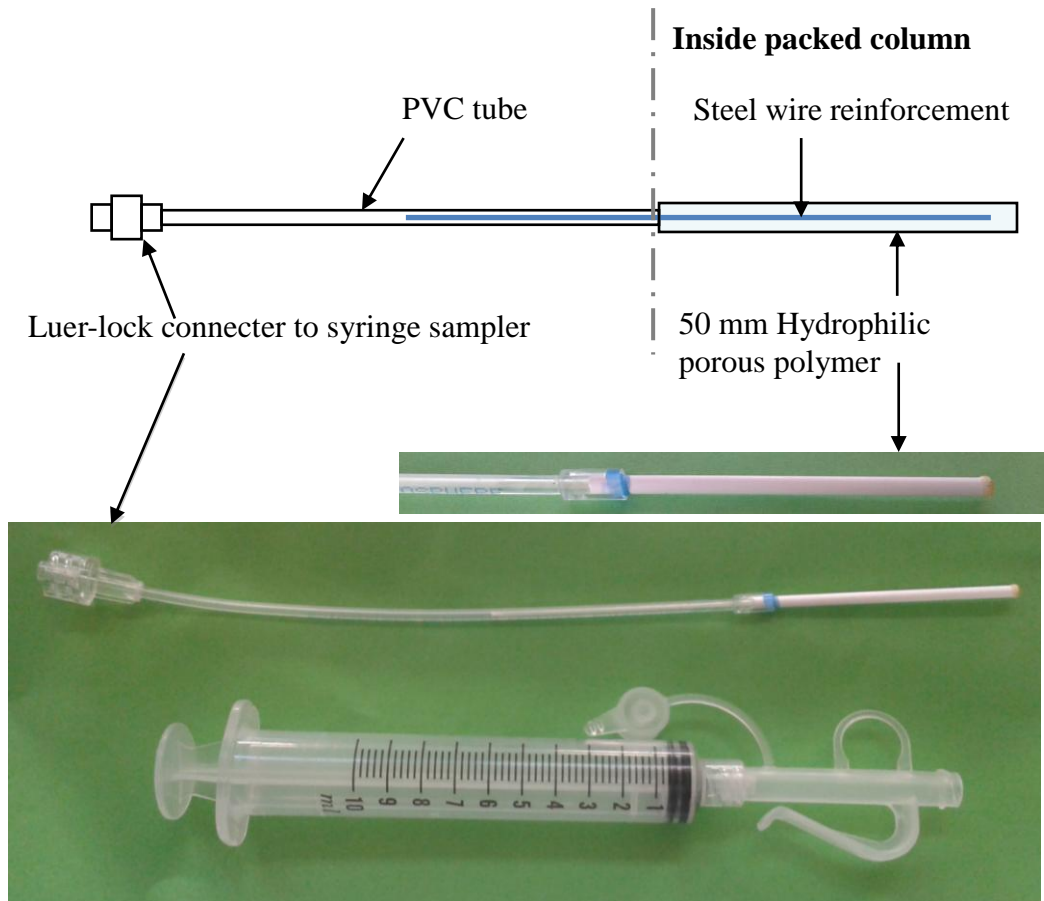
Figure 3.10 Rhizon Sampler.

After 4 weeks all *E. nitens* grown in the perlite/ Kettering Loam and CLO/Kettering Loam in saturated conditions were deceased. Of the unsaturated columns with perlite one out of three survived, and those planted in CLO two out of three survived. The reason for premature death was unknown. All columns were dismantled and soil and plant material were dried and ground as described in section 3.3.1. Plant material was analysed for total heavy metal content using XRF analysis (section 3.3.7).

Figure 3.11 Column Growth trials set up with Rhizon and base sampling points.

Preliminary growth trials had shown the sensitivity of *E. nitens* to replanting (section 4.2) and it was previously proven that CLO at application rates equivalent to 3000 kg N/Ha was not detrimental to the plant growth (Chapter 5). It was hypothesised by the author that the stress of replanting as well as immediate saturation might have caused the premature death of the *E. nitens.*

To investigate if root establishment was required before saturation of *E. nitens*, all columns were repacked with CLO / Kettering Loam and saturated after different time periods from the point of planting. As previously discussed columns were packed with CLO and Kettering Loam at total nitrogen application rates equivalent

to 3000 kg N / Ha and *E. nitens* were planted. Three columns were saturated on day 0, and all other columns (9) were unsaturated. A further three columns were saturated after 3 and 6 weeks of unsaturated conditions and three columns remained unsaturated for the 12 weeks growth trials. Unsaturated columns were watered with 200 ml of tap water weekly and were free draining at all times.

Leachate samples were collected from Rhizons and base drainage points weekly for pH and EC (section 3.3.3) analysis. Soluble metal (section 3.3.7), anion (section 3.3.9) and ammonium (section 3.3.10**Error! Reference source not found.**) analysis were assessed fortnightly.

3.8 SUMMARY

This chapter has described the materials and methods used in this thesis. Three ROWs were characterised using a number of different standard tests to understand the components and behaviour of the materials and the difference in waste stream source and biological pre-treatment.

Growth trials were conducted assessing the effect of the three different ROWs on the growth SRC/ SRF energy crop species. Preliminary growth trials investigated three ROWs on the growth of three different tree species. The findings from the preliminary experiments were developed further in the optimum nitrogen application rate growth trials, which reduced the variables by investigating two ROWs and two SRC tree species. The optimum nitrogen application rate growth trials investigated the characteristics of the leachate from the different ROWs over time.

From the optimum nitrogen application rate experiments, further questions were established and the column growth trials were designed. The column experiments intended to extend the knowledge and understanding of the effect of environmental conditions on CLO and two SRC species. This experiment resulted in further experimental redesign in response to the findings within the first four weeks for *E. nitens*.

Chapter 4 ROW CHARACTERISATION AND PRELIMINARY GROWTH TRIAL RESULTS

Characterisation of different ROWs and growth trials were conducted to identify the effect of the materials and compare the differences between waste source and biological treatment. This chapter presents the characterisation of three different ROWs and the results of the preliminary growth trials.

Three ROWs were sourced from different pre-treatment plants in the UK as described in section 3.2. Two materials were source segregated by households and are certified under the British Standard PAS. A third ROW known as CLO was aerobically composted from a mixed MSW stream.

Preliminary growth trials were conducted to understand the sole influence of the ROWs on the growth of three different energy crop species at two different total nitrogen application rates based on field trials being conducted at the time by Forest Research. *S. viminalis* was investigated as a standard SRC energy crop grown in the UK. *E. nitens* and *P. tomentosa* were also investigated as potential new energy crop species to be used in the UK due to their fast growth and biomass production.

Complete data sets can be found in Appendix 6.

4.1   THE EFFECT OF WASTE SOURCE AND BIOLOGICAL TREATMENT ON ROW CHARACTERISTICS

Three ROWs were investigated; two source segregate (PAS 100 and PAS 110) and one from a mixed MSW stream (CLO). Of these materials PAS 100 and CLO were aerobically composted in windrows, whereas PAS 110 was the solid residue from AD. The different waste stream and biological pre-treatment could influence the characteristics of the material.

Characterisation tests such as pH, EC, MC and BD were conducted, as well as total, available and water soluble heavy metal content. Batch and sequential extraction techniques were conducted to identify the heavy metal binding and speciation within the ROWs. All analyses were quality controlled against standard calibrations and repetition of samples within batches.

The characteristics of each ROW are shown in Table 4.1 with the PAS quality limits for the total heavy metal concentrations. The pH values for all materials are neutral excluding PAS 100, which were 9.26. Electrical conductivity values were higher in the CLO samples compared to the PAS materials as a result of the mixed MSW source. Aerobically composted materials had lower MC compared to anaerobically digested materials. There was little difference in the compacted BD of all materials whereas source segregated material had slightly lower loosely packed BD compared to CLO. The bulk density loose values of all ROWs were near the recommended ranges of 400- 500 kg / m^3 to reduce transport costs (WRAP, 2011).

The total nitrogen content was similar between treatments and waste streams, whereas total carbon concentrations were greater in the source segregated than the mixed source streams. The C:N ratios for all ROW materials were all below 22.

The total heavy metal content of each material was identified using a 1: 3 *aqua regia* digestion method as described in section 3.3.7. Source segregated waste certified under the PAS standard must not exceed the total heavy metal concentrations in Table 4.1. The PAS 110 samples did not exceed the heavy metal concentrations whereas the PAS 100 composted material exceeded the zinc concentrations required under the PAS certification.

The total mercury concentrations of all ROWs were below 1.0 mg / kg maximum limit. Mercury analysis was conducted after *aqua regia* digestion with 6 replicates of each sample to ensure accuracy in the mercury analysis.

The concentrations of available nutrients and heavy metals from each material were identified after extraction using calcium chloride/ DTPA (section 3.3.11). There were higher concentrations of heavy metals associated with the CLO followed by PAS 100 and lowest concentrations extracted from PAS 110. The available heavy metal concentrations follows the trend Zn > Pb > Cu > Ni > Cr. Water soluble extraction analysis identified similar trends to the available concentrations whereby CLO exceeded PAS 100 and PAS 110 samples. The water soluble heavy metal concentrations followed a slightly different trend whereby Cu > Zn > Ni > Pb > Cr for CLO samples compared to PAS 100 in which Zn > Cu > Pb > Ni = Cr = Cd. PAS 110 samples were very low or less than the ICP-OES dection limits (Appendix 6).

Perlite and Kettering Loam samples were also characterised to identify the low concentrations associated with the mixing mediums used in the growth trials compared to the ROWs.

4.1.1 SEQUENTIAL METAL EXTRACTION

To assess the heavy metal speciation within each ROW, sequential extraction analysis was conducted to identify the mass of heavy metals within the exchangeable, reducible, oxidisable and residual fractions of each ROW.

Table 4.2 shows the masses of cadmium, chromium, copper, lead, nickel and zinc in each fraction. The metal masses as a percentage of the total metal masses for each fraction are shown in Figure 4.1. Cadmium was below detection levels in all fractions for all ROWs. Heavy metal masses were greatest from the CLO as a result of the mixed waste stream. Similar trends in the percentage of heavy metal fractions are evident between the two PAS 110 samples and the three CLO samples, with some difference between the aerobically composted (PAS 100 and CLO) and anaerobically digested materials (PAS 110). Copper was mainly bound to the residue and the oxidisable fraction for the aerobically composted materials, whereas there was no copper in the residue fractions and the majority bound within the reducible fraction of the PAS 110, anaerobically digested materials. Zinc concentrations were greater within the exchangeable fraction compared to other heavy metals (excluding lead, PAS 110[b]), whereas lead was greater within the reducible fraction, copper within the oxidisable fraction and chromium the least mobile with the majority held within the residue fraction; these trends are evident across all the ROWs.

Table 4.1 Characterisation of all ROW.

Analysis	SOMs limits	PAS limits	PAS 100 [a]	PAS 110 [a]	PAS 110 [b]	CLO [a]	CLO [b]	CLO [c]	Perlite [a]	Kettering Loam [b, c]
pH			9.29	7.05	7.13	7.37	7.89	7.55	7.03	7.73
Electrical Conductivity (mS / cm)			2.15	0.58	0.68	5.82	6.99	6.51	0.05	0.27
Moisture Content (%)			64.5	89.1	87.7	42.5	45.4	38.8	0.6	4.72
Dry Matter Content (%)			35.5	10.9	12.3	57.5	54.6	61.2	99.4	95.3
Bulk Density- Compacted (kg /m^3)			802	817	710	879	821	832	123	916
Bulk Density- Loose (kg / m^3)			389	371	321	400	448	439	104	872
Total Carbon (%)			30.9	48.9	50.9	20.5	24.4	24.0	2.56	2.45
Total Nitrogen (%)			2.66	2.51	2.37	1.37	2.07	2.05	0.06	0.32
Carbon: Nitrogen Ratio		20:1	11.6	19.5	21.5	14.9	11.8	11.7	42.7	7.66
Total Cadmium (mg / kg) [*]	20	1.5	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00
Total Chromium (mg / kg) [*]	1000	100	30.3	29.0	13.6	38.8	75.2	70.6	0.49	0.20
Total Copper (mg / kg) [*]	1000	200	139	83.5	59.8	196	251	190	0.00	1.05
Total Lead (mg / kg) [*]	750	200	160	50.9	43.5	279	289	278	21.1	1.40
Total Nickel (mg / kg) [*]	300	50	7.24	15.6	4.25	29.7	49.2	44.3	0.00	0.12
Total Zinc (mg / kg) [*]	2500	400	440	63.2	49.2	433	517	508	8.58	3.27
Total Mercury (mg / kg) [*]	16	1.0	0.001	0.001	0.001	0.003	0.002	0.002	0.00	0.00
Available Cadmium (mg / kg) [*]			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Available Chromium (mg / kg) [*]			0.09	0.00	0.00	0.83	0.06	0.04	0.00	0.00
Available Copper (mg / kg) [*]			15.2	9.49	7.63	123	49.5	44.9	0.00	0.75
Available Lead (mg / kg) [*]			51.8	0.76	1.22	101	182	161	0.00	0.22
Available Nickel (mg / kg) [*]			0.60	0.00	0.00	4.82	2.43	1.61	0.00	0.37
Available Zinc (mg / kg) [*]			186	36.1	29.9	374	264	222	0.65	2.91
Water soluble Cadmium (mg / kg) [*]			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Water soluble Chromium (mg / kg) [*]			0.00	0.00	0.00	0.1	0.00	0.00	0.00	0.00
Water soluble Copper (mg / kg) [*]			0.83	0.27	0.00	18.1	4.66	3.96	0.00	0.00
Water soluble Lead (mg / kg) [*]			0.47	0.00	0.00	0.89	0.31	0.22	0.00	0.00
Water soluble Nickel (mg / kg) [*]			0.00	0.00	0.00	1.67	0.31	0.67	0.00	0.00
Water soluble Zinc (mg / kg) [*]			2.32	0.00	0.00	7.80	3.46	2.82	0.00	0.13

[a] Samples collected in 2011
[b] Samples collected in 2012
[c] Samples collected in 2012, re-analysed in 2013
[*] mg/ kg dry matter

Table 4.2 Sequential Metal Extraction of ROWs.

Sequential Extraction	(mg / kg) Dry Matter	PAS 100 [a]	PAS 110 [a]	PAS 110 [b]	CLO [a]	CLO [b]	CLO [c]	Perlite [a]	Kettering Loam [b, c]
STEP 1: Exchangeable	Cadmium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Copper	0.901	3.041	0.727	19.36	9.405	10.40	0.011	0.068
	Chromium	0.000	0.094	0.000	1.023	0.359	0.000	0.113	0.000
	Nickel	0.000	0.721	0.000	4.557	2.575	3.200	0.000	0.038
	Lead	2.193	4.337	10.77	13.96	4.819	4.000	0.000	0.000
	Zinc	64.55	31.68	7.082	162.6	132.8	143.2	0.150	0.860
STEP 2: Reducible	Cadmium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Copper	23.36	26.28	21.01	61.02	73.72	53.02	1.260	0.121
	Chromium	2.955	0.440	3.557	10.02	24.62	24.50	1.157	0.052
	Nickel	2.000	2.567	0.897	13.77	3.34	34.78	0.417	0.035
	Lead	125.9	45.67	34.15	215.8	216.2	209.3	1.119	0.266
	Zinc	268.9	24.29	19.88	212.0	293.4	306.4	0.137	0.314
STEP 3: Oxidisable	Cadmium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Copper	44.53	7.344	7.061	27.18	46.01	42.85	0.486	0.094
	Chromium	6.929	0.270	1.915	4.118	11.12	9.993	0.178	0.032
	Nickel	0.164	1.729	0.226	2.815	5.785	4.918	0.598	0.112
	Lead	11.54	2.483	1.289	12.93	12.22	11.99	1.152	0.864
	Zinc	22.73	4.895	3.477	9.323	34.85	32.25	0.067	0.046
STEP 4: Residue	Cadmium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Copper	24.74	0.000	0.000	161.1	148.3	138.6	0.000	1.711
	Chromium	36.42	32.61	17.33	80.64	51.03	48.04	0.000	0.046
	Nickel	2.329	13.50	11.83	46.52	28.04	28.62	0.505	0.147
	Lead	0.000	0.000	0.000	11.66	12.28	17.59	0.000	0.000
	Zinc	6.178	6.178	10.88	82.35	62.85	72.72	0.000	2.917

[a] Samples collected in 2011
[b] Samples collected in 2012
[c] Samples collected in 2012, re-analysed in 2013
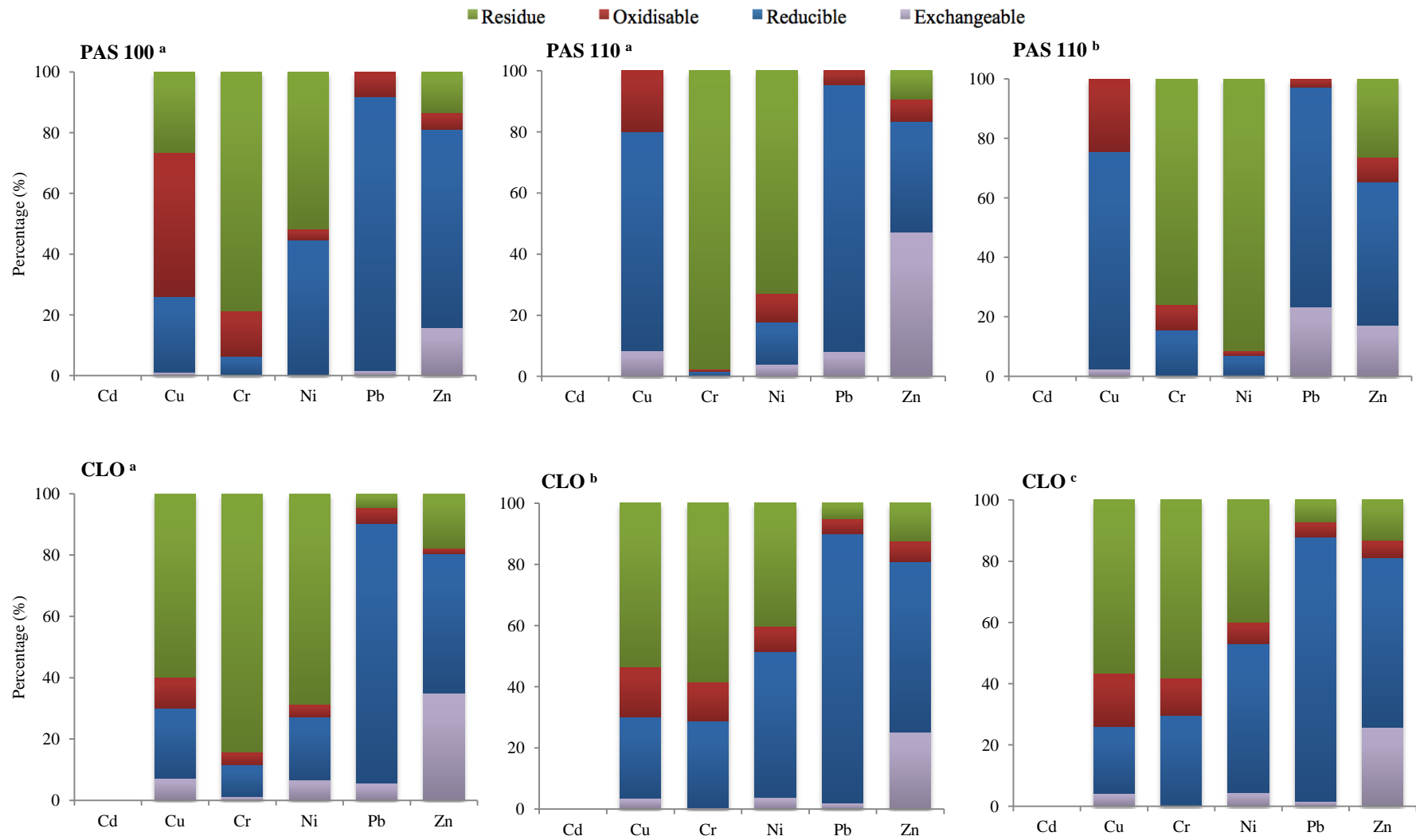
Figure 4.1 Sequential extraction heavy metal masses as a percentage of total masses leached.

[a] Samples collected in 2011, [b] Samples collected in 2012, [c] Samples collected in 2012, re-analysed in 2013

4.1.2   BATCH LEACHING

Compliance batch leaching tests were conducted to characterise each ROW and allow comparison to other waste materials. PAS 110 samples were unsuitable for the batch leaching analysis as a result of the high moisture content of the ROW and the relatively high moisture absorbency restricting the available leachate to be extracted after 24 hours of agitation.

Table 4.3 shows the concentration of leached heavy metals and soluble anions.

The heavy metal leaching varied between CLO and PAS 100 samples. CLO[a] heavy metal leaching exceeding PAS 100 for chromium, copper and lead, nickel and sulfate whereas PAS 100 exceeded CLO for zinc, chloride and nitrate concentrations. CLO samples [b] and [c] were generally lower than the PAS 100 samples excluding copper, lead and sulpate. Nitrite concentrations were undetectable in all samples. The mobility of heavy metals may vary between materials due to the strength of binding and the rate of equilibrium of reactions within the 24 hours of agitation.

Perlite and Kettering Loam indicated low concentrations of all metals leached. Zinc was the most leached heavy metal in all materials, with zinc concentrations slightly greater in PAS 100 than all of the CLO samples.

Table 4.3 Batch leaching analysis.

	PAS 100 [a]	CLO [a]	CLO [b]	CLO [c]	Perlite [a]	Kettering Loam [b, c]
pH	8.184	7.411	7.655	7.508	7.029	7.034
Electrical Conductivity (mS / cm)	1.891	5.192	6.307	6.001	0.004	0.149
Cadmium (mg / kg)	0.00	0.00	0.00	0.00	0.00	0.00
Chromium (mg / kg)[*]	0.57	0.78	0.49	0.44	0.00	0.03
Copper (mg / kg)[*]	0.69	4.15	1.03	0.97	0.00	0.00
Lead (mg / kg)[*]	0.98	1.31	1.30	1.32	0.13	0.25
Nickel (mg / kg)[*]	0.68	1.05	0.58	0.56	1.79	0.08
Zinc (mg / kg)[*]	3.61	3.59	1.95	2.23	0.76	0.09
Chloride (mg / kg)[*]	4341	3984	2558	2320	25.4	244
Nitrite (mg / kg)[*]	BD	BD	BD	BD	BD	BD
Nitrate (mg / kg)[*]	9078	828	1068	1151	133	58.0
Sulfate (mg / kg)[*]	1322	7984	6276	6616	656.3	1777

[a] Samples collected in 2011
[b] Samples collected in 2012
[c] Samples collected in 2012, re-analysed in 2013
BD -Below Detection limit of 0.001mg/ l.
* mg / kg dry matter

4.1.3   PARTICLE SIZE DISTRIBUTION

Particle size distribution analysis was conducted on each ROW to identify the mass of material associated with different fractions of the material. Once separated into fractions as described in section 3.4.4, each sample was ground and the heavy metal content was analysed using XRF analysis to assess the heavy metal partitioning within material fractions (section 3.3.7).

Table 4.4 and Table 4.5 present the percentage of each particle size fraction for each ROW and the average heavy metal content of each sample. Aerobically composted material (PAS 100 and CLO) had the largest fraction within the 6 - 10 mm fraction size compared to anaerobically digested material (PAS 110), which was predominantly in the 4-6 mm fraction.

The heavy metal concentrations of each fraction are shown in Table 4.4 and Table 4.5. The mass of heavy metals within each fraction were calculated (Appendix 6) and totaled to calculate the heavy metal concentration within the 150g samples and compared to the total heavy metal analysis in Table 4.1. The XRF values were higher than the ICP-OES analysis although all showed similar trends in comparison to other heavy metals and materials. The overestimation suggests slight variation in the accuracy of the XRF analysis in comparison to complete aqua regia digestion and ICP-OES analysis.

Source segregated materials (PAS 100/110) had lower heavy metal concentrations than mixed waste stream, CLO. In all materials and metals, there is a general trend for higher concentrations present within the smaller particle size fraction of less than 2 mm. The heavy metal masses (Appendix 6) within each fraction indicate that particle sizes between 10 and 4 (varied between ROW) also had high masses of heavy metals due to the higher masses of material.

Table 4.4 Particle size distribution and total heavy metal content of PAS 100 and PAS 110 samples.

ROW	Sieve Size (mm)	Fraction (%)	Total Cadmium[*]	Total Chromium[*]	Total Copper[*]	Total Lead[*]	Total Nickel[*]	Total Zinc[*]
PAS 100 [a]	19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	23.7	0.00	4.06	10.3	99.4	1.20	285
	6.3	44.8	0.00	7.62	259	187	3.80	352
	4	15.4	0.00	58.9	197	152	14.2	571
	2	8.22	0.00	32.1	232	213	10.8	847
	1.18	4.03	0.00	40.3	281	358	20.6	922
	Residue	3.82	0.00	519	225	452	262	1130
PAS 110 [a]	19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.68	0.00	2.80	5.27	18.9	12.3	23.1
	6.3	17.0	0.00	6.34	65.1	7.41	10.1	52.7
	4	35.4	0.00	80.9	21.8	12.5	13.8	38.8
	2	24.8	0.00	61.3	156	81.1	39.5	87.2
	1.18	12.3	0.00	39.7	141	104	35.6	162
	Residue	9.83	0.00	41.1	198	131	41.2	149
PAS 110 [b]	19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	1.78	0.00	0.14	2.31	1.45	0.20	8.40
	6.3	16.1	0.00	7.10	47.8	6.84	5.80	12.4
	4	39.5	0.00	6.90	66.8	9.07	7.80	48.1
	2	19.9	0.00	14.8	109	98.4	10.3	78.4
	1.18	13.0	0.00	21.3	111	101	14.7	96.2
	Residue	9.67	0.00	30.7	125	128	20.8	162

[a] Samples collected in 2011
[b] Samples collected in 2012
[c] Samples collected in 2012, re-analysed in 2013
[*] Heavy metal concentrations (mg/kg dry matter)

Table 4.5 Particle size distribution and total heavy metal content of CLO samples.

ROW	Sieve Size (mm)	Fraction (%)	Total Cadmium[*]	Total Chromium[*]	Total Copper[*]	Total Lead[*]	Total Nickel[*]	Total Zinc[*]
CLO [a]	19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	11.3	0.00	1.03	120	184	10.4	128
	6.3	31.9	0.00	19.9	208	257	38.5	287
	4	11.5	0.00	34.2	202	414	37.1	429
	2	19.3	0.00	79.7	219	391	45.2	874
	1.18	2.12	0.00	97.3	238	459	84.1	1050
	Residue	23.8	0.00	111	294	602	71.3	1290
CLO [b]	19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	10.4	0.00	4.17	187	176	56.6	159
	6.3	29.1	0.00	65.2	256	291	40.5	297
	4	15.3	0.00	70.9	317	395	48.5	477
	2	19.3	0.00	97.8	397	415	90.5	936
	1.18	10.7	0.00	181	458	487	113	1170
	Residue	15.2	0.00	245	519	628	126	1680
CLO [c]	19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	10.8	0.03	3.74	8.91	164	43.2	135
	6.3	29.5	0.05	54.2	206	246	48.9	327
	4	13.8	0.08	65.5	214	369	45.6	591
	2	19.5	0.08	82.9	301	376	80.9	1010
	1.18	10.4	0.11	168	375	462	104	1140
	Residue	16.0	0.16	189	383	594	117	1480

[a] Samples collected in 2011
[b] Samples collected in 2012
[c] Samples collected in 2012, re-analysed in 2013
[*] Heavy metal concentrations (mg/kg dry matter).

4.2   PRELIMINARY GROWTH TRIALS

Preliminary growth trials were conducted for 22 weeks to investigate the growth of three different tree species; *S. viminalis, E. nitens, P. tomentosa* in three different ROWs; PAS 100, PAS 110 and CLO as described in section 3.5.5. An inert material perlite was used as a mixing medium and bulking agent with the ROWs in 1-litre pots. Control pots containing perlite only were watered with 25 % Hoagland nutrient solution (section 3.7.5).

To assess the effect of the ROWs and application rate on each species the percentage change in dry plant biomass was calculated assuming the moisture content had remained constant. Figure 4.2 shows the percentage change in biomass, negative percentage change in dry biomass indicates plant demise during the growth trial. Error bars indicate the standard deviation between replicate trees in each material and application rate. It must be noted that there were some limitations in the collection of plant material as a result of plant demise and natural degradation of root material particularly for those planted in CLO and PAS 100. All plant masses and weekly observational results are presented in Appendix 6 with unavailable data identified.

After 1 week there was evidence of plant wilting and stress within all tree species grown in CLO at both 1500 and 3000 kg N / Ha applications. *P. tomentosa* grown in CLO were dry and crispy by week 3, *E. nitens* had died by week 5 and *S. viminalis* by week 6.

There was evidence of greater variability between replicates in control and PAS 110 trials, with *S. viminalis* having greater range compared to *E. nitens* and *P. tomentosa*. Overall only controls plants and those grown in PAS 110 at both 1500 and 3000 kg N / Ha application rates had positive changes in dry biomass. *S. viminalis* and *E. nitens* grown in PAS 100 (3000 kg N / Ha) and CLO (1500 and 3000 kg N / Ha) had negative percentage change in dry biomass after 22 weeks. *P. tomentosa* grown in CLO had a positive percentage change in dry biomass, with greater biomass change in 1500 kg N / Ha compared to the 3000 kg N / Ha, although there was negative percentage change in dry biomass when grown in PAS 100.

Statistical analysis of the percentage change in dry biomass over the growth trial was assessed using SPSS v 20 (IBM Corporation, 2011). Individual data sets were split by tree species before univariate analysis was conducted to identify the effect of the application rates and ROWs on the biomass production within tree species. The application rates had no significant effect on biomass production for all species ($p > 0.05$), however *P. tomentosa* did have a lower significance value ($p = 0.276$) compared to *E. nitens* and *S. viminalis* $p = 0.557$ and $p = 0.559$ respectively, indicating some difference in application rate for *P. tomentosa* but not of significance levels. The application of any ROW had a significant effect on the biomass production for *E. nitens* and *S. viminalis* ($p < 0.01$) but not for *P. tomentosa* ($p > 0.05$). There was no significant effect from the application rate and ROW on biomass production for any tree species.

To identify the effect of tree species grown in the different ROWs, data sets were split by ROW and univariate analysis conducted. Tree species had a significant effect on the percentage change in dry biomass grown in PAS 100, CLO and the control pots ($p < 0.05$). Biomass production from trees species grown in PAS 110 exceeded the significance level ($p = 0.056$) however there is some evidence of difference between tree species.
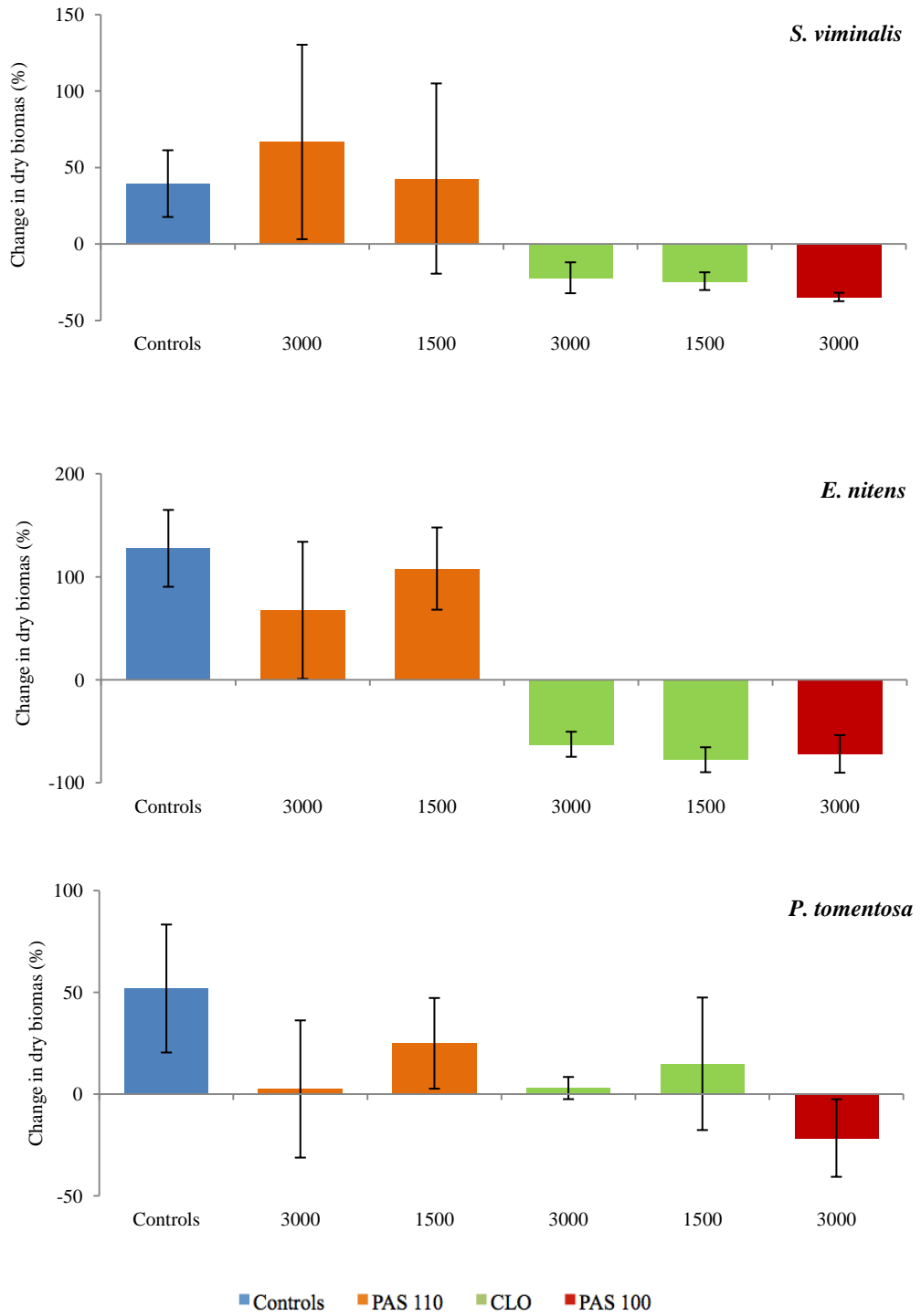
Figure 4.2 Percentage change in dry biomass after 22 weeks of growth.

The heavy metal concentration in to each plant was identified by XRF analysis of dried and ground plant material. Figure 4.3, Figure 4.4 and Figure 4.5 show the heavy metal speciation and accumulation in each tree species root, stem and leaf material after 22 weeks of growth in the ROWs. Values represent the average metal concentration between replicates with one-way standard deviation error bars indicating the variability. Due to natural demise and biological degradation some root material was unattainable in some pots and therefore error bars are not present for those samples where only one sample could be analysed. For example no root material was available for *S. viminalis* PAS 100 pots. Root material from *S. viminalis* and leaf material from *P. tomentosa* were mainly affected and limited data from replicate pots as a result of unavailable sample material restricted the ability to conduct consistent statistical analysis.

Heavy metal accumulation within the plant material varied between species, *S viminalis* root > leaf > stem, *E. nitens*, root > stem = leaf, and *P. tomentosa*, root = stem > leaf.

In general control pots contain lower concentrations of heavy metals compared to the ROWs pots. Heavy metal concentrations were greatest in the CLO pot trials for all species, with *S. viminalis* having the highest uptake and *E. nitens* the lowest. Zinc was present in all material with highest concentrations within root material across all materials and species.

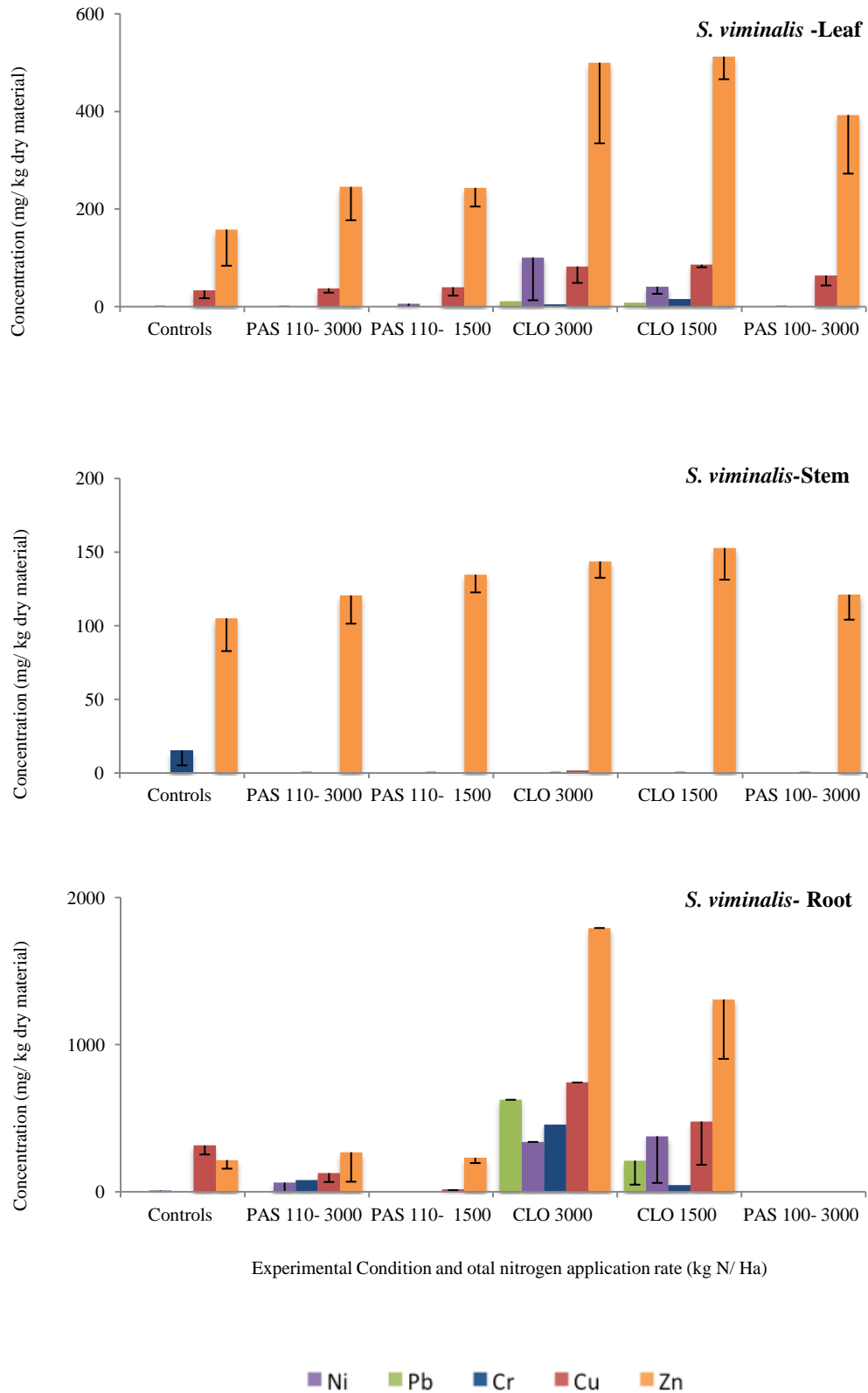Figure 4.3 *S. viminalis,* XRF heavy metal content in leaf, stem and root material. No error bar represents only one sample analysed due to limited material collection.
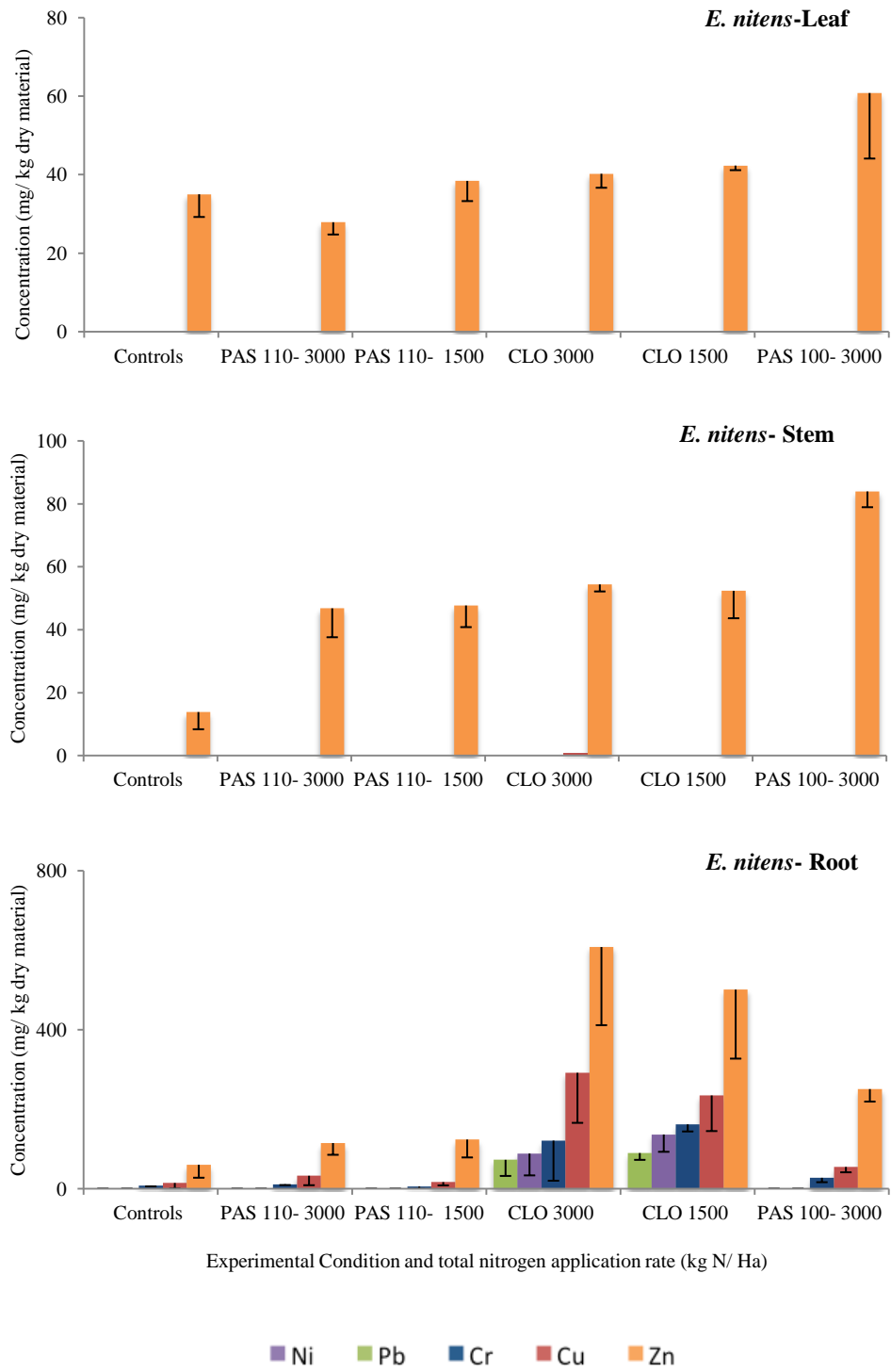
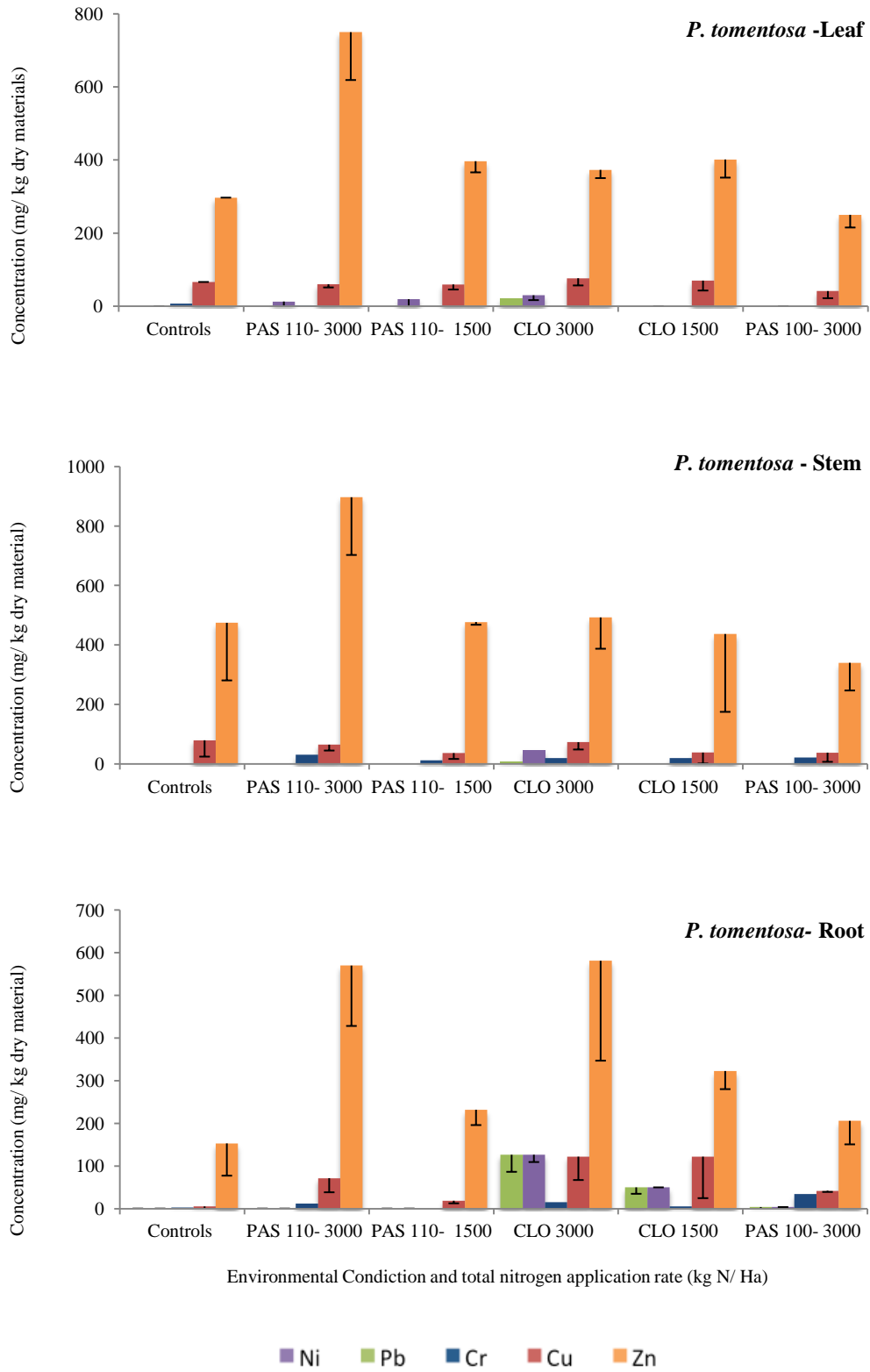Figure 4.4 *E. nitens*, XRF heavy metal content in leaf, stem and root material.

Figure 4.5 *P. tomentosa*, XRF heavy metal content in leaf, stem and root material.

There was evidence of some translocation of metals, particularly zinc, copper, nickel and lead (*E. nitens* zinc only) from the root material to the leaf material. The translocation factor for zinc concentrations from CLO for each tree species is shown in Table 4.6 (Zacchini *et al*., 2009). The average mass of zinc within the leaf and stem material was added together to calculate the concentration of zinc within the arieal plant material for trees grown in CLO only. The translocation factor is the ratio of the aerial concentrations to the root concentrations multiplied by 100. Translocation factor figures indicate higher translocation rates for lower application rates of CLO for all tree species, with greatest translocation of zinc evident in *P tomentosa*.

Table 4.6 CLO zinc translocation factors for all tree species.

CLO (kg N / Ha)	*S. viminalis*	*E. nitens*	*P. tomentosa*
1500	12.22	9.08	133.81
3000	8.32	7.34	76.27

4.3   PLANT IMAGE ANALYSIS

A plant image analysis tool was developed using Matlab (Mathworks, 2011) to allow quick, simple and non- intrusive analysis of plant growth over time. Manual height measurements of each plant were taken weekly along with 3 photos from 3 fixed horizontal positions as described in section 3.7.4. Plant photos were uploaded to the program and estimated heights from the photos were compared to the weekly manual measurements. Figure 4.6 shows the manual heights compared to the estimated heights from the plant image analysis tool. An R^2 value of 0.9989 indicated good correlation between manual and estimated heights for all species. When separated by species, R^2 values were 0.9782, 0.9914 and 0.9971 for *S. viminalis, P.tomentosa* and *E. nitens* respectively. Variability and error in the results were from a number of sources such as human error, variability in plant shape, size and colour as well as simplistic calculations within the plant image analysis program. Human error during manual measurements as well as pale plant material resulting in images being cropped too early, poor light and contrast between plant material and the background were also issues during image upload and analysis.

The plant image analysis program also estimated weekly plant volume although these values could only be assessed after week 22. The volume of each plant was estimated at the end of the growth trial by measuring the displacement of water and comparing the manual measurement to the Plant Image Analysis volume measurement. Figure 4.7 shows the manual volumes measured by the displacement of water against the estimated plant volumes. There was lower correlation between the manual volumes and the estimated volume as shown by the R^2 value of 0.2293. Outlying points included two control *E. nitens* pots with lots of larger leaves that are evident in the pictures. When plotted by species, *S. viminalis* and *P. tomentosa* showed no correlation with R^2 values of less than 0.2. *E. nitens* showed slightly stronger correlation of R^2 value of 0.43 (including control outliers) and R^2 value of 0.66 (excluding control outliers). Due to limitations in the method of measuring the volumes manually, the manual volumes may be underestimated due to plant frictional resistance, which was a result of plant material touching the side of the container and simplistic calculations (assumed cylindrical shape) within the program limiting the correlation.

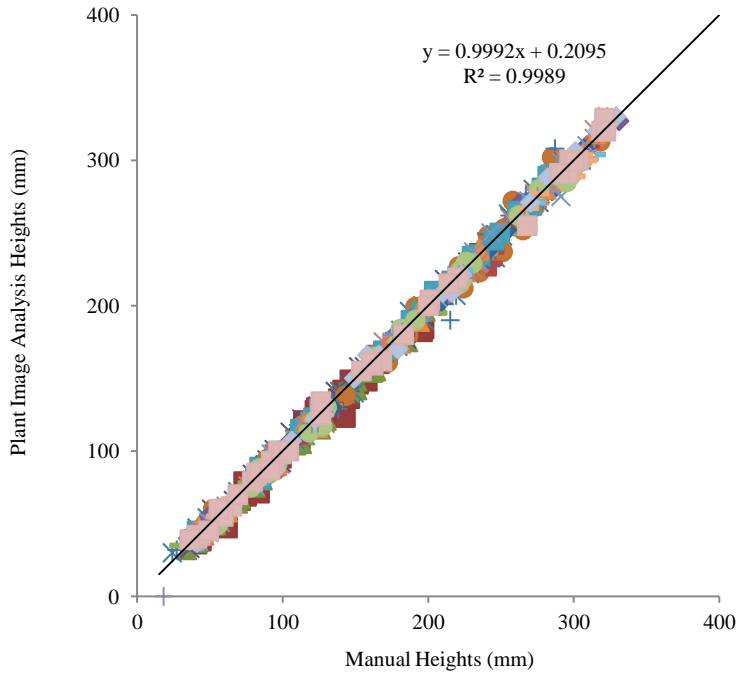$$y = 0.9992x + 0.2095$$
$$R^2 = 0.9989$$

Figure 4.6 Measured and estimated weekly plant heights using Plant Image Analysis tool.
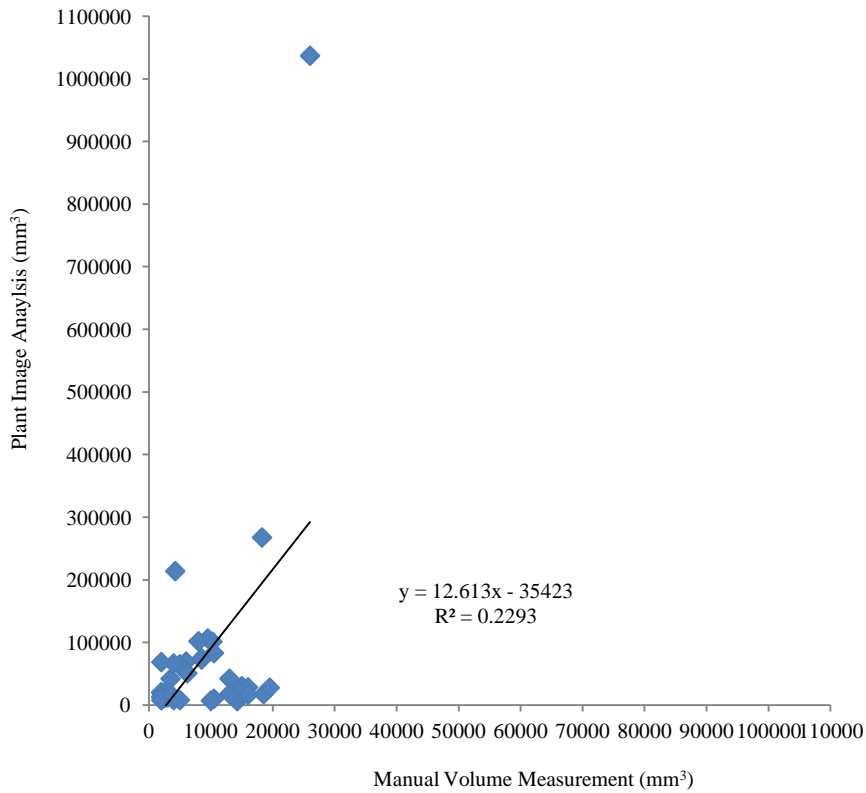

$$y = 12.613x - 35423$$
$$R^2 = 0.2293$$

Figure 4.7 Measured and estimated plant volumes using Plant Image Analysis tool.

4.4 SUMMARY

The aim of this chapter was to characterise different ROWs from source segregated and mixed waste streams and assess the influence of aerobic and anaerobic stabilisation treatments. Three different ROW were investigated; PAS 100 (source segregated, aerobically composted), PAS 110 (source segregation, anaerobically digested) and CLO (mixed waste, aerobically composted).

These materials were characterised to identify the pH, EC, total nitrogen and total carbon content. The total, available and water soluble heavy metal content as well as sequential extraction and batch analysis were also conducted. Source segregated materials had lower heavy metal concentrations compared to CLO materials which exceeded the PAS quality limits. The sequential extraction of each material identified the heavy metal content of different fractions. Zinc was the most available heavy metal in all materials compared to chromium, which was mainly present in the residue fraction. The concentrations of copper were mainly present in the residue fractions for the aerobically composted material, however anaerobically digested material had no copper present in the residue fraction but was bound within the reducible and oxidisable fractions. Particle size distribution and heavy metal analysis within fractions identified highest heavy metal concentrations within the finer particles of less than 2 mm.

Preliminary growth trials were conducted to investigate the ability of three different tree species to grow in all the materials when mixed with an inert medium, perlite. Within the first 6 weeks all trees grown in CLO and PAS 100 suffered wilting and premature necrosis. *S. viminalis* and *E. nitens* grown in PAS 110 survived at both application rates with the percentage change in dry biomass exceeding 40 % after 22 weeks of growth.

A plant image analysis program was developed using Matlab (Mathworks, 2011) by an undergraduate student as part of a CUROP placement. The aim of the Matlab program was to allow quick assessment of plant growth over time. There was good correlation between the Matlab estimated height and the manual height measurements. The program also estimated the plant volume, which was compared to the actual plant volumes in week 22, however correlation was weak.

Chapter 5 IDENTIFICATION OF THE OPTIMUM NITROGEN APPLICATION RATE OF ROW AND THE CHARACTERISATION OF SOIL LEACHATE

This chapter presents the results of the optimum nitrogen application rate growth trials conducted to identify the optimum total nitrogen application rate and the tolerance of *E. nitens* and *S. viminalis* trees to heavy metals and contaminants as described in section 3.7.7.

The aims of the growth trial were to a) identify the optimum application rate of PAS 110 and CLO based on the total nitrogen content for *E. nitens* and *S. viminalis*, b) understand the soluble components of leachate from each ROW and the difference between application rates and species on the leachate, c) investigate the heavy metal uptake by each species and the effect of application rates on metal uptake and biomass production.

Kettering Loam was used as a mixing medium with the ROWs due to its low nitrogen and carbon content and repeatability. Growth trials were conducted as described in section 3.7.7.

Complete data sets can be found in Appendix 6.

5.1 BIOMASS PRODUCTION

The percentage change in dry biomass of *E.nitens* and *S. viminalis* grown in PAS 110 and CLO are shown in Figure 5.1. Double planting for the first 3 weeks ensured that natural demise did not influence the results, however the biomass values (week 3) represent the weaker of the two plants present in each pot.

Negative changes in dry biomass indicates plant demise or substantial loss of leaf material during the growth trial. The percentage change in dry biomass was averaged between the three replicate pot conditions; error bars represent the standard errors between replicates. Comparison of week 3 and week 12 percentage change in dry biomass is limited, as week 3 represents the naturally weaker plant that was removed whereas the stronger plant remained until week 12. Biomass

production in week 12 represents a longer growing period, as well as greater natural tolerance and survival.

E. nitens and *S. viminalis* percentage change in dry biomass were greater in PAS 110 than CLO. The percentage change in dry biomass of *S. viminalis* exceeded biomass production by *E. nitens* in both PAS 110 and CLO at all application rates. Error bars indicate there were some high variability between the replicate pots in both materials and species. The average change in dry biomass of both species in control pots containing Kettering Loam (watered with 25 % Hoagland solution) had similar biomass production to the 3000 kg N / Ha for both ROWs.

After 12 weeks of growth, the average biomass production suggests that the optimum nitrogen application rate of PAS 110 was 1000 kg N / Ha for *E. nitens* and 10,000 kg N / Ha for *S. viminalis*. The average biomass production suggests the optimum nitrogen application rate of CLO for *E. nitens* was 1000 kg N / Ha, however there was a high variability between replicates. *E. nitens* 3000 kg N / Ha showed similar biomass percentage changes to the 1000 kg N / Ha however there was smaller variability between replicates. Similar trends were identified in *S. viminalis* grown in CLO; the highest average change in dry biomass after 12 weeks was 6000 kg N / Ha. Although the 1000 and 3000 kg N / Ha had slightly lower average dry biomasses, the smaller error suggests greater consistency.

Univariate analysis of biomass after 12 weeks growth for PAS 110 and CLO indicated the application rate of either PAS 110 or CLO had no significant effect on biomass production ($p > 0.05$). Tree species had a significant influence on the biomass production, $p = 0.004$ (PAS 110) and $p \leq 0.0001$ (CLO). To identify the effect of ROW on each tree species biomass after 12 weeks of growth, the data set was split by tree species and univariate analysis conducted. For each species, there were no significant different on biomass production between PAS 110 and CLO pot trials suggesting the higher heavy metal content in CLO may not have influenced the biomass production significantly. The fairly high variably between 3 replicates may also have influenced the results and statistical significance of the results.
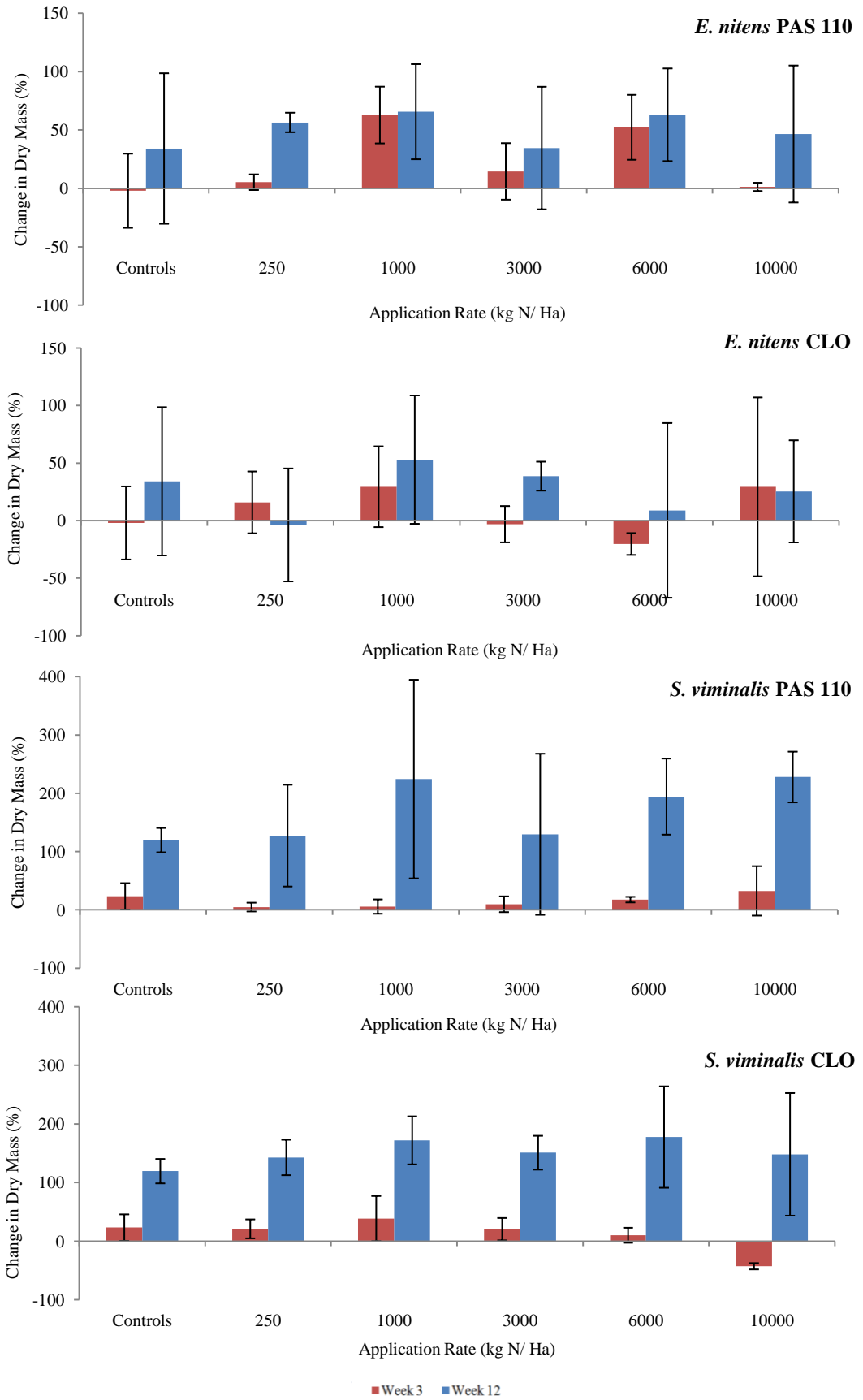
Figure 5.1 Percentage change in dry biomass for *E. nitens* and *S. viminalis* grown in PAS 110 and CLO at different total nitrogen application rates.

5.2 LEACHATE

Leachate samples were collected from each pot in order to identify the soluble components of the ROWs, assess the potential risk to the wider environment with increasing application rates, as well as identify the influence of plants on the concentration of heavy metals. The pH, EC and metal cation analyses were conducted weekly, with fortnightly analysis of soluble anions and ammonium concentrations (section 3.3).

The leachate values represent the average of the replicates; standard errors between experimental conditions are presented with the complete data sets available in Appendix 6.

5.2.1 PH AND EC

Figure 5.2 shows the average weekly pH values for *E. nitens* and *S. viminalis* grown in PAS 110 and CLO at different total nitrogen application rates. Leachate pH values were between 6.5 and 9 for all application rates and both species. The higher pH values are generally associated with the higher application rates for both PAS 110 and CLO. *E. nitens* pH values have greater variability over time for both ROWs compared to *S. viminalis* trials, particularly noticeable in control pots and 250 kg N / Ha application rates dipping in week 3 and peaking in week 5. The changes in pH may be linked to the plant health and the plant root interaction with the pore water around the rhizopshere and processes such as ROL (Hillel, 2008; Yang *et al*., 2014). There were general increases in pH values from week 0 to week 8 and 9 before reaching a plateau with only slight changes evident. *S. viminalis* also showed gradual increases with less variability in weekly values. Standard error values for pH over the 12 weeks ranged between 0.132 - 0.394 for PAS 110, 0.098 - 0.220 for CLO and between 0.183 - 0.248 for control pots (25 % Hoagland solution).
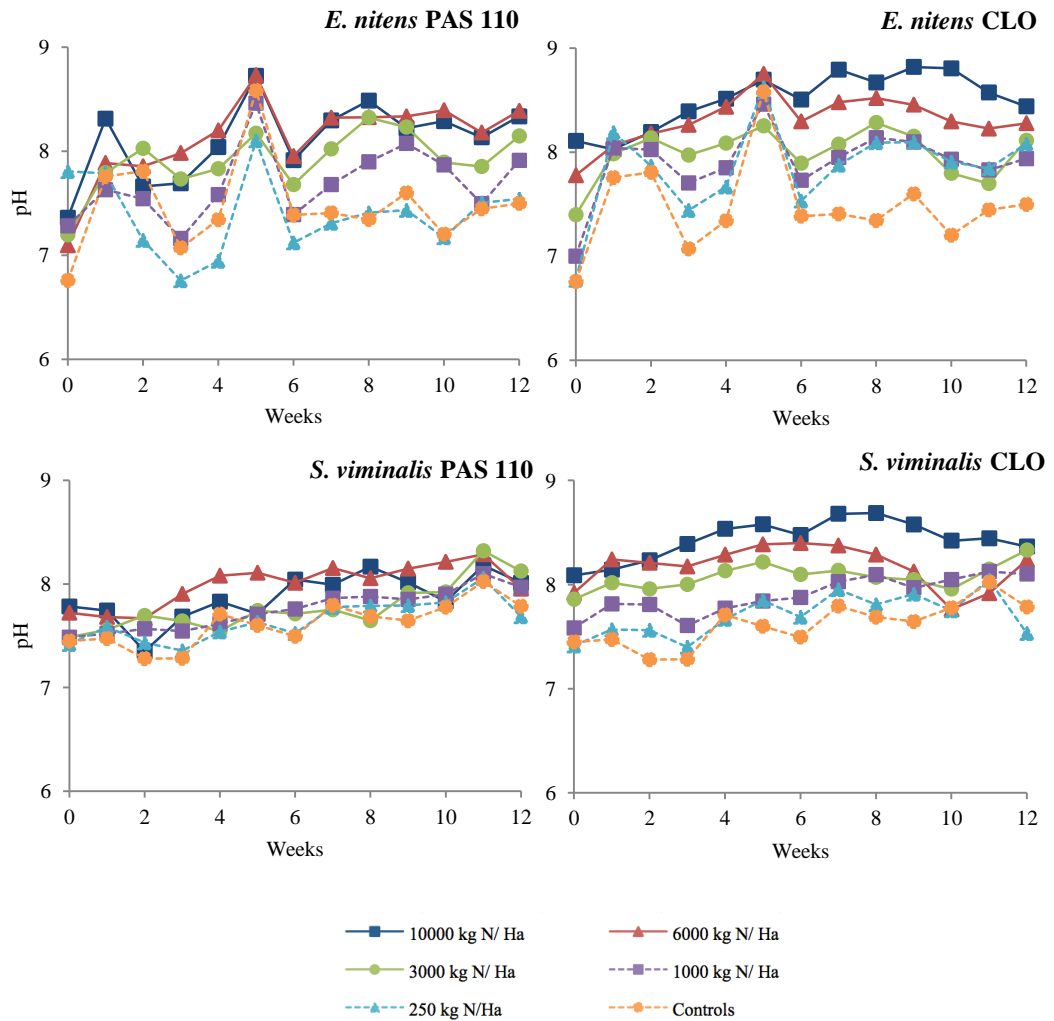
Figure 5.2 Average weekly pH values for *E. nitens* and *S. viminalis* grown in PAS 110 and CLO at different total nitrogen application rates.

Figure 5.3 shows the weekly average electrical conductivity of leachate samples collected from growth trials. Leachate samples from PAS 110 initially had lower EC levels than CLO due to the source segregated waste stream. Higher application rates generally had higher EC results, particularly evident in the CLO trials due to the increased ratio of ROW to Kettering Loam. There were gradual decreases in EC values for all experimental conditions over the 12 weeks. Exceptions to those trends were *E. nitens*; PAS 110 for application rates 250 and 1000 kg N / Ha peaking in week 5 and week 8. A rapid increase in EC values were also evident for *S. viminalis* grown in CLO at application rates of greater than 3000 kg N / Ha between week 10 and 12. Standard error values for EC over the 12 weeks ranged between 0.078 - 0.772 mS / cm for PAS 110, 0.175 - 0.499 mS / cm for CLO and between 0.144 -

0.179 mS / cm for control pots (25 % Hoagland solution). Higher standard errors between replicate pots were evident at the higher application rates for both tree species and ROWs. There were rapid increases in the EC values for CLO pots ($\geq$ 3000 kg N / Ha) between weeks 10 and 12, from the replicate pots there were standard errors ranging between 0.063 and 0.334 mS / cm, with the errors increasing with application rate.
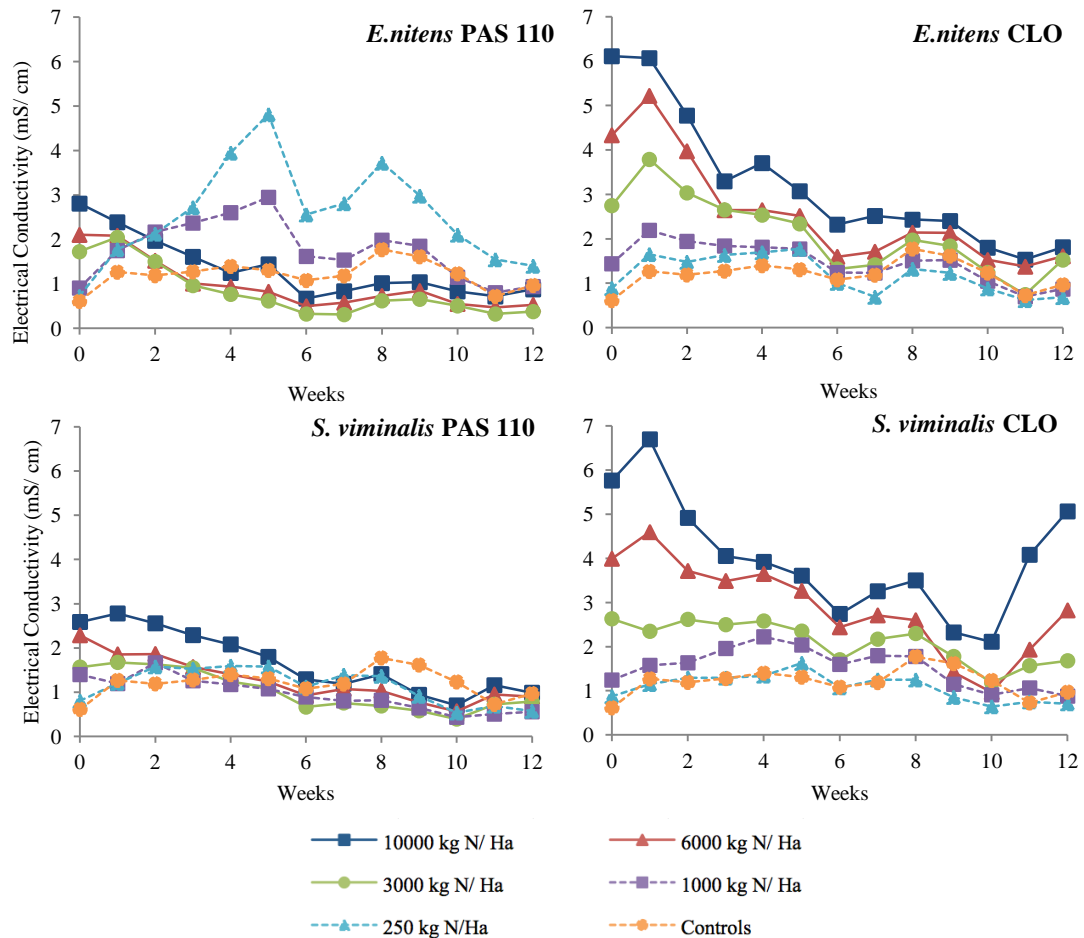
Figure 5.3 Weekly average electrical conductivity for *E. nitens* and *S. viminalis* grown in PAS 110 and CLO.

5.2.2   SOLUBLE METAL CATIONS

Leachate samples were analysed to assess the potential risk of metal leaching to the wider environment. The heavy metals considered under the PAS standard were measured in the leachate samples and the heavy metal concentrations were compared to water quality concentration limits for aquatic life (Environment

Agency, 2013). Other soluble cations analysed included calcium, sodium, magnesium and aluminium to assess changes within the soil and pore water over time. Cadmium was below detection limits in all leachate samples taken from all application rates of PAS 110 and CLO.

Chromium, copper, nickel, lead and zinc water quality concentration levels are dependent upon water hardness (between 0 to > 250 mg $CaCO_3$ / l) and the heavy metal concentration ranges are shown in Table 5.1 (Environment Agency, 2013). Horizontal lines in the heavy metal leachate results (Figure 5.4, 5.5, 5.7 and 5.8) indicate the minimum water quality limits with calcium carbonate concentrations between 0 - 50 mg / l. The maximum water quality limits are only presented in the leachate results when required and with suitable concentration scales.

Table 5.1 Environment Agency (2013) water quality concentration ranges dependent upon water hardness (calcium carbonate concentrations)

Calcium carbonate (mg / l)	Cr (mg / l) a	b	Cu (mg / l) c	Pb (mg / l) a	b	Ni (mg / l) c	Zn (mg / l) a	b
0 - 50	0.005	0.15	0.005	0.004	0.05	0.05	0.03	0.3
50 - 100	0.01	0.175	0.022	0.01	0.125	0.1	0.2	0.7
100 - 150	0.02	0.2	0.04	0.01	0.125	0.15	0.3	1.0
150 - 200	0.02	0.2	0.04	0.02	0.25	0.15	0.3	1.0
200 - 250	0.05	0.25	0.04	0.02	0.25	0.2	0.3	1.0
250 +	0.05	0.25	0.112	0.02	0.25	0.2	0.5	2.0

[a] *Protection of sensitive freshwater aquatic life such as salmonid fish (Environment Agency, 2013).*
[b] *Protection of other freshwater aquatic life such as cyprinid fish (Environment Agency, 2013).*
[c] *Protection of all types of freshwater aquatic life (Environment Agency, 2013).*

Figure 5.4 and Figure 5.5 shows the average weekly PAS 110 leachate concentrations for heavy metals for both tree species. Average concentrations of chromium and nickel for all application rates of PAS 110 were below the minimum water quality concentration limits (horizontal lines). The concentrations of copper, lead and zinc were within the water quality concentration ranges (Table 5.1), however the calcium carbonate concentrations were not investigated therefore the exact heavy metal concentrations limits within water quality ranges are unknown.

Chromium and nickel concentrations remained below the minimum water quality limits for the duration of the growth trial, with similar peaking patterns in week 7 shown within the *E. nitens* and *S.viminalis*. The overall concentrations of copper in

E. nitens pots exceed *S. viminalis*. Concentrations of copper exceeded the minimum water quality limits at least once during the growth trials from *E. nitens* (250, 1000, 6000 and 10,000 kg N / Ha) and *S. viminalis* (250 kg N / Ha) pots. The copper concentrations are generally less than 0.04mg/l which is near the ICP-OES detaction limits (Appendix 6) therefore peaks evident from the 250 kg N/Ha pots may be low level detection errors.

Lead concentrations in leachate from *S. viminalis* pots were very low, with slight peaks all below the minimum water quality limit and although *E. nitens* concentrations were generally below the minimum water quality limit, peaks in weeks 7 and 8 were evident (Control and 6000 kg N / Ha pots). As seen with copper, the detection limits of the ICP-OES for lead was 0.005 mg/l therefore peaks evident in weeks 7 and 8 may be due to low level detection errors.

Soluble concentrations of zinc showed a general decrease in both species and all application rates. Higher zinc leaching was associated with 250 kg N / Ha application rate in both species. Zinc concentrations peaked in week 5 for *S. viminalis* trials (10,000 kg N / Ha) whereas zinc concentrations peaked in week 7 for *E. nitens* trials (6000 and 10,000 kg N / Ha). Zinc concentrations in leachate varied in weeks 11 and 12 from *E. nitens* pots at all application rates excluding 3000 kg N / Ha. Heavy metal standard error values from PAS 110 leachate concentrations were 0.0002 mg / l for Cr, Ni, Pb and Zn and 0.0032 mg / l for Cu. Figure 5.6 shows the average weekly concentrations of calcium, sodium and magnesium in leachate collected from PAS 110 trials. Calcium concentrations in leachate gradually decreased over the course of the growth trials for *S. viminalis* trials. *E. nitens* pots showed a gradual decrease of calcium concentrations in leachate from higher application rates ($\geq$ 3000 kg N / Ha), however lower application rates (<3000kg N / Ha) showed increases during the course of the growth trials, with calcium concentrations peaking in weeks 5 and 8. Similar trends were evident in the magnesium concentrations in leachate from *E. nitens* pots, with peaks in week 5 and 8 for lower application rates (1000 and 250 kg N / Ha). There were slight increases in magnesium concentrations in leachate from *S. viminalis* trials in weeks 2, 8 and between week 10 and 12. *E. nitens* and *S. viminalis* showed similar decreasing trends of sodium concentrations in all application rates, with

higher concentrations associated with higher total nitrogen application rates and gradual decreases in concentrations during the course of the growth trial.

Figure 5.4 PAS 110 average weekly leachate concentrations. The dotted lines represent water quality concentration limits dependent on the calcium carbonate concentration ranges for chromium (0.005-0.25 mg / l), copper (0.005 - 0.112 mg / l) and nickel (0.05 - 0.2 mg / l) as seen in table 5.1.

Figure 5.5 PAS 110 average weekly leachate concentrations. The dotted lines represent water quality concentration limits dependent on the calcium carbonate concentration ranges for lead (0.004 - 0.25 mg / l) and zinc (0.03 - 2.0 mg / l) as seen in table 5.1.
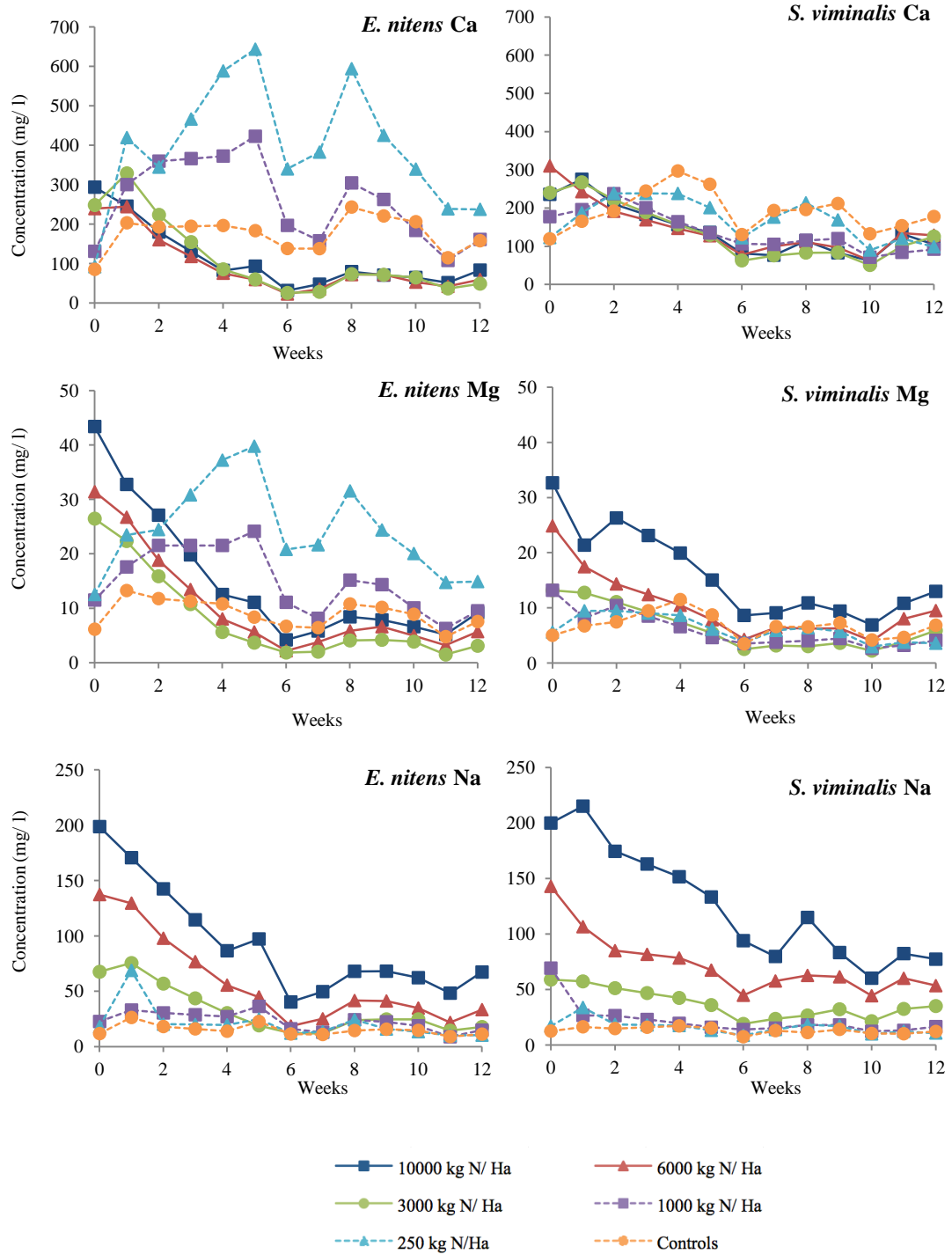
Figure 5.6 PAS 110 averaged weekly leachate concentrations for calcium, magnesium and sodium from the different total nitrogen application rate pot trails.

Figure 5.7 and Figure 5.8 show the averaged weekly concentrations of soluble heavy metals for *E. nitens* and *S. viminalis* grown in all application rates of CLO.

Metal cation concentrations leached from CLO trials were greater than the concentrations leached from the PAS 110 pots, with metal concentrations within or exceeding the water quality concentration limits for application rates of ≥ 3000 kg

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N / Ha. Concentrations of chromium, lead and zinc fell within the water hardness quality limits for both species. At application rates equivalent to 3000 and 6000 kg N/Ha for copper and 10,000 kg N/ Ha for nickel concentrations initially exceeded the maximum limit decreasing within the first 2 weeks before falling within water hardness ranges. Pot trials with total nitrogen application rates equivalent to 10,000 kg N / Ha for both tree species exceeded the copper water quality limits for the duration of the growth trial.

Metal cation concentrations peaked in week 1 (excluding copper and zinc) for both *S. viminalis* and *E. nitens*. There were overall decreases with only slight variations between week 2 and 11 in both species. Metal cation concentrations increased in week 12 in *S.viminalis* trials at application rates of ≥ 3,000 kg N / Ha and a slight increase was evident for copper concentrations from *E. nitens* 10,000 kg N / Ha trials.

Standard error values for PAS 110 and CLO trials ranged between 0.0001 - 0.001 mg / l for chromium, 0.001 - 0.047 mg / l for nickel, 0.001 - 0.16 mg / l for copper, 0.001 - 0.07 mg / l for lead and 0.0005 - 0.26 mg / l for zinc. Higher standard errors were associated with CLO trials particularly at higher application rates (≥ 6000 kg N / Ha) within the first 4 weeks.

The averaged concentrations of calcium, magnesium and sodium from CLO trials are shown in Figure 5.9. Calcium concentrations were similar for all conditions, with lower application rates generally associated with higher concentrations of calcium. There were increases in calcium leaching from week 10 to week 12, particularly *S. viminalis* trials applied with 6000 and 10,000 kg N / Ha.
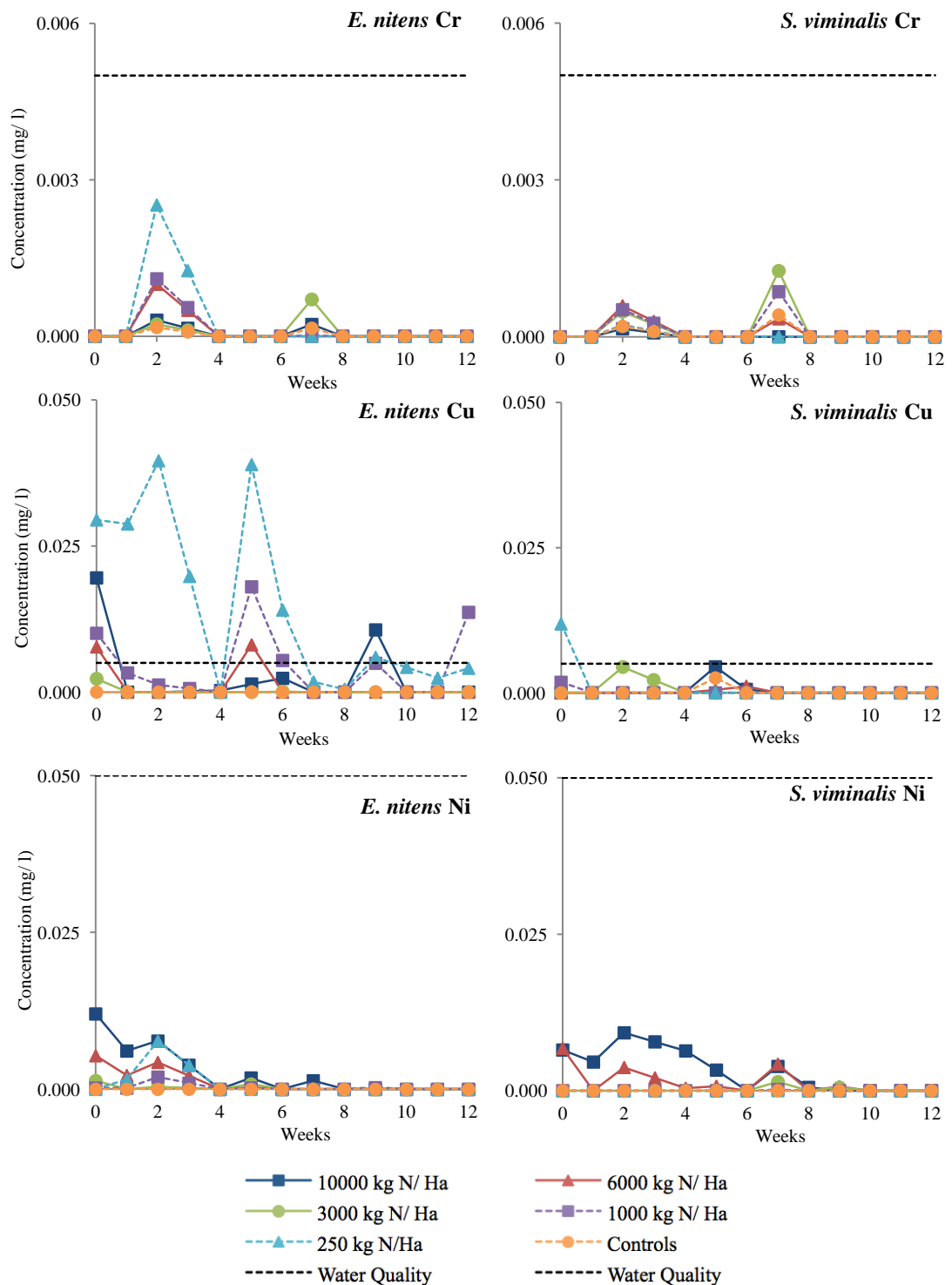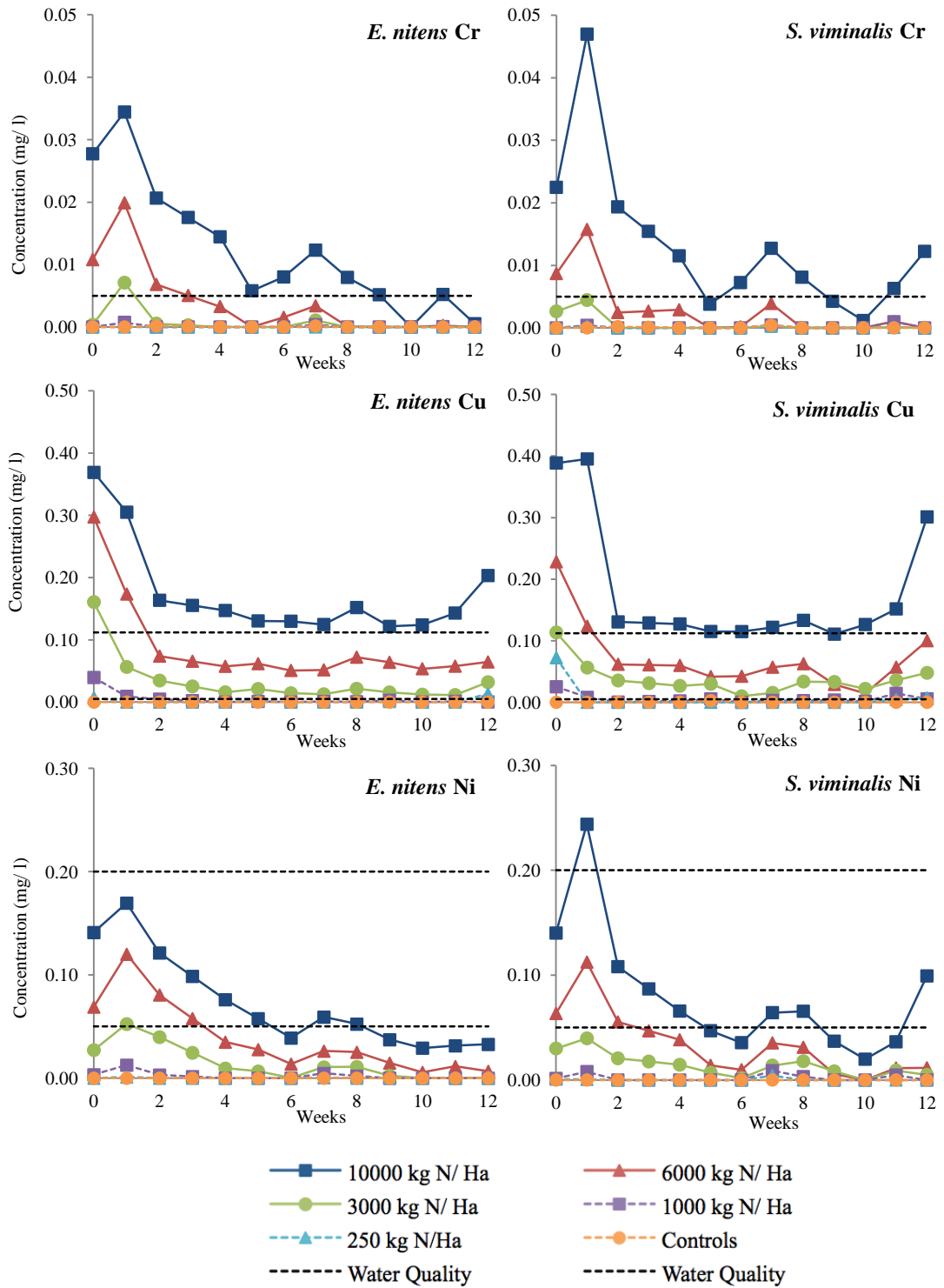
Figure 5.7 CLO average weekly leachate concentrations. The dotted lines represent water quality concentration limits dependent on the calcium carbonate concentration ranges for chromium (0.005 - 0.25 mg / l), copper (0.005 - 0.112 mg / l) and nickel (0.05 - 0.2 mg / l) as seen in table 5.1.
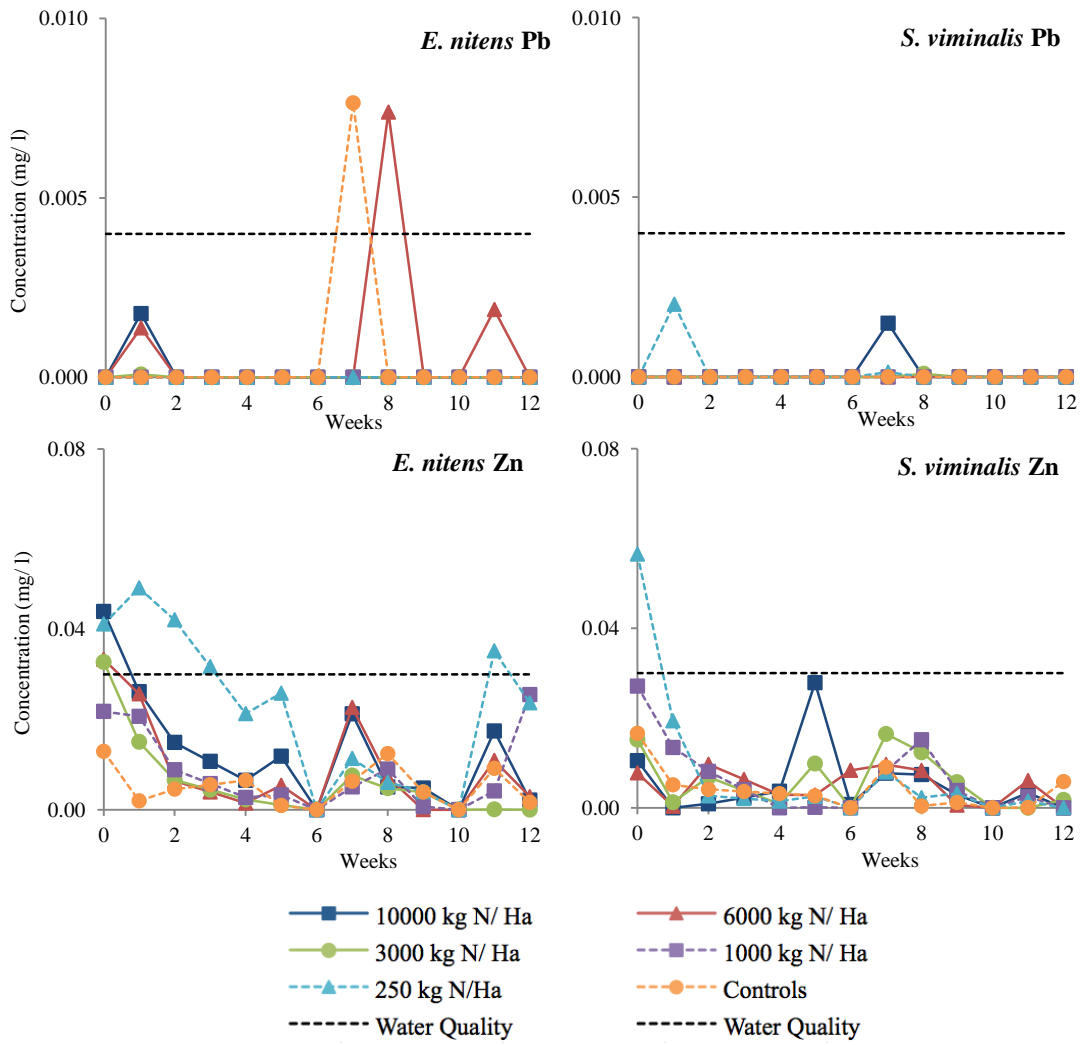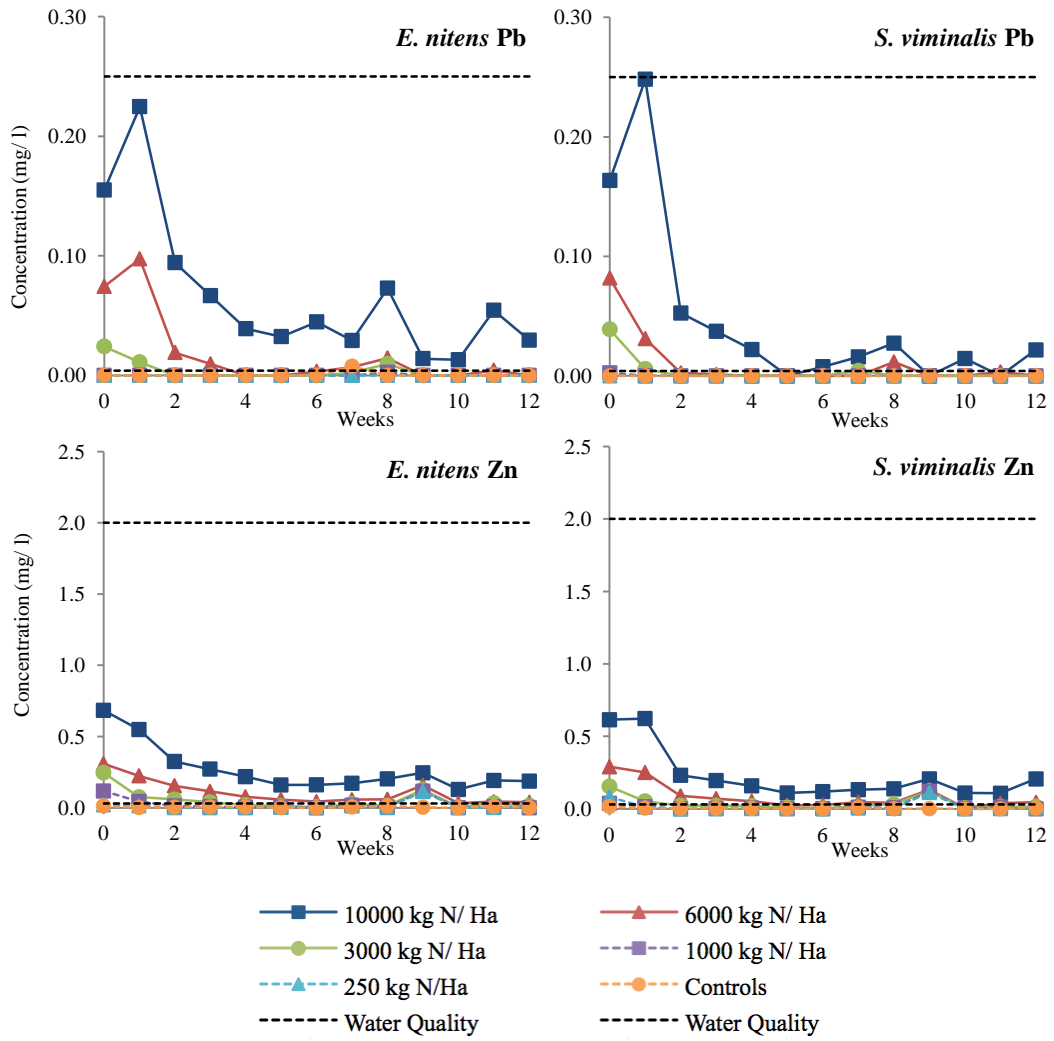
Figure 5.8 CLO average weekly leachate concentration. The dotted lines represent water quality concentration limits dependent on the calcium carbonate concentration ranges for lead (0.004 - 0.25 mg / l) and zinc (0.03 - 2.0 mg / l) as seen in table 5.1.
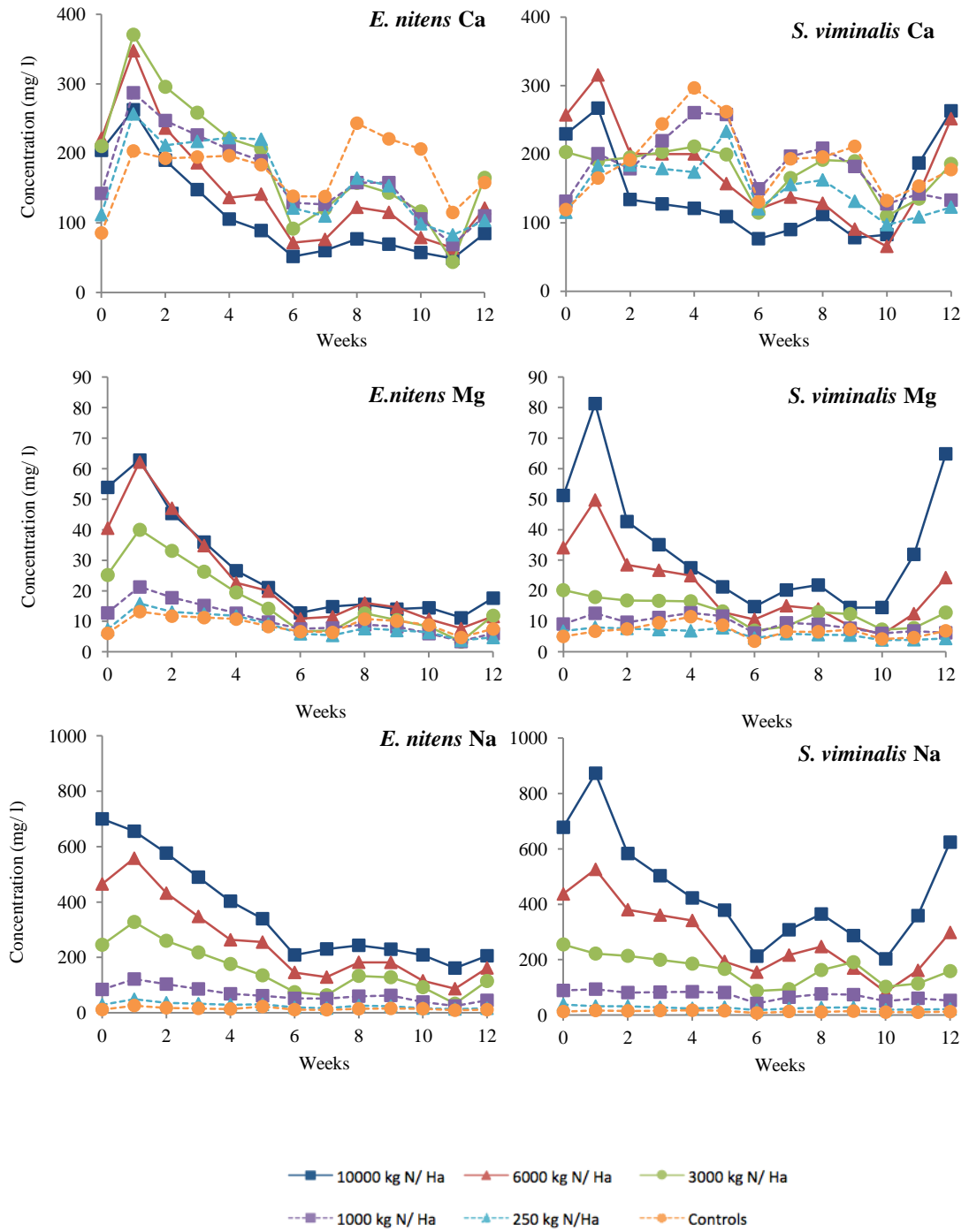
Figure 5.9 CLO averaged weekly leachate concentrations for calcium, magnesium and sodium from the different total nitrogen application rate pot trails.

5.2.3 SOLUBLE ANIONS

Leachate collected fortnightly was analysed using IC to identify the concentration of soluble anions; chloride, sulfate, nitrite and nitrate (section 3.3.9). Nitrite was undetectable in all samples.

Figure 5.10 shows the averaged soluble anion concentrations from PAS 110 trials for both *E. nitens* and *S. viminalis*. Higher concentrations of chloride and sulfate were generally associated with higher application rates and generally decreased between week 0 and week 12 in both species. Nitrate leaching showed higher concentrations were associated with application rates of ≤ 1000 kg N / Ha, with slightly higher concentrations leached from *E. nitens* pots. Application rates of ≥ 3000 kg N / Ha had lower nitrate concentrations in both species that decreased within the first 4 weeks before reaching steady leaching concentrations of approximately 10 mg / l.
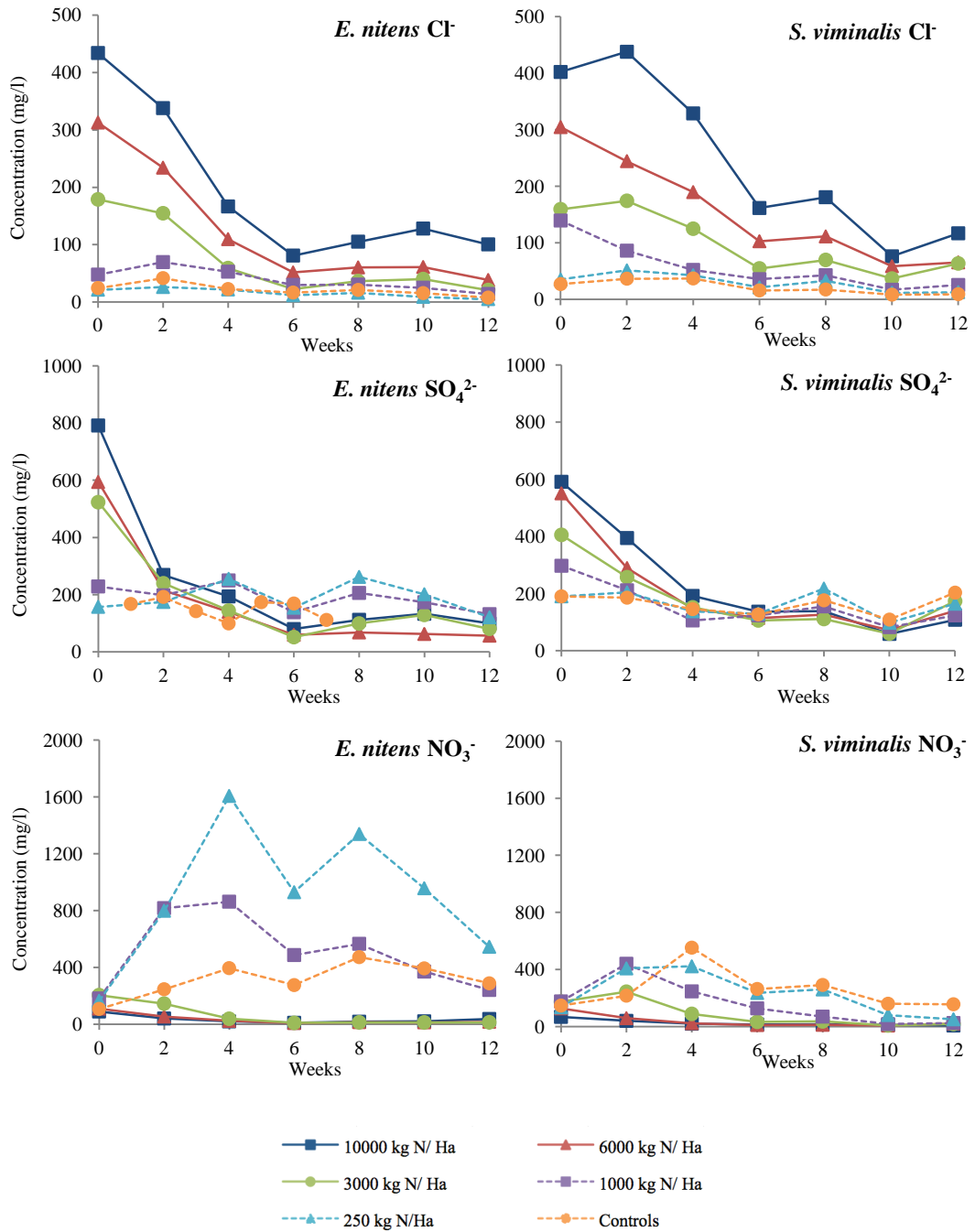
Figure 5.10 PAS 110 averaged fortnightly leachate concentrations for chloride, sulfate and nitrate from the different total nitrogen application rate pot trails.

The average fortnightly soluble anions from CLO pot trials are presented in Figure 5.11. Concentrations of chloride and sulfate in CLO exceed concentrations leached from PAS 110 pot trials in both species. Higher concentrations of chloride and sulfate were generally associated with higher application rates. Chloride concentrations gradually decreased for the duration of the growth trial from *E.*

nitens trials, however *S. viminalis* pots trials showed a gradual decrease until week 6 before peaks were evident in weeks 8 and 12 ($\geq$ 3000 kg N / Ha).

Sulfate concentrations leached from CLO pots showed a rapid decrease in concentrations in the first 2 weeks. *E. nitens* trials showed a slight increase in sulfate concentrations in weeks 4 and 8 for application rate 3000 kg N / Ha. *S. viminalis* however showed increases in sulfate concentrations in week 12 at application rates of 3000 kg N / Ha and above.

The standard errors in the PAS 110 and CLO samples ranged between 0.36 – 197 mg / l for chloride, 3.6 - 262 mg / l for sulfate and 0.31 - 571 mg / l for nitrate.

Concentrations of nitrate within leachate were lower than those measured in PAS 110 leachate, although similar trends are evident. There were negative correlation between nitrate concentrations and total nitrogen application rate for both *E. nitens* and *S. viminalis*. Pot trials with application rates of less than 6000 kg N / Ha showed peaks in nitrate concentrations for both species in week 4 and 8. The higher application rates (6000 and 10,000 kg N / Ha) had fairly steady nitrate concentrations between weeks 0 and 10 for both species. In week 12 there were slight increases in nitrate concentrations from *E. nitens* pot trials and a rapid increase from *S. viminalis* pots with application rates $\geq$ 3000 kg N / Ha.
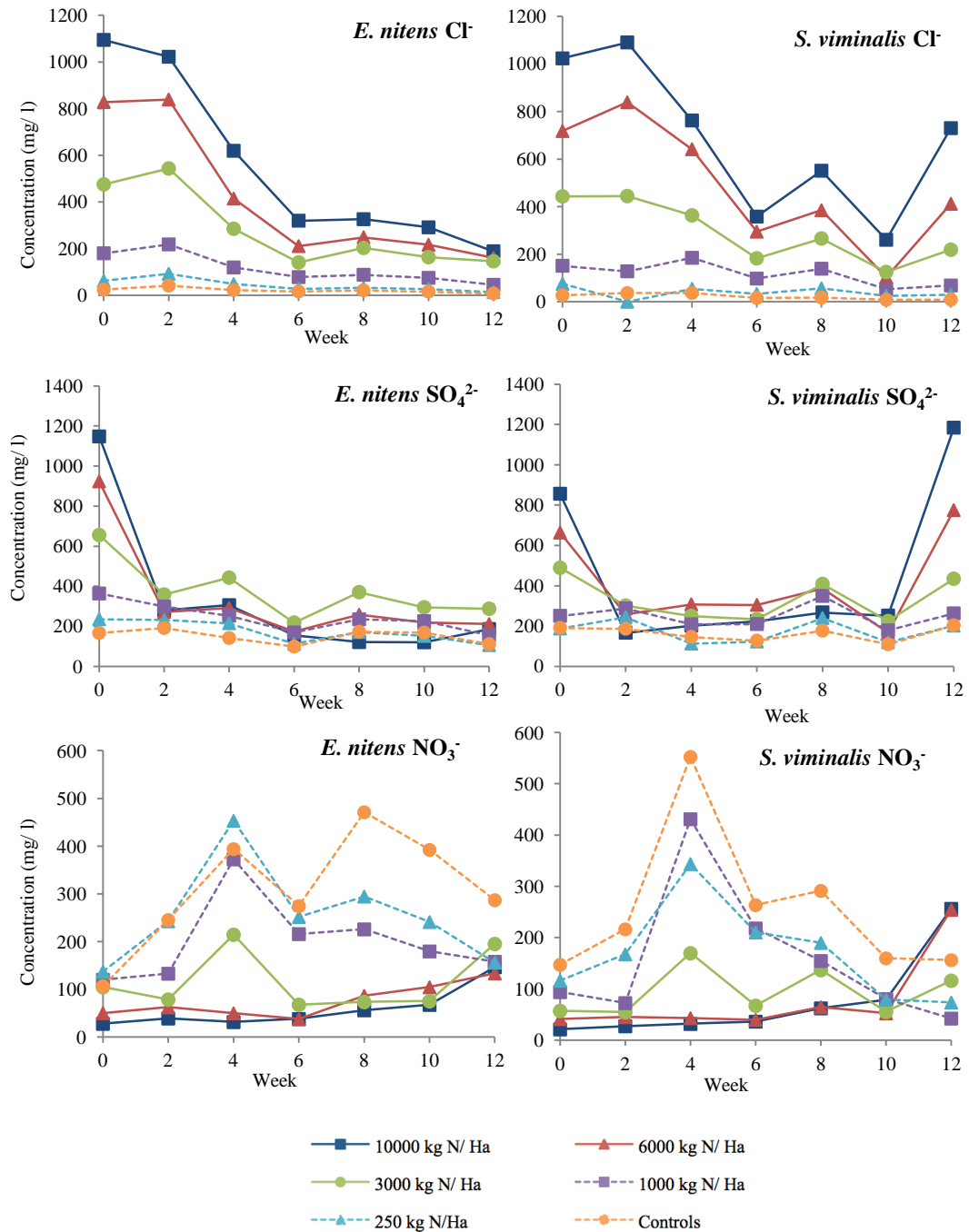
Figure 5.11 CLO averaged fortnightly leachate concentrations for chloride, sulfate and nitrate from the different total nitrogen application rate pot trails.

5.2.4 AMMONIUM

The ammonium content of leachate samples were analysed fortnightly to assess ammonium release from the different ROWs and the influence of different application rates on the concentrations. Colourmetric analyses of samples were compared to calibration curves using known standards (section 3.3.10).

Figure 5.12 shows the averaged fortnightly ammonium concentrations (log) for both *E. nitens* and *S. viminalis* grown in PAS 110 and CLO at different total nitrogen application rates.
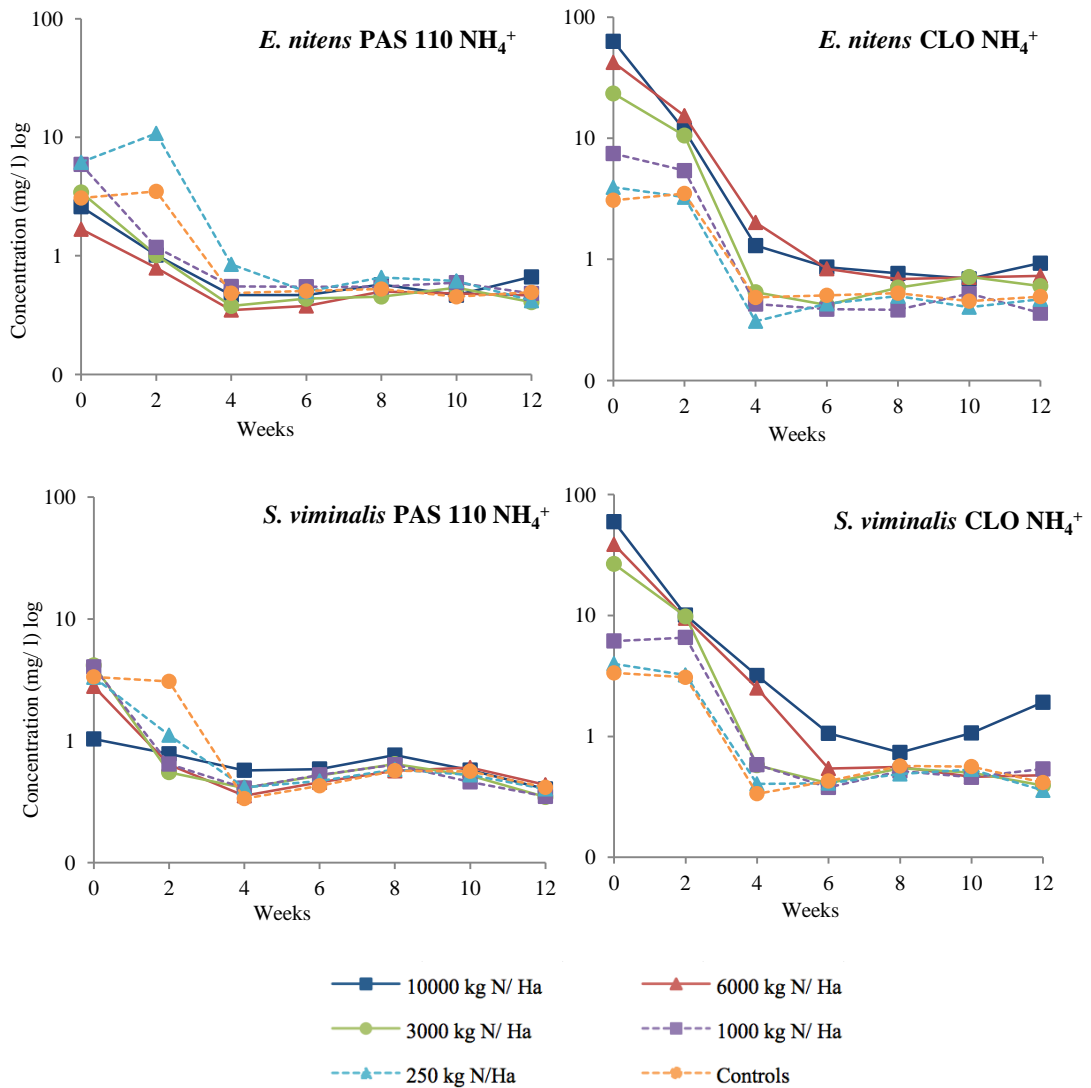
Figure 5.12 Average Fortnightly ammonium (log) analysis for PAS 110 and CLO from the different total nitrogen application rate pot trials.

Ammonium analysis identified higher leachable concentrations in CLO trials compared to PAS 110. From CLO trials, higher ammonium concentrations were associated with higher application rates, however within the PAS 110 trials there was little difference in the ammonium leaching between application rates excluding peaks in week 2 from control and 250 kg N / Ha application rates. Standard error between replicates ranged between 0.007 - 14.5 mg / l for both CLO and PAS 110. Similar trends were identified between species grown in each ROW at each application rate. Overall decreases were evident in both PAS 110 and CLO at all application rates; with a slight increase in week 12 for *S. viminalis* grown in CLO at an application rate equivalent to 10,000 kg N / Ha.

5.3   HEAVY METAL PHYTOREMEDIATION

E. nitens and *S. viminalis* grown in PAS 110 and CLO at different application rates were analysed using XRF analysis to assess the heavy metal concentrations within the trees roots, stems and leaf material after 12 weeks growth.   The total concentrations of cadmium, chromium, copper, nickel, lead and zinc found in the roots, stem and leaves for both tree species grown in PAS 110 and CLO are shown in . *E. nitens* and *S. viminalis* were also analysed on day 0 to identify background heavy metal concentrations, which identified some natural accumulation pre-growth trial in both species. Error bars represent one-way standard error between replicate pots for the total heavy metal accumulation in either the root, stem or leaf material. Larger error bars were generally associated with the root material, where highest concentrations of heavy metals were identified in both materials and tree species. The overall heavy metal uptake was lower in the PAS 110 compared to CLO for both species as a result of the different waste streams. Total heavy metal uptake by *S. viminalis* was greater than *E. nitens* in both PAS 110 and CLO.

The total heavy meal uptake in both tree species grown in PAS 110 varied between application rates; whereas there was clear correlation between application rate and total heavy metal uptake for both species grown in CLO. There was a significant different between the total heavy metal concentration of trees grown in PAS 110 compared to CLO for 12 weeks ($p < 0.0001$). Univariate analysis of total heavy metal uptake for PAS 110 indicated the application rate of PAS 110 had no significant effect on total heavy metal uptake ($p > 0.05$) whereas the tree species

had a significant influence on the total heavy metal uptake, $p < 0.0001$. The application rate of CLO and tree species had significant effect on the total heavy metal uptake into the plant material ($p < 0.0001$).
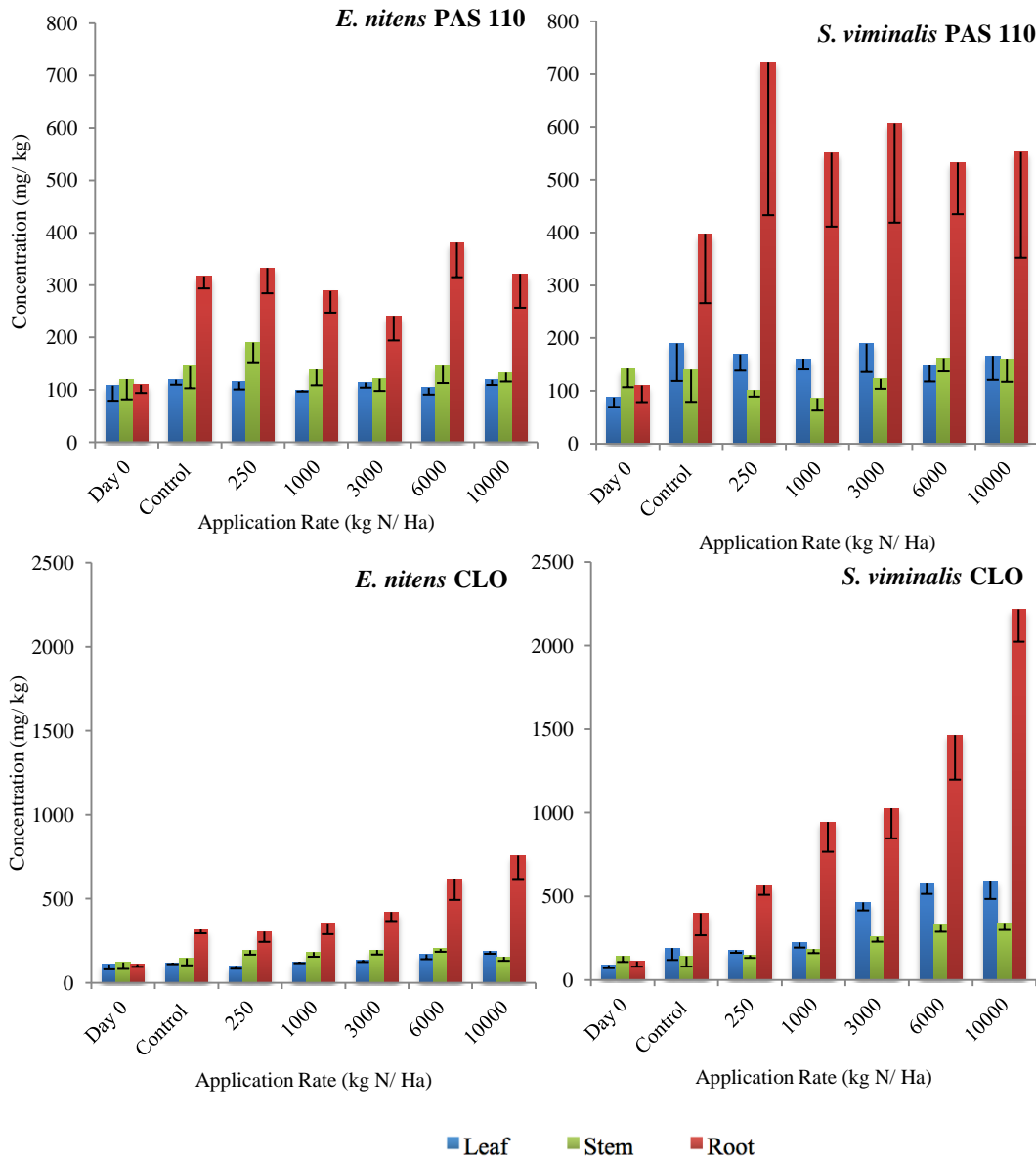
Figure 5.13 Total heavy metal (sum of the mean concentrations Cd, Cr, Cu, Pb, Ni and Zn) of accumulation in root, stem and leaf material.

Figure 5.14 and Figure 5.15 show the heavy metals speciation within the root, stem and leaf material between species grown in PAS 110 and CLO. The heavy metal concentrations within root material were greatest across all experimental conditions. *S. viminalis* had higher concentrations within the leaf material compared to stems in both PAS 110 and CLO, whereas *E. nitens* had similar concentrations within leaf and stem material for both ROWs.

E. nitens and *S. viminalis* grown in PAS 110 accumulated chromium, copper and zinc within the roots and stem material compared to lower concentrations of copper and zinc within the leaf material. There was variability between the application rates of PAS 110 and the concentrations of heavy metals from all material.

Higher concentrations of heavy metals were identified in both *E. nitens* and *S. viminalis* grown in CLO compared to PAS 110. Chromium, copper, zinc and lead were identified within root material for both species grown in CLO, however lead was not accumulated within the stem and leaf material and there were lower concentrations of chromium, copper and zinc.
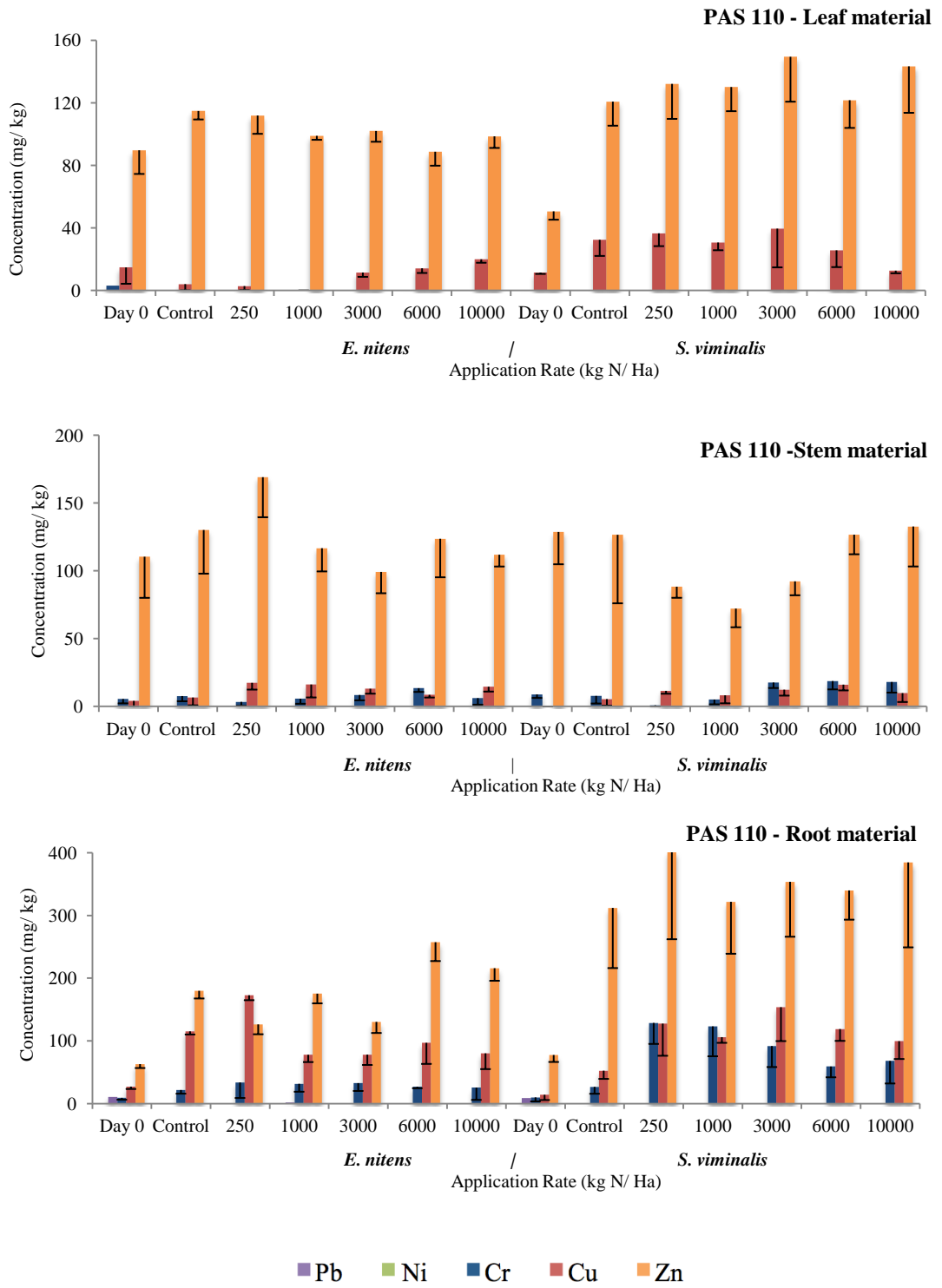
Figure 5.14 Heavy metal speciation within plant root, stem and leaf material grown in PAS 110.
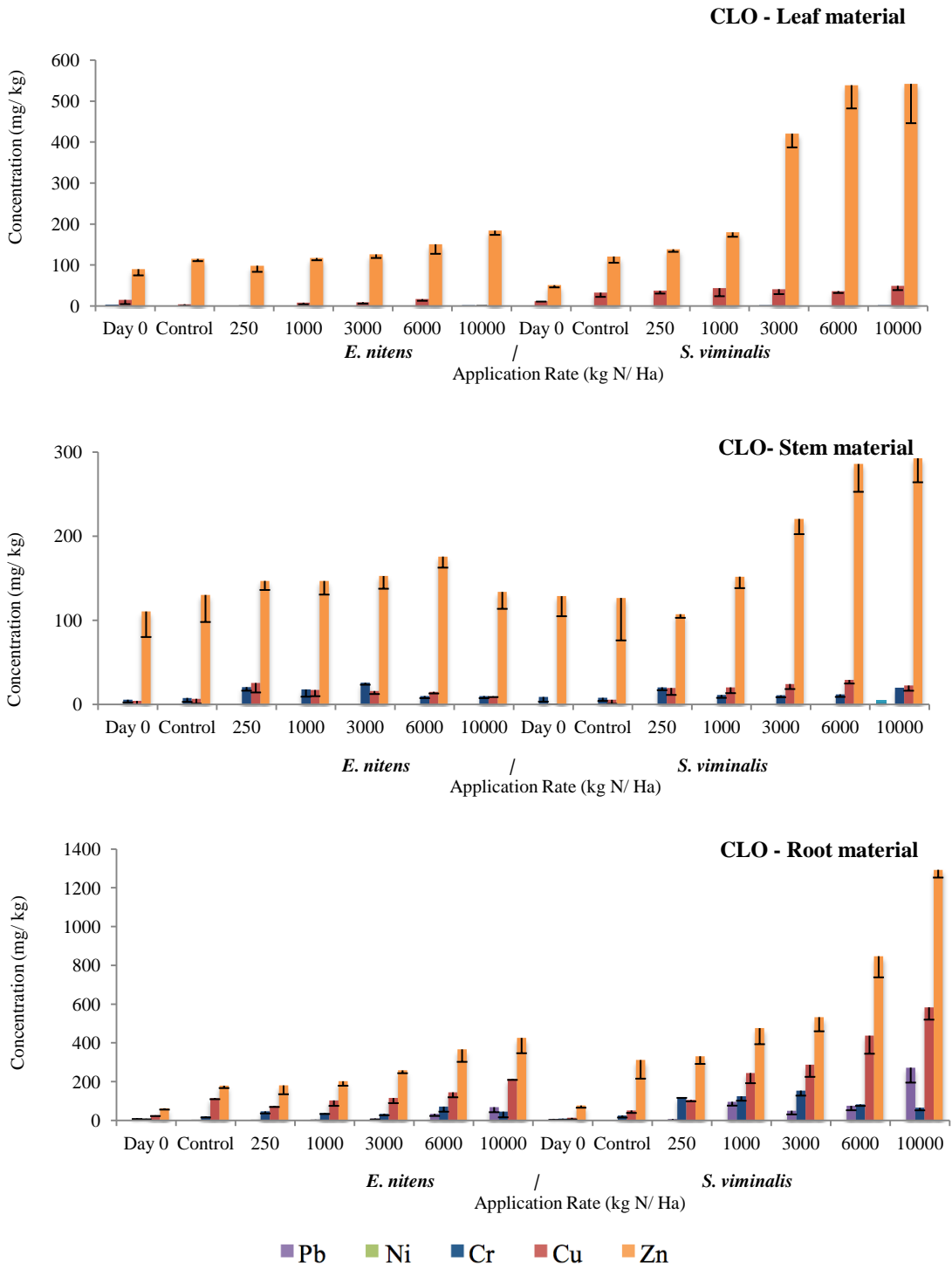
Figure 5.15 Heavy metal speciation in plant root, stem and leaf material grown in CLO.

5.4   SUMMARY

Plant growth trials have been conducted to investigate the effect of different total nitrogen application rates of two ROWs on the growth of *E. nitens* and *S. viminalis*. Weekly leachate analysis assessed the soluble components and the influence of higher application rates on leachate composition and the potential risk to the wider environment. Biomass production and heavy metal uptake into plant material was assessed after 12 weeks of growth to identify the natural remediation effects of each tree species.

Weekly leachate identified steady pH levels ranging between 6.5 and 9 in both PAS 110 and CLO trials. The EC analysis identified initially high values from both ROWs with higher EC values associated with higher application rates. There was evidence of soluble salts increasing in *S. viminalis* in week 12 at the higher application rates, which was also evident in cation and anion analysis.

Heavy metal concentrations in weekly leachate from PAS 110 trials were below water quality limits for chromium and nickel, whereas copper, lead and zinc fell within water quality concentration limits dependent upon water hardness (calcium carbonate concentration). Concentrations of heavy metals leached from CLO pots were higher than PAS 110 pots, with chromium, lead and zinc falling within water quality ranges and copper and nickel exceeding the maximum water quality concentrations limits for aquatic life at some point during the growth trial.

Nitrate concentrations were greater in PAS 110 pots than CLO and higher concentrations were generally associated with application rates of less than 3000 kg N / Ha. Ammonium concentrations were higher in CLO trials and the higher application rates.

The application rate of both PAS 110 and CLO showed no significant effect on the biomass production of either *E. nitens* or *S. viminalis*. The average biomass change suggested greatest change in 1000 kg N/ Ha and 10,000 kg N/ Ha for *E. nitens* and *S. viminalis* grown in PAS 110, and 1000 kg N/ Ha for both species in CLO. The greatest average change however showed high variability, whereas the 3000 kg N/ Ha for PAS 110 and CLO showed less variability for both tree species. Biomass production was influenced by tree species more than ROW and application rate.

Due to the greater consistency between replicates and field trials being conducted by Forest Research an application rate 3000 kg N/ Ha of CLO was used in the column growth trials as described in Chapter 3 and the results presented in Chapter 6. Heavy metal uptake was greatest within *S. viminalis* grown in CLO pots, with highest concentrations within the root material for all experimental conditions. There was some evidence of heavy metal translocation and variability between the species accumulation in leaf or stem material.

Chapter 6 THE EFFECT OF DIFFERENT REDOX CONDITIONS ON SOIL LEACHATE, PLANT BIOMASS AND PLANT PHYTOREMEDIATION IN COLUMN GROWTH TRIALS

Previous chapters have characterised different ROWs, presented the results of preliminary growth trials and identified the optimum total nitrogen application rate for CLO and PAS 110 to *E. nitens* and *S. viminalis*.

This chapter presents the results of column growth trials conducted to identify the effect of saturated conditions compared to unsaturated conditions on plant growth, biomass production and the influence on nitrogen and heavy metal leaching from CLO. Columns were packed with a mixture of CLO and Kettering Loam at the optimum total nitrogen application rate identified in Chapter 5. Control columns contained perlite in replacement of CLO. Saturated and unsaturated columns containing perlite/Kettering Loam and CLO/Kettering Loam were allowed to equilibrate for 1 week before *E. nitens* and *S. viminalis* were planted and grown for a further 12 weeks. To assess the effect of reducing conditions on the leachate composition, leachate samples were collected fortnightly from the middle and base of the column (Figure 3.9).

Leachate samples were analysed for soluble metal cations (ICP-OES), anions (IC) and ammonium concentrations. Plant growth was assessed with weekly manual observations, height measurements and leaf counts before the biomass production of each plant was assessed at the end of the 12 weeks growing period. Dry biomass was assessed using XRF to identify heavy metal uptake and accumulation within plant material.

Premature demise of all *E. nitens* within 4 weeks of saturation resulted in the development of an additional experiment to identify if plant root development was required before saturation as described in section 3.7.8.

6.1   BIOMASS PRODUCTION

E. nitens and *S. viminalis* were grown in CLO/Kettering Loam mixes at an application rate equivalent to 3000 kg N / Ha in columns of 400 mm in depth. Control columns aimed to investigate the effect of Kettering Loam only and CLO was replaced with an equivalent volume of perlite (an inert mixing medium). Plant-free controls were also included within the experimental design to identify the impact of trees on the leachate chemistry.

Figure 6.1 shows the percentage change in dry biomass for *E. nitens* (4 weeks after premature death) and *S. viminalis* (12 weeks) in unsaturated and saturated conditions. Results represent the average percentage change in dry biomass change between replicate conditions with error bars indicating the standard errors.

Although the percentage change in dry biomass of *E. nitens* indicated a positive change in biomass within saturated conditions, weekly observational results presented in Appendix 6 showed premature death of the majority of *E. nitens* after 4 weeks excluding two unsaturated columns (perlite/Kettering Loam and CLO/Kettering Loam).

In unsaturated conditions there was little difference between the perlite/Kettering Loam and CLO/Kettering Loam columns, however in the saturated condition there was greater variability between the perlite/Kettering Loam and CLO/Kettering Loam columns. Two way ANOVA univariate analyses assessed the effect of ROW application (perlite or CLO) and environmental conditions (saturated or unsaturated) on biomass production for each species. There was no significant difference between the use of perlite or CLO, although a *p* value of 0.058 suggests some relationship. The effect of unsaturated and saturated conditions significantly affected the percentage change in dry biomass ($p < 0.001$). However there was no significant relationship between the application of CLO and environmental conditions ($p > 0.05$).

S.viminalis grown in saturated conditions in both control (perlite) and CLO had a greater change in dry biomass compared to the unsaturated conditions after 12 weeks growth. The standard errors were greater within the saturated conditions compared to the unsaturated conditions indicating greater variability between

replicates. Two way ANOVA analysis of *S. viminalis* identified the application of either perlite or CLO and environmental conditions (unsaturated or saturated) had no significant effect on the percentage change in dry biomass ($p > 0.05$).
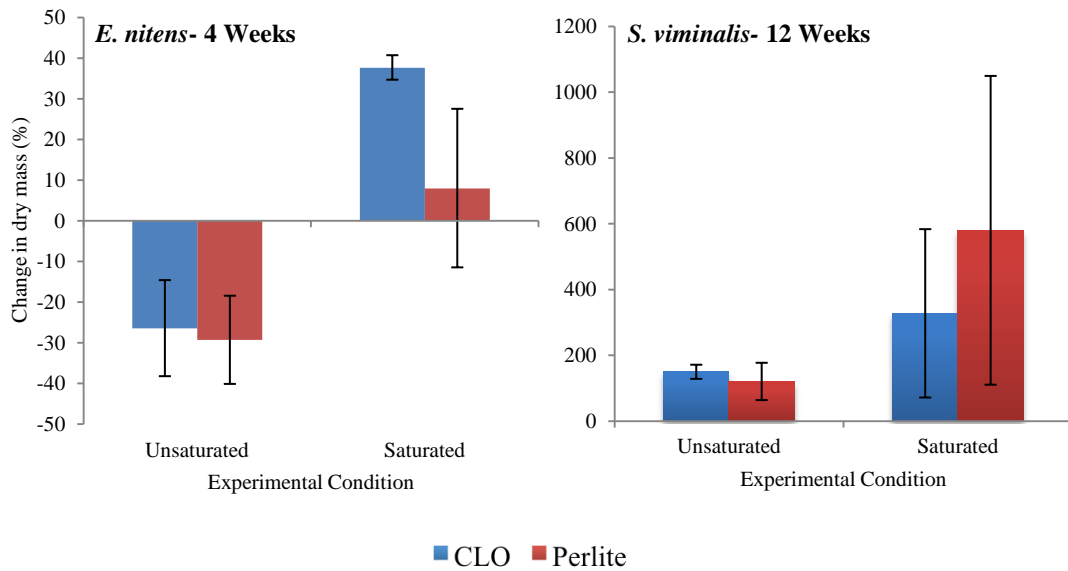
Figure 6.1 Average percentage change in dry biomass for *E. nitens* after 4 weeks growth and *S. viminalis* after 12 weeks growth.

6.2 LEACHATE

Leachate samples were collected and analysed after 24 and 96 hours of saturation and thereafter fortnightly. Figure 6.2 shows the average pH and EC of leachate samples collected weekly from the Rhizon samplers and base samples (section 3.7.8) against the average liquid to solid ratio. Leachate pH values were overall neutral to slightly alkaline, ranging between 6.43 and 8.36 and remained fairly consistent for the duration of the growth trial. Unsaturated columns containing both perlite/Kettering Loam and CLO/Kettering Loam mixes generally had slightly more alkaline pH readings from the Rhizon and base samples compared to the saturated columns. The standard errors of pH values ranged between 0.0065 and 0.44 for all conditions.

The EC values were greater from the CLO/ Kettering Loam mixed columns (unsaturated and saturated) compared to the perlite/ Kettering Loam control columns. Saturated columns showed slightly higher EC values compared to unsaturated columns, with gradual decreases in CLO/ Kettering Loam EC reading during the growth trial in all columns. Leachate samples collected from the base of

all columns showed slightly higher EC values compared to the leachate samples collected from the Rhizons in the middle of the column (data available in Appendix 6). The EC of the weekly leachate had standard errors ranging between 0.0053 and 3.2 mS/ cm for both conditions.
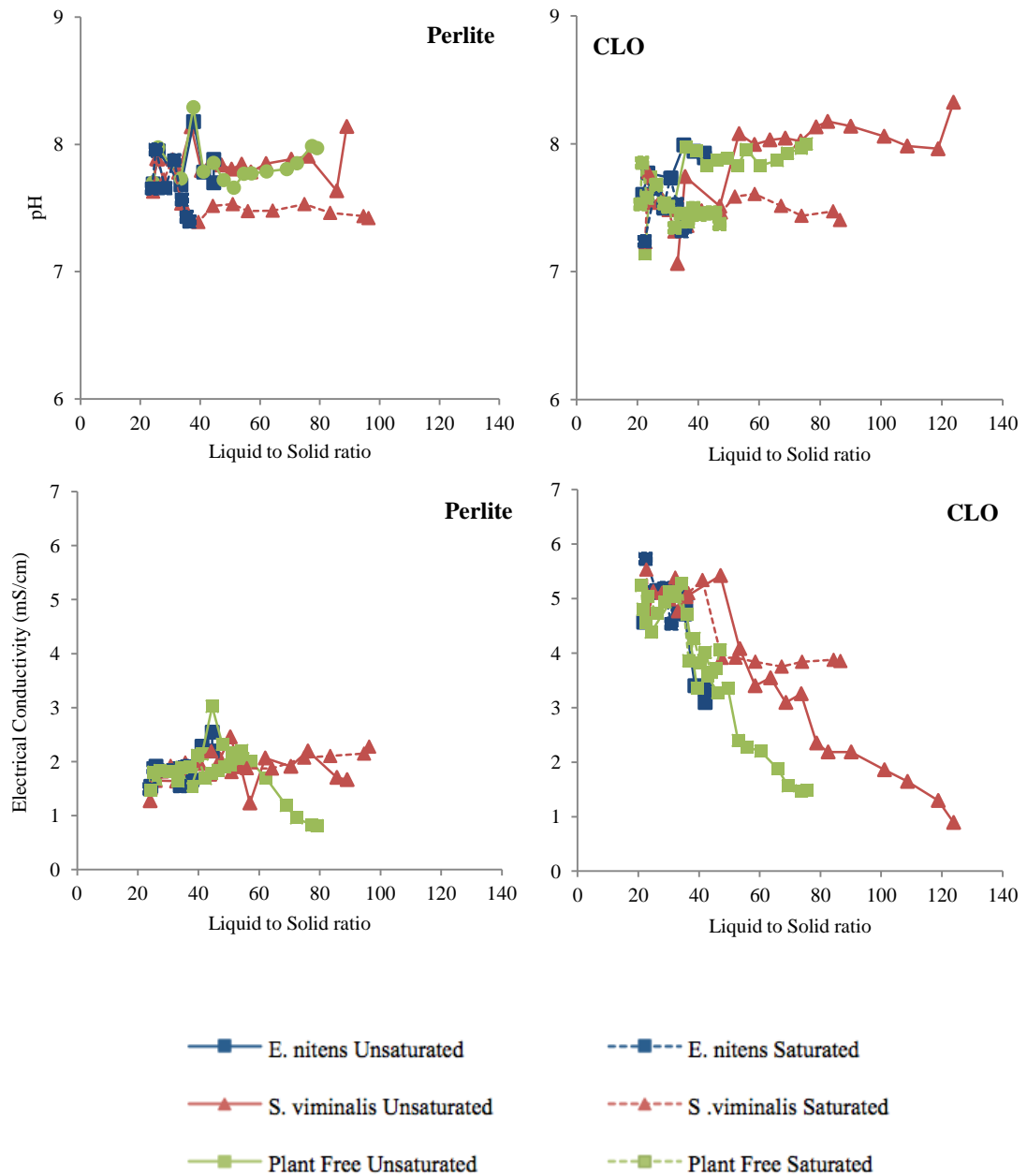
Figure 6.2 Average weekly pH and EC values for perlite and CLO columns in saturated and unsaturated conditions.

Figure 6.3 and Figure 6.4 present the average cumulative masses of chromium, copper, nickel, iron, lead and zinc leached from the columns during the growth trial against the average cumulative liquid to solid ratio. Perlite/Kettering Loam columns showed lower leached masses compared to the CLO/ Kettering Loam columns. All *E. nitens* columns showed similar trends to the *S. viminalis* and plant free columns before the premature demise of the trees after 4 weeks of planting.

There was evidence of rapid leaching of heavy metals (excluding lead) from the CLO/ Kettering Loam columns where L: S ratios were <40, before leaching reached a plateau or steady release of metal cations over the rest of the growth trial. After the initial saturation and flushing of the unsaturated columns (L: S ratio <30) there was high leaching from all columns however the saturation columns leached greater masses of heavy metals compared to the unsaturated columns. The L: S ratios were similar at the beginning of the growth trials and therefore the greater leaching from the saturated columns at the start maybe a result of the greater surface area of CLO incontact with water compared to the unsaturated columns. As the L: S ratios increase the unsaturated columns generally reached a steady state whereas the saturated columns continued to leach higher masses of heavy metals due to greater contact time and changes within the soil pore water chemistry (particularly evident in CLO Cr, Ni and Fe).

The cumulative mass of lead from CLO/ Kettering Loam columns remained very low or zero until the L: S ratio was > 30 and lead was leached from saturated conditions only.

All saturated columns showed greater leaching of all heavy metals (excluding copper) compared to the unsaturated columns and zinc concentrations were only greater in the *S. viminalis* saturated CLO/Kettering Loam columns. Of the CLO saturated columns, chromium, nickel and zinc concentrations were greater in the *S. viminalis* columns compared to the plant free columns.

Copper showed opposite trends to other heavy metals and greater cumulative masses of copper were leached from unsaturated columns compared to the saturated columns with slightly lower masses leached from columns containing *S. viminalis*.

Iron concentrations were analysed as the result of an orange residue developing within the Rhizon and base sampling points as shown in Figure 6.5. Iron is not assessed under the PAS certification, however the release and mobility of iron indicates environmental conditions. Iron leaching from saturated columns occurred in perlite/ Kettering Loam and CLO/ Kettering Loam columns, which exceeded the iron leaching from unsaturated columns and did not have orange deposits at sampling points (Figure 6.4).

Unsaturated columns showed overall much lower cumulative leached masses after an initial release of mobile ions before plateaus were reached, particularly evident in chromium, nickel and iron. Zinc leaching from saturated and unsaturated columns differed slightly from other metals. Saturated *E. nitens* and plant free controls showed lower masses of zinc leaching than the unsaturated columns and compared to *S. viminalis* saturated columns.

Standard errors ranged between 0.0002 - 0.13 mg / l for chromium, 0.03 - 13.3 mg / l for copper, 0.0008 - 8.5 mg / l for nickel, 0.12 - 332 mg / l for iron, 0.002 - 2.39 mg / l for lead and 0.002 - 58 mg / l for zinc for all experimental conditions.

Leachate samples were also analysed for calcium, sodium and magnesium, the average results are shown in Figure 6.6. Calcium, sodium and magnesium cumulative leached masses from the CLO/ Kettering Loam columns were greater from the saturated conditions compared to the unsaturated columns. As seen with the heavy metal leaching there were rapid releases of soluble ions where L: S ratios were <40, before leaching rates reached steady states.

Calcium leaching from perlite/ Kettering Loam columns showed similar cumulative leached masses to the CLO/ Kettering Loam columns suggesting high calcium leaching from the Kettering Loam. Sodium and magnesium showed lower cumulative masses leached from the perlite/ Kettering Loam columns, with little difference between the saturated and unsaturated conditions. Standard errors for all environmental conditions ranged between 315 - 28435 mg / l for calcium, 64 - 11264 mg / l for sodium and 14.9 - 2076 mg / l for magnesium.

Figure 6.7 shows the average cumulative masses of chloride and sulfate released from the columns. Lower levels of leaching were evident in the perlite/ Kettering

Loam columns compared to the CLO/ Kettering Loam columns. A rapid release of chloride and sulfate ions was evident in all conditions where L: S ratios were <40, before fairly steady release of ions over the remaining experimental period. Chloride leaching from saturated columns was slightly higher compared to the unsaturated columns, whereas there was little difference within the sulfate leaching between conditions. Standard errors between replicates ranged between 0.002 - 135 mg / l for chloride and 0.5 - 127 mg / l for sulfate for all experimental conditions.
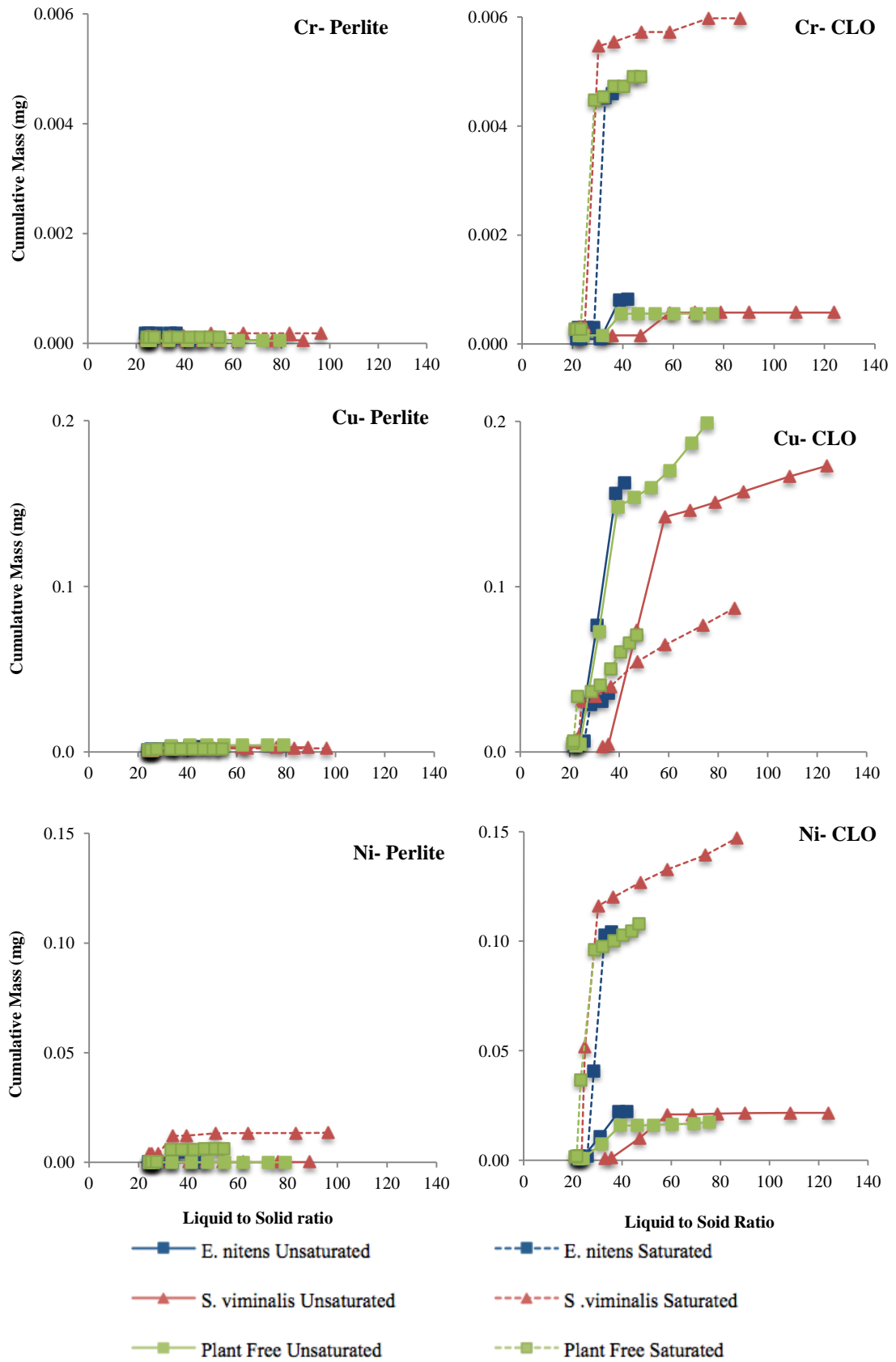
Figure 6.3 Cumulative leaching of chromium, copper and nickel from saturated and unsaturated columns packed with perlite/ Kettering Loam or CLO/ Kettering Loam mixes.
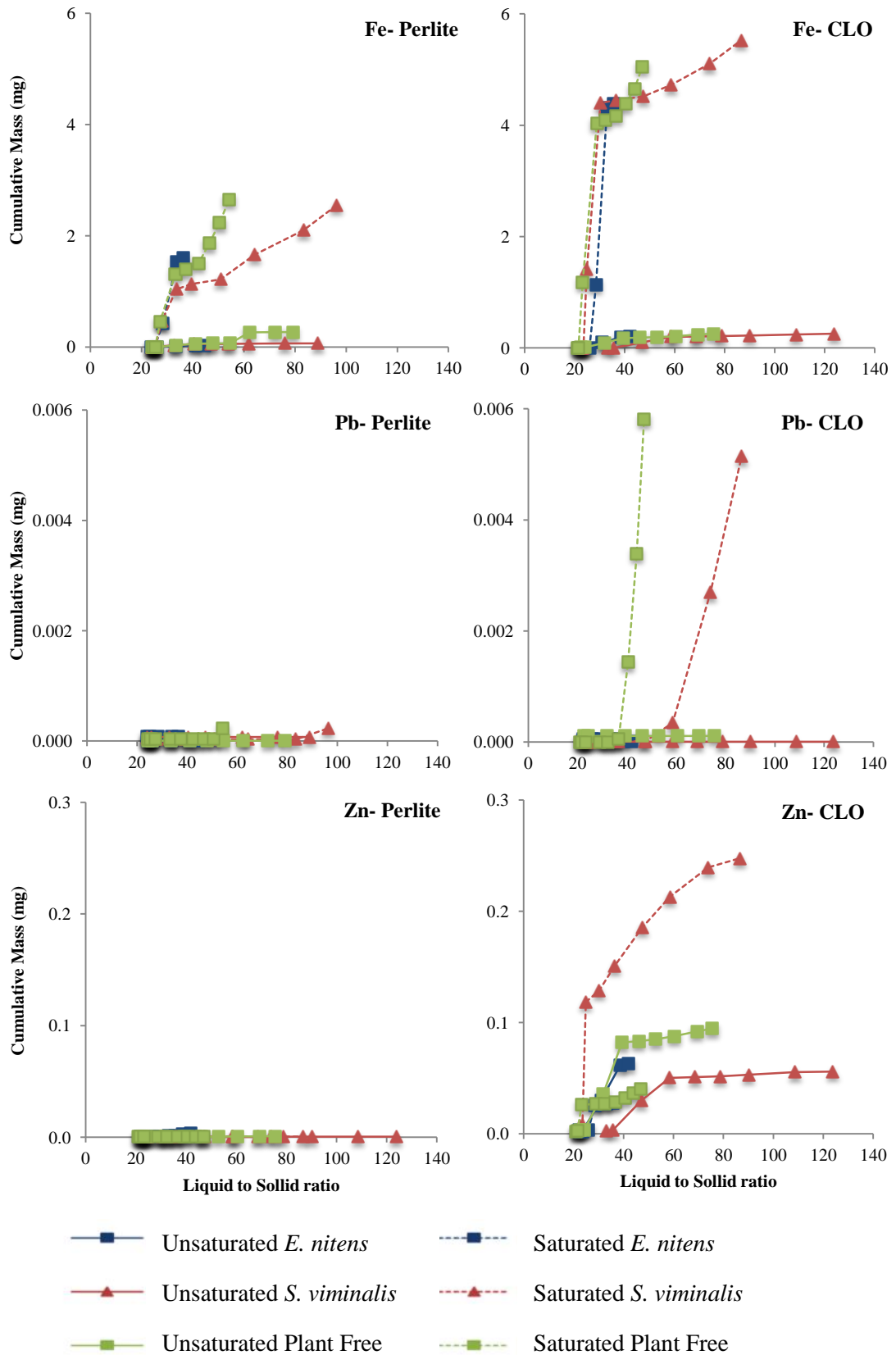
Figure 6.4 Cumulative leaching of iron, lead and zinc from saturated and unsaturated columns packed with perlite/ Kettering Loam or CLO/ Kettering Loam mixes.

Figure 6.5 Orange deposit evident only in the saturated columns Rhizon samplers (Left). Clear Rhizon sampler in the unsaturated columns.

Figure 6.8 represents the nitrogen leaching from the columns and gives an indication of the redox conditions within the unsaturated and saturated columns. Higher nitrate leaching was evident within the unsaturated columns whereas higher ammonium leaching was associated with the saturated columns. There was initial nitrate leaching from all columns where L: S ratios were <40, before a plateau was reached in the saturated columns and nitrate was released steadily from the unsaturated columns. A similar trend was seen in the ammonium; however it is the unsaturated columns that reached a plateau, while ammonium was continually leached from the saturated columns. Nitrate leaching was much greater in the CLO/ Kettering Loam columns compared to the perlite/ Kettering Loam columns however the perlite/ Kettering Loam plant free columns leached fairly high cumulative masses of nitrate by the end of the growth trial. Ammonium concentrations were similar in the CLO/Kettering Loam and perlite/Kettering Loam columns. Nitrogen leaching from *S. viminalis* columns was generally lower than the plant free columns. Standard errors between replicates ranged between 0.0068 - 325 mg / l for nitrate and 0.0013 - 1.38 mg / l for ammonium for all experimental conditions.
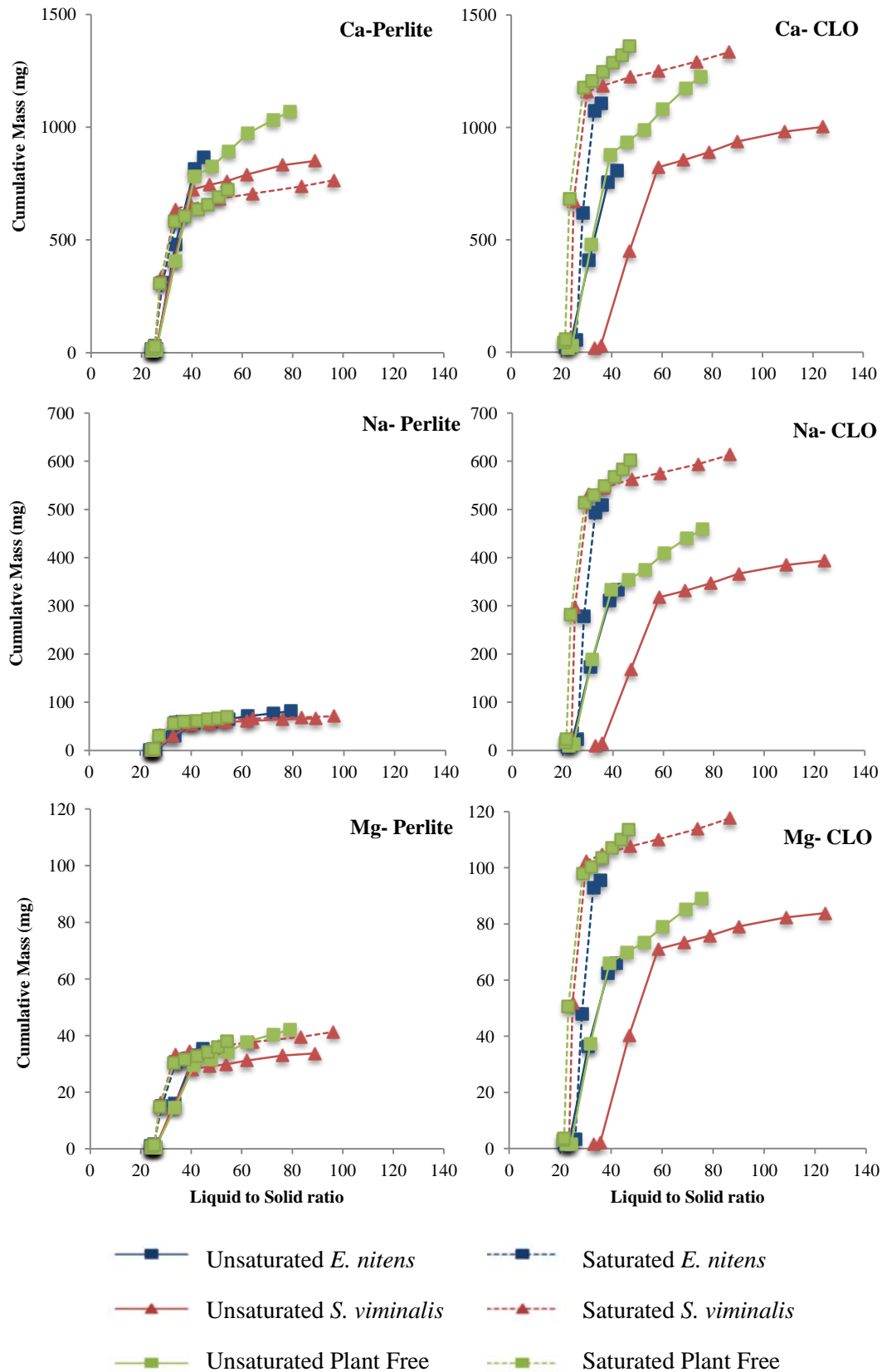
Figure 6.6 Cumulative leaching of calcium, sodium and manganese from saturated and unsaturated columns packed with perlite/ Kettering Loam or CLO/ Kettering Loam mixes.
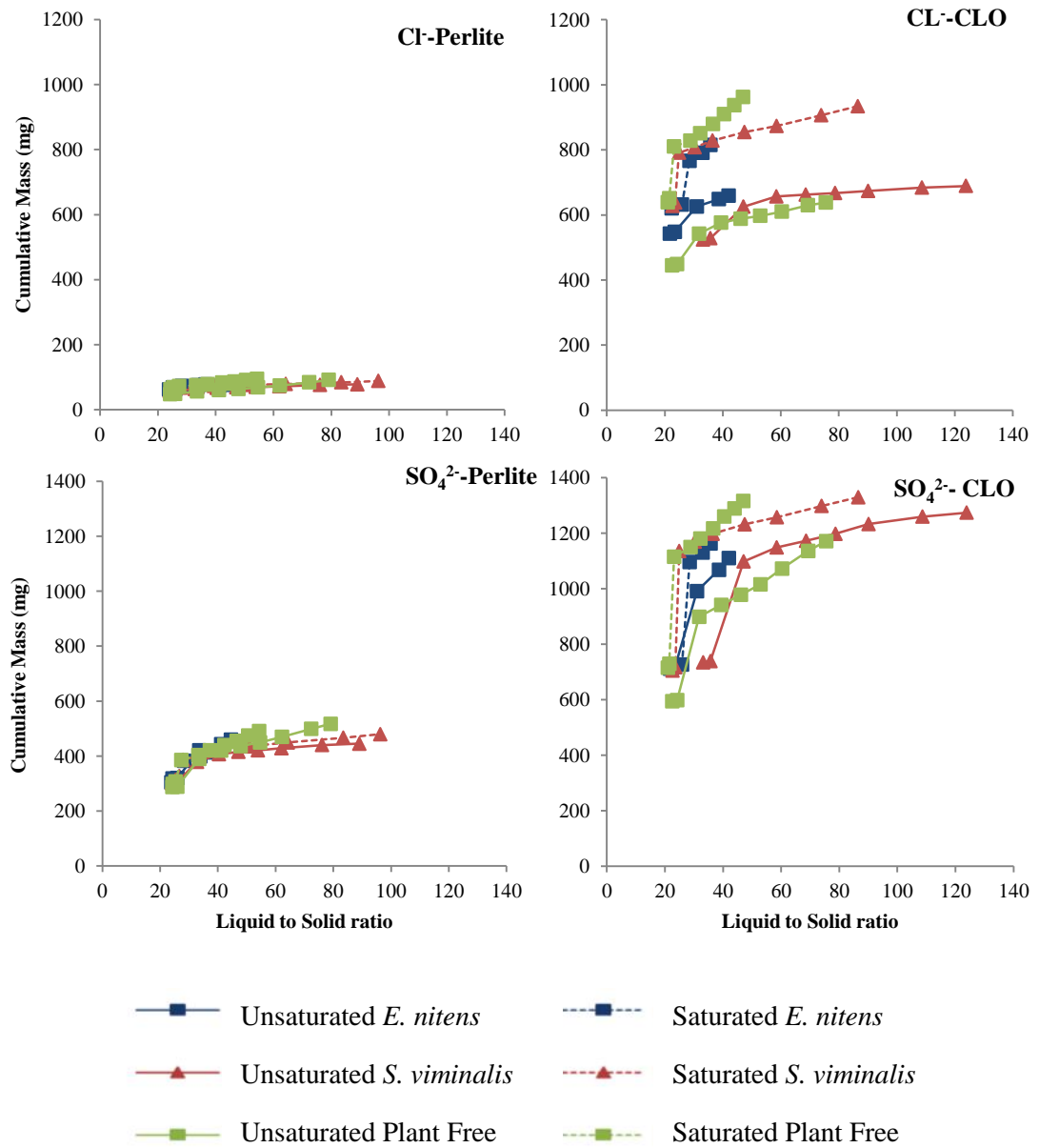
Figure 6.7 Cumulative leaching of chloride and sulfate from saturated and unsaturated columns packed with perlite/ Kettering Loam or CLO/ Kettering Loam mixes.
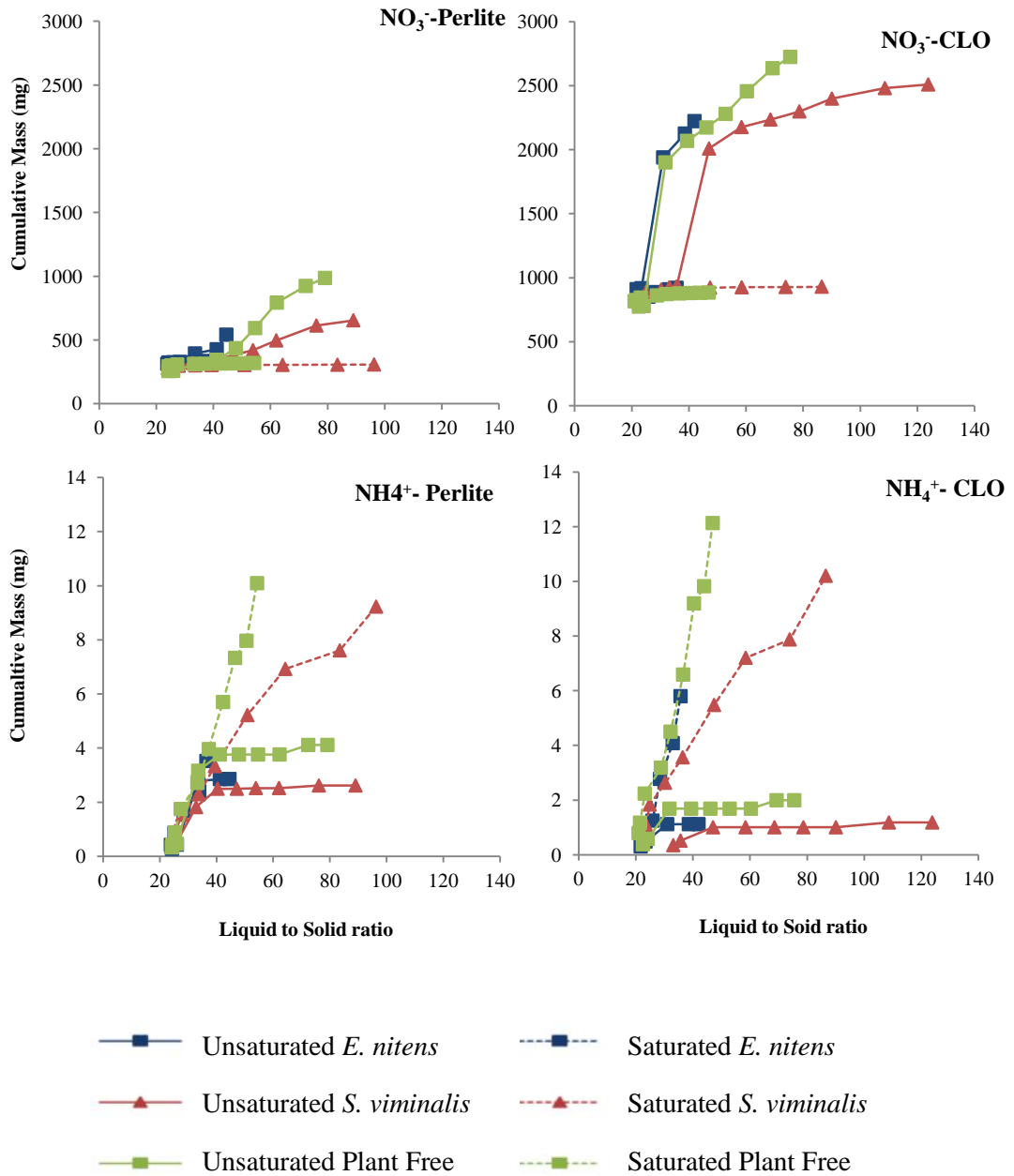
Figure 6.8 Average cumulative masses of nitrate and ammonium leached from perlite/ Kettering Loam and CLO/ Kettering Loam column in saturated and unsaturated conditions.

6.3   HEAVY METAL UPTAKE

To assess the effect of saturated and unsaturated conditions on heavy metal uptake and accumulation within plant material, each plant was divided into leaf, stem and root material before XRF analysis. Figure 6.9 and Figure 6.10 show the average heavy metal content for each species including pre-test background levels. Standard

errors (one-way) between replicates are shown for each metal by negative error bars only to prevent error bars over lapping.

Heavy metal concentrations within the *E. nitens* represent 4 weeks of potential uptake and accumulation due to premature death. Heavy metal concentrations and accumulation within plant material followed the trend of root >leaf > stem. Total heavy metal concentrations were greater within *E. nitens* grown in perlite/ Kettering Loam unsaturated columns, compared to the *E. nitens* grown in saturated conditions. *E. nitens* grown in CLO/ Kettering Loam showed the opposite, with greater heavy metal concentrations within plants grown in saturated columns compared to the unsaturated columns. Zinc and copper were present in all *E. nitens* organs, whereas lead was only present within the root material and chromium accumulation varied between treatment and environmental condition. Statistical analysis identified no significant difference ($p > 0.05$) between the application of perlite/ Kettering Loam and CLO/ Kettering Loam concerning the total heavy metal uptake within the root, stem and leaf material; this maybe a result of the short growing period. There was also no significant difference ($p > 0.05$) in total heavy metal uptake between unsaturated and saturated conditions.

S. viminalis showed similar accumulation trends to *E. nitens*, root> leaf> stem. Heavy metal concentrations were greater within the plants grown in saturated perlite/Kettering Loam compared to those grown in unsaturated perlite/Kettering Loam. Within the CLO/ Kettering Loam columns, heavy metal accumulation within saturated *S. viminalis* root material exceeded the concentrations present within *S. viminalis* root material grown in unsaturated columns. Heavy metal accumulation within *S. viminalis* stem and leaf material showed greater accumulation within the plants grown in unsaturated conditions compared to the saturated conditions. Zinc and copper were present in all *S. viminalis* organs; however chromium and lead were generally stored within the root material. There were significant differences ($p = 0.001$) in the total heavy metal uptake within root, stem and leaf material between perlite/ Kettering Loam and CLO/ Kettering Loam applications, however there was no significant difference between saturated and unsaturated conditions.
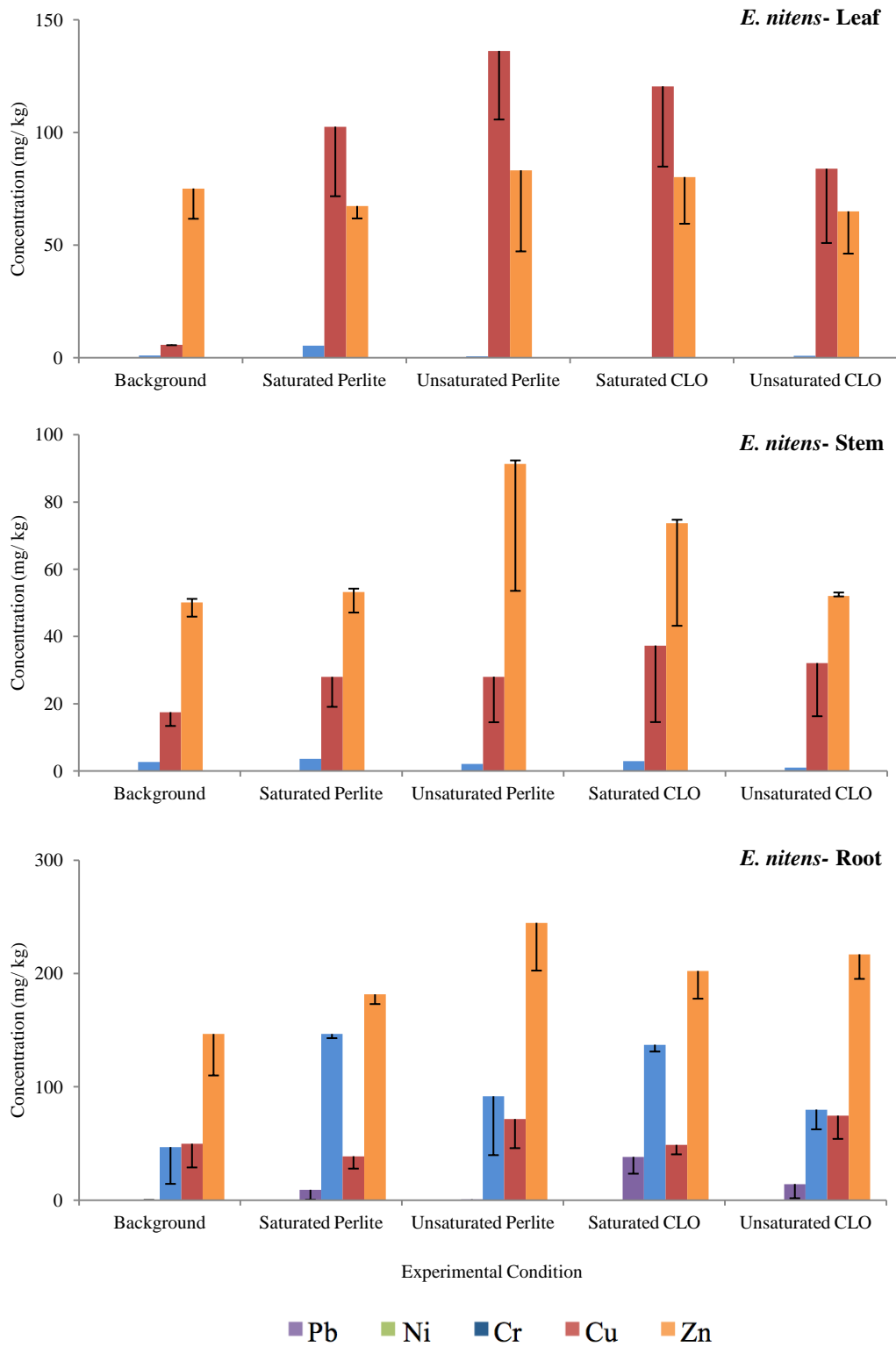
Figure 6.9 Average heavy metal uptake and accumulation within *E. nitens* after 4 weeks growth and the background levels before growth trials.
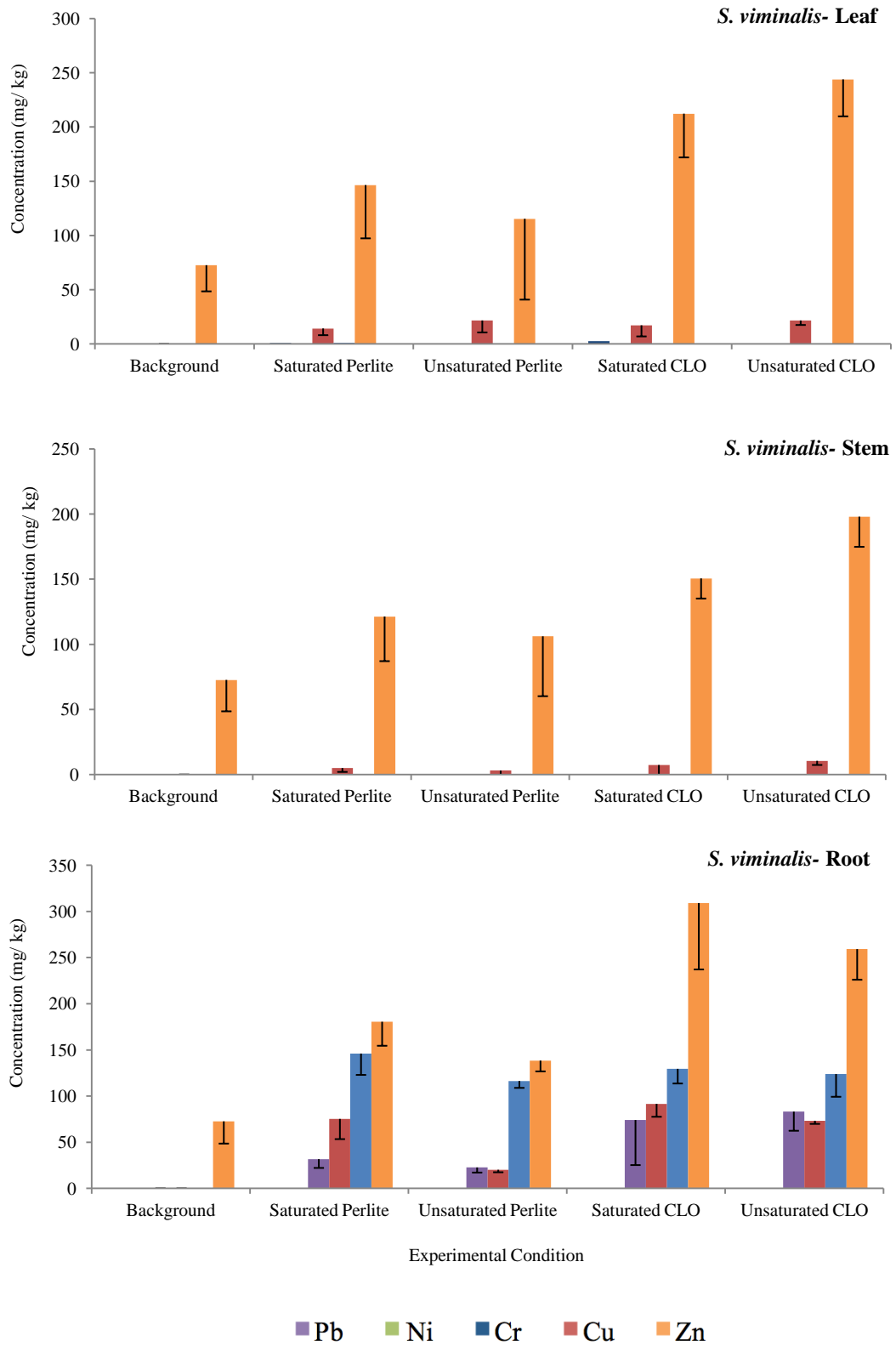
Figure 6.10 Average heavy metal uptake and accumulation within *S. viminalis* after 12 weeks growth.

As a result of increased iron mobility within saturated columns as seen in section 6.2, iron uptake and accumulation within *E. nitens* and *S. viminalis* were compared to identify the difference between saturated and unsaturated conditions. Figure 6.11 shows the iron uptake within the root, stem and leaf material of the *E. nitens* and *S. viminalis*. Background levels of iron were less than 6000 mg/ kg for *E. nitens* and less than 200 mg/ kg for *S. viminalis*. Iron predominantly accumulated within the root material for both species and all experimental conditions. The environmental condition had a significant effect on the iron uptake for both *E. nitens* ($p = 0.013$) and *S. viminalis* ($p = 0.01$), although the application of perlite/Kettering Loam or CLO/Kettering Loam did not have a significant effect on iron accumulation.
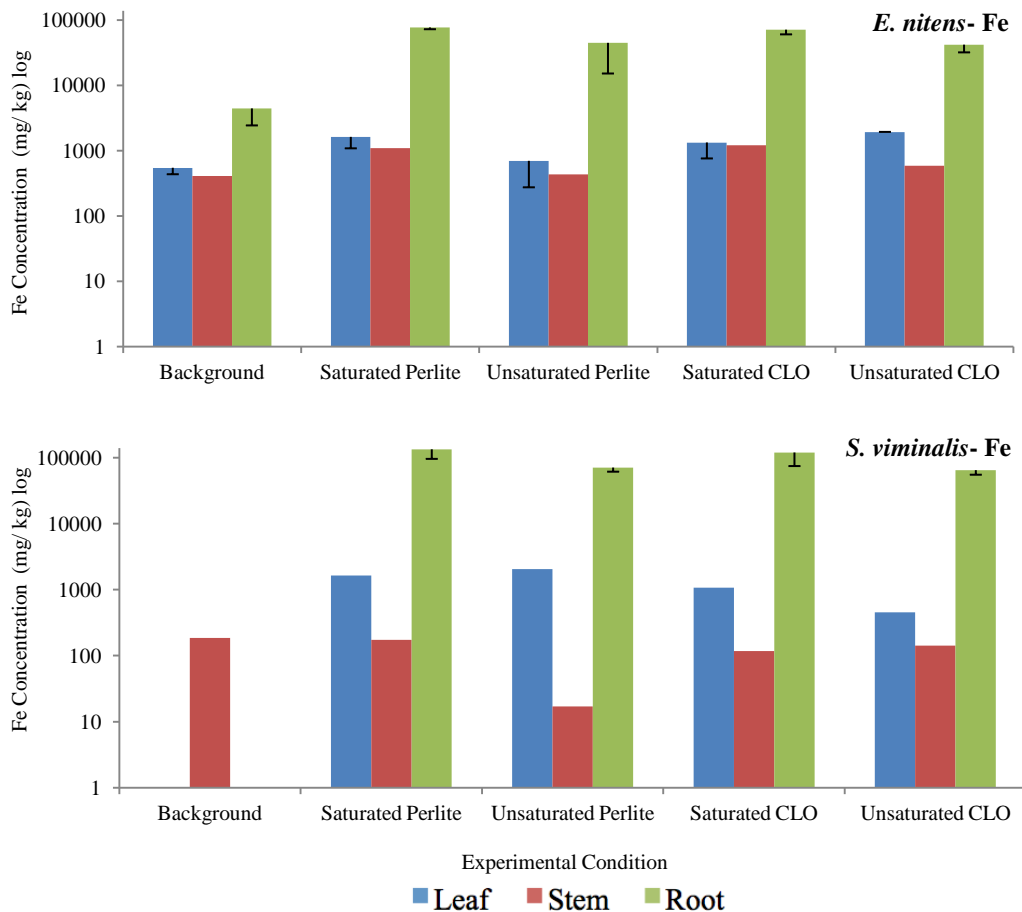
Figure 6.11 Average iron uptake and accumulation within *E. nitens* after 4 weeks growth and *S. viminalis* after 12 weeks growth.

6.4   *E. NITENS* POINT OF SATURATION

As a result of the premature demise of *E. nitens* within the column growth trials (section 3.7.8) it was hypothesised that root development of *E. nitens* was required before saturation to ensure plant survival. To investigate *E. nitens* columns were replanted with CLO/ Kettering Loam mixes at an application rate equivalent to 3000 kg N / Ha. The columns were either saturated on day 0, after 3 weeks of unsaturated root development or after 6 weeks of unsaturated root development. Control columns remained unsaturated for the duration of the repeat trial as described in section 3.7.8.

Figure 6.12 shows the average percentage change in dry biomass of *E. nitens* grown in CLO/Kettering Loam, which were saturated at different times. Negative changes in dry biomass indicate plant demise, with error bars showing the standard error between replicates. The weekly observational results are presented in Appendix 6. *E. nitens* saturated on day 0 showed evidence of demise within the first 2 weeks. Those saturated after 3 weeks of unsaturated root development died after 3 and 4 weeks of saturation. Columns saturated after 6 weeks of root development showed evidence of deterioration after 2 weeks of saturation, however 1 plant out of three saturated in week 6 survived and showed good healthy growth in week 12.
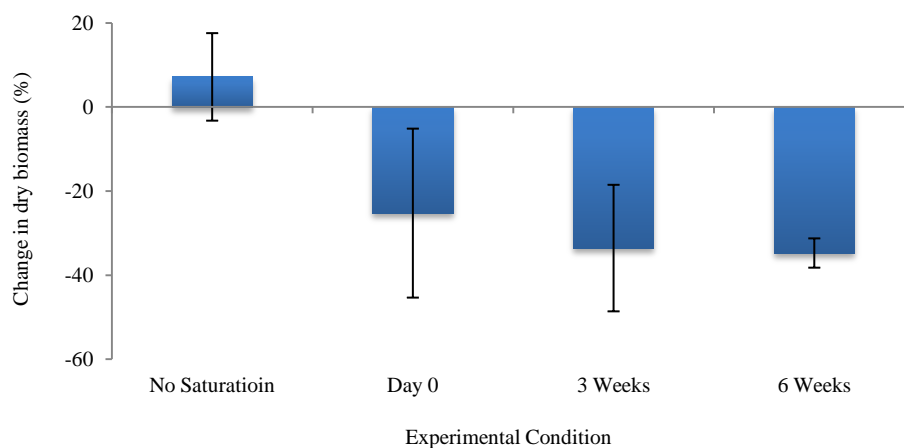
Figure 6.12 Average percentage change in dry biomass in *E. nitens* after different saturation times.

6.4.1 E. NITENS LEACHATE

Leachate samples were collected weekly from the Rhizon samplers in the middle of the columns and from the base of the columns. The pH and EC were monitored weekly, with soluble metal cation, anion and ammonium concentrations analysed fortnightly. Figure 6.13 presents the average Rhizon and Base leachate pH and EC of leachate samples compared to the cumulative L: S ratio. The average pH values ranged between 6.7 and 8.3 with small variations between experimental conditions. The columns that remained unsaturated for 12 weeks showed a gradual increase in average pH after the L: S ratio was >5. There were slight decreases in pH values after saturation particularly noticeable in columns saturated after 3 and 6 weeks.

There was an overall decrease in EC values during the growth trials with rapid decreases evident after saturation (3 weeks and 6 weeks). Average standard errors ranged between 0.009 - 0.64 for pH and 0.055 - 1.7 mS/ cm for EC.
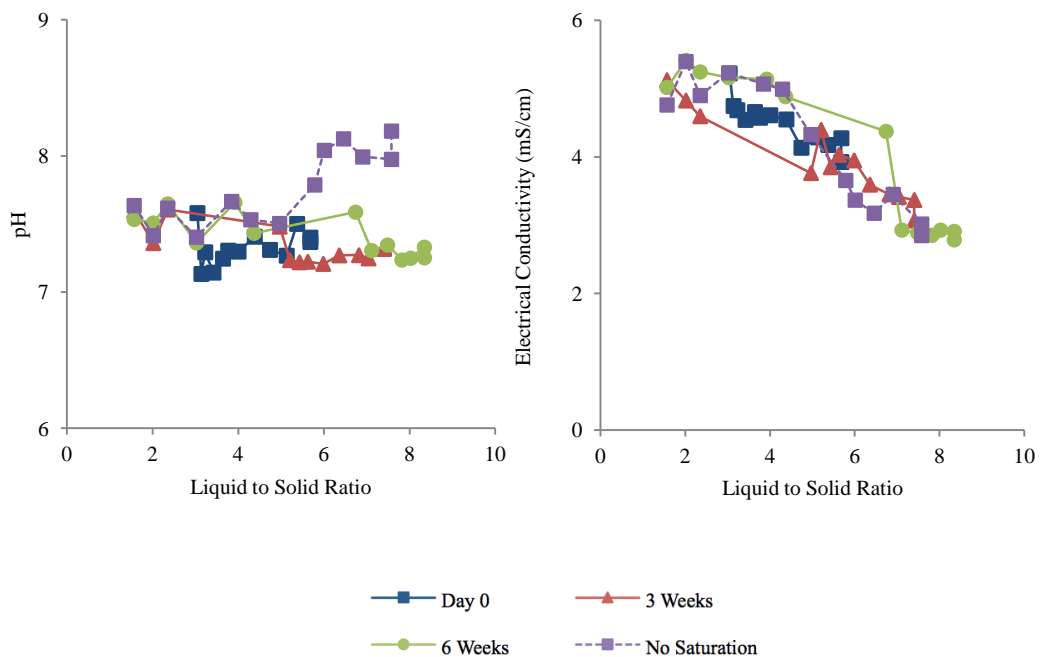
Figure 6.13 Average weekly pH and EC of *E. nitens* leachate samples of columns saturated at different times.

The average cumulative masses leached of metal cations, anions and ammonium against the cumulative L: S ratios are shown in Figure 6.14, 6.15 and 6.16. Chromium, nickel, lead and iron showed greater leaching concentrations from the columns saturated on day 0. Once columns were saturated after 3 and 6 weeks, there was a rapid increase in leached metal masses (partciulary noticeable in Cr, Ni and Fe. The high release of metals maybe linked to the increase surface area contact with the CLO once saturated as seen in section 6.2. The L: S ratio is much lower in the point of saturation growth trials compared to the initial column growth trials (6.2) and therefore the initial release seen in section 6.2 has greater visibility in Figure 6.14.

Copper and zinc showed greater leaching from the unsaturated columns and those saturated after 6 and 3 weeks. There was a delayed release in chromium and lead after saturation compared to other metal cations, which showed a relatively rapid response to saturation. Standard errors between replicates ranged between 0.0000002 - 0.00034 mg / l for cadmium, 0.00013 - 0.015 mg / l for copper, 0.000002 - 0.003 mg / l for nickel, 0.000056 - 0.12 mg / l for iron, 0.00006 - 0.001 mg / l for lead and 0.00003 - 0.007 mg / l for zinc in all conditions.

Chloride, sulfate and nitrate leaching showed similar trends with greatest cumulative leaching associated with the columns which remained unsaturated or those saturated after 3 and 6 weeks and lower overall leached masses from the columns saturated on day 0. There was a decrease in leached anions once columns were saturated after 3 and 6 weeks of unsaturated growth. Standard errors between replicates ranged between 0.50 – 84 mg / l for calcium, 0.24 - 35 mg / l for sodium and 0.027 - 5.36 mg / l for magnesium.

Ammonium leaching was only associated with the columns saturated on day 0. The remaining columns showed an initial release of ammonium within the first week before ammonium was undetected or very low concentrations for columns saturated after 3 and 6 weeks. The standard error between replicate pots for ammonium ranged between 0.0009 - 2.98 mg / l for all experiment conditions.
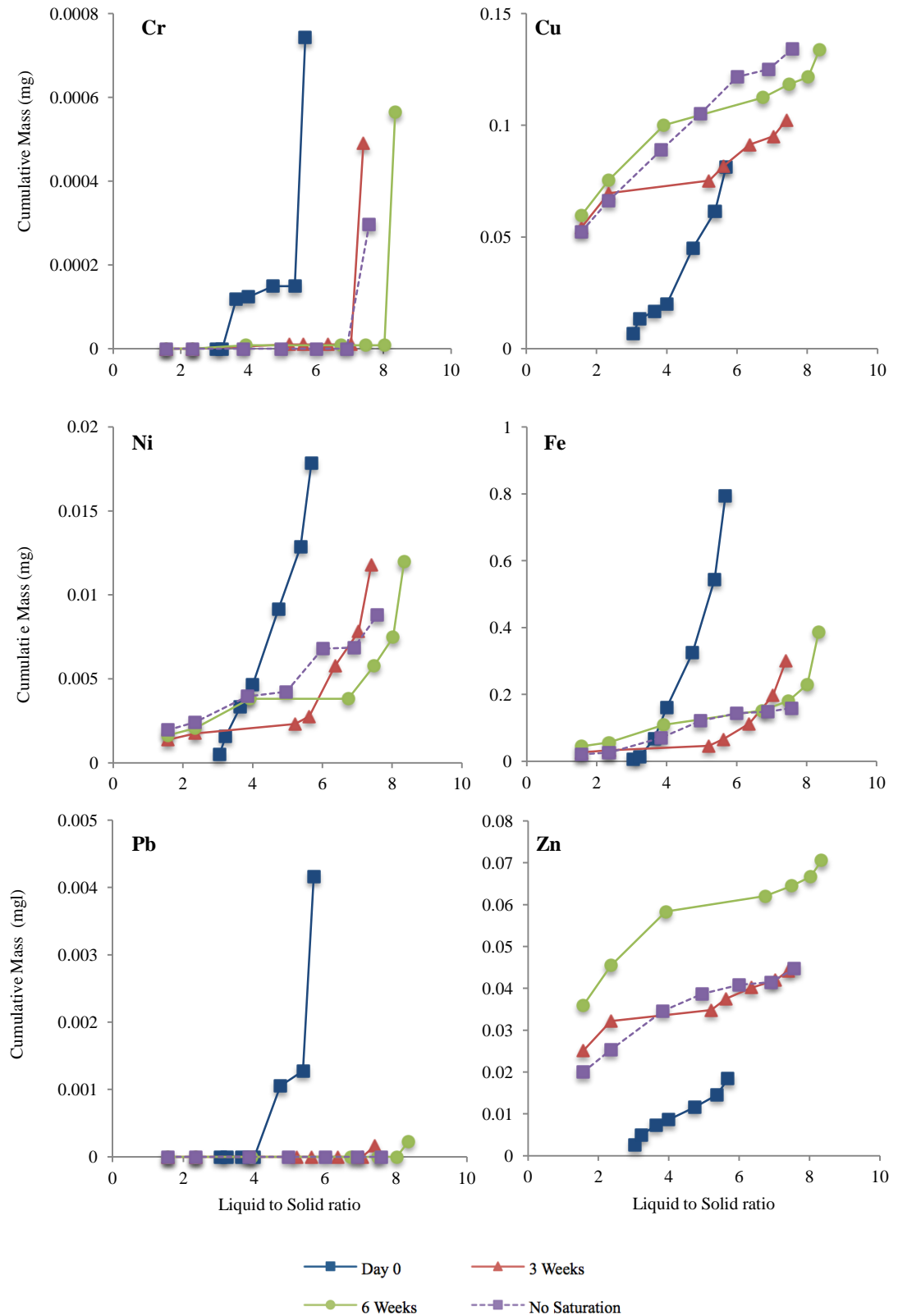
Figure 6.14 Average cumulative masses of chromium, copper, nickel, iron, lead and zinc leached from *E. nitens* after different saturation times.
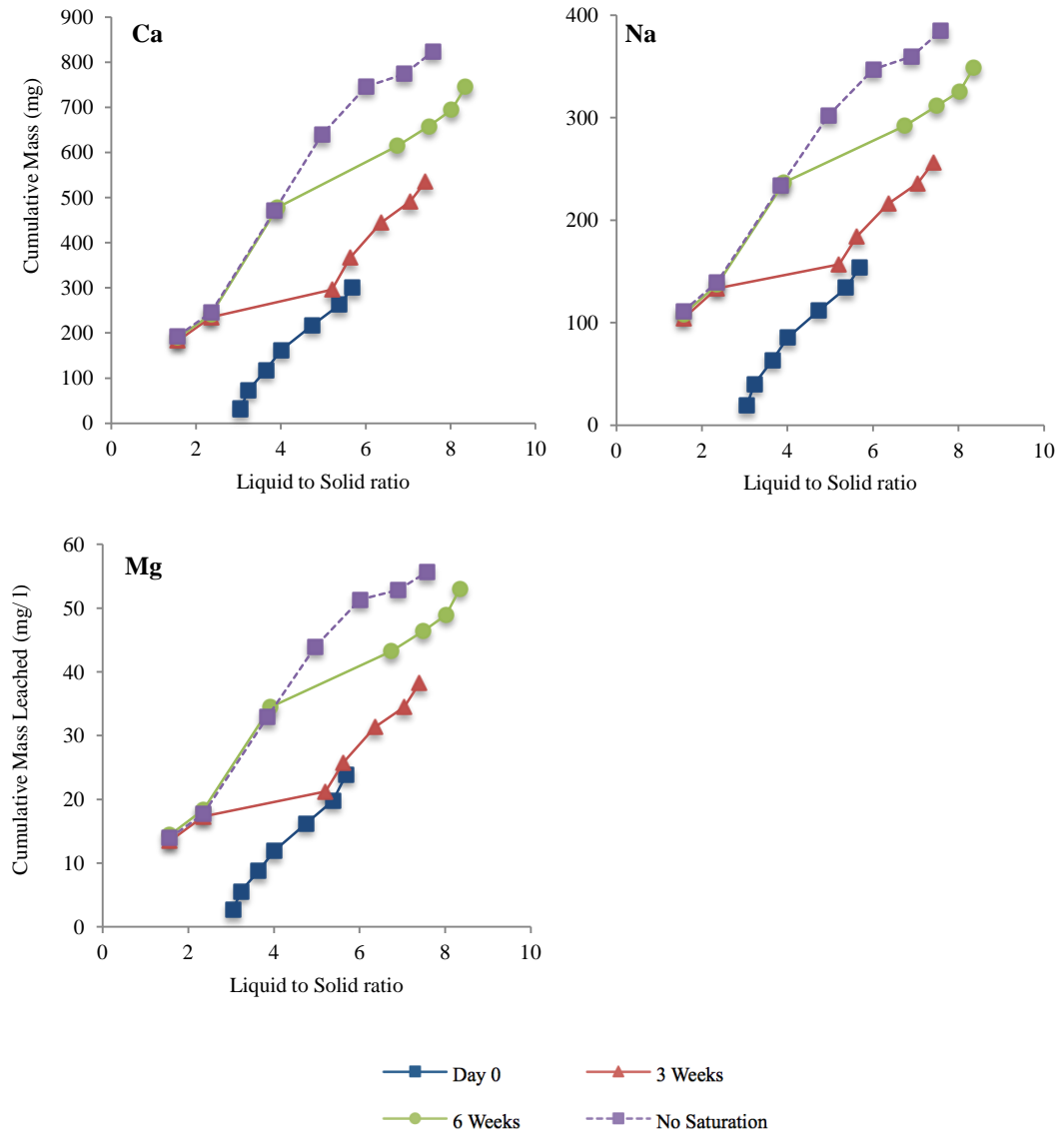
Figure 6.15 Average cumulative aluminum, calcium, sodium and magnesium leached from *E. nitens* after difference saturation times.
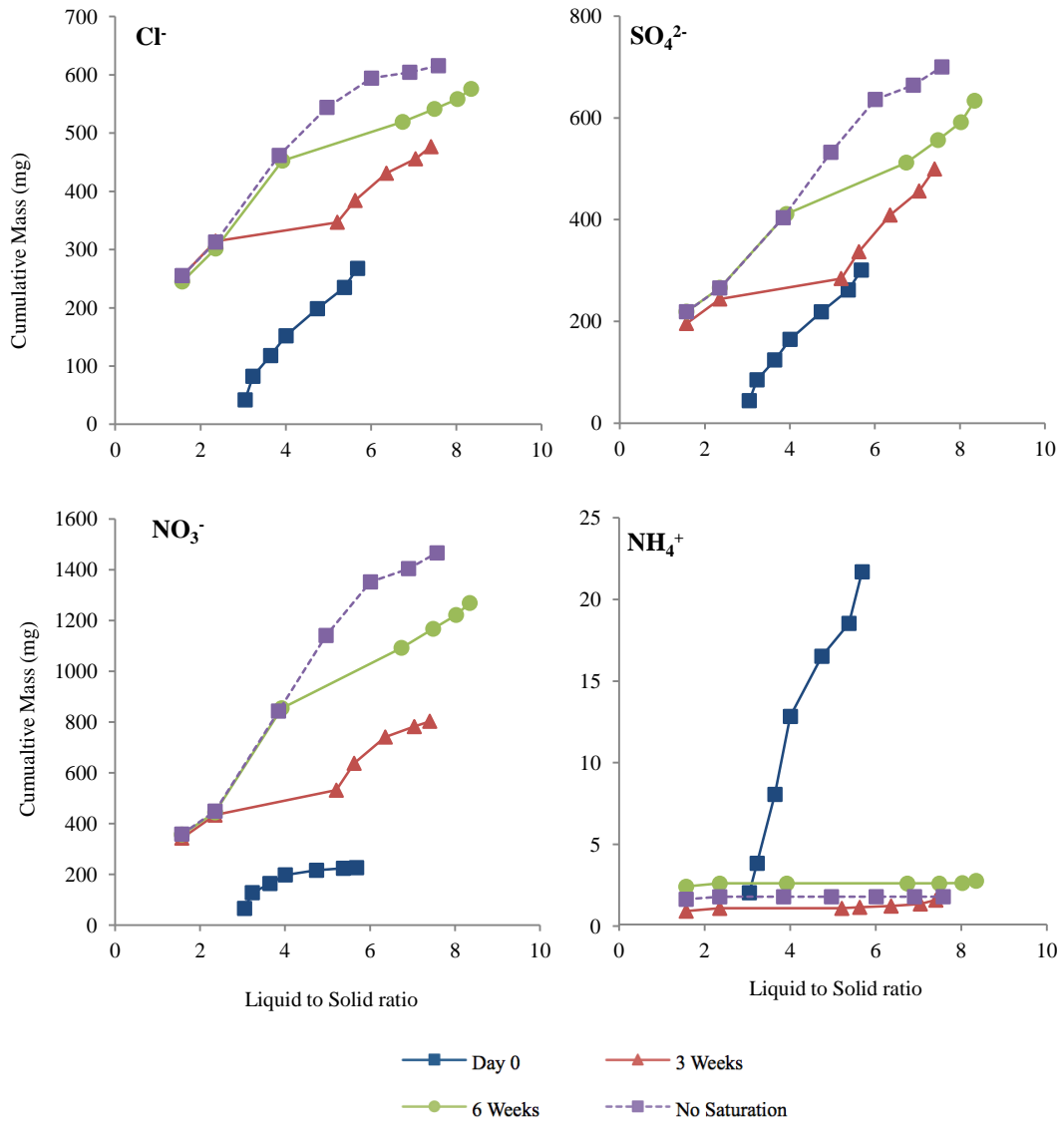
Figure 6.16 Average cumulative chloride, sulfate, nitrate and ammonium leached from *E. nitens* after different saturation times.

6.4.2   *E. NITENS HEAVY METAL UPTAKE*

E. nitens were analysed to assess the heavy metal concentration and translocation within the root, stem and leaf material. Figure 6.17 shows the heavy metal speciation within the plant material and the preliminary background levels within *E. nitens*. Error bars indicate the standard error (one way) of replicate trials to limit overlapping of error bars.

Of the background material there was a general trend for root > leaf = stem, similar to those seen in the previous growth trials (sections 4.2, 5.3 and 6.3). Unsaturated columns showed a similar trend with root = leaf > stem material. Of the columns saturated, there was a trend for leaf > root > stem, particularly evident within the *E. nitens* saturated on day 0. Although leaf accumulation of heavy metal decreased the longer the plant was grown in unsaturated conditions (6 weeks), root accumulation increased with unsaturated growing period (6 weeks). The translocation of copper was greater within plants grown in saturated conditions, particularly evident in the *E. nitens* saturated on day 0 (Figure 6.17).

As seen in section 6.3 and Figure 6.11 iron mobility and uptake increased in saturated columns. Figure 6.18 showed the iron uptake and accumulation within *E. nitens* saturated after different periods of unsaturated growth. Background concentrations of iron were mainly associated within the root material, which was also evident within the saturated and unsaturated columns. There was some evidence of iron translocation to the stem and leaf material, particularly *E. nitens* saturated after 3 weeks unsaturated growth, although there were fairly high standard errors between leaf material replicates. Overall there were lower iron concentrations present within the root, stem and leaf material of the *E. nitens*, which remained unsaturated for the duration of the growth trial. Iron accumulation was generally lower within this *E. nitens* experiment compared to the initial *E. nitens* saturation trials presented in section 6.3.

The potential uptake of heavy metals within the *E. nitens* maybe limited due to the premature dealth, although the premature death could be linked to the high heavy metal uptake once pore water soil chemistry changes in saturated conditions.
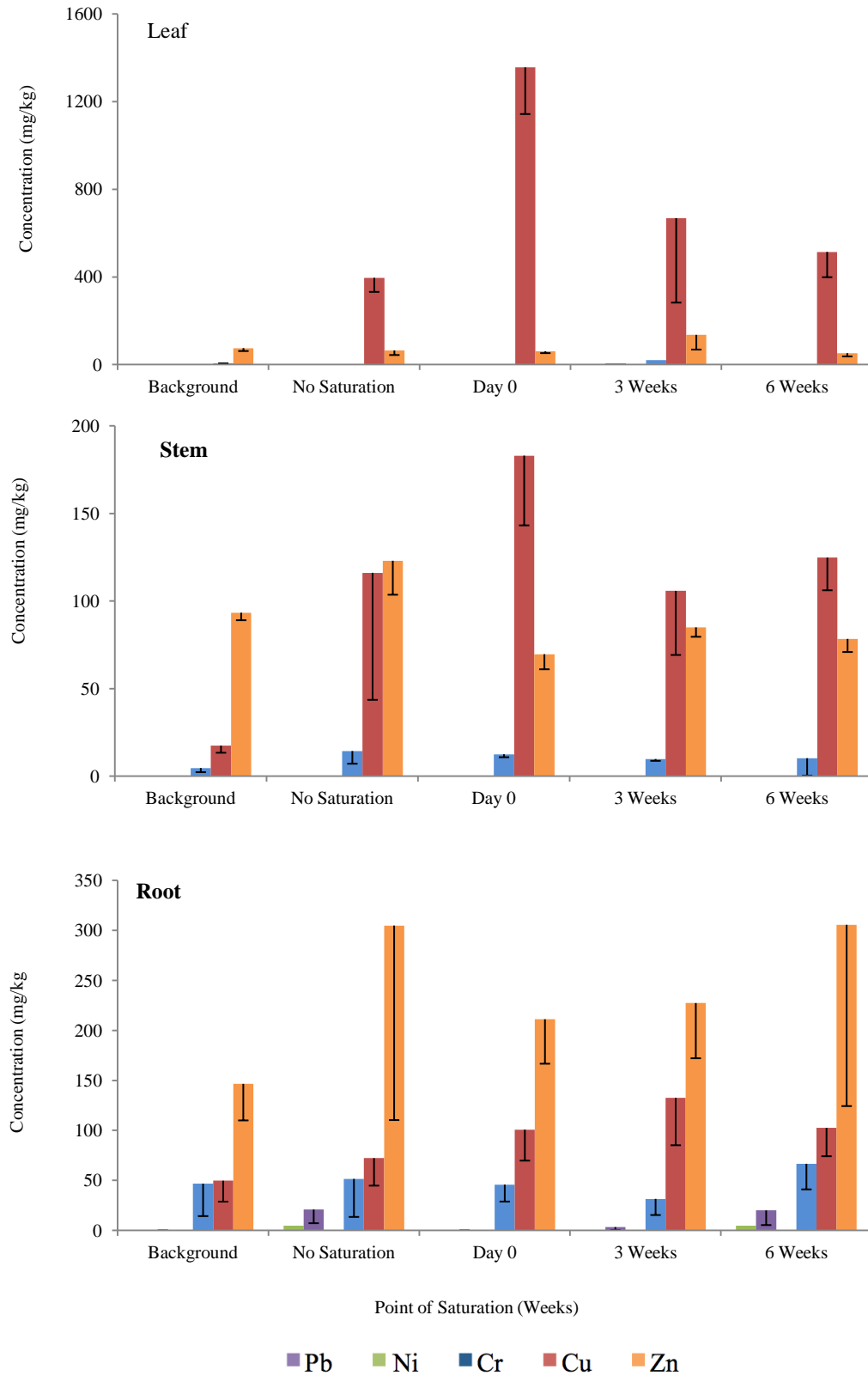
Figure 6.17 Average heavy metal uptake and accumulation in *E. nitens* after different saturation times.
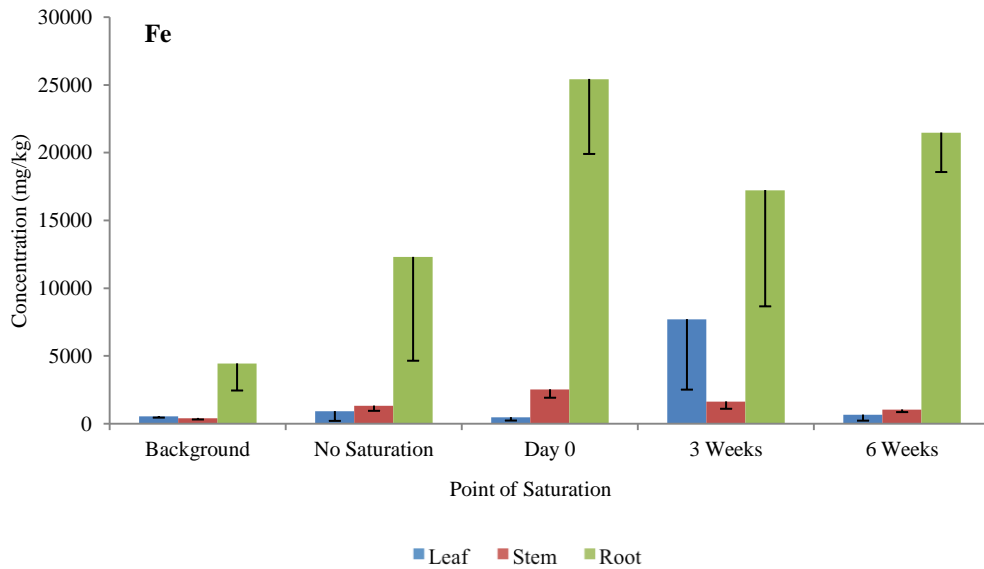
Figure 6.18 Average iron uptake and accumulation in *E. nitens* after different saturation times.

6.5   SUMMARY

E. nitens and *S. viminalis* were grown in unsaturated and saturated columns at the optimum application rate of CLO identified in Chapter 5.

Within 4 weeks all *E. nitens* in saturated columns and 9 out of 12 unsaturated were black and dry as a result of premature demise. Saturated *S. viminalis* grown in perlite/Kettering Loam and CLO/Kettering Loam both had greater percentage changes in dry biomass compared to unsaturated columns.

Leachate samples were collected mid-way down the column using Rhizon soil samplers and at the base of each column. Metal cation, anion and ammonium concentrations were analysed fortnightly and used to calculate the average cumulative masses of solutes over 12 weeks. Under saturated conditions greater masses of chromium, nickel and lead were leached compared to the unsaturated columns. Copper however had greater leached masses in the unsaturated columns. Iron was analysed during these experiments as a result of the precipitation of an orange residue at the sampling points of the saturated columns only. The concentrations of iron were greater within the saturated columns compared to the unsaturated columns as a result of changes in the redox conditions.

Nitrate cumulative leaching was greater within the unsaturated columns, with higher ammonium leaching associated with the saturated columns.

Heavy metal accumulation within *E. nitens* and *S. viminalis* generally followed the trend roots > leaf > stem material. There was an overall trend of greater heavy metal uptake and accumulation associated with the saturated columns compared to the unsaturated columns. Iron uptake was also assessed as a result of increased iron leaching associated with the saturated columns, which identified higher concentration of iron accumulated within the saturated plants compared to those unsaturated.

Due to the premature death of *E. nitens*, columns were dismantled after 4 weeks while the *S. viminalis* continued for a total of 12 weeks plant growth. It was hypothesised that *E. nitens* required root development before saturation. To investigate this *E. nitens* were replanted and saturated on either day 0 or after 3 or 6 weeks of unsaturated root growth with control *E. nitens* that remained unsaturated for the duration of the trial. All *E. nitens* saturated on day 0 and after 3 weeks died within 2 to 4 weeks. Of those saturated after 6 weeks, one survived out of the three replicates until week 12.

As seen with the initial column growth trial, cumulative masses of some metals were greater in the day 0 saturated columns compared to those saturated at a later time giving some indication of solubility changes within the changing environmental conditions.

Chapter 7 DISCUSSION

This thesis investigates the use of ROWs as nutrient sources for energy crops by characterising three different materials and conducting three different growth trials. ROWs from source segregated wastes streams (PAS 100 and PAS 110) were compared to mixed MSW material (CLO). The source of waste and the biological degradation process; aerobic composting or anaerobic digestion can affect the characteristics of the final product and the future use of the organic wastes.

Three ROWs (PAS 100, PAS 110 and CLO) were investigated in preliminary growth trials using *S. viminalis, E. nitens* and *P. tomentosa*. Based upon these preliminary results, further growth trials were conducted to identify the optimum nitrogen application rate of two ROWs (PAS 110 and CLO) for *S. viminalis* and *E. nitens*. To understand the potential risk of mixed MSW derived CLO to the environment, column growth trials were conducted to identify the effect of different redox conditions on the leachate composition.

This chapter discusses the results from the experimental work conducted and presented in Chapters 4, 5 and 6. This will include the characterisation of each ROW and the effect of waste source and pre-treatment on the final material, before comparing the results from the growth trials. To discuss and compare the findings from the growth trials, three common themes have been identified; biomass production, leachate composition and heavy metal phytoremediation.

7.1 ROWS CHARACTERISATION

Chapter 4 presented the characterisation of three different types of ROWs; PAS 100 (source segregated, aerobically composted), PAS 110 (source segregated, anaerobically digested) and CLO (mixed MSW stream, aerobically composted). To characterise the materials a number of standard parameters were identified such as pH, EC, BD and MC. The total, available and water-soluble heavy metal concentrations were assessed as well as sequential extraction to identify heavy metal fractions.

The results showed all materials were suitably decomposed and matured when compared to values recommended by Edwards *et al.* (2010), with pH values

between 6 - 8 (excluding PAS 100, 9.26) and C:N ratios of less than 20 excluding one PAS 110 sample although it was near the recommended levels. There were substantial differences between source segregated and mixed waste source EC values as a result of excess soluble salts. Previous studies have identified EC values ranging between 4 and 8 mS/ cm for both source segregated and mixed waste material. Excess soluble salts can be detrimental to some plants at values as low as 1.4 mS/ cm by affecting water availability to roots and inhibiting soil biological activity, which may in turn impact upon yield productions (Cameron *et al.*, 2009; Hargreaves *et al.,* 2008). Although ROW amendments can increase EC values of native soils, soluble salts have been found to decrease over time as a result of plant uptake and leaching (Hargreaves *et al.,* 2008).

The total heavy metal analysis identified greatest concentrations within the CLO compared to the source segregated material (PAS 100, PAS 110), consistent with previous studies due to the original mixed MSW stream (Smith, 2009; Cameron *et al*., 2009; Hargreaves *et al*., 2008; Veeken and Hamelers, 2002). All CLO samples were considerably lower than the maximum SOMs limits (ORG, 2013) with values closer to the PAS limits. Copper, lead and zinc exceeded the PAS quality limits in all CLO samples, as also seen by Paradelo *et al*. (2011). There was evidence of variability between the total copper concentrations measured within the CLO (b and c) samples, which maybe due to variability within the bulk sample as the replicate samples identified low standard errors. This indicates the variability within CLO and the importance of regular analysis before use and land application. The PAS 100 compost exceeded the zinc requirements, as such; this material would not be sold as PAS 100 certified compost without further mixing, composting and re-analysis.

Under the PAS certification total mercury content must be less than 1.0 mg/ kg, however all materials (CLO, PAS 100 and PAS 110) had mercury concentrations of less than 0.005 mg/ kg. Cameron *et al*. (2009) identified much higher concentrations ranging between 0.3 and 3.6 mg/ kg, whereas Merrington *et al*. (2010) could not detect mercury in any MBT derived CLO samples indicating the variability in CLO as a result of the particular source waste stream. Veeken and Hamelers (2002) identified the variation in heavy metal concentrations and the

effect of geographical collection sites as well as seasonal variations, which can influence the final waste stream composition.

Available and water soluble heavy metal extractions (section 4.1) showed similar results, with greater concentrations of copper, lead and zinc present in the ROWs compared to metals such as chromium, cadmium and nickel. Paradelo *et al*. (2011) also found greater available concentrations of copper, lead and zinc within mixed MSW, which pose greater risk to the environment due to the higher initial concentrations and greater solubility and availability compared to other heavy metals such as chromium and nickel. Bucher and Schenk (2000) found the concentrations of available heavy metals (calcium chloride/ DTPA extraction) such as copper had good correlation with the copper uptake and accumulation with plant material. Copper and zinc are essential nutrients for plant growth and high accumulation of copper and zinc within *S. viminalis* and *E. nitens* presented in sections 4.2, 5.3 and 6.3 may be linked to the higher water soluble and available concentrations present within the ROWs.

The modified BCR sequential extraction was conducted to identify the heavy metal concentrations within the exchangeable, reducible, oxidisable and residual fractions. Farrel and Jones (2009) used the same modified BCR method to investigate the heavy metal fractions within MSW derived compost during the degradation process as discussed in Chapter 2. This study found that zinc was predominantly present within the exchangeable and reducible fractions this showed zinc was fairly available compared to chromium and nickel that were strongly bound within residual fractions, similar binding patterns were also seen by Paradelo *et al*. (2011). Lead was mainly present within the reducible fraction bound to iron and manganese oxides. The mobility of lead within the reducible fraction can be influenced by redox conditions, as iron and manganese oxides can become terminal electrons releasing the bound metals in anaerobic conditions (Vink *et al.,* 2010). Lead was also bound within the residual fraction of CLO samples only, suggesting differences within the initial material or the decomposition process influencing the binding of lead to the residual organic matter (Smith, 2009).

Copper showed differences in partitioning between aerobic and anaerobic processes. Copper was mostly present in the oxidisable and residual fractions for

aerobically treated materials (PAS 100 and CLO) consistent with previous studies as copper has a strong affiliation with organic matter (Paradelo *et al.*, 2011; Farrell and Jones, 2009). Anaerobically treated PAS 110 samples showed copper predominantly present in the reducible fractions indicating a difference in copper binding to iron and manganese oxides. The results indicated copper has the potential to pose a greater risk to the environment from anaerobically digested PAS 110 material compared to PAS 100 and CLO due to greater availability. Shao *et al.* (2013) showed the difference between aerobic and anaerobic processes in the degradation of sludge and found aerobically composted sludge had a greater degree of humification. Humic substances have a high capacity to bind heavy metals due to oxygenated reactive functional groups, which can influence the different binding of heavy metals. The degradation efficiency of organic matter was greater within the aerobic processes due to the rate of hydrolysis and the degradation of proteins compared to the anaerobic conditions. Shao *et al.* (2013) found the anaerobic processes were limited which affected the humification of organic matter. The differences in heavy metal fractions between the composted (PAS 100 and CLO) and anaerobic digestion (PAS 110) maybe a result of different rates of humification and therefore influencing the binding potential within different fractions.

Batch leaching analysis was conducted on each material to identify the soluble components of the waste materials assuming equilibrium conditions were reached within 24 hours. All materials were tested, however the PAS 110 samples were unsuitable for analysis as a result of the high absorbance of the material and insufficient effluent collection. The concentration of zinc leached from the PAS 100 samples exceed the CLO zinc concentration even though the total zinc in PAS 100 was lower than the CLO. This suggests the PAS 100 zinc is very mobile and available, compared to the CLO zinc binding. It is difficult to compare batch leacing analysis to water soluble leaching and the exchangeable fraction of sequesntial extraction due to the different solvents (deionioned water or slightly acidic solvents) used and the different periods of agitation and contact time which influence the equilibrium of reactions in solution.

When compared to previous batch leaching conducted by Godley *et al.* (2007) source segregated waste and mixed MSW compost showed similar heavy metal concentrations identified within the PAS 100 compost and CLO samples

respectively collected as part of this study. There was greater variability between Godley's samples as a result of sample collection from different facilities and changes in the waste collection areas. Samples collected for this investigation were from the same facilities and geographical area but were collected at different times of the year, which may have influenced the characteristics of waste and variability within CLO to a lesser degree than between facilities and collection areas as seen by Godley *et al*. (2007).

The particle size distribution analysis identified differences between particle size fractions in the ROWs due to the pre-treatment and processing methods. This was particularly evident with PAS 110 material that was shredded before treatment to ensure a pumpable mixture, which showed greater consistency between size fractions. Composted material had a greater percentage of material associated with slightly larger fractions due to the presence of woody materials and contaminants such as nails and plastic within the CLO. Additional shredding and screening of output does occur at some sites although it is not a requirement. Higher heavy metal concentrations were identified in the finer fractions of the PAS 100 compost and CLO samples; this was also identified by Bardos (2004) and Smith (2009). Zhao *et al*. (2012) found the greatest heavy metals were associated with particles of less than 0.8 mm, whereas greater nutrient value was found in particles of greater than 0.8 mm suggesting compost quality could be improved with greater screening and the removal of the fines. When the mass of heavy metals was calculated for each fraction generally higher masses were associated with fines due to the higher concentrations as well as some larger fractions due to the higher mass of materials. The heavy metal content of each particle fraction was anaysised use XRF and total material concentrations were calculated and compared to the total aqua regia digestion values. The comparison of heavy metal analysis indicated some over estimation in heavy metal concentriaton from the XRF anlaysis.

The leaching analyses (section 4.1) conducted on all materials identified consistently lower available metals concentrations compared to the total concentrations used to determine the risk of ROWs when applied to land. The long term release of heavy metals within the organically bound and residual fractions needs to be assessed to identify the point of release to ensure the use of ROWs are sustainable and do not impact the environment negatively over time. Numerous

studies have also identified the variability within ROW materials; particularly MSW derived CLO. A land restoration end use standard developed Organic Recycling Group (2013) (section 1.2.2) uses fairly high limits to allow for high variability within the final product. The standard ensures the use of CLO in restoration projects are not restricted, however high heavy metal limits do not encourage producers to develop a high quality product and the overall heavy metal input to land should be limited where possible. Potential improvements to the processing procedures such as the removal of fines may improve the overall total concentrations of heavy metals, reduce soluble salt concentrations and improve soil structure to reduce the risk of water logging, which in turn could aid consistency between facilities and quality of final CLO.

7.2   GROWTH TRIALS

To investigate the use of ROWs as nutrient sources for energy crops three growth trials were developed. Preliminary growth trials investigated the impact of ROWs on the growth of three different tree species (*S. viminalis, E. nitens, P. tomentosa*). From this further pot trials were conducted to identify the optimum total nitrogen application rate for *S. viminalis* and *E. nitens* and the effect on the leachate composition. Finally, to assess the effect of different environmental conditions column growth trials were conducted in saturated and unsaturated conditions. Individual results from each growth trial and comparison between trials are discussed based on the biomass production, leachate composition and heavy metal phytoremediation.

7.2.1   THE IMPACT OF ROW ON BIOMASS PRODUCTION

The biomass production of each tree was assessed at the end of each growth trial in order to understand the effect of different experimental conditions on tree growth and potential yield production. It was assumed the moisture content of each plant was constant from the start to evaluate the percentage change in dry biomass.

7.2.1.1   PRELIMINARY GROWTH TRIALS

The preliminary trials identified high rates of plant demise and negative changes in dry biomass for trees grown in PAS 100 and CLO. This was attributed to the excessive soluble salts and high EC values measured in PAS 100 and CLO samples.

The ROWs were mixed with perlite, an inert medium, and as a result there were limited binding sites available for excess soluble salts from the ROWs. The high EC values (> 2 mS/ cm) associated with the ROWs were therefore exacerbated by using an inert medium which impacted upon plant survival by restricting water availability to roots and inhibiting soil biological activity (Cameron *et al*., 2009; Hargreaves *et al*., 2008). Literature identifies EC values as low as 1.4mS/cm can be detrimental to plant growth, however it was important to understand the tolerance point of *S.viminalis, E. nitens* and *P. tomentosa* as part of this study. The use of perlite identified the direct impacts of the ROWs on plant growth; however it did not simulate the natural environmental. ROWs will be used as soil amendments and would be mixed with natural soils with a greater adsorption ability and CEC, reducing the effect of the soluble ions.

The weekly observations gave a greater indication of the differences between species and the potential of trees to survive in ROWs. All tree species grown in PAS 110 and controls pots showed positive biomass production, although there was high variability between replicate pots and differences between tree species. *S. viminalis* showed rapid growth within the PAS 110 trials compared to the CLO trials, although a pest (Red spider mite) was identified on all healthy plants, increasing leaf loss and may have affected the end biomass values. *E. nitens* showed initial signs of stress after planting (red leaves), but showed fairly consistent growth during the trial. *P. tomemtosa* showed premature demise as the above ground material dropped leaves and appeared brown. During pot dissection at the end of the growth period, root material appeared white and healthy suggesting some evidence of potential for recovery.

As shown in section 4.2 there were lower or negative percentage changes in dry biomass of trees grown in CLO and PAS 100 trials as a result of the high EC values and limiting binding sites. The ability of species to survive varied and factors other than the ROWs, such as the pests could have affected the plant survival within the preliminary growth trials. *S. viminalis* and *P. tomentosa* showed greater resilience and potential to recovery compared to *E. nitens* which did not show any signs of recovery once the plant had begun to demise within the CLO and PAS 100 pots. *Salix* spp. are traditional species used in the UK, suitable to many different climatic environments (Nixon *et al*., 2001). *Paulownia* spp. are also suitable in different

climates and can be grown in altitudes of up to 2000 m and latitudes 40° north and 40° south (Woods, 2008). Although *Eucalyptus* spp. are found in many different climates, there is variation between *Eucalyptus* clones and some are better adapted to cooler or wetter environments than others (Leslie *et al*., 2012). A *Eucalyptus* clone should be selected on the ability to survive in the UK climate while being able to produce high biomass yields.

7.2.1.2 OPTIMUM NITROGEN APPLICATION

The optimum nitrogen application growth trials used Kettering Loam as a mixing medium rather than perlite. Kettering Loam had greater binding potential than perlite to reduce the effect of excess soluble ions on plant growth as seen in the preliminary growth trials. The variables investigated were also focused to *S. viminalis* and *E. nitens* grown with CLO and PAS 110 at 6 different total nitrogen application rates (equivalent to 0, 250, 1000, 3000, 6000 and 10,000 kg N / Ha).

Biomass production after 12 weeks of growth identified *S. viminalis* and *E. nitens* had optimum application rates of 10,000 and 1000 kg N / Ha respectively for PAS 110 digestate. CLO had an optimum application rate of 3000 kg N / Ha for both species, supporting the preliminary findings that the low absorbance capacity of perlite affected the growth and survival of both species as all plants had previously died at application rates of > 1500 kg N / Ha. *S. viminalis* and *E. nitens* survived at higher application rates (≥ 6000 kg N / Ha) but the biomass production was reduced compared to lower application rates (≤ 3000 kg N / Ha) this may be attributed to the concentrations of heavy metals or soluble salts reaching phytotoxic levels.

Biomass production was significantly affected by the tree species rather than the ROW and application rate. *S. viminalis* have been shown to grow up to 4 meters within the first year with crop rotations every three years (Dawson, 2007), and *E. nitens* up to 12 meters within 4 years with harvesting taking place between 8 and 20 years once the plant stem has reached 15 cm in diameter (Mckay, 2011). Comparison between species biomass production within the pot trials is limited due to the naturally slower growth rate of *E. nitens* and the short experimental periods. *E. nitens* are commonly used for SRF, which have longer growing periods whereas *S. viminalis* are commonly used in SRC with repeated rotations.

7.2.1.3 COLUMN GROWTH TRIALS

The optimum nitrogen application rate of CLO identified in section 5.1 was used to assess the effect of saturated and unsaturated conditions on *S. viminalis* and *E. nitens* and the potential environmental risk from CLO. Brownfield sites can have poor soil structure and drainage, and although the application of amendments can improve this, sites can be prone to water logging (Nixon *et al.*, 2001). Saturated soils become anaerobic and reducing condition may influence soil solution chemistry and heavy metals mobility. Low oxygen concentrations within the rhizosphere can affect root respiration and the availability of nutrients for uptake by plants. Phytotoxic materials such as sulfides also have the potential to build up in the root zone influencing plant health (Pezeshki and DeLaune, 2012).

From the column growth trials, saturated conditions improved biomass production in *S. viminalis* significantly compared to unsaturated conditions as a result of the naturally high water uptake required by *Salix* spp., as well as the ability of *Salix* spp. to adapt to saturated conditions. Control columns containing perlite in replacement of CLO showed greater average change in biomass production within saturated conditions suggesting CLO had a negative impact upon plant growth compared to the control columns.

Li *et al.* (2006) found *Salix nigra* were able to survive in continuous and partial flooding as a result of aerenchyma formation and rhizosphere oxygenation. Aerenchyma tissue allows the transportation of atmospheric oxygen from leaves to the roots so as to maintain aerobic respiration within the rhizosphere by ROL. The oxygenation of the rhizosphere allows the transformation of reduced compounds such as ammonium to plant available forms such as nitrate. Li *et al.* (2006) found no significant difference in the height and shoot biomass production, although the root biomass was reduced in continuously flooded plants. The above ground biomass production was improved by saturation in this study, however there were noticeable differences in the root depth between saturated and unsaturated columns. Unsaturated *S. viminalis* had much longer root systems (> 30 cm) through the entire column into the drainage layer, whereas *S. viminalis* grown in saturated conditions had much shorter root material concentrated within the top 20 cm. This difference could be a result of saturation affecting root growth as seen by Li *et al.* (2006) or

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due to limited water availability in the unsaturated columns suggesting root growth in search of water. The percentage change in dry biomass production of *S. viminalis* in the unsaturated columns ranged between 132 % - 173 %, similar to biomass production in the optimum nitrogen application trials (3000 kg N / Ha) that ranged between 119 – 174 % suggesting good repeatability between the trials conducted.

E. nitens showed evidence of stress and premature death within the first 3 weeks in saturated and unsaturated conditions grown in CLO and perlite control columns. The optimum application growth trials identified that *E. nitens* were capable of succeeding in CLO at a total nitrogen application rate equivalent to 3000 kg N / Ha suggesting the premature death was a result of other factors. Previous studies have identified *E. camaldulensis* and E. *globulus* seedlings as being able to tolerate waterlogged soils for 40 days (Sena Gomes and Kozlowski, 1980), and *E. nitens* to develop an aerenchymatious root system when saturated for 8 months (Close and Davidson, 2003).

7.2.1.4 EUCALYPTUS POINT OF SATURATION

An additional experiment was designed to investigate if *E. nitens* required roots to develop before saturation in order to survive. *E. nitens* were planted in unsaturated conditions and columns were then saturated on either day 0, after 3 weeks and 6 weeks of unsaturated root development. As seen with the initial saturated *E. nitens* columns (section 7.2.1.3), those plants saturated on day 0 and after 3 weeks unsaturated growth died within 2 - 3 weeks of saturation. Those saturated after 6 weeks of root develop showed slower demise with one plant surviving until the end of the growth trials. The ability to adapt to saturated environments may vary between *Eucalyptus* spp. as Sena Gomes and Kozlowski (1980) observed morphological changes within 10 - 15 days of flooding *E. camaldulensis* and *E. globulus* seedlings however this was not apparent with *E. nitens* in this investigation.

E. nitens that remained unsaturated for 12 weeks had a noticeably lower biomass production when compared to the optimum nitrogen application rate growth trials (section 7.2.1.2) when both had a total nitrogen application rate equivalent to 3000 kg N / Ha. Optimum nitrogen application rate growth trials average biomass production was 39 % compared to 7.1 % in the unsaturated column trials suggesting

other factors may have affected *E. nitens* biomass and premature demise within the column growth trials.

7.2.1.5 BIOMASS ASSESSMENT AND ANALYSIS

During the growth trials potential errors were unavoidably present and may have affected the final biomass values. Deciduous trees such as *S. viminalis* and *P. tomentosa* naturally lost leaf material during the growth trials and could not be accounted for at the end of the growth trial potentially under estimating the biomass production. Although *E. nitens* are evergreen trees and do not naturally loose leaves, leaf material had the potential to dry and become damaged increasing above ground material loss and biomass error.

The percentage change in dry biomass was calucated at the end of each growth trial by assuming the moisture content was constant throughout the growth trials. This method is commonly used by Forest Research and was used throughout the trial to allow consistence and comparison between trials.

The biomass production from all the growth trials represents a very short growth period in optimum temperatures and light conditions and a prediction of biomass production for field trials are limited. In general the biomass production was increased when grown with ROWs compared to control trials. Long term trials would be required to fully understand the biomass yields expected from ROW application and the impact of repeated rotations.

Weih and Nordh (2005) used pot trials to predict field trials with some success, although changes in the growing patterns over time can affect the relationship to pot trial results. The ability to measure and predict biomass using non-destructive methods has been investigated by numerous studies (Nordh and Verwijst, 2004; Hangs *et al*., 2011; Rico-García *et al*., 2009; Lindsey and Bassuk, 1992). This study also tried to improve the collection of weekly plant growth data by using a plant image analysis tool tested during the preliminary growth trials. Plant height was calculated from three photos and showed strong correlation to the manual height measurements. The plant image analysis tool also estimated the volume of the plant, which was compared to manual measurements of the plant volume at the end of the growth trials. Weak correlation was identified between the program estimated and

manually measured volumes. Plant volumes may have been under or over estimated as a result of the program design and methods used to calculate the volume as well as errors in the collection of manual volume measurements. The program design and methods were fairly simple assuming a cylindrical shape from the three photos taken, if a photo was unsuitable for analysis, only two photos were used and one photo repeated. This may have affected the results by under or over estimating the plant volume. Manual volume measurements from the displacement of water may have incurred errors as a result of plant material touching the container side causing plant frictional resistance underestimating plant volume and human error. Harrington *et al*. (1994) used volume displacement to measure new root production in seedlings. Volume displacement showed strong correlation ($R^2 > 0.9$) when compared to the dry weight of seedlings. When the total dry biomass of the leaf and stem material from the preliminary growth trials were compared to the manual volumes there was evidence of correlation ($R^2 = 0.76$) whereas when dry biomass was compared to plant image analysis volume estimation there was no correlation ($R^2 = 0.01$) (Appendix 6). This suggests the program design and assumptions requires greater accuracy in calculating the volume from the plant images to generate reliable results.

7.2.2 *The potential risk from PAS 110 and CLO leachate*

Leachate generated as a result of watering the plants was collected during the optimum and column growth trials for pH, EC, metal cations, anions and ammonium analysis. The release of heavy metals and pollutants within leachate pose a risk to the wider environment by reaching watercourses and ground water. It is essential to understand the mechanisms and processes that generate leachate in order to protect human and environmental health.

7.2.2.1 Optimum application

Leachate analysis identified pH values ranging between 6.5 and 9 in both *S. viminalis* and *E. nitens* grown in PAS 110 and CLO. All pots were watered with deionised water with an average pH of 5.64 suggesting both ROWs had buffering potential as a result of the high humus content (Garcia-Gil *et al*., 2004). Higher pH values were generally associated with the higher application rates with higher ratios of ROW and organic material compared to Kettering Loam. Increases in soil pH

have been attributed to the production of OH⁻ ions by ligand exchange and the introduction of large amounts of basic cations such as calcium and magnesium (Mkhabela and Warman, 2005). *E. nitens* grown in PAS 110 and CLO at application rates equilavent to 250kg N/ Ha, 1000 kg N/ Ha and control pots showed pH values peaking in week 5. This is not evident in the *S. viminalis* suggesting plant specific changes. Changes in soil pore water pH can be influenced by a number of factors as previously discussed in Chapter 2, however the increases in *E. nitens* at lower applications rates is unknown. The plant health and root interaction may influence the soil pore water as *E. nitens* grown at lower application rates had healthy plant growth compared to the higher applications that died prematurely.

CLO leachate had overall higher EC values than PAS 110 leachate with higher values generally associated with the higher application rates as a result of the original mixed MSW stream and the ratios of ROWs to Kettering Loam. *E. nitens* ground in PAS 110 at an application rate of 250 kg N/ Ha and 1000 kg N/ Ha showed higher EC values compared to the higher application rates. The peaks are associated with high Ca and Mg ions release which may be linked to the higher perlite application rates. *S. viminalis* and *E. nitens* were not visibly affected by excess soluble salts as seen in the preliminary trials this maybe a result of perlite being replaced with Kettering Loam. The gradual decreases and rises of EC evident in both ROWs are consistent with increases in soluble cations and anions. The decrease suggests natural leaching and losses, although the increases evident in CLO *S. viminalis* and PAS 110 *E. nitens* suggest changes in the soil system causing the release of ions.

The concentrations of soluble heavy metal cations were compared to water quality limits for aquatic life (Environment Agency, 2013). Greater concentrations of heavy metals were leached from the CLO than PAS 110 as a result of the source waste stream as also seen by Cameron *et al*. (2009), Farrell *et al*. (2010) and Hargreaves *et al*. (2008).

Heavy metal concentrations within the PAS 110 leachate samples show irregular patterns as a result of the low concentrations and the sensitivity of analytical equipment reaching its detection limits. The ICP-OES detection limits can be found

in Appendix 6. Clearer patterns are evident in the concentration of soluble cations such as calcium, magnesium and sodium. Peaks within calcium and magnesium are consistent with the EC peaks (*E. nitens* 250 kg N/ Ha) and pH increases (*E. nitens* PAS 110 and CLO, 1000 kg N/ Ha) suggesting potential buffering within the pots releasing calcium and magnesium ions (Flyhammar and Håkansson, 1999; Tamir *et al*., 2013).

Soluble metal cation concentrations in CLO leachate samples showed noticeable patterns, with higher concentrations associated with higher applications and overall decreases in concentrations suggesting ions were leached as seen in the EC analysis. Leachate collected from trial pots with application rates of $\geq$ 3000 kg N / Ha exceeded the Environment Agency (2013) water quality levels for aquatic life limits for chromium, copper and nickel and would therefore have the potential to pollute ground and surface water on uncontained sites.

In CLO pots there was a clear increase in cation concentrations between weeks 10 and 12, which are also consistent with increases with the EC and soluble anions. The increase in soluble ions may be a result of metal sulfides being oxided, releasing metal ions, hydrogen ions and sulfate (Bozkurts *et al*., 2000). The release of hydrogen ions can lower pH levels, however this maybe buffered by the high humus content of the higher application rates of CLO.

Flyhammar and Håkansson (1999) investigated the release of heavy metals in stabilised MSW under aerated and anaerobic conditions. Oxidation of the MSW resulted in increases in calcium, magnesium, manganese and sulfate concentrations, with a drop in pH from 9.0 to 8.0. The increased leaching of sulfate was attributed to the oxidation of sulfide phases and increased concentrations of calcium, magnesium and manganese indicated the dissolution of carbonate solids and buffering against proton addition to the solution. The neutral to alkaline pH limited the heavy metal concentrations in the leachate (zinc and cadmium) however under acidic conditions 30% of the total heavy metals contents could be dissolved (Flyhammar and Håkansson, 1999). The increased solubility of calcium and magnesium was also evident in this study as well as increases in sodium, chloride and nitrate concentrations, all associated with EC of a solution.

Increased metal and sulfate leaching, possibly due to sulfide oxidation, was only evident in the *S.viminalis,* CLO pot trials. This could be a result of the poor health or premature death of *E. nitens* at applications rates of greater than 6000 kg N / Ha limiting root activity or a *Salix spp.* specific influence. Vervaeke *et al.* (2004) investigated the effects of *S. viminalis* on metal extractability in anoxic contaminated dredged sediment. The extractability of cadmium, zinc, and copper increased in the root zone of *S. viminalis* compared to the bulk soil as a result of increased oxidation at the root zone by aerenchyma tissue and ROL. Long term field trials indicated lower metal concentrations in the root zone suggesting metal leaching over 2 years. This was linked to the increased mobility of metals, as well as a general improved soil structure from the application of an organic amendment, reducing compaction, increasing soil flow pathways and allowing metal leaching vertically though the profile (Vervaeke *et al.*, 2004). The ability for plant roots to release oxygen supports the theory of oxidation processes and changes in the solubility of cations and anions over time presented within this study.

The rate of mineralisation, nitrification and denitrification can influence the water-soluble concentrations of nitrate and ammonium. PAS 110 pots (*E. nitens* and *S. viminalis*) showed higher nitrate concentrations; particularly the lower application rates ($\leq$ 1000 kg N / Ha) compared to the CLO pots in which there were variable nitrate concentrations during the course of the growth trial. The majority of PAS 110 nitrogen forms are present in available forms compared to composted material (WRAP, 2011), which may explain the greater concentrations of soluble nitrogen forms within the leachate.

Nitrate leaching within the PAS 110 and CLO pots showed peaks in week 4 for both species indicating excess nitrate concentrations that may be attributed to the removal of the double planting (week 3) resulting in reduced nitrate uptake, thereby leading to an increase in nitrate leaching. Control and 250 kg N / Ha (PAS 110) pots for *E. nitens* also peaked in week 8; this cannot be attributed to the removal of double planting however may simply be excess nitrate not required by the individual plants. Ammonium concentrations decreased and remained steady from PAS 110 pots suggesting overall lower concentrations and sufficient binding capacity within the PAS 110/ Kettering Loam mixes (Cameron *et al.,* 2009).

Initial concentrations of ammonium were greater within the CLO pots, particularly higher application rates, indicating negative correlation to the concentrations of nitrate. Ammonium leaching suggests low nitrification within the CLO samples after windrow and bag storage as well as limited binding sites (Cameron *et al.,* 2009) due to greater CLO to Kettering Loam ratios within the higher application pots (≥ 3000 kg N / Ha). Nitrate concentrations increased in week 12 for application rates ≥ 3000 kg N / Ha for both species, with greater concentrations associated with *S. viminalis*. This may be attributed to nitrogen initially being immobilised in the higher application rates pots, limiting nitrification and nitrate concentrations in leachate compared to lower application rates and control pots. Increased nitrate concentrations (week 12) at the higher applications suggest stabilisation and nitrification, which is consistent with oxidation evidence present from the increased sulfate concentrations previously discussed. Similar patterns were seen Burgos *et al.* (2006) during incubation trials where nitrate was initially immobilised after MSW compost amendment, after 16 weeks nitrate leaching concentrations increased once stabilisation had occurred.

The high solubility of nitrate poses a potential risk to the wider environment at all application rates for both PAS 110 and CLO as concentrations of > 40 mg/ l are considered high by the Environment Agency's nutrient classification system (Environment Agency, 2013).

The leachate collected from the pot trials indicated the effect of different application rates on the quality of leachate. The water-soluble fraction of heavy metals decreased after the initial application of ROWs soil amendment. The application of organic amendments have previously been shown to reduce metal mobility by adsorption, complexation and precipitation processes (Hee Park *et al*., 2011; Farrell *et al*., 2010), however changes in soil chemistry can influence the solubility of salts and increase heavy metal mobility. Although heavy metal concentrations in the leachate were greater for higher application rates, the nitrate concentrations pose greater risk in the lower application rates for both materials irrespective of the source waste stream.

7.2.2.2 COLUMN GROWTH TRIALS

Leachate samples were collected from saturated and unsaturated columns containing perlite/ Kettering loam and CLO/ Kettering Loam mixes and compared to the average cumulative L: S ratio in order to compare the effect of unsaturated columns against saturated columns. Leachate pH values were similar between perlite controls and CLO columns although lower pH values were generally associated with the saturated columns. Anaerobic soils will naturally reach neutral pH values as a result of either reduction reactions consuming H^+ ions in acidic soils or the presence of carbonates buffering the solution (Kashem and Singh, 2001). CLO leachate had significantly greater EC values as a result of the source waste stream compared to inert perlite/ Kettering Loam mixes. The gradual EC decreases in unsaturated CLO columns represent the leaching and loss of soluble ions through the soil profile. In order to maintain the anaerobic conditions within the saturated column, water flow was restricted and the water level maintained throughout the growth trial. The limited water flow through the saturated columns may have reduced the leaching of soluble ion as greater EC values were associated with the saturated columns. Higher EC values were generally seen in the base samples as a result ions leaching within the columns from the top to the base of the column as seen by Farrell *et al*. (2010).

Leachate concentrations of cations, anions and ammonium were plotted based on the total average cumulative masses leached over the 12 weeks from the Rhizon and base sampling points. Control columns containing perlite and Kettering loam had lower masses leached compared to the CLO columns as a result of the inert perlite compared to the mixed MSW stream of CLO. There was generally a rapid release of cations (excluding lead) and anions when the L: S ratio was < 40, before the rate of leaching decreased. Ammonium was leached steadily over time whereas lead did not leach until the L: S ratio was >30.

There was a notable difference between the total cumulative masses of metal cation leached between unsaturated and saturated conditions. The L: S ratios were fairly similar at the beginning of the growth trial and rapid flushing of metals and ions were evident in all columns. The cumulative masses of some metals (Cr, Ni, Fe) continued to increase in the saturated columns, whereas unsaturated columns

leveled off even with increasing L: S ratios and vice versa for metals such as copper. The mobility and release of ions can be affected by pH, redox potential, contact time and binding processes (Bozkurt *et al*, 2000). Acidic soil pH values can increase the mobility of metal cations, however the high humus content and buffering capacity ensured the pH values were fairly neutral implying changes in the solubility are not pH related. Redox conditions can influence the oxidation state of some ions and in turn affect the solubility and toxicity to the wider environment. For example chromium III (+3) is non-toxic and fairly immobile in soils compared to chromium VI (+6), which is readily soluble and toxic. The transformation of ions is dependent upon factors such organic content, biological activity and environmental conditions (Mclean and Beldsoe, 1992; Ma and Dong, 2004).

Chromium, nickel, iron, calcium, sodium and magnesium showed greater cumulative leached masses from the saturated columns compared to the unsaturated columns. This suggests the redox conditions may have influence the ion valancy and in turn the solubility and risk to the wider environment. During the degradation of organic material in anaerobic conditions, nitrate, iron and manganese oxides and sulfate are alternative terminal electron acceptors in replacement of oxygen (Vink *et al*., 2010). Within the saturated columns nitrate availability reduced significantly within the first few days, whereas iron concentrations increased indicating iron was being reduced from insoluble iron III to soluble iron II within the saturated columns. The reduction of iron oxides could potentially release previously bound ferric hydroxide metals into solution (Bozkurts *et al*., 2000; Ma and Dong, 2004; Vink *et al*., 2010). After 4 weeks of saturation, Rhizon samplers had an orange deposit develop as shown in Figure 6.5. This was potentially the precipitation of iron III due to oxidation of iron II during sampling. This was only present in CLO and perlite saturated columns. During the dissection of the columns at the end of the growth trial the base of saturated columns showed evidence of orange and black precipitants within the perlite filter layer as seen in Figure 7.1. The orange precipitate is potentially iron III deposits due to oxidation near the sampling point as seen in the Rhizon samplers and the black deposit may be iron sulfide precipitate due to anaerobic conditions (Hansen *et al*., 2011)

Heavy metals bound to iron and manganese oxides can become mobile in reducing conditions; the metal ions have the potential to bind to DOM and remain fairly

mobile. The reaction with DOM may occur before reducing conditions reduce metals to form insoluble sulfide precipitates therefore metals can remain mobile in anaerobic conditions (Vink *et al*., 2010). However iron and manganese oxide reduction can occur simultaneously to sulfate reduction (Vink *et al*., 2010). Sulfate concentrations showed similar patterns of rapid leaching before leveling off within the saturated columns suggesting either the leaching of the majority of sulfate ions or the majority of soluble sulfate ions were reduced to sulfides within 7 days. Sulfides are capable of immobilising soluble metals such as iron to form ferrous sulfides which can precipitate as black sludge deposits similar to that seen in the base of the saturated columns (Figure 7.1) (Hansen *et al*., 2011). The black deposits could potentially be mold that has grown along the edge of the columns rather than ferrous sulfides, however unsaturated columns were also damp environments, ideal for mold growth but did not show any evidence of black deposits. Kashem and Singh (2001) found similar results, after flooding high concentrations of metals were initially leached due to the dissolution of iron and manganese oxides, however after sulfate reduction, metal sulfides reduced the solubility of metals and concentrations leveled off after 20 days of saturation as seen in this investigation.
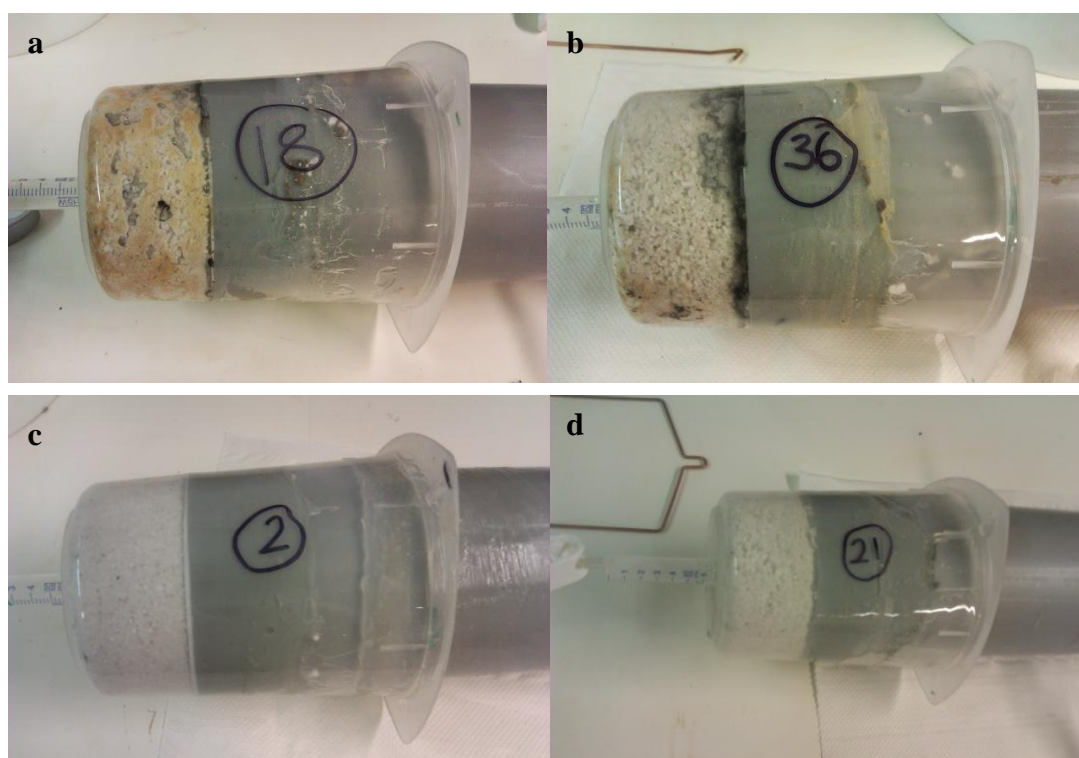

Figure 7.1 Base filter layers. a, b, Saturated columns with orange and black precipitate. c, d, unsaturated columns with 'clean' white perlite layer.

Lead leaching did not occur until the L: S ratio was > 30 within the CLO columns only. The solubility of iron and lead are linked as a result of similar affinities to complex with ligands and the absorption of lead to ferrous hydroxides (Dong *et al*., 2000). During the reduction of ferrous hydroxides lead would be released, increasing solubility (Dong *et al*., 2000), however lead was not leached until after day 40 once the majority of iron leaching had occurred. Interestingly the majority of lead was identified as being bound to the reducible fraction from sequential extraction (Chapter 4 and section 7.1) and therefore high leaching of lead would be expected suggesting other factors affecting lead mobility.

Copper was the only cation to show greater masses leached from the unsaturated columns than the saturated columns. Copper forms strong bonds with organic matter as shown from the sequential extraction in Chapter 4 where the majority of copper extracted was within the residual or oxidisable fraction. Previous studies have identified the solubility of copper in relation to its binding with soluble organic matter (van Herwijnen *et al*., 2007) or increased metal leaching with soluble salts such as chloride (Farrell *et al*., 2010). The degradation rate of organic matter within the unsaturated columns may have influence the amount of copper released and the greater availability compared to saturated columns. Copper leaching from the saturated columns was reduced within 7 days, which may also be linked to sulfide binding and rapid immobilisation (Fulda *et al*., 2013).

Zinc is the only cation that does not follow any particular pattern between unsaturated and saturated conditions as seen with other cations, anions and ammonium analysis. After an initial pulse of zinc from all columns including the unsaturated columns, concentrations leveled off excluding the saturated *S. viminalis*. Saturated *S. viminalis* trials showed greater leaching of zinc than the plant free saturated controls implying *S. viminalis* affected its solubility. Considering the high initial concentrations of zinc within CLO and the fractionation of zinc within the reducible and exchangeable fractions, zinc leaching is low potentially indicating stronger binding and immobilisation by other factors. The mobility of zinc was not influenced by redox conditions and changes in the oxidation state. The binding capacity of humic material, as well as available sulfide and iron/manganese oxide binding has a greater influence on the mobility of zinc (Du Laing *et al*., 2009).

The mobility of other cations such calcium, sodium and magnesium followed similar patterns to the heavy metals with high initial leaching rates before leveling off with greater cumulative masses leached from saturated columns compared to unsaturated columns. Sodium and magnesium masses were much greater within the CLO/ Kettering Loam columns compared to the perlite/ Kettering Loam columns. However calcium masses were similar suggesting a substantial amount of calcium ions were available from the Kettering loam and that CLO isn't a significant source of calcium. Calcium release was greater from the saturated CLO/ Kettering Loam columns compared to the unsaturated CLO/ Kettering Loam columns, which may be linked to the soil solution buffering in reducing conditions increasing the concentration of available calcium ions (Du Laing *et al*., 2009). The perlite/ Kettering Loam saturated columns had lower calcium leaching compared to the unsaturated perlite/ Kettering Loam indicating potential differences between the perlite and CLO in terms of buffering and calcium ion release.

As expected nitrate concentrations were greater within the unsaturated columns as ammonium could be oxidised to nitrate compared to higher ammonium concentrations within the saturated columns due to limited oxygen restricting the nitrification of ammonium to nitrate. The growth of *S. viminalis* did not appear to be affected by the presence of ammonium as the main source of nitrogen, although it is unknown if ammonium was converted to nitrate within the rhizosphere as a result of ROL (Cronk and Fennessy, 2001). Wetland plants can develop aerenchyma tissue to transport oxygen from aerial plant tissue to roots to allow respiration and oxidation of the rhizosphere zone (Li *et al*., 2006; Yang *et al*., 2010). The oxidation of the rhizosphere has the potential to aid the uptake of essential and non-essential nutrients that may be unavailable within the bulk reduced soil. For example, zinc, nickel and chromium leaching was greater within the saturated *S. viminalis* columns compared to the plant free and *E. nitens* pots, which may be attributed to ROL from the living *S. viminalis* oxidising complexed metals increasing metal mobility within the rhizosphere.

The continuous flooding of the saturated columns identified greater cumulative masses leached compared to the unsaturated columns by the end of the growth trial. As discussed this is linked to the reduced conditions, however the release of contaminants from the CLO can also be linked to surface area contact and

equilibrium time (Rennert and Rinklebe, 2010). Leachate collected from the saturated column was continually in contact with the CLO mix for longer periods allowing reaction equilibriums to be reached. The unsaturated column may have developed preferential flow paths (Farrell *et al*., 2010), limiting the contact time and surface area with contaminants reducing the masses of contaminants leached during the growth trial.

The column trials identified the impact of saturation of CLO amended soils and the general increase in leaching of many metal cations, anions and ammonium. Although the leaching reduced after approximately 20 days (L: S ratio < 40) the potential for brownfield sites to become saturated will affect the risk to the wider environment. Although the specific speciation of ions and mobility are unknown in this study, predictions can be made concerning the binding with sulfides reducing the mobility of heavy metals. As seen in the optimum nitrogen application rate growth trials when metal sulfides are oxidised, heavy metal mobility increases. There is potential for cycles of metal release and metal immobilisation to occur as soils become saturated and unsaturated during seasonal changes with water levels.

7.2.2.3 Eucalyptus Point of Saturation

As a result of the premature death of *E. nitens* an additional experiment was conducted to investigate if root development of *E. nitens* was required before saturation. The pH/EC and the cumulative masses were compared to the cumulative L: S ratio of each column. The cumulative L: S ratios were lower during this additional trials because the unsaturated columns were not initlaly flushed through with water as seen in the column growth trials in section 7.2.2.2. The L: S ratio represents the rapid release seen in 7.2.2.2 in saturated and unsaturated columns.

As seen in the previous growth trials pH values were between neutral and slightly alkaline, although the unsaturated columns did show slightly higher pH values compared to the saturated columns which naturally became neutral due to H^+ ions being consumed by reducing reactions or the presence of carbonates buffering the solution as previously discussed (Section 7.2.2.2). Leachate EC values all gradually decreased over time due to soluble salts leaching through the columns. There are notable drops in EC values after saturation as a result of the increased L: S ratio Mand leaching to the base of the column as seen by Farrell *et al*. (2010).

Overall greater masses of heavy metals were leached from the columns saturated day 0, excluding copper and zinc as seen in section 7.2.2.2. The changes in metal mobility once saturated maybe linked to the anaerobic conditions, reducing iron oxides releasing soluble iron II ions and bound metals as previously seen in section 7.2.2.2. There was little evidence of metal leaching reducing over time as previously identified; instead there was a fairly steady constant leaching over time. Sulfate levels decreased once columns were saturated, particularly evident in the 6 week columns compared to the unsaturated column. Sulfate reduction to sulfide is potentially reducing the metal mobility, particular metals such as copper and zinc. However concentrations of iron are lower than previously seen in section 7.2.2.2 and did not appear to decrease within the saturated columns over time.

There was little difference in metal leaching between columns saturated after 3 and 6 weeks for metals such as chromium, nickel, lead and iron. For copper, calcium, sodium and magnesium there was evidence of greater concentrations leaching from unsaturated columns followed by those saturated after 6 weeks. Greater leaching of heavy metals was previously seen in the saturated columns (section 7.2.2.2). The potential changes during the aerobic period before saturation may have influenced the mobility of metals once saturated. The degradation of organic matter differs between aerobic and anaerobic conditions as a result of different micro-organisms and chemical processes (McLatchey and Reddy, 1998). The difference in rate of degradation between aerobic and anaerobic conditions can vary, with factors such as composition of organic matter, environmental conditions and rate of mineralisation all influencing the degradation rate (Dauwe *et al.,* 2001). Columns saturated after 6 weeks of aerobic conditions may have achieved different rates of degradation, and once saturated had different availability and mobility of some elements.

As seen in the column growth trial (section 7.2.2.2) there was a delayed release of lead (L: S ratio > 4). Lead has a low solubility within aerobic soils and its slow release imply it is still relatively immobile in anaerobic conditions, even though the sequential extraction procedure identified the majority of lead associated with the reducible fraction. It has been suggested that the solubility of lead in anaerobic conditions is related to iron II solubility as both have a high affinity to bind with ligands. Dong *et al.* (2000) found that lead solubility was related to the ratio of

soluble iron to sorbed iron. The solubility of lead may change over time as a result of competition with other ions, however without understanding of the speciation of ions and binding occurring particularly in the anaerobic columns, interpretation is limited.

Chromium concentrations increased once saturated on day 0 and there was a notable increase in all columns at the end of the growth trials. In reducing conditions toxic chromium (VI) can be reduced to insoluble chromium (III), however mobility appears to increase over time within all columns. The concentrations of chromium are very low and below the minimum water quality limit of 0.005 mg/ l (Environment Agency, 2013), therefore slight increases maybe errors and variations.

Soluble anion analysis showed lowest cumulative masses leached from the columns saturated on day 0. All columns excluding those saturated on day 0, were initially flushed through with water resulting in the initial higher masses leached. There were clear decreases in the concentrations of anions after saturation and a lower leached masses within columns saturated after 3 weeks and 6 weeks compared to the unsaturated columns. This represents a decrease in water flow through the columns, decreasing the leachate concentrations and the change in chemical processes as columns became reduced.

Nitrate leaching decreased once columns were saturated, although only the columns saturated on day 0 show substantial ammonium production. It would be expected that the week 3 columns would begin to show an increase in ammonium concentrations as nitrification was restricted. This may be a result of sufficient binding sites for ammonium, or restricted mineralisation of organic nitrogen and immobilisation within the columns once saturated. There was a gradual release of nitrate after saturation from day 0 and week 3 columns before a steady state was reached after approximately 5 L: S ratio and 7 L: S ratio respectively suggesting some nitrification occurred.

As seen with the initial column growth trials (section 7.2.2.2), *E. nitens* showed a high death rate and an inability to adapt and survive in saturated conditions. As with the initial column trial, without specific speciation of elements such as iron and sulfur as well as concentrations of DOM definitive conclusions are limited.

7.2.3   POTENTIAL FOR ENERGY CROPS TO PHYTOREMEDIATE HEAVY METALS FROM ROW

The ability of the different tree species to uptake and accumulate heavy metals was investigated at the end of each growth trial. Phytoremediation of heavy metals from material such as CLO represents a long-term remediation method with the potential to reduce the over all heavy metal contamination to sites. However heavy metal accumulations varied between species and have the potential to affect yield production. Previous studies have shown the potential of *S. viminalis, E. nitens* and *P. tomentosa* to uptake and accumulate heavy metals (Pulford and Watson, 2003, Rockwood *et al.,* 2004; Kim and Owens, 2010).

7.2.3.1 PRELIMINARY GROWTH TRIALS

The survival rate of trees from the preliminary growth trials varied between the ROWs, which also affected the collection of some plant material for analyses. The growth trials indicated plant uptake of heavy metals, specifically within the CLO would have occurred fairly quickly within the first 3 to 6 weeks due to premature death of all trees grown in CLO. *S. viminalis* had the longest growing period before premature demise after 6 weeks, which may have influenced the heavy metal accumulation as *S. viminalis* had the greatest total heavy metal concentrations compared to *E. nitens* and *P. tomentosa*.

Total heavy metal concentrations within ROWs followed the trend CLO > PAS 100 > PAS 110 and this was reflected in the total heavy metal concentrations identified within plant material. All trees grown in CLO showed higher accumulation rates as a result of greater initial metal concentrations within the CLO material compared to the control and source segregated materials. As well as higher total concentrations, higher available concentrations were also identified within the CLO material compared to the PAS 100 and PAS 110. The total, available, water soluble and sequential extraction for each ROW found zinc was present in the highest concentrations out of all heavy metals analysed with a substantial proportion readily available. Zinc is an essential nutrient taken up by roots with the aid of membrane transport proteins (Lasat, 2001) and therefore high concentrations of available zinc may explain the high uptake and translocation of zinc within all plant material.

Highest metal accumulation was identified within the root material of all tree species, followed by leaf and stem material indicating some translocation of heavy metals. Pulford and Watson (2003) stated that lead, chromium and copper were commonly accumulated within the root material, and cadmium, nickel and zinc were translocated to above ground material. Zinc and copper were mainly present within root material although they were also present within the stem and leaf material similar to Pulford and Watsons (2003) observations. Chromium, nickel and lead, which are non essential nutrients, were translocated by *S. viminalis* and *P. tomentosa* in CLO trials, differing from Pulford and Watson (2003). Hasselgren (1999) found copper concentrations in above ground biomass increased over time as a result of roots becoming saturated with copper. This suggests some metal concentrations may have saturated the root material and been translocated to above ground material. Hasselgren (1999) also found copper concentrations within leaf material increased during autumn suggesting compartmentalisation in preparation for defoliation in order to remove excess nutrients from the plant. There was evidence of leaf loss particularly from the *S. viminalis*, however it could not be determined which tree the leaves had originated from and therefore difficult to assess if leaf loss was due to high metal translocation.

7.2.3.2 OPTIMUM NITROGEN GROWTH TRIALS

The survival rate of both *S. viminalis* and *E. nitens* was greater within the optimum growth trials compared to the preliminary growth trial, which may have influenced the uptake and translocation of metals within the plants.

There was strong correlation between application rate and heavy metal uptake within the CLO pots, with tree species also having a significant effect. The application rate of PAS 110 was only influenced by species and not application rate, potentially as a result of the overall lower heavy metal concentrations and limited bioavailability (Chapter 4). Assareh *et al.* (2008) investigated the response of *Eucalyptus* spp. to copper and zinc and found heavy metal uptake and accumulation correlated with the metal concentrations in the soils as seen in this investigation.

S. viminalis showed greater accumulation of heavy metals compared to *E. nitens*, similar to that seen in the preliminary growth trials. *S. vimnialis* and *E. nitens* accumulated fairly low concentrations of heavy metals when compared to

hyperaccumulating species that are classified as plants that can potentially accumulate over 1000 mg/ kg of copper, chromium, nickel or lead, or over 10000 mg/ kg of zinc (Yoon *et al*., 2006). Although the concentrations accumulated may not be as high as hyperaccumulating species, woody species including *Salix* and *Eucalyptus* species have the potential to remove similar or greater quantities of pollutants as a result of the high biomass production and repeated harvesting (Mughini *et al*, 2013).

The majority of heavy metals were accumulated within the root material although there was translocation to above ground material, particular zinc, copper and chromium. Lead was only present within the root material, as lead is not an essential nutrient, lead can be immobilised within the roots (Pulford and Watson, 2003).

Copper and chromium uptake and accumulation was greater in both tree species and ROWs compared to the preliminary growth trials. This may be attributed to higher biomass production and longer survival rates. Although *E. nitens* 10,000 kg N / Ha died within the first 2 weeks, there was evidence of heavy metal accumulation within the roots, potentially before concentrations became toxic. The ability for plants to acclimatise to different soils is a significant factor affecting survival rate in metal- contaminated soils. Native plants that naturally colonise contaminated soil are likely to have greater survival rates, as well as mature trees which are less sensitive to adverse conditions; however seedlings and cuttings are commonly used to assess tolerance and survival rates (Pulsford and Watson, 2003) consistent with the methods undertaken in this study.

The accumulation of heavy metals within root material with limited translocation to the harvestable biomass has potential to impact upon biomass production in latter rotations. Long term trials and yield production assessments would be required to ensure there is not a negative influence upon biomass production over multiple rotation crops.

7.2.3.3 COLUMN GROWTH TRIALS

The column growth trials showed overall trends similar to those seen in the preliminary and optimum nitrogen growth trials with the majority of heavy metals

accumulated within the root material. The column growth trials showed overall greater accumulation rates followed by the optimum growth trials and then the preliminary growth trials.

Zinc was the predominate metal accumulated within the root, stem and leaf material, followed by copper and chromium, with some lead accumulation within the roots. Statistical analysis identified no significant difference ($p > 0.05$) in heavy metal uptake between saturated and unsaturated conditions even though leachate analysis generally identified greater soluble concentrations within the saturated columns pore water. The accumulation of similar concentrations suggests plant uptake mechanisms were not significantly influenced by the higher soluble concentrations within the soil solution in the first 20 days of saturation.

The ability for plants to tolerate flooding is linked to the ROL and amount of aerenchyma tissue (Yang *et al.*, 2014). The release of oxygen by ROL as previously discussed (section 7.2.2.2) has the potential to oxidise nutrients and phytotoxic compounds (ferrous ion, manganese ion, hydrogen sulfide and methane) within the rhizosphere. There were no obvious differences if the root tissue between those grown in the saturated and unsaturated conditions apart the extent of the roots. It was observed that the plants grown in the saturated columns had shorter root systems compared the long root systems that extended the length of the unsaturated columns. The longer root systems in the unsaturated columns would have had greater contact with the CLO/ Kettering Loam mixes which may have influenced the heavy metal uptake.

Iron uptake by plants however was significantly greater from the saturated columns compared to the unsaturated as a result of greater concentrations of soluble iron from the reduced environmental conditions as discussed in sections 7.2.2.2 and 7.2.2.3. The iron uptake and accumulation was plotted for the column growth trials as a result of the orange precipitate evident within the saturated columns. Yang *et al*. (2014) suggests the oxidation of iron near the roots from the ROL forms an iron oxyhydroxide plaque, which can affect the uptake of metals by acting as a barrier, forming bonds with heavy metals and immobilising them at the roots. Greater ROL from some wetland species have been found to increase iron plaque and metal accumulation within root material and limit translocation from roots to shoots

(Yang *et al.*, 2014). The heavy metal accumulation by the *E. nitens* showed similar patterns to the *S. viminalis* even with premature death of the *E. nitens* in less than 4 weeks suggesting potential rapid uptake within the first few weeks after saturation before phototoxic conditions prevailed.

7.2.3.4 EUCALYPTUS POINT OF SATURATION

The replacement *E. nitens* column growth trial (Chapter 6.6), that investigated the point of saturation and root development on survival rate showed much higher accumulation rates than any previous growth trial. *E. nitens* saturated on day 0 showed much higher metal accumulation within the leaf material, with copper uptake particularly high compared to the optimum and preliminary growth trials. Heavy metal uptake within root material was greatest in the 6 weeks saturation followed by the unsaturated columns, which also have the lower leaf accumulation indicating less translocation, compared to the day 0 saturated columns. As suggested in section 7.2.2.3, variations between the week 6 and the saturated columns maybe linked to the aerobic period before saturation affecting the mobility and uptake of metals as seen with the leached masses.

Copper concentrations within the leachate were lower within the day 0 and 3 weeks saturated columns, potentially as a result of greater uptake within plant material. However as seen with the initial *E. nitens* growth trials, plants died within 2 - 3 weeks of saturation suggesting fast initial uptake or additional factors affecting *E. nitens*. Shukla *et al*. (2011) investigated the heavy metal uptake from tannery sludge of *E. tereticornis* and *E. teretocornis*. Both Eucalyptus species showed potential for accumulating substantial amounts of iron, copper, manganese and zinc. Although *E. nitens* showed high concentrations of accumulation, the survival rate of *E. nitens* has the potential to be substantially effect the growth of biomass and yield production.

Shukla *et al*. (2011) found after 3 months of growth, copper concentrations were less than 10 mg/ kg dry weight within root and leaf material, compared to over 1300 mg/ kg (day 0 saturation) and nearly 400 mg/ kg (No saturation) within leaf material identified in this investigation (section 6.6). There are limited studies into *Eucalyptus* phytoremediation abilities, with scarce data on *E. nitens* specifically in the UK. *Eucalyptus* spp. are grown globally and have adapted to many different

environments due to the high genetic variability, which in turn has resulted in diverse phytoremediation abilities (Mughini *et al.*, 2013).

7.3 SUMMARY

This chapter has interpreted and discussed the results of ROW characterisation and three growth trials conducted to address the aims and objectives of this thesis.

Characterisations of the ROWs have shown they are valuable resources containing high organic matter and a source of slow release nutrients. However the source of biodegradable material can affect the quality of the final product with a clear difference between source segregated (PAS 100/ 110) and mixed waste CLO heavy metal concentrations.

All ROWs were sufficiently decomposed and matured from either aerobic or anaerobic treatments with some slight differences in the heavy metal binding as a result of the decomposition processes. Aerobic composting had higher concentrations of heavy metals within the residual fraction compared to the anaerobically digested PAS 110, which may influence heavy metal mobility.

CLO samples were all below the maximum heavy metal quality limits required under the land restoration end use standards (ORG, 2013) and exceeded the PAS quality limits for copper, lead and zinc. The quality limits for CLO use in restoration projects provides guidance and clarity for producers and users to ensure restoration projects can be completed (ORG, 2013). High heavy metal limits allow for the high variability commonly identified within mixed MSW CLO, however the CLO samples investigated within this thesis identified heavy metal concentrations closer to the PAS limits than the restoration limits. Reducing the CLO restoration limits will encourage producers to improve processes to generate higher quality CLO. Bardos *et al.* (2007) identified that presently the engineering processes are determining the quality of CLO whereas the required quality of CLO should determine the engineering processes. For example particle size analysis of CLO samples found the highest concentrations of heavy metals within the fines of less than 1 mm, similar to results found by Bardos (2004) and Zhao *et al.* (2012). To improve CLO quality, finer fractions could potentially be screened to reduce the overall heavy metal concentrations.

The preliminary growth trials were designed to assess the overall impact of ROWs on tree growth as a sole material, from this the optimum nitrogen application rate growth trials were developed. From the optimum growth trials and an understanding of the leachate composition associated with CLO application, the effects of environmental conditions were investigated in the column growth trials. Discussion of the growth trials were divided into common themes across each study; biomass production, leachate composition and phytoremediation to allow comparison between different trials.

The optimum nitrogen application rate growth trials represent the greatest value in terms of evaluating the biomass production of trees when grown in ROWs. The application ROWs at different nitrogen loading rates identified the increased biomass production compared to limited inorganic nutrient amendments as well as the point of toxicity which impacts biomass production. Interestingly column growth trials identified that saturation conditions further improved biomass production in *S. viminalis* identifying water availability as potential limiting factor for biomass production for some species. *S.viminalis* was consistently successful in all growth trials whereas *E. nitens* showed greater variability and survival rates during the growth trials, which also affected reliable biomass production within the pot trials. Although pot trials using young saplings are common practice, field trials are necessary to fully understand the biomass production over time and repeated rotations.

The composition of leachate from ROWs can be considered as the most important aspect when assessing the use of ROWs as soil amendments and its environmental risk. Leachate contains mobile pollutants capable of reaching groundwater and water courses, which can pose risk to human and environmental health. The optimum nitrogen application rate trials identified the impact of higher amendment rates on the concentrations of heavy metals and nitrogen leaching from lower amendment rates. Changes within the soil chemistry also allowed concentrations of heavy metal leaching to increase over time. The column trials identified the impact of redox conditions on leachate composition and the increased cumulative leaching of some heavy metals within saturated conditions. The leachate analysis from the optimum and column growth trials identified the impact of changing environmental conditions and the interaction of plants. There was evidence of metal sulfide

oxidation potentially increasing the solubility of heavy metals in the optimum growth trials due to ROL from the *S. viminalis* roots. The saturated columns and reducing conditions released metals bound to iron and manganese oxides compared to the unsaturated conditions. This highlights the potential risk from seasonal changes and the importance CLO application to contained landfills to ensure leachate risk to the wider environment is limited.

High biomass production is an essential requirement for the selection of energy crop species and the potential to phytoremediate soils is an additional advantage. All tree species exhibited the ability to uptake and accumulation heavy metals within root and above ground material. Phytoremediation represents a long-term treatment method of CLO by removing soluble heavy metals as well as complexed heavy metals over time. There is potential for heavy metal to be permanently removed from soils due to the translocation of heavy metals from the root material to the above ground organs before harvesting, providing harvesting occurs before leaf loss which can return metals to soil. The heavy metal accumulation within root material may influence biomass productions over time and reduce yields in later rotations resulting in replacement crops more often.

The fate of heavy metals within the biomass should be considered even if it has not been investigated as part of this thesis. The accumulation of heavy metals within leaf material has the potential to be returned to soil by leaf loss depending on tree type and harvesting time. Once harvested, biomass is incinerated for energy production resulting in the release of heavy metals within flue gases and incineration ash. Modern incinerators ensure flue gases are cleaned and emissions meet stringent standards under the European Waste Incineration Directive (2000/76/EU) (European Council, 2000). Bottom ash contains ferrous and non ferrous metals, which are removed before bottom ash is reprocessed to produce aggregate material. The potential extraction of heavy metals and valuable resources from incinerator bottom ash is known as phytomining and has the potential to add further value to development of energy crops on brownfield sites (Keller *et al*., 2005; Padmavathiamma and Li, 2007).

Chapter 8 CONCLUSIONS AND FURTHER WORK

The aim of this thesis was to investigate the use of ROWs as soil amendments to aid the growth of energy crops on landfill sites and assess the risk to the wider environment in order to confirm a potential market for CLO.

This research has added to the academic understanding into the use of ROWs to aid the growth of energy crops on brownfield sites in a number of ways.

- Characterising different ROWs has identified key differences as a result of the initial waste stream and the effect of the biological degradation process on the heavy metal binding and fractionation.
- Identified the optimum application rates of ROWs to *E. nitens* and *S. viminalis*, which can be used to inform field trials and improve biomass yields.
- From the leachate composition it has been identified that pH cannot be used to assess the mobility of heavy metals, due to the high buffering capacity associated with ROWs.
- Evidence of oxidation maybe a result of ROL influencing changes in the oxidation of insoluble metal sulfides increasing sulfate and metal ions within ROW leachate.
- Available nitrate concentrations within the lower applications are a significant risk to watercourses.
- There was no previous research into the effect of saturation and reducing conditions on CLO and the associated leachate. The reducing conditions increased the release of heavy metals compared to the unsaturated conditions
- Heavy metal accumulation and translocation potential of *S. viminalis*, *E.nitens* and *P. tomentosa* when grown in ROWs indicate potential long term remediation of heavy metals.

8.1 ROWs CHARACTERISATION

Characterisation of three different ROWs, source segregated (PAS 100/110) and mixed MSW (CLO), found all materials were well matured and biologically stable.

Heavy metal concentrations and electrical conductivity values were noticeably different between the source segregated samples and the mixed waste CLO as a result of the nature of the initial waste streams. There were also slight differences in the heavy metal binding between aerobic and anaerobic decomposition methods due to the decomposition processes and the humification of organic material.

The British Standard PAS certifications for source-segregated materials ensures a minimum quality of organic material, with maximum permissible heavy metal concentrations and traceability from source to final product. This certification ensures a market and use for organic material within agricultural, horticultural and landscaping projects. The Organic Recycling Group (ORG) developed a quality standard for mixed MSW waste materials such as CLO for the use in land restoration projects. Heavy metal limits identified in the standard originate from the Sludge (Use in Agricultural) Regulations (Organic Recycling Group, 2013) and are much higher than the PAS maximum heavy metal limits to allow for the high variability of the CLO material. All CLO samples investigated within this thesis were below the ORG limits and were much closer to the PAS limits. The ORG standard ensures restoration projects are not hindered by planning permission and uncertainties in the use of CLO materials. However the high limits enable higher heavy metal concentrations to be applied to the land when the heavy metal input should be limited wherever possible. The high ORG heavy metal limits allow the quality of the product to be determined by the engineering process, whereas the quality standard should determine the engineering processes (Bardos *et al.,* 2007). By reducing the heavy metal limits for CLO, producers can improve CLO quality reducing the impact on the environment and encourage a more consistent product. As identified by previous studies (Bardos, 2004; Zhao *et al*., 2012) the highest concentrations of heavy metals were within the fines of less than 1 mm. Additional screening methods to remove the fines could be trialed to improve the total heavy metal concentrations. Removing the fines may also improve overall soil structure and reduce the risk of water logging. Greater product consistency will give greater confidence to site owners to apply CLO to brownfield sites, particularly landfills as part of the restoration of closed sites thus increasing the value of CLO.

8.2 ROWs as Soil Amendments

ROWs have an economic value as an organic matter and nutrient source to replace the use of inorganic fertilisers. The application of ROWs to land have been shown to improve soil structure and drainage, water holding capacity, increase the buffering of soil as well as increasing the organic content and providing a source of slow release nutrients (Kilbride, 2006; Merrington *et al*., 2010; Smith 2009).

Presently CLO application to land is restricted to brownfield sites only as the heavy metal content and contaminants pose a risk to the environment and human health. The Environment Agency (England) is responsible for regulating over 2000 landfills, of which over 800 stopped accepting waste in 2001 and have since closed (Environment Agency, 2014). Closed or restored areas of landfills represent a significant area of land available for energy crop growth. The UK aims to have 350,000 hectares of energy crops by 2020 to meet its sustainable energy targets (Lindegaard, 2012). The National Non-Food Crops Centre (NNFCC) recently published a report concerning AD capacity in the UK and predicted 2.7M tonnes of energy crops maybe required by 2017, this is equivalent to 60,000 ha or 1% of arable land (NNFCC, 2014 cited in Farmers Interactive Weekly, 2014). Energy crop development as part of landfill restoration programs will work towards targets while not encroaching upon agricultural land essential for food production. CLO application to landfill caps will improve soil quality and nutrient content ensuring biomass production is not limited.

Growth trials were designed to assess the effect of ROWs at different application rates on biomass production by three tree species; *Salix viminalis, Eucalyptus nitens* and *Paulownia tomentosa*. *S. viminalis* is a traditional SRC species used in the UK and northern Europe, while *E. nitens* and *P. tomentosa* are relatively new species used for biomass production within the UK. Preliminary growth trials identified the negative impact of CLO and PAS 100 on tree growth due to excess soluble salts and the limited binding with an inert mixing medium, perlite. The use of perlite identified the direct impact of ROWs, however the results did not simulate the natural environment as ROWs will be used as soil amendments and mixed with natural soils with greater cation exchange capacities to reduce the effect of soluble salts.

The optimum nitrogen growth trials aimed to assess the ideal ROWs loading to ensure maximum yields without becoming phytotoxic to plant growth. Kettering loam replaced perlite to allow natural adsorption of soluble ions while ensuring consistency and repeatability in the mixing medium. *S. viminalis* and *E. nitens* were grown at 6 different total nitrogen application rates of CLO and PAS 110 with weekly leachate analysis to assess the risk to the wider environment. The growth trials identified the optimum nitrogen loadings of PAS 110 was 1000 kg N / Ha for *E. nitens*, 10,000 kg N / Ha for *S. viminalis* and 3000 kg N / Ha of CLO for both species. Application rates affected leachate composition with higher applications leaching higher concentrations of metal cations, anions and ammonium. Source segregated PAS 110 had lower heavy metal concentrations compared to the CLO as a result of the mixed MSW. Leachate concentrations were compared to Environmental Agency water quality limits for aquatic life and found PAS 110 concentrations all within or below acceptable concentrations. CLO leachate samples identified concentrations of chromium, lead and zinc fell within quality limits and copper and nickel at application rates of greater than 3000 kg N / Ha exceeded water quality limits. Higher heavy metal concentrations were associated with higher application rates, whereas higher nitrate concentrations were evident in the lower application rates of both PAS 110 and CLO. Potential nitrogen immobilisation within higher application rates may have limited the soluble forms available for leaching however nitrate leaching to the wider environment represents a significant risk of eutrophication in water courses. Leachate analysis also identified potential oxidation of metal sulfides, increasing the solubility of heavy metals with little change in pH values as a result of the high buffering capacity of ROWs. It was concluded the use of ROWs, particularly CLO at application rates of greater than 250 kg N / Ha to landfill sites may be restricted to those with collection and containment systems, not solely for heavy metal leaching but also nitrogen leaching. Alternatively a compromise of lower application rates and therefore lower biomass production is required to protect the environment.

As seen in the optimum nitrogen growth trials, slight changes in the environmental conditions can influence significant changes in the leachate compositions and risk to the environment. Evidence of oxidation within the higher application rates lead to the oxidation of metal sulfide releasing soluble heavy metals and other metal

cations, such as calcium, in response to increase H^+ ions. It is important to understand the potential effect of changing environments on the characteristics of CLO. For example landfills and other brownfield sites can be prone to water logging as a result of poor soil structure and low moisture holding capacity within normal rainfall years. However the high rainfalls seen in November 2012 and the winter of 2013/2014 have shown the potential for excessive rainfall and the increased risk of saturated ground across the UK (Met Office, 2014).

Column growth trials were designed to investigate the effect of reducing conditions on leachate composition and biomass production by saturating *S. viminalis* and *E. nitens*. *S. viminalis* biomass production increased with saturated columns compared to unsaturated due the naturally high water uptake by *Salix* spp. and the development of aerenchyma tissue to allow oxygen transfer from the leaves to the roots. However *E. nitens* showed limited acclimatisation to survive within saturated conditions. The column growth trials identified the initial increase of heavy metal mobility within saturated columns potentially due to the reduction of iron and manganese oxides releasing previously bound heavy metals. As reducing conditions continued heavy metal mobility decreased which may be linked to binding with DOM or sulfate reduction and insoluble heavy metal sulfides forming. Saturated conditions generally increased the total masses of heavy metals leached compared to unsaturated conditions as a result of the initial release during changing redox conditions. As seen in both the optimum and column growth trials, changes in the soil chemistry and environmental conditions have the potential to release bound metals into the environment.

S. viminalis, E. nitens and *P. tomentosa* are all known for their fast growth and high biomass production, however all species showed evidence of heavy metal uptake and accumulation. The ability for species to accumulate and translocate heavy metals represents a long term remediation method to ensure heavy metal release and build up within the environment from CLO application is limited. Although the species are not classified as hyperaccumualtors with the ability to take up high concentrations, over time, repeated harvests, the energy crops have the potential to remove substantial amounts of heavy metals from CLO. Long term field trials would be required to assess the impact of heavy metal accumulation within root material on biomass production in repeated rotations and harvests. Heavy metals

can be translocated from roots to above ground material which once harvested are permanently removed from the site soil. Once biomass has been incinerated, heavy metals may accumulate in higher concentrations within bottom ash, which in turn can be processed to remove valuable metals.

It is not only the physical and chemical benefits and risks to the environment that should be considered when assessing the use of ROWs as soil amendments, but also the ecological and social impacts. Coleby *et al.* (2012) suggested site-specific ecosystem services assessments could be integrated into the Environmental Impact Assessments (EIA). The use of ROWs and the change of land-use for the growth of energy crops on brownfield sites can be considered using an ecosystem services framework. General factors that would be assessed during an ecosystem services assessment include the development of habitats and greater biodiversity, as well as the aesthetic improvements to the local horizon and the perceptions of local communities. The impact of land use changes may also be assessed, for example brownfield sites represent large areas of land with little or no vegetation and therefore the development of crops have the potential to reduce surface runoff, intercepting rainfall which in turn will increase lag times reducing the risk of local flooding. Although the increased water uptake by energy crops also has the potential to limit water supply to aquifers and increase the risk of droughts in some areas. Ecosystem services assessments can aid decision makers and planners to ensure the best projects are identified rather than solely focusing on the negative impacts from a project at local, regional and national levels.

Waste management companies have the potential to develop and manage their facilities and resources in a sustainable and low carbon way by using ROWs as a nutrient source to aid the growth of energy crops on restored landfill sites to supply biomass for local energy from waste plants. An example of biomass production on landfills is currently being undertaken by FCC Environment and Drax power station. Restored landfills and quarries in the north of England are being used to grow energy crops to supply a sustainable biomass to Drax power station to generate low carbon electricity (FCC Environment, 2013). FCC Environment currently has 200 hectares of biomass crops over 24 former landfills (FCC Environment, 2013). Similar trials are currently being conducted by WRAP using PAS compost and digestate to aid the growth of willow and miscanthus crops on

landfill sites in the UK. However it could be argued that PAS certified material has a greater market value when used on agriculture land to replace costly inorganic fertiliser and therefore its use on landfills and brownfield sites is unnecessary. CLO which has limited end uses can be applied as a soil amendment to landfills improving soil structure and nutrient content to support fast growing energy crops at a lower cost than PAS certified material.

The overall aim of this thesis was to identify a viable market for CLO and assess the risk to the wider environment from the use of ROWs as soil amendments to aid the growth of energy crops on landfill sites. Through experimental work characterising ROWs and growth trials assessing the composition of leachate it is concluded that CLO is a valuable resource and can improve the biomass production on landfill sites without damaging human and environmental health providing the risks are mitigated or managed. Site specific risk assessments before use are essential to ensure suitable land management during and after land application. It would also be advisable to incorporate ecosystem services assessments in order to aid decision-making rather than purely assessing negative impacts from the use of CLO as a soil amendment to landfills sites.

8.3 LIMITATIONS

It is important to identify the limitations experienced during this thesis, particularly during the experimental growth trials, and how these were overcome. The most significant limitation was time; in terms of lab available time in the collection and analysis of samples to ensure the integrity of samples were not compromised. Due to the diverse analytical techniques and limited sample preservation methods, sample collection and analysis would often have to take place within a few hours (pH, EC) or within 24 hours (IC analysis) of sampling. To overcome these issues the number of variables and repetitions were kept to a minimum, however the results showed relatively small deviations and consistency between replicates. Effective planning and efficient working practices were also implemented by staggering planting and sampling days to ensure sampling and analysis was achievable within the specific time periods.

As with all analytical equipment the accuracy was checked inhouse using known calibration solutions and repeating sample anlaysis within batches. The cation

analysis of some leachate samples may have reached the ICP-OES detection limits due to the low concentrations present particularly evident in the PAS 110 leachate samples (Chapter 5). The best available technology was used for the analysis of the leachate samples. For the solid samples an alternative method of heavy metal analysis was used (XRF) which allowed for large number of samples to anlaysed quickly and cost effectively. The compost materials were anaylsed using ICP-OES but not XRF and the accuracy of the analytical methods cannot be compared.

Redox was measured as part of the leachate analysis collected from the column growth trials however the redox meter even after calibration showed varied results and were discarded as unreliable by the author and are therefore not presented as part of this thesis. The high nitrate concentrations within the unsaturated columns and the high ammonium concentrations within the saturated columns were used to indicate the effect of the aerobic and anaerobic conditions with the columns.

An inevitable risk and limitation of conducting growth trials is the health of the plants and any premature death not related to the experimental condition affecting the results. For example the presence of a natural pest (Red Spider mite) may have influenced the survival of *S. viminalis* in the preliminary growth trials. To overcome this limitation and ensure plant survival, plant insecticide was applied and double planting took place during the optimum growth trials to distinguish between natural plant demise and the effect of the experimental condition. There were still instances of plant demise affecting the collection of plant material and therefore gaps within the results even with additional measures.

The column growth trials were unsuccessful for the *E. nitens* and it was hypothesized by the author that the immediate saturation of *E. nitens* roots may have influenced the survival rate of plants. The results also indicated that the unsaturated *E. nitens* had poor survival rates too with no saturation. As a result of experience and observational results the premature death of the *E. nitens* even in unsaturated conditions could be attributed to the limited tolerance level of *E. nitens* and the inability to recover from stress as identified in section 7.2.1.1. The results indicated unsaturated conditions did not adversely affect the health of *E. nitens*, whereas those saturated after 3 and 6 week generally died within a few weeks excluding 1 plant (saturated after 6 weeks unsaturated growth). This indicates

further time steps may have been required to fully understand the effect of saturation on *E. nitens*.

8.4   FURTHER WORK

This thesis focused upon the risk of ROWs as soil amendments based on the heavy metal content and nitrogen forms associated with source segregated and mixed MSW organic material. Changes within the soil chemistry that influenced the changes in mobility were particularly evident within the column growth trials. Further experimental work could be conducted to assess metal speciation and valence to investigate the theories suggested in this study concerning heavy metal mobility from iron and manganese oxides and metal sulfides.

Further work could be conducted to address the limitations mentioned in section 8.3. Further investigation into the mechanisms that effect heavy metal uptake within the porewater and rhizosphere and the difference between species ability to mobilise heavy metals as well as the development of iron plaque formation on roots as discussed in chapter 7 is required. The heavy metal concentrations in plant material could be assessed futher to ensure the XRF results are accurate. This study has identified the sensitivity of *E. nitens* saplings to saturation and further work is required to understand the implications this has on the use of *E. nitens* in the UK as a biomass species.

Experimental data collected as part of the thesis and further metal speciation data could be incorporated with present computational models to understand long term binding and leaching associated with ROWs as well as the potential impacts from climate change. Models can potentially investigate the interactions between plant roots and ROL in the mobility of essential nutrients and heavy metals to further inform land application permits for decision makers and influence national policy.

Other factors that have not been considered as part of this thesis include organic pollutants and micro-organism populations. Under the PAS certification organic pollutants are not investigated, although other studies have identified organic pollutants such as polycyclic aromatic hydrocarbons, pesticides and polychlorinated biphenyl (Hough *et al.*, 2012). Organic pollutants can be broken down during biodegradation processes and continue to decompose over time once applied to

land. There is also potential for organic pollutants to be remediated as *Salix* spp. have been shown to phytoremediate petroleum derived hydrocarbons (Cunha *et al.*, 2012).

A number of changes within ROWs and organic materials are dependent upon microorganisms such as immobilisation of nitrogen restricting nitrification and nitrate for plant uptake as well as changes in microorganism populations within aerobic and anaerobic soils. Further research into the types and amounts of microorganisms could be used to understand rate of change and release of pollutants. Understanding microbial populations also have the potential to allow manipulation of microorganisms, in order to manage heavy metal release and bioremediation of soils (Gadd, 2004).

The scientific understanding gained from this research can be used to inform improvements in engineering processes to reduce the heavy metal content of final ROWs, particularly CLO which can also influence the future use and markets of CLO. Source segregated materials certified under the PAS British Standard have developed markets within the agricultural and horticultural sectors however this research has the potential to develop the market for the use of CLO. This research can be used to guide policy and develop best practice guidance in the use and application rate of CLO to brownfield sites. Further research into the use of CLO will continue to provide confidence to land owners and ensure restoration projects are achieved while aiding the UK to meet landfill diversion targets and generate renewable biomass for electricity production.

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APPENDIX 1: CONFERENCE AND JOURNAL PAPERS.

16th European Biosolids and Organic Resources Conference, 2011.

The use of recycled organic waste as a soil amendment for growing short rotation coppice

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Abstract

This paper presents initial results of an investigation of the potential of using PAS 100 graded compost and Compost-Like Output (CLO) as a nutrient source for short rotation coppice energy crops. Initial analysis of the PAS 100 compost and CLO measured; electrical conductivity, pH, total nitrogen and total heavy metal content. Pot trials using both PAS 100 compost and CLO were then used to assess the growth rates of Willow (*Salix viminalis)* at application rates equivalent to 1500 and 3000 kg N / Ha mixed with an inert growing medium (perlite). A plant image analysis program was designed as a quick, simple and non-destructive plant growth estimation method to be compared to manual measurements.

Initial results showed higher total metal content in the CLO than PAS 100 compost, with both showing electrical conductivity levels of over 2.0 mS/cm. Excess soluble salts thought to be the cause of reduced initial growth of *Salix viminalis*. There are however indications of re-growth after leaching of the excess salts from the main body of compost.

Keywords

Recycled Organic Waste, PAS 100 Compost, Compost-Like Outputs, Soil Amendments, Short Rotation Coppice.

Introduction

The European Landfill Directive (1999/31/EC) was introduced to reduce the dependence on landfills for the disposal of waste and to drive waste up the waste hierarchy by reducing, re-using and recycling materials. In particular as part of the Landfill Directive, strict limits have been introduced to control the amount of biodegradable municipal waste reaching landfills and by 2020 quantities of

biodegradable municipal waste must be reduced to 35% of the levels reaching landfill in 1995 (Stretton-Maycock and Merrington, 2009).

The diversion of biodegradable waste from landfills results in the beneficial reduction in generation of methane from the decomposing organic material in anaerobic conditions. To enable this diversion local waste disposal authorities encourage source segregation of recyclable waste by households as well as biodegradable waste suitable for anaerobic digestion and energy production (CIWM, 2010; Environment Agency, 2010a). As an additional measure mixed Municipal Solid Waste (MSW) is sent to Mechanical Biological Treatment (MBT) plants to mechanically sort and segregate waste before biologically treating biodegradable material (Merrington *et al*. 2010).

The source and pre-treatment of waste streams determines the characteristics and future potential uses of the Recycled Organic Wastes (ROW). PAS 100 compost and Compost-Like Output (CLO) have received increased commercial interest as MBT by-products (WRAP, 2008; Stretton-Maycock and Merrington, 2009). Compost created from source segregated organic waste streams can be commercially sold as PAS 100 compost under a British Standards certification scheme to demonstrate a minimum quality. In contrast, CLO is the biodegradable fraction from mixed MSW and has the potential to contain heavy metals, glass and plastics (Merrington *et al.* 2010); currently there is no standard specification for CLO so its characteristics can vary widely. CLO use is currently limited to a temporary landfill cover, or habitat restoration of landfill sites if it can be demonstrated that it brings ecological benefit to the restoration.

The uses of ROWs as soil amendments has two main benefits; 1) Use as a plant nutrient source, 2) as a soil conditioner to improve the soil structure (Britt *et al.* 2002). Source segregated ROWs which pass the PAS 100 Standard can be applied to land as an agricultural or horticultural nutrient source. The use of CLO however is restricted to brownfield sites only by England and Wales Environment Agency because of the potential heavy metal content from the MSW (Environment Agency, 2011). It has been suggested that CLO can be used on brownfield sites as a fertile soil amendment to aid the growth of short rotation coppice crops (Bardos *et al.* 2007; Chapman 2007).

Short rotation coppice has been identified as a suitable renewable biomass for use as a low carbon fuel to produce renewable heat and /or electricity. This is current of high relevance as the UK is committed to producing 20% of electricity from renewable fuel sources under the Climate Change Act 2008 (Environment Agency, 2010b). To be economically effective short rotation coppice crops require fast growing species to produce high yields every 2 to 4 years (Tubby and Armstrong, 2002).

The plantation of short rotation coppice crops on brownfield sites with ROW amendments to improve the growing medium and ensure sufficient nutrients are available for rapid growth has been identified as being potentially beneficial (Bardos *et al*. 2007). In particular ROWs are a significant source of nitrogen which is one of the most common limiting factors to the growth of plants (Madrid *et al*. 2007; Fircks *et al.* 2001). It should be noted that fertiliser application to agricultural land is restricted to 250 kg N / Ha due to the potential leaching risks associated with soluble nitrates and this restricts the usage of ROW on a site-by-site basis. Furthermore whilst ROW total nitrogen content typically ranges between 1.1% and 2.1% (Merrington *et al.* 2009ab) it is present mainly in organic forms which are less soluble because organic nitrogen must be mineralised by bacteria to become available to plants as nitrate or ammonium (Amlinger *et al*. 2003).

Currently the Environment Agency grant bespoke permits for the application of CLO to land for scientific trials (Environment Agency, 2011). In such cases the heavy metal, nitrogen and electrical conductivity content of the CLO is often the main limiting factor in restricting the use of CLO to brownfield sites because of evidence from previous studies that repeated CLO application to soil results in the build up of heavy metals in soil (Ayari *et al*. 2010; Madrid *et al.* 2007; Smith, 2009; Lee *et al*. 2004).

This paper presents an investigation into the potential use of two ROW products as nutrient sources for Willow (*Salix viminalis)* to be grown as energy crops in the UK. PAS 100 compost and CLO was analysed to compare the material characteristics. Pot trials are used to assess the ability for *S. viminalis* to be grown at the equivalent of 1500 and 3000 kg N / Ha of CLO and 3000 kg N / Ha of PAS 100 compost to control pots.

Methodology

ROW samples

PAS 100 compost was collected from a recycling centre in South Wales which is PAS 100 accredited by the Composting Association. The compost was derived from segregated food wastes which had been in-vessel composted for between 7 and 21 days before being further maturation for up to 10 weeks.

The CLO was sourced from a MBT in the south of England from a mixed MSW stream. The Biodegradable fraction of MSW is separated from the mixed waste and composted in bio-stabilisation halls for 6 weeks with repeated aeration and irrigation to maintain optimum composting conditions.

ROW sample preparation

Both compost materials were stored at <4°C in sealed containers before analysis and use in the growth trials. Each material was tested to determine the moisture content, electrical conductivity, pH, bulk density, total nitrogen and total metal content. The bulk density of ROW was determined using wet mass weight and volume. Approximately 500g of each material was weighed out in 250g batches, dried for 16 hours at 80°C and weighed again to calculate the moisture content. Electrical Conductivity (EC) and pH of each sample was tested by using 1:5 ratio of material to ionised water before using mettle Toledo Seven Multi (UK) testing probes, See BS EN 13038 and BS EN 13037 for testing methods respectively.

To determine the total nitrogen and total heavy metal content of ROW, 100g of dried sample was ground using a gyro crusher to grind and homogenise the sample material to a ,2mm fraction. Total nitrogen content was analysed using a total nitrogen analyser (Shimadzu, UK). The total heavy metal content used a full *aqua regia* digestion of the samples before ICP-OES (Perkin Elmer Optima 2100 DV, UK) analysis.

Pot Trials

S. viminalis cuttings approximately 150mm in length were planted in commercial compost for 4 weeks to allow roots and shoots to develop. For replanting with ROW, the root systems were washed to remove all compost soil. *S. viminalis* saplings root length, number of shoots, sapling length and weight were recorded.

S. viminalis was grown in CLO at concentrations equivalent to 1500 kg N / Ha and 3000 kg N / Ha with 3 replicates each, PAS 100 compost pots were grown at concentrations equivalent to 3000 kg N / Ha with 3 replicates each. For material input calculations average total nitrogen, bulk density and dry mass content were used for each material; the field mix depth was assumed as 150mm. Perlite was used as an additional inert growing medium mixed with the ROW. Control pots containing perlite only were watered weekly with 100ml of quarter strength Hoagland solution (Sigma Aldrich, UK) as a nutrient supply, and additional 100ml deionised water. ROW pots were watered weekly with 200ml of deionised water. Pot trials were grown under daylight spectrum fluorescent bulbs for 16:8 hours of light: dark, at temperatures ranging between 15-28°C on a diurnal temperature cycle, for 5 weeks.

The growth rates of *S. viminalis* were monitored weekly by manual height and leaf measurements, and observational notes. Weekly photographs were taken of each plant from 3 fixed points.

Plant analysis program

Matlab (Mathworks, UK) was used to create a plant analysis program using three photographs taken from fixed points at 0°, 45° and 90° on each pot. Plant height was calculated from a datum established at the top of the plant pot from a cropped image. Weekly photos of each pot were imported to Matlab and the final calculated data exported into Microsoft Excel.

Results

ROW characteristics

Initial ROW were analysed to compare the differences between source segregated (PAS 100) and mixed MSW stream (CLO) composts. Table 1 shows the initial characteristics of PAS 100 and CLO alongside the PAS 100 heavy metal compliance concentrations for BSi certification (BSi, 2011).

There is a notable difference in the results between PAS 100 compost and CLO. PAS 100 compost has a fairly alkaline pH compared to a neutral pH in the CLO. The EC of the CLO was over twice as much as the PAS 100 compost. The moisture content of the ROWs differed significantly, with the CLO having a moisture content of 10.94% compared to PAS 100 of 58.05%, as a result of

different storage conditions. PAS 100 compost has a greater total carbon and nitrogen content than CLO, although they both have similar carbon: nitrogen ratios.

The heavy metal concentrations identified in the PAS 100 compost sample were all below the BSi compliance limits except zinc which was 464.73 mg/ kg^{-1} when the limit is 400 mg/ kg^{-1} limit. The concentrations of cadmium and chromium in CLO were below the PAS 100 certification limits, however the copper, lead, nickel and zinc all exceeded the limits. In particular the copper and zinc concentration in CLO were higher than the standard limits.

Table 1: BSi PAS 100 minimum quality limits, PAS 100 compost and CLO initial characteristics

Parameter	PAS 100 Limits	PAS 100	CLO
pH		9.29	7.37
Electrical Conductivity (mS/cm)		2.15	5.82
Moisture Content (%)		58.05	10.94
Total Carbon (%)		38.71	23.2
Total Nitrogen (%)		2.59	1.49
Carbon: Nitrogen Ratio*	20:1	14.95	15.57
Total Cadmium (mg/kg)	1.5	<1.0	<1.0
Total Chromium (mg/kg)	100	41.02	85.2
Total Copper (mg/kg)	200	133.00	419.05
Total Lead (mg/kg)	200	92.16	275.55
Total Nickel (mg/kg)	50	11.02	60.16
Total Zinc (mg/kg)	400	464.73	1667.83

Recommended C: N of soil improvers (Edwards et al. 2011).

Pot Trials

Within the first week of planting there were visible signs of distress shown by all trial pots. All *S. viminalis* shoots on specimens grown in CLO and PAS 100 compost were pale green with yellowing and wilting leaves. The lower leaves on the control plants had also turned yellow, however upper leaves remained green. In the third week there was evidence of new bud growth in both CLO pots 4 and 6 pots (1500kg N / Ha) and in one PAS 100 (PAS 2, 3000kg N / Ha) pot. The remaining PAS 100 and CLO specimens showed no evidence of new growth, with the remaining yellow and wilted leaves curling. In contrast, the cuttings in the control

pots had green and healthy shoots with no visible signs of distress. Table 2 shows the results.

After 5 weeks of growth the CLO (CLO 4) specimen containing equivalent to 1500 kg N / Ha nitrogen loading showed evidence of new growth and at week 3 had developed small buds in place of the previously wilted stems. Specimen CLO 6 (1500 kg N / Ha) had no evidence of new growth. The PAS 100 specimen (PAS 2) which showed new growth at week 3 did not fully recovery and showed no evidence of new growth; although the replicate [PAS 100 (PAS 3]) specimen with 3000 kg N / Ha of nitrogen rate had two small buds growing below the pot level at week 5. There was no evidence of new growth on any of the remaining ROW pot. The control pots were healthy with dark green leaves.

Plant analysis program

Due to initial wilting and limited re-growth of the CLO and PAS 100 compost pots; there were negative height changes after 1 week with little change over the following weeks. Control pots showed good growth as shown in Table 2. The relationship between the manual height measurements and the image analysed heights using the Matlab program showed good correlation with a R^2 value of 0.9832.

Table 2: Manual height measurements, Observations and Plant image analysis height measurements for week 0, week 1, week 3 and week 5.

Plant ID	Week 0		Week 1			Week 3			Week 5		
	Manual Height (mm)	Matlab Height (mm)	Manual Height (mm)	Matlab Height (mm)	Observations	Manual Height (mm)	Matab Height (mm)	Observations	Manual Height (mm)	Matlab Height (mm)	Observations
CLO 1	122	126	115	119	Yellow, wilted	99	97	Wilted, NNG	96	97	NNG
CLO 2	164	166	162	163	Yellow, wilted	146	148	Wilted, NNG	143	136	NNG
CLO 3	162	163	151	149	Yellow, wilted	129	135	Wilted, NNG	126	126	NNG
CLO 4	121	116	107	101	Yellow, wilted	98	97	Wilted, SNG	96	94	2 small shoots
CLO 5	114	114	130	124	Yellow, wilted	111	110	Wilted, NNG	99	95	NNG
CLO 6	146	149	132	137	Yellow, wilted	124	129	Wilted, SNG	118	116	NNG
PAS 1	203	191	187	196	Yellow, some wilting	185	178	Wilted, NNG	185	-	NNG
PAS 2	188	-	185	143	Yellow, some wilting	146	148	Wilted, SNG	123	120	NNG
PAS 3	166	156	159	163	Yellow, some wilting	126	120	Wilted, NNG	124	114	2 small shoots
Control 1	138	142	141	148	Lower leaves yellow, top leaves green	227	-	Healthy growth	318	307	Health growth
Control 2	182	-	164	172	Lower leaves yellow, top leaves green	152	158	Healthy growth	229	226	Healthy growth
Control 3	157	-	151	155	Lower leaves yellow, top leaves green	156	161	Healthy growth	230	-	Healthy growth

CLO 1 -3 Total N application equivalent to 3000 kg N / Ha, CLO 4- 6 Total N application equivalent to 1500kg N / Ha
NNG No New Growth evident, SNG some new growth evident, - Image could not be processed.

Discussion

ROW

The initial measured pH of PAS 100 compost and CLO are consistent with previous studies that have shown the pH of ROW to range between neutral and slightly alkaline (Cameron *et al*. 2009). The impact of pH variations can be significant with the application of ROW to soils having a potential liming effect by increasing pH and soil buffering capacity (Cameron *et al*. 2009). Also increasing the bulk pH and buffering capacity of soils reduces the solubility of heavy metals by forming stable metal-humic complexes (Smith, 2009).

The total carbon and nitrogen concentrations present in the CLO were 23.2 and 1.49 % respectively; these are consistent with values measured by Merrington *et al.* (2010). An indication of the maturity of ROW is the carbon: nitrogen ratio. High C: N ratios of >30:1 can cause the immobilisation of inorganic nitrogen. Microorganisms immobilise the available nitrogen in ROW to decompose the high concentrations of organic matter thus reducing the available nitrogen for plant uptake. Once the organic matter is decomposed, the organic nitrogen within the organic matter can be mineralized to inorganic available forms (ammonium and nitrate). Edwards *et al.* (2011) suggest the threshold above which immobilisation occurs is 20:1, the C: N of PAS 100 compost and CLO were 14.95 and 15.57 respectively suggesting suitable maturity.

The EC of both ROWs was high, in particularly the CLO being classed as 'slightly saline' (4.0-8.0 mS/cm) (Cameron *et al*. 2009). Excessive EC is known to restrict the yield of crops and even levels of 1.4mS/cm can affect root develop and plant growth (Cameron *et al*. 2009). The high concentration of soluble salts affect the osmotic pressure created by roots to uptake water causing plants to wilt and restricting growth rates (Duggan, 2005; Cameron *et al.* 2009). Brady and Weil (1996, Cited in Hargreaves *et al*. 2008) identified MSW compost EC to range between 3.69 to 7.49 mS/cm consistent with the CLO concentration (5.82 mS/cm). The EC for the PAS 100 compost was measured at 2.15 mS/cm, slightly above the recommended range for PAS 100 compost as a soil improver of 2.0 mS/cm (Edwards *et al*. 2011).

The PAS 100 compost contained low concentrations of heavy metals; all metal concentrations were below the PAS 100 limits except zinc. The concentration

of zinc exceeded the upper concentration to be certified as PAS 100 and as such would not be suitable for application to agricultural land.

The CLO heavy metal concentrations were higher than PAS 100 for 4 out of the 6 heavy metals tested. Cadmium and Chromium were below the PAS 100 limits of 1.5 and 100 mg/ kg respectively. The concentration of zinc in the CLO was over four times the PAS 100 limit, and significantly higher than previously reported values from MSW stream composts. Cameron *et al*. (2009) reported values between 492 and 585 mg/ kg and Smith (2009) between 130 and 757mg/ kg from UK sites.

Copper concentrations in CLO were 419 mg/ kg, over twice the 200mg/ kg PAS 100 limit. This value is similar to the values reported by Cameron *et al.* (2009) ranging between 240 and 481 mg/ kg, whereas Smith (2009) reported slightly lower levels of 25 to 306 mg/ kg. The levels of lead (275.55 mg/ kg) detected were only slightly above the PAS 100 limit of 200 mg/ kg. This was much lower than lead concentrations identified by Cameron *et al.* (2009) ranging between 612 and 630 mg/ kg.

The literature suggests zinc and lead are the most abundant heavy metals associated with both segregated and mixed waste compost (Smith, 2009). The results of the PAS 100 compost and CLO are consistent with this statement for zinc, however excess levels of copper was identified within the CLO, with lower than average concentrations of lead. A possible cause of these variations is the result of waste stream variability between households, across regional waste collections and seasonal differences as suggested by Stretton-Maycock and Merrington (2009).

Pot Trials

Growth trials using *S. viminalis* showed significant wilting and yellowing of leaves in all ROW pots and slight wilting in control pots after one week. This was initially attributed to the stress of replanting and damage incurred to the roots while removing commercial compost. Control pots recovered with good healthy growth after 3 weeks; however the remaining ROW pots still exhibited phytotoxic effects suggesting other contributing factors were affecting the growth.

The wilting and necrosis of *S. viminalis* in ROW was attributed to the high EC and excess soluble salts; although high concentrations of specific metals such as zinc may also have had a phytotoxic effect. The new growth of 2 buds in 2 ROW pots suggest after repeated watering some of the excess salts may have been leached

from the pots. Only one pot containing CLO at equivalent to 1500 kg N / Ha and one PAS 100 compost at equivalent to 3000 kg N / Ha showed new growth after initial wilting at week 5. Initial growth trials are inconclusive as recovery was not evident in any of the replicates or the remaining ROW containing CLO equivalent to 3000 kg N / Ha. Understanding the composition of the leachate produced from the ROW will identify excess concentrations of specific soluble ions. Previous studies have applied landfill leachate with an EC of 8.89 mS/ cm to willow saplings (Cureton *et al*. 1991, cited in Duggan, 2005), this exceeds the levels measured in the composts which ranged between 2.15 and 5.82 mS/ cm. Chlorosis or yellowing of leaves due to a lack of chlorophyll, necrosis and leaf desiccation were observed after leachate application (Cureton *et al*. 1991, cited in Duggan, 2005) similar to the phytotoxic effects observed in the *S. viminalis* grown in the PAS 100 and CLO composts.

Plant image analysis

The plant image analysis program showed good correlation ($R^2 = 0.9832$) with the manual height measurements of the *S. viminalis*. There is limited previous work on height estimation for tree growth. Lindsey and Bassuk (1992) investigated the use of images to calculate the leaf area of an intact tree by Delta-T video image analysis. Photos of entire trees were taken against a contrasting background and processed to produce negative images for surface area analysis. Positive correlation of tree silhouette area and actual leaf area was shown for 3 different tree species ranging between 0.920 and 0.977 (Lindsey and Bassuk, 1992).

Further development of the Matlab program aims to calculate the tree mass volume and volume occupied by a plant (envelope volume). From the plant volume and the envelope volume a density factor can be calculated to estimate density of leaves which will vary between tree species in future testing.

Conclusions

This paper presented the initial results of ROW as a nutrient source for short rotation coppice crops. Initial analysis of the ROW materials identified differences in the pH, EC and total heavy metal content due to different sources of waste streams. The EC values from both materials showed excess soluble salts were present in both PAS 100 compost and CLO.

Growth trials of *S. viminalis* indicated significant phytotoxic effects from the excess soluble salts present in the ROW. This is supported by the recovery of only two plants after leaching of salts. The results are therefore currently inconclusive and further investigations into the composition of leachate from each material and the effect on plant growth are required.

The plant image analysis program showed good correlation with the manual height measurements. Further development of the program will estimate the plant volume, however new plant growth below pot levels or large leaves lower than the pot level may lead to under and over estimations. Further work will be required to ensure consistent volume results and testing with other tree species.

Acknowledgements

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Heavy Metal Content of Recycled Organic Wastes and Potential Bioaccumulation in Short Rotation Coppice When Used as a Nutrient Source

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EXECUTIVE SUMMARY
This paper investigates both the heavy metal content of three Recycled Organic Wastes (ROWs) and metal accumulation in two Short Rotation Coppice (SRC) energy crops grown in ROWs. In particular *Salix viminalis* and *Eucalyptus nitens* were grown in Compost-Like Output (CLO), PAS 100 compost and PAS 110 at concentrations equivalent to 3000kg N/ha.
The heavy metal content of each ROW was investigated and growth trials used to assess potential movement and uptake of heavy metals from the compost to SRC species. The individual ROW mixes were separated from the plant material and the latter was divided into stem, leaves and root materials and all material analysed using an X-ray Fluorescence machine (XRF).
Initial characterisation of the ROW materials identified higher heavy metal concentrations in CLO compared to PAS 100 and PAS 110 and electrical conductivity levels of over 2.0 mS/cm in both the CLO and PAS 100. Trees grown in CLO and PAS 100 had negative height changes as a result of heavy metal concentration and electrical conductivity levels. There were positive height changes in the PAS 110 and control plants that had low electrical conductivity and heavy metal concentrations. Plant material analysis identified heavy metal concentration of the plant material and were in the order of; root > leaves > stems. Zinc was translocated to stems in both species although heavy metal uptake was greater in *S. viminalis* compared to *E. nitens*.

1.0 INTRODUCTION
Biodegradable material collected from source segregated and mixed waste streams can be biologically treated to produce material known as Recycled Organic Waste (ROW). In the UK source segregated waste can be certified under the British Standards as either PAS 100 (aerobic compost) or PAS 110 (Anaerobic Digestate) ensuring a minimum quality is achieved and commercially sold as a nutrient source (Merrington *et al.* 2010).
Compost-Like Output (CLO) produced from the aerobic composting of the biodegradable fraction of mixed Municipal Solid Waste (MSW) has the potential to contain heavy metals, glass and plastics (Merrington *et al.* 2010). There is currently no standard specification for CLO so its characteristics vary widely. CLO is only permitted for use as temporary landfill cover or in habitat restoration of landfills providing that an ecological benefit to the site can be demonstrated. It has been suggested that CLO can be used on brownfield sites as a fertile soil amendment to aid the growth of Short Rotation Coppice (SRC) crops (Bardos *et al.* 2007). Also ROWs have been identified as having the potential to be used as soil amendments as a nutrient source and to improve soil structure (Merrington *et al.* 2010).

To be economically effective SRC crops require fast growing species able to produce high yields every 2 to 4 years (Tubby and Armstrong, 2002).The addition of ROW to poor soils may improve the soil structure and provide sufficient nutrients for rapid growth of SRC potentially ensuring that crop yields are not limited. The application of fertilisers to agricultural land is restricted to 250 kg N/ha so as to reduce potential risks of leaching to the surrounding environment (Merrington *et al*. 2009). The Environment Agency (England & Wales) presently grant bespoke permits for the use of CLO to land and for higher application rates for scientific trials based on site specific assessments (Environment Agency, 2011). Previous studies by Ayari *et al*. 2010, Madrid *et al.* 2007, and Smith, 2009, have shown the potential for heavy metals to accumulate in soils that has had repeated CLO application. It has also be found that the bioavailability of heavy metals to plant in the environment is dependent upon metal concentration in soil, soil pH, the binding strengths of metals to soil and the uptake mechanisms of plants (Pulford & Watson, 2003; Smith, 2009).

Labile forms of heavy metals within material have been identified as being readily taken up by roots, however, the ability to translocate metals to above ground plant material varies. Zacchini *et al* (2008) identified the ability of *Salix* clones to translocate cadmium to above ground material, whereas *poplar* species accumulated cadmium in the roots. The ability of fast growing trees such as *Eucalyptus* trees to uptake heavy metals as a form of soil and water remediation has been investigated by Shukla *et al* (2011). *E. tereticomis* indicated effective accumulation of lead, copper, zinc and manganese within the whole plant material. However the accumulation of heavy metals can also have a detrimental effect on plant productivity (Pulford & Watson, 2003).

This paper investigates the potential for ROWs from source segregated and mixed waste streams to be used as a nutrient source for two UK SRC species *Salix viminalis* and *Eucalyptus nitens*. The initial characteristics of CLO, PAS 100, and PAS 110 were analysed. *S. viminalis* and *E. nitens* were then grown at concentrations equivalent to 3000 kg N/ha of each material for 22 weeks. ROW from each pot and plant material was analysed to assess the movement and uptake of heavy metals.

2.0 METHODOLGY
2.1 ROW samples
 The CLO was sourced from a Mechanical Biological Treatment (MBT) plant in the south of England from a mixed MSW stream. At this facility the biodegradable fraction of MSW is separated from the mixed waste and composted in bio-stabilisation halls for 6 weeks with repeated aeration and irrigation to maintain optimum composting conditions.
PAS 100 compost was collected from a recycling centre in South Wales which is PAS 100 accredited by the Composting Association (BSi. 2011a). The compost was derived from segregated food wastes which had been in-vessel composted for between 7 and 21 days before being further matured for up to 10 weeks.
Solid Anaerobic Digestate (PAS 110) was collected from an anaerobic digestion plant in South Wales which treats source segregated food waste only. Three different feed stocks; effluent sludge, potato sludge and food waste, are blended at different ratios and fed into the anaerobic digestion system. The site is working towards PAS 110 accreditation and currently applies the solid digestate to local

fields to replace chemical fertilisers and disposes of the liquid fraction to waste water drains.

2.2 ROW sample preparation

All ROW materials were stored at < 4°C in sealed containers before analysis and use in the growth trials. Each material was tested to determine the moisture content, electrical conductivity (EC), pH, bulk density, total nitrogen and total metal content. The bulk density of ROW was determined using wet mass weight and volume. Approximately 500 g of each material was weighed out in 250 g batches, dried for 16 hours at 80°C and weighed again to calculate the moisture content. The EC and pH of each sample were tested by using 1:5 ratio of material to ionised water (BS EN 13038 and BS EN 13037 for testing methods respectively).

To determine the total nitrogen and total heavy metal content of ROW, 100 g of dried sample was ground using a gyro crusher to homogenise the sample material to < 2 mm fraction. Total nitrogen content was analysed using a total nitrogen analyser (Shimadzu, UK). The total heavy metal content was determined by full *aqua regia* digestion of the samples before ICP-OES (Perkin Elmer Optima 2100 DV, UK) analysis (BS EN 13650:2001 test method).

2.3 Pot Trials

Two different tree species were investigated; *Salix viminalis* (Willow), and *Eucalyptus nitens* (Shinning Gum). *S. viminalis* cuttings approximately 150 mm in length were planted in commercial compost for 4 weeks to allow roots and shoots to develop. *E. nitens* saplings were approximately 280 mm with well established root systems around commercial compost. For replanting with ROW, the root systems were washed to remove the majority of compost material around the roots. The plant weight, plant length, and root length were recorded.

Both tree species were grown in the ROW at nitrogen concentration equivalent to application rates of 3000 kg N/ha based on current field trials being run by Forest Research, with 3 replicates of each. For material input calculations average total nitrogen, bulk density and moisture content were used; field mix depth assumed as 150 mm. Perlite was used as an additional inert growing medium mixed with the ROW. Control pots containing perlite only were watered weekly with 100 ml of quarter strength Hoagland solution (Sigma Aldrich, UK) as a nutrient supply, and additional 100 ml deionised water. ROW pots were watered weekly with 200ml of deionised water. Pot trials were grown under daylight spectrum fluorescent bulbs for 16:8 hours of light: dark, at temperatures ranging between 15-28°C on a diurnal temperature cycle, for 22 weeks. The growth rates of all species were monitored weekly by manual height and leaf measurements, and observational notes.

At the end of the 22 weeks each plant was weighed and measured before being divided in to root, stem and leaf material. Plant material was dried at 80°C for 16 hours before being ground to < 4mm. Plant material was tested using an Innov-X System X-ray Fluorescence (XRF) system to identify the heavy metal content of the roots, stem and leaves (Pietrini *et al*. 2009).

ROW + perlite mixes from each pot was collected, and dried at 80°C for 16 hours before grinding to < 2 mm for XRF analysis of heavy metal content (Kilbride, 2006).

3.0 RESULTS

3.1 ROW Characteristics

The characteristics of three ROWs were investigated, two of the materials were produced from source segregated waste (PAS 100 and PAS 110) and one from a mixed MSW (CLO). The PAS 100 and CLO were aerobically composted and the PAS 110 was anaerobically digested. Table 3.1 shows characteristics of the materials, the PAS BSi certification quality limits (BSi, 2011a,b) and the control material perlite.

The composted materials (CLO and PAS 100) had a neutral pH and high electrical conductivity compared to a slightly acidic pH and low EC for the PAS 110. The moisture content varied between materials as a result of difference treatment processes and storage conditions. The source segregated material had greater total carbon and nitrogen content than CLO but all materials had C: N ratios of less than 20.

The total heavy metal (*aqua regia*) concentrations of the ROW were compared to the BSi PAS quality standards. The CLO exceeded the PAS limits for copper, lead, nickel and zinc, the PAS 100 compost exceed zinc levels by 64.73ppm whereas the PAS 110 material was below the required concentrations. The control material perlite had low heavy metals concentrations.

Table 3.1 ROW Characteristics

Parameter	PAS 100/110 Limits	CLO	PAS 100	PAS 110	Perlite
pH		7.37	9.29	7.05	7.30
EC (mS/cm)		5.82	2.25	0.574	0.012
MC (%)		10.94	58.05	85.80	N/A
Total Carbon (%)		23.20	38.71	48.90	2.56
Total Nitrogen (%)		1.49	2.59	2.51	0.06
C:N Ratio	20:1	15.52	14.94	19.48	N/A
Total Cd (ppm)	1.5	<1.00	<1.00	<1.0	1.0
Total Cr (ppm)	100	85.20	41.02	13.15	0.60
Total Cu (ppm)	200	419.05	133.00	41.20	2.31
Total Ni (ppm)	50	60.16	11.02	8.20	1.54
Total Pb (ppm)	200	275.55	92.16	10.38	4.79
Total Zn (ppm)	400	1667.83	464.73	72.21	40.18

3.2 Pot Trials

S. viminalis and *E. nitens* were grown in CLO, PAS 100, and PAS 110 at concentrations equivalent to 3000 kg N/ha with perlite. All species showed evidence of distress within the first week as a result of replanting. *S. viminalis* sample pots had wilted leaves and turned pale green/ yellow, whereas *E. nitens* leaves turned red when stressed. Re-growth and recovery was evident in the majority of pot samples after week 2.

After 5 weeks all trees grown in PAS 110 and perlite recovered from the stress and leaves returned to their original colours. *S. viminalis* grown in CLO and PAS 100 all died back completely between week 5 and 6 with no evidence of new growth. *E. nitens* grown in CLO and PAS 100 had black stems and dried red leaves at week 5 and 6.

The growth trials ran for 22 weeks before individual pot ROW + perlite mixes were collected and plant material was divided into root, stem and leaves for analysis. All material were analysed using XRF for efficient and cost effective indication of the heavy metal distributions in growing medium and plant material to an accuracy of

1ppm. Figure 3.2.1 shows the average heavy metal concentration present in the ROW + perlite mixes and the average above ground height change.

All species grown in CLO and PAS 100 died and had a negative height change compared to the PAS 110 and control plants that survived and gained height over the 22 weeks. The graph indicates there is some correlation between the heavy metal concentration within the pots and the survival rate. The heavy metal concentration of zinc was compared to the plant height change and indicated greater correlation for *S. viminalis* ($R^2 = 0.46$) than *E. nitens* ($R^2 = 0.20$).

The plant material was divided into stem, leaves and roots to be analysed to identify potential heavy metal accumulation and any variation between species as shown in Figures 3.2.2, 3.2.3 and 3.2.4 respectively. Relative heavy metal concentration levels of the plant material was in the order of; root > leaves > stem. The average mass of stems, leaves and roots are shown to indicate the metal concentration changes between plant species and plant organs. Error bars indicate the highest and lowest values detected.
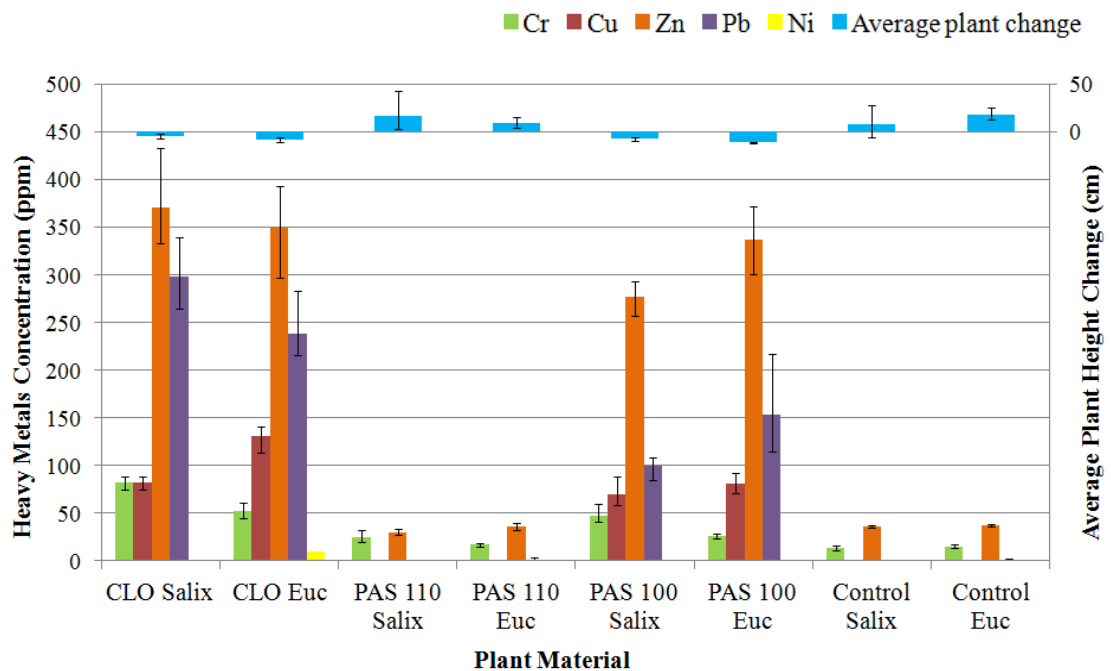

Figure 3.2.1 Average heavy metal content of ROW + perlite mixes compared to the average change in plant height.

Zinc was the dominant heavy metal present in the stem with greater levels present in *S. viminalis* than *E. nitens*, concentrations between materials ranged between 105 to 141 ppm and 14 to 84 ppm including the control samples respectively. The stem mass of *E. nitens* is naturally smaller than *S. viminalis*. Low levels of copper were detected in CLO *S. viminalis* and chromium was present in the control *S. viminalis*.
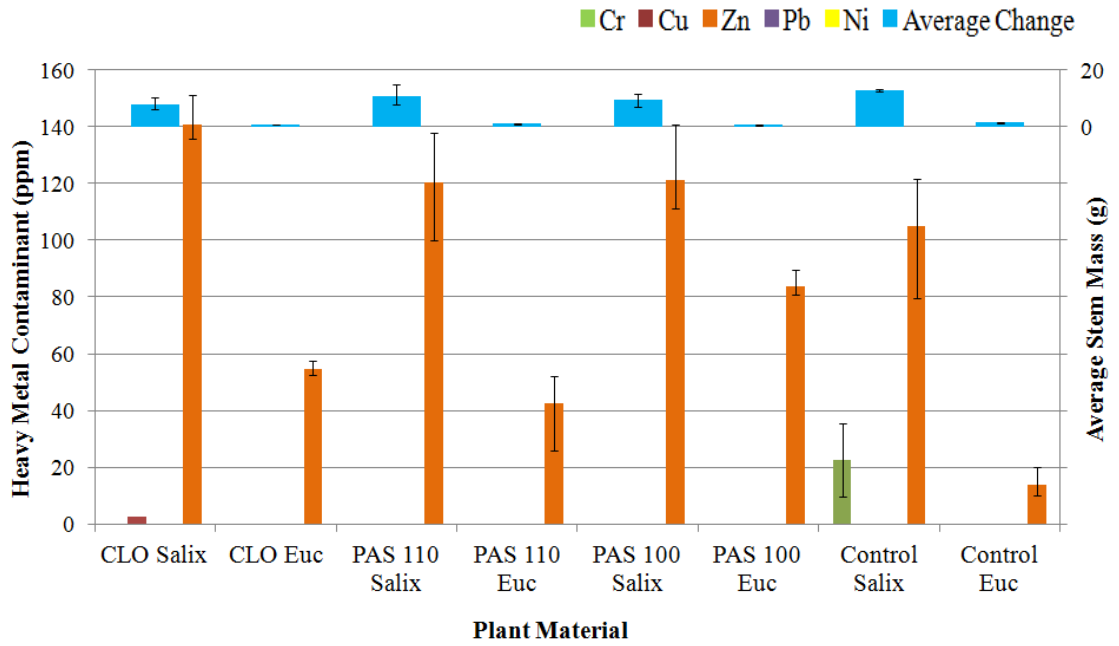
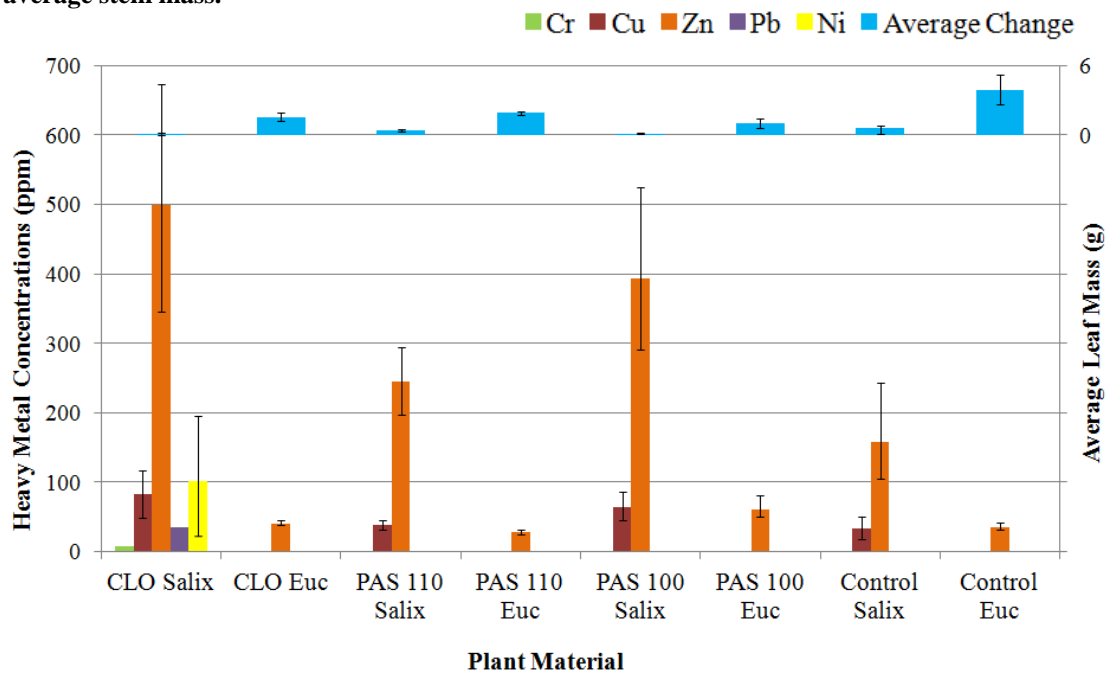**Figure 3.2.2 Average heavy metal concentrations present in stem material compared to the average stem mass.**

Figure 3.2.3 Average heavy metal concentrations present in leaf material compared to the average leaf mass.

The heavy metal concentrations identified in the plant leaves were greater than those in the stem material as shown in Figure 3.2.3. As previously seen in the stem mass, zinc is the only heavy metal present in *E. nitens* leaf matter with levels ranging between 28 and 61 ppm including the control pots. Low levels of copper (33- 82 ppm) were present in all *S. viminalis* pots including the control samples, however the CLO *S. viminalis* pots also contained lead and nickel. Lead was only present in one pot sample at 34 ppm, and nickel averaged from 3 pot samples at 101 ppm. Leaf mass was greater in *E. nitens* than *S. viminalis*.

The greatest heavy metal concentrations were identified in the root material of both *S. viminalis* and *E. niten* (Figure 3.2.4). Chromium, copper, zinc, lead and nickel

were present in both species grown in CLO, although lower levels identified in *E. nitens*. Only one root sample was collected from the *S. viminalis* CLO pot samples due to material loss and decomposition within the compost.

Root samples collected from *S. viminalis* and *E. nitens* grown in the PAS 110 had lower concentration of heavy metals compared to CLO pots. Copper and zinc were present in *E. nitens* roots, whereas chromium and nickel were present in *S. viminalis*. No root material was collected from *S. viminalis* grown in PAS 100. The control *S. viminalis* had greater levels of copper present than the *E. nitens* controls and of those levels identified in the *S. viminalis* grown in the PAS 110. Root mass collected was slightly higher for the *E. nitens* than *S. viminalis,* root masses were similar to those grown in CLO.
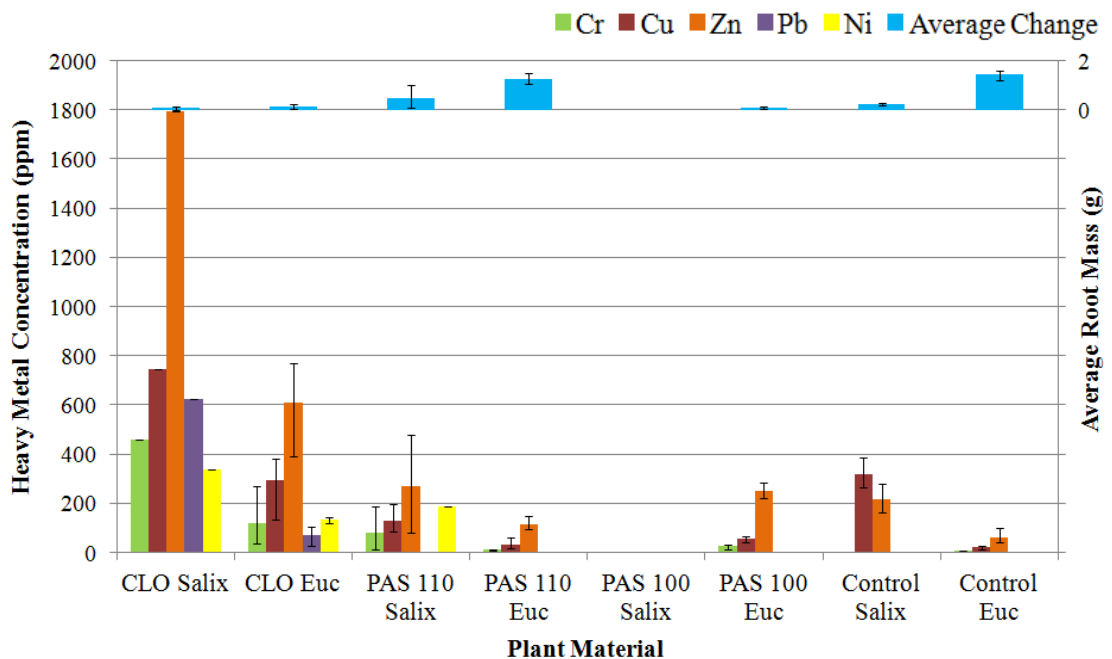

Figure 3.4.4 Average Heavy metal accumulation in root material compared to the average root mass.

4.0 DISCUSSION
4.1 ROW Characteristics
From the initial analysis of each ROW the pH, EC, total carbon and nitrogen and the total heavy metal concentrations were identified. The pH of the composted material (PAS 100 and CLO) was consistent with previous studies (Cameron *et al.* 2009) and the 7.05 pH of the PAS 110 is within the 6.0-8.0 range recommended by WRAP (2011).The total carbon and nitrogen content of the CLO was lower than that of the source segregated material, although all materials have C: N ratios of less than 20 suggesting sufficient maturity (Edwards *et al.* 2011).

The composted materials had high EC values, in particularly the CLO (5.82 mS/cm) which is classified as 'slightly saline' (4.0-8.0 mS/cm) (Cameron *et al.* 2009). EC levels of over 1.4mS/cm can affect root development and growth in plants, restricting the yields of crops (Cameron *et al.* 2009). Previous studies by Brady and Weil (1996, Cited in Hargreaves *et al.* 2008) identified MSW compost EC to range between 3.69 to 7.49 mS/cm consistent with the CLO concentration (5.82 mS/cm). The BSi standards recommend PAS 100 compost used as a soil improver to have EC less than 2.0mS/cm (Edwards *et al.* 2011), levels detected in this material were slightly above the recommended values (2.25mS/cm).

The heavy metal concentration of the CLO exceeded the PAS limits as a result of the mixed waste stream. The concentration of zinc present in the PAS 100 compost exceeded the required levels, the batch would require further processing and analysis before being certified and sold. The PAS 110 material was below the required standards and would be suitable for application to agricultural land.

4.2 Pot Trials

The average heavy metal concentration in each ROW + perlite mixes were compared to the average plant height change over the growing period. There was evidence of some correlation between the heavy metal concentrations in CLO and PAS 100 pots and the reduction in plant height compared to plant height increase in the PAS 110 and controls pots with lower heavy metals concentrations.

Although initial wilting and leaf colour change in all trials was a result of replanting stress, continued necrosis of both species grown in CLO and PAS 100 can be attributed to the higher EC and heavy metal content. Cureton et al. (1991, cited in Duggan, 2005) applied leachate with an EC of 8.89mS/cm to *Salix* sapling, the plants showed evidence of chlorosis, necrosis and leaf desiccation as a result of the high EC as seen in the CLO and PAS 100 pot trials at lower EC levels. After 2 weeks there was evidence of plant recovery which may be a result of soluble salts and metals being leached from the material weekly. Understanding of the composition of the leachate produced from the different ROW will identify excess concentrations of specific soluble ions.

The heavy metal concentrations within stem, leaf and root material was compared between species and ROW material. Heavy metal concentrations were in the order of root> leaves> stems, suggesting some translocation of metals once taken up by root mechanisms (Pulford & Watson, 2003).

S. viminalis stems grown in all materials contained greater levels of zinc compared to *E. nitens*. The concentration of zinc present in *S. viminalis* controls was similar to the ROW materials suggesting a natural background level of zinc within the trees as previously shown by Mleczek *et al*. (2009). The zinc levels in the *E. nitens* stems grown in PAS 100 was greater that the levels in the controls, PAS 110 and CLO. This indicated potential different forms and complexities of heavy metals within PAS 100 with greater bioavailability and plant uptake (Pulford & Watson, 2003). Chromium was present in all 3 *S. viminalis* replicated control sample indicating possible contamination of the controls.

The heavy metal concentrations present in leaves were greater in the *S. viminalis* species compared to the *E. nitens* for all materials including the controls. Only low levels of zinc were detected in all the *E. nitens* trials, where as *S. viminalis* contained traces of zinc and copper in all trials with chromium, lead and nickel present in the CLO pots. The ability of metals to be translocated is dependent upon the metal, with copper having a greater binding potential than zinc and zinc being more readily taken up by plants (Smith, 2009). The concentration of heavy metals within the plants is consistent with the levels of heavy metal present in the initial material, i.e CLO> PAS 100> PAS 110.

Root material collected from both *S.viminalis* and *E. nitens* had higher heavy metal concentrations than other parts of the plant material. Species grown in CLO had traces of chromium, copper, lead, nickel and zinc, with lower levels present in *E. nitens* than *S.viminalis*. The increased concentrations present in the root material of all pot trials suggest some immobilisation of heavy metals within the roots, but greater translocation of metals by *S.viminalis* as seen by Zacchini *et al* (2008).

Assareh *et al* (2008) investigated the toxicity of copper and zinc to three Eucalyptus species. As seen in this study there was little accumulation of copper in the stem and leaves, low levels of zinc accumulation in the stem with greater levels present in the leaves and significant accumulation of copper and zinc in the roots.

Hasselgren (1999) investigated the metal uptake of *S. viminalis* grown in sludge amended soils and identified that chromium, copper and lead accumulated in stems and zinc, whilst cadmium and nickel accumulated in leaves.

The analysis of the *S. viminalis* and *E. nitens* suggests there is some immobilisation of chromium, copper and lead within the roots with greater translocation of zinc in particular. There are some inconsistencies from these trends such as the presence of copper and lead within the leaves, this maybe a result of the roots becoming saturated with heavy metals due to the high concentrations within the material causing greater movement within the plant tissue than in less contaminated soils such as the PAS 110.

The mass of material present for analysis and the short growing period for both *S. viminalis* and *E. nitens* grown in the CLO and PAS 100 could have influenced the heavy metal uptake and translocation within the plant material.

5.0 CONCLUSION

This study investigated the use of three ROWs as nutrient sources for the growth of *Salix viminalis* and *Eucalyptus nitens* as SRC energy crops in the UK.

The initial characteristics of the materials identified higher metal concentrations in the CLO as a result of the mixed waste stream. High values of electrical conductivity in the CLO and PAS 100 were both above the recommended levels.

S. viminalis and *E. nitens* were grown with the ROW at concentrations equivalent to 3000kg N/ha and mixed with inert perlite. All plants grown with CLO and PAS 100 died within the first 6 weeks as a result of the high electrical conductivity in the material and the limited adsorption available from the perlite. The use of perlite allowed the direct impacts of the ROWs to be assessed however it does not simulate the natural environment as ROW will be used as soil amendments and will be mixed with natural soils with greater cation exchange capacities reducing the effect of soluble salts. Further work will investigate the leaching characteristics of the materials and analyse the soluble salts present.

S. viminalis accumulated higher concentrations of heavy metals than *E. niten* in the stem, leaves and roots material. There was evidence of metal translocation particularly of zinc from roots to the stems. The availability of heavy metals to be taken up is dependent upon soil characteristics, metal binding and plant mechanisms. To understand the chemical distribution of heavy metals within ROW sequential extraction analysis can be conducted to identify differences between the effect of waste stream (source segregated or mixed) and the effect of the biological treatment (aerobic and anaerobic conditions). The effect of the restricted growing period for CLO and PAS 100 trials is unknown and further study is required. There is limited work on the use of Eucalyptus as a biomass crop and the ability to accumulate heavy metals, so further study into the effect on yield is required.

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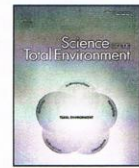
Heavy metal leaching and environmental risk from the use of compost-like output as an energy crop growth substrate

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HIGHLIGHTS

- *S. viminalis* and *E. nitens* grown in CLO at 5 different nitrogen application rates.
- Optimum application rate of CLO was 3000 kg N/Ha for each species.
- Excess heavy metal concentrations in weekly leachate at higher application rates.
- Excess nitrate and ammonium in leachate at lower application rates.
- Heavy metal accumulation in root material, some translocation to leaf material.

ARTICLE INFO

ABSTRACT

Conversion of productive agricultural land towards growth of energy crops has become increasingly controversial. Closed landfill sites represent significant areas of brownfield land, which have potential for the establishment of energy crops. Increasingly composts are now being produced from the degradable fraction of mixed municipal solid waste (MSW) and are commonly referred to as Compost-Like-Output (CLO). However, leaching of heavy metal and other elements due to the use of CLO as soil amendment has the potential to pose a risk to the wider environment as a diffuse pollution source if not managed correctly. *Salix viminalis* and *Eucalyptus nitens* were grown at 5 different CLO application rates (equivalent to 250, 1000, 3000, 6000, 10000 kg N/Ha) with weekly leachate analysis to assess the solubility of heavy metals and the potential release into the environment. The change in plant total dry mass suggested 3000 kg N/Ha as the optimum application rate for both species. Weekly leachate analysis identified excess soluble ions within the first 4 weeks, with heavy metals concentrations exceeding water quality limits at the higher application rates (>3000 kg N/Ha). Heavy metal uptake and accumulation within each species was also investigated; *S. viminalis* accumulated greater levels of heavy metals than *E. nitens* with a general trend of metal accumulation in root > stem > leaf material. Heavy metal leaching from soils amended with CLO has the potential to occur at neutral and slightly alkaline pH levels as a result of the high buffering capacity of CLO. The use of CLO at application rates of greater than 250 kg N/Ha may be limited to sites with leachate collection and containment systems, not solely for the heavy metal leaching but also excess nitrogen leaching. Alternatively lower application rates are required but will also limit biomass production.

1. Introduction

In the UK the amount of biodegradable waste reaching landfills must be reduced to 35% of the 1995 levels by 2020 under the European Landfill Directive (1999/30/EC) (European Council, 1999). In order to meet these targets mechanical biological treatment (MBT) plants have been developed to segregate, sort and pre-treat mixed municipal solid waste (MSW). There are 30 active MBT plants in the UK, which treated an estimated 2.52 million tonnes of mixed waste, from which 390,000 tonnes of organic fraction outputs were generated in 2012 (Horne et al., 2013). The organic fraction of the MSW is biologically treated using aerobic processes to produce a Compost-Like-Output (CLO). Currently CLO is not permitted to be used as an organic amendment on agricultural land due to the mixed source waste stream it stems from and

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the potential to contain heavy metals, plastics and glass. However the use of CLO is permitted on brownfield sites providing that an ecological benefit to the site can be demonstrated (Environment Agency, 2011). It has been suggested that CLO can be applied to landfill sites as a fertile soil amendment to aid the growth of Short Rotation Coppice (SRC) crops for use as a renewable biomass source (Bardos et al., 2007).

Recycled organic composted material is a valuable source of slow re-lease organic matter and nutrients that can be used to improve soil structure and soil quality (Businelli et al., 2009; Merrington et al., 2010). Brownfield sites commonly have poor soil structure and low nu-trient content which can be a significant barrier in the development of plant growth (Nixon et al., 2001). The addition of CLO to poor soils may improve the soil structure and provide sufficient nutrients for rapid growth of SRC species helping to alleviate potential nutrient limi-tations on crop yields (Forest Research, 2008). It has been identified that many land reclamation projects require greater quantities of nitrogen in order to improve poor soils (Edwards et al., 2012). Currently CLO is permitted to be used on brownfield sites by the Environment Agency (England) at application rates greater than the agricultural limit of 250 kg N/Ha for scientific trials with approval based on site specific conditions (Environment Agency, 2011). However the effect on soil chemistry from the application of CLO and the impact on SRC biomass yields are largely unknown.

Previous studies have investigated the effect of municipal waste composts (MWC) on the soil by increasing pH and improving soil texture and organic content (Bardos et al., 2007; Hargreaves et al., 2008; Smith, 2009). Businelli et al. (2009) investigated the fate of heavy metals from MWC applications over 10 years and the mobility through the soil profile. Initial metal concentrations increased after MWC application compared to non-amended soils and organo-metal complexes were found distributed vertically through the soil profile. The metal concentrations in the lower horizons after 10 years returned to background concentrations suggesting further leaching of the metals into the underlying soils beyond the investi-gated horizons. Plant material found on site was also analysed to identify heavy metal uptake, however there was limited metal phytoavailability and uptake was species dependent (Businelli et al., 2009).

Salix spp. are well known for SRC, however they also have the po-tential to phytoremediate contaminated water and soils (McKendry, 2002; Weih and Nordh, 2002; Aronsson et al., 2010). Previous stud-ies have investigated the ability of *Salix spp.* to tolerate high metal concentrations as well as their potential to uptake and accumulate metals (Pulford and Watson, 2003; Zacchini et al., 2009; Hangs et al., 2011). The use of *Eucalyptus spp.* as energy crops in the UK has gained interest due to the high productivity over short rotations, straight stem growth and the ability to tolerate a wide range of soil types (Leslie et al., 2012). Previous studies investigating heavy metal uptake from tannery sludge, landfill leachate and municipal solid waste compost have found that *Eucalyptus spp.* have also indi-cated beneficial phytoremediation characteristics (Rockwood et al., 2004; Shukla et al., 2011; Rockwood et al., 2012). Therefore by using biomass crops that also have known phytoremediation bene-fits, there is potential to limit the release of heavy metals into the en-vironment by taking up bioavailable forms and accumulating them in plant tissue that is then harvested as biomass. However the incin-eration of biomass containing heavy metals leads to other questions concerning metal released to the atmosphere or metal recovery from fly ashes (Keller et al., 2005).

This study investigates the effect of different application rates of CLO used for soil improvement on the leaching of soluble heavy metals and the risk to the wider environment when used as a nutrient source for two tree species *Salix viminalis* and *Eucalyptus nitens*. The initial charac-teristics of CLO were analysed. *S. viminalis* and *E. nitens* were grown on CLO amended soils with the CLO applied to give 5 different total

nitrogen application rates for 12 weeks to assess the biomass produc-tion, soil pore water chemistry changes and heavy metal leaching.

2. Methodology

2.1. Material collection and storage

The CLO used in this study was sourced from a MBT plant in the south of England from a mixed MSW stream. At this facility the biode-gradable fraction of MSW is separated from the mixed waste and composted in bio-stabilisation halls for 6 weeks with repeated aeration and irrigation to maintain optimum composting conditions. After composting and stabilisation of the biodegradable material, the CLO is sanitised by a pasteurisation process to destroy pathogens before being stored outside in windrows, with turning taking place every 2–3 weeks. It was collected in March 2012, and stored at <4 °C in sealed containers before analysis and use in the growth trial.

2.2. Soil material characterisation

Initial analyses quantified the moisture content (MC), electrical con-ductivity (EC), pH, and the bulk density (BD) of the CLO and Kettering Loam. The total nitrogen and total heavy metal content were also determined.

Approximately 500 g of CLO and Kettering Loam were weighed and dried for 16 hours at 75 °C and weighed again to calculate the MC in accordance with BS EN 13040 (British Standards Institution, 2007). The pH and EC of both materials were tested by using a 1 : 5 ratio of material to deionised water (British Standards Institution, 2000a, 2000b). The BD (loose) of the CLO and Kettering Loam were determined based on lower compaction and packing of pots (British Standards Institution, 2001a).

To determine the total nitrogen and total heavy metal content of the CLO and Kettering Loam, 100 g of dried sample was ground using a Labtech Essa grinder to homogenise the sample material to <2 mm frac-tion. Total nitrogen content was analysed using a total nitrogen analyser (Shimadzu, UK). The total heavy metal content was determined by *aqua regia* digestion using a 1 : 3 ratio of nitric acid and hydrochloric acid. Digestate liquid was analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Perkin Elmer Optima 2100 DV, UK) to determine the concentrations of chromium, cadmium, copper, lead, nickel and zinc (British Standards Institution, 2001b). The heavy metal concentrations were compared to the British Standard, PAS 100 limits for source segregated composted waste (British Standards Institution, 2011).

2.3. Growth trials

S. viminalis cuttings approximately 150 mm in length were planted in Kettering Loam for 2 weeks to allow roots and shoots to develop. *E. nitens* saplings were approximately 220 mm in length and were sup-plied with well-established root systems in commercial compost. For replanting in CLO/Kettering Loam, the root systems were washed to re-move the majority of commercial compost material around the roots.

Pot trials were used to identify the optimum application rate of CLO to ensure maximum biomass yield and point of toxicity of two tree species. *S. viminalis* and *E. nitens* were grown for 12 weeks with 5 different CLO total nitrogen loading rates (250, 1000, 3000, 6000 and 10000 kg N/Ha). The CLO and Kettering Loam mixes had average wet mixing ratios of 0.015, 0.069, 0.24, 0.69, and 2.65 with total masses of 894, 854, 848, 800 and 749 g corresponding to appli-cation rates 250, 1000, 3000, 6000 and 10000 kg N/Ha. The increas-ing applications rates of CLO reduced the bulk density of pots compared to the control pots containing Kettering Loam only (933 g). Kettering Loam is a uniform (<3 mm) clay loam with organic content of approximately 2%, less than 1% total nitrogen and low

weed seed content with consistent characteristics suitable for repeatability. Each application rate and species had 3 replicate pots. Application rates were calculated using the total nitrogen content (%) and moisture content of the CLO while assuming a mixing depth of 150 mm. Control pots (3 replicates) containing Kettering Loam only were treated with a standard nutrient solution to assess the difference in plant growth to the CLO application rates. The experimental matrix consisted of a total of 36 1 litre pots, with 6 different total nitrogen loading rates and two different tree species with 3 replicates per condition.

Each pot was initially double planted in order to ensure that natural death of a plant within the first 3 weeks did not limit or affect the results from different CLO application rates. After 3 weeks, one plant from each pot was removed ensuring the healthiest plant remained; this was based on the number of shoots/number of leaves rather than height alone. The remaining plants were monitored for a further 9 weeks.

All pots containing CLO were watered on the first day of each week with 100 ml of deionised water and control pots (no CLO) were watered weekly with 100 ml of 25% Hoagland nutrient solution (Punshon and Dickinson, 1995; Jenson et al., 2009). Control pots therefore received 58.82 mg of nitrogen over the 12 weeks, equivalent to an application rate of 87 kg N/Ha (Sigma Aldrich, 2013). An additional 100 ml of deionised water was added to all pots weekly on the fourth day of each week and allowed to drain for 30 minutes. Excess leachate from this operation was collected from pot saucers placed under each pot and filtered (8 μm Whatman Grade 40) under vacuum. The purpose of collecting leachate for analysis from the second watering event was to ensure excess Hoagland solution was not collected from control pots.

On completion of the growth trials the plant and soil material were separated with excess soil washed from the plant root mass. The soil material was dried at 75 °C for 16 hours to limit excessive drying and loss of volatiles, before being ground to homogenise the sample material using a Labtech Essa grinder to a particle size of < 2 mm. All plant material was divided into root, stem and leaf components before drying at 75 °C for 16 hours and ground to < 4 mm.

To assess plant growth and the biomass production of each species the moisture content of each plant was measured at the end of each growth trial and was assumed constant through the test period. This was used to estimate the initial dry biomass to calculate the percentage change in plant biomass over the growth trial and the effect of different variables on species growth.

2.4. Leachate analysis

The leachate pH and EC were measured using a Mettler Toledo SevenMulti meter. The metal contents (chromium, cadmium copper, lead, nickel and zinc, as well as additional soluble cations calcium, magnesium and sodium) of the leachate samples were analysed using ICP-OES (Perkin Elmer Optima 2100 DV, UK). Calcium, magnesium and sodium concentrations were used to calculate the Sodium Adsorption Ratio (SAR) using Eq. (1) expressed in milli-equivalents per litre. The SAR is a measure of the dissolved solids used to assess soil dispersal characteristics based on the concentrations of dissolved salts (Bell, 2007).

$$SAR = \frac{[Na^+]}{\sqrt{\frac{1}{2}\left([Ca^{2+}] + [Mg^{2+}]\right)}} \tag{1}$$

Fortnightly leachate samples were also analysed using Ion Chromatography (ICS-2000, AS40 auto sampler, AS11 Column) for soluble anions (nitrate, nitrite, chloride and sulphate).

The ammonium content in the fortnightly leachate was analysed using a modified Berthelot spectrophotometer colorimetric method (Rhine et al., 1998). This involves the reaction of ammonium with hypochlorite and sodium salt of 2-phenylphenol under alkaline conditions to produce a blue-green colour. The intensity of the colour change indicates the ammonium concentration and was measured using a Hitachi spectrophotometer U-1900 at a wavelength of 660 nm. Standard solutions of known ammonium concentrations were used to generate a linear calibration with an r^2 value of 0.9939. Samples of > 1.0 absorbance unit were diluted to ensure results were within calibration values.

Individual leachate pot data was averaged between the 3 replicates for each application rate and species.

2.5. Heavy metal accumulation

To assess the ability of *S. viminalis* and *E. nitens* to uptake and translocate heavy metals to plant tissues, the dried and ground root, stem and leaf material at week 3 and week 12 were analysed via X-ray fluorescence (XRF) (Innov-X System 5000, 3 beam soil program used) to identity the heavy metal concentrations within individual plant organs. Each sample was tested 3 times and averaged to determine final values. Plant material analysis using XRF has been reported as being an efficient and cost effective method to indicate the distribution of heavy metals in plant material (Kilbride et al., 2008; Pietrini et al., 2009).

2.6. Statistical analysis

SPSS v. 20 (IBM Corporation, 2011) was used to assess any statistical significance of tree species and application rate on the biomass and heavy metal accumulation within plant material. Univariate general linear model, ANOVA procedures were used to assess the effect of tree species and application rate on the percentage change in biomass and the total heavy metal accumulation. Multivariate general linear model procedures were conducted to assess the heavy metal accumulation within root, stem and leaf material.

3. Results

3.1. Soil material characterisation

CLO and Kettering Loam properties are shown in Table 1. The CLO heavy metal concentration can be compared to the British Standards PAS 100 total heavy metals limits to indicate the difference in source segregated limit requirements and the elevated levels found in mixed MSW streams. In the CLO mixed waste stream, copper, nickel, lead and zinc all exceeded the PAS 100 limits. The EC of the CLO was high, however the neutral pH and C: N ratios were within the recommended ranges. Kettering Loam showed neutral pH, and low EC values as well as a low C: N ratio and heavy metal total concentrations below PAS 100 standards (British Standards Institution, 2011).

3.2. Growth trials

The biomass of each plant was assessed as a percentage of the change in dry mass from the start of the experiment. All pots were initially double planted and the weaker plants were dried and weighed after 3 weeks of growth. The final biomasses of all the remaining plants were assessed after 12 weeks of growth. Fig. 1 shows the average dry mass change between the application rates. Error bars indicate the standard deviation mass change of the individual plants with negative change in dry mass indicating the death of a plant during the 3 or 12 weeks growth. It should be noted that the change in dry biomass at week 3 may represent natural deterioration due to planting stress and is therefore the weaker of the double-planted samples.

The average change in the dry mass of *S. viminalis* after 3 weeks indicates an optimum application rate of 250 kg N/Ha. There was a negative mass change in the 10,000 kg N/Ha application rate, with control pots (3 weeks Hoagland application equivalent to 22.1 kg N/Ha) showing a similar mass change to the 3000 kg N/Ha application rate. The optimum application rate at 12 weeks based on the average change is

Table 1
Soil material characteristics.

Parameter	PAS 100 quality limits[a]	Water quality limits[b]	CLO	Kettering loam
pH			7.89	7.42
EC (mS/cm)			6.02	0.28
MC (%)			83.3	4.96
Wet BD (kg/l)			717.8	916.2
Total carbon (%)			24.4	2.45
Total nitrogen (%)			2.07	0.32
C:N ratio	20:1		11.8	7.76
Total Cd (mg/l)	1.5	0.0025–0.005	0.65	BDL[c]
Total Cr (mg/l)	100	0.005–0.250	75.2	70.6
Total Cu (mg/l)	200	0.005–0.112	250.9	19.6
Total Ni (mg/l)	50	0.05–0.2	59.2	24.3
Total Pb (mg/l)	200	0.004–0.250	289.3	8.68
Total Zn (mg/l)	400	0.08–2.0	516.6	109.0
Total Ca (mg/l)			48734	8314
Total Na (mg/l)			11409	3687
Total Mg (mg/l)			6587	4039

[a] British Standards Institution, 2011.
[b] Environment Agency Water quality levels for Protection of Aquatic Life. Water Hardness dependent, site-specific assessment 0–250 mg CaCO3/l (Environment Agency, 2013).
[c] BDL Below Detection limit of 0.01 mg/l.

6000 kg N/Ha, however the individual plant data shows a high standard deviation. Application rates of 1000 and 3000 kg N/Ha had lower average change in dry mass compared to the 6000 kg N/Ha application rate however the standard deviations were lower.

The optimum application rate for *E. nitens* after 3 weeks was 1000 kg N/Ha. After 12 weeks of growth the optimum application based on average change in dry mass was 1000 kg N/Ha however the 3000 kg N/Ha application rate had a smaller variability between replicates.

Comparison of the two species considered indicates that *S. viminalis* had a greater change in dry biomass at all application rates at week 12. The tree species had a significant influence ($p < 0.001$, 2-way ANOVA) on biomass production whereas the application rate did not significantly affect the percentage change in biomass over 12 weeks ($p > 0.05$, 2-way ANOVA). There was no significant relationship between the tree species and the application rate evident after 12 weeks ($p > 0.05$, 2-way ANOVA).

Weekly leachate samples from each pot were analysed for pH, EC and metal concentrations. Fig. 1. Average dry percentage change in mass after 3 weeks and 12 weeks growth at different CLO application rates.

Fig. 2 shows the average pH, EC and SAR readings for each application rate and species. The pH readings for all application rates ranged between 6.76 and 8.82 for *E. nitens* and 7.20 and 8.68 for *S. viminalis* over the 12 weeks. Higher pH values were generally associated with the higher application rates. The EC values correlated to the application rates for both *S. viminalis* and *E. nitens* with a decreasing EC measured with decreasing CLO application rate. The *E. nitens* EC values gradually decreased over time with slight weekly deviations to levels of less than 2.0 mS/cm at week 12. The *S. viminalis* EC also reduced steadily between week 1 and 10 to approximately 2.0 mS/cm before increasing in the 3000, 6000 and 10,000 kg N/Ha application rates. Similar patterns are evident in the SAR of the *S. viminalis* leachate weekly samples.

The metal concentrations of the weekly leachate were analysed using ICP-OES. Individual pot leachate values were averaged for each application rate and species. Standard deviations were calculated based on the individual leachate data for each week, and the average standard deviation for the 12 weeks was identified for each application rate. Standard deviation values ranged between 0.002 and 0.01 mg/l for chromium and nickel, 0.01 and 0.04 mg/l for copper, 0.001 and 0.02 mg/l for lead, and 0.003 and 0.07 mg/l for zinc. Higher standard deviation errors were associated with higher application rates (6000 and 10,000 kg N/Ha) within the first 4 weeks.

Fig. 3 shows the average concentration for the heavy metals assessed in the PAS 100 criteria and Fig. 4 shows the average concentration of
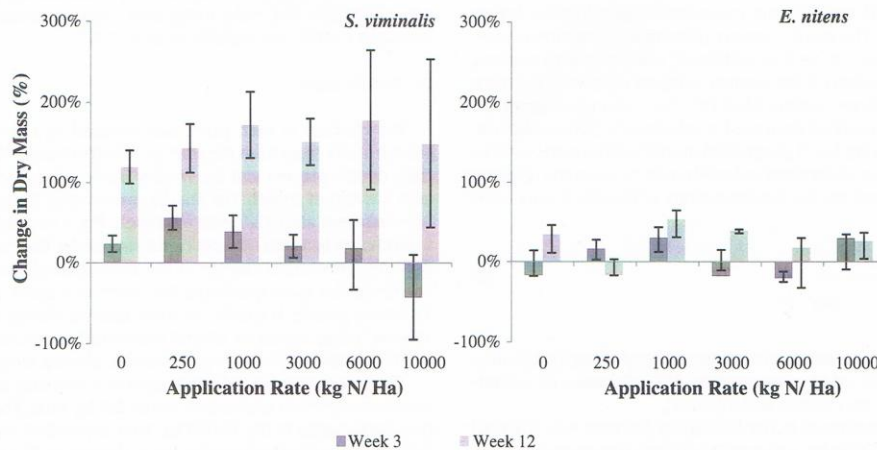

Fig. 1. Average dry percentage change in mass after 3 weeks and 12 weeks growth at different CLO application rates.
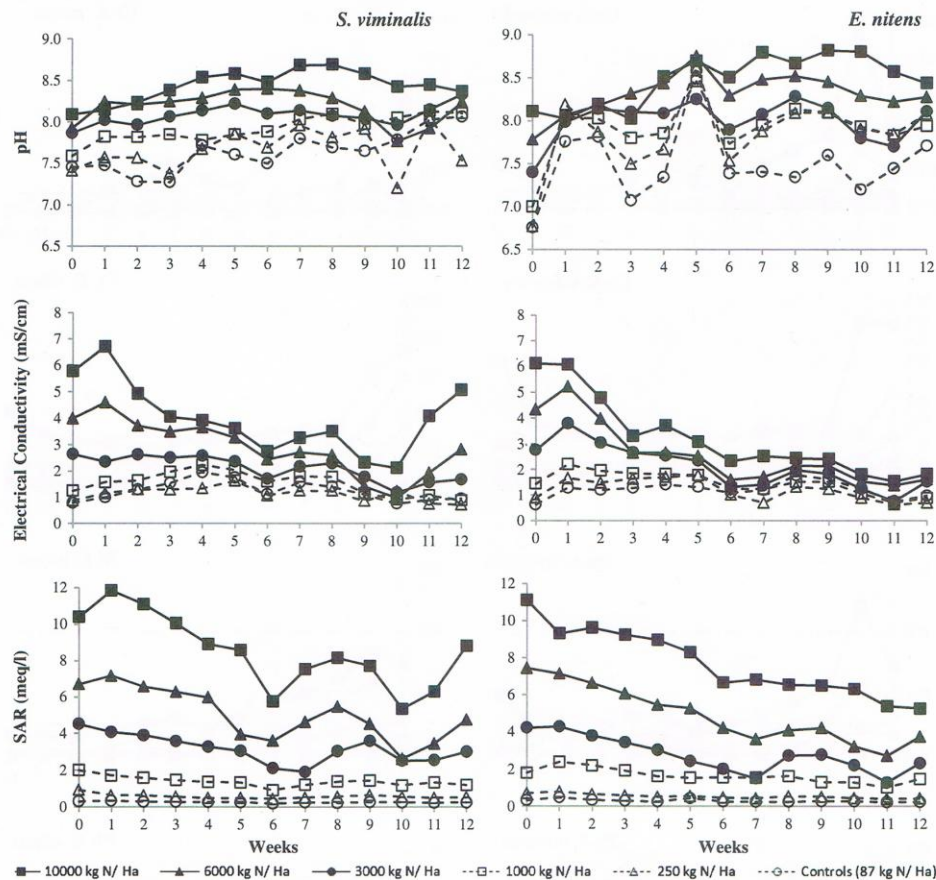
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Fig. 2. Average weekly leachate pH, electrical conductivity and sodium adsorption ratio for *S. viminalis* and *E. nitens* at different CLO total nitrogen application rates (kg N/Ha).

other additional metal cations present in the weekly leachate for each species and application rates. Cadmium concentrations were below detectable levels with both species and for all application rates (data not shown). Horizontal lines indicate water quality levels for aquatic life as stated by Environment Agency (England) (Environment Agency, 2013). The Environment Agency water quality levels for chromium, copper, nickel, lead and zinc are dependent upon water hardness (between 0 to > 250 mg $CaCO_3$ /l) and the heavy metal concentration ranges are shown in Table 1 and in Fig. 3 (Environment Agency, 2013).

Leachate analysis indicated the higher concentrations of heavy metal were generally associated with the higher application rates of greater than 6000 kg N/Ha. Concentrations of lead and zinc fell within the water hardness quality limits for both species; however chromium; copper and nickel exceeded the maximum limit for higher application rates.

Peaks were evident in week 1 for heavy metals in pots containing both species, excluding copper and zinc with *E. nitens* for higher application rates. There was an overall decrease with only slight variations of heavy metals between week 2 and 11 in both species. Heavy metal concentrations (excluding lead) increased in week 12 in *S. viminalis* at application rates of 10,000 kg N/Ha and a slight increase was evident for copper with *E. nitens* at 10,000 kg N/Ha.

High magnesium and sodium concentrations gradually decreased over time for both species, with low constant levels in the lower application rates. There were increases in the 3000, 6000 and

10,000 kg N/Ha (*S. viminalis*) and 3000 kg N/Ha (*E. nitens*) application rates from week 10 to week 12. Calcium concentrations varied during the 12 weeks between application rates with some consistency between the two species. A gradual decrease in calcium concentration for application rates of greater than 3000 kg N/Ha occurred before an increase in concentrations between week 10 and 12 as seen in the magnesium and sodium concentrations.

Fig. 5 shows results of the soluble anion and ammonium analysis. At week 0 sulphate concentrations for *E. nitens* application rates were higher than *S. viminalis* for all applications rate $\geq$ 3000 kg N/Ha. Between weeks 2 and 10 all application rates had decreased with little variation between species. In week 10 with *S. viminalis*, sulphate concentrations increased to levels greater than or similar to week 0 concentrations for application rates > 3000 kg N/Ha.

Chloride concentrations decreased generally for both species, with higher chloride concentrations corresponding to higher application rates of CLO. Constant concentrations of chloride were present at the lower application rates and control pots for both species. *S. viminalis* pots of 6000 and 10,000 kg N/Ha application rates showed increased concentrations from week 0 to week 2, before gradually decreasing until week 6. Concentrations of chloride peaked in week 8 and week 12 for *S. viminalis* pots at application rates were greater than 3000 kg N/Ha.

In general *S. viminalis* and *E. nitens* pots showed similar nitrate patterns during the 12 week growth trials for all CLO loading rates. There
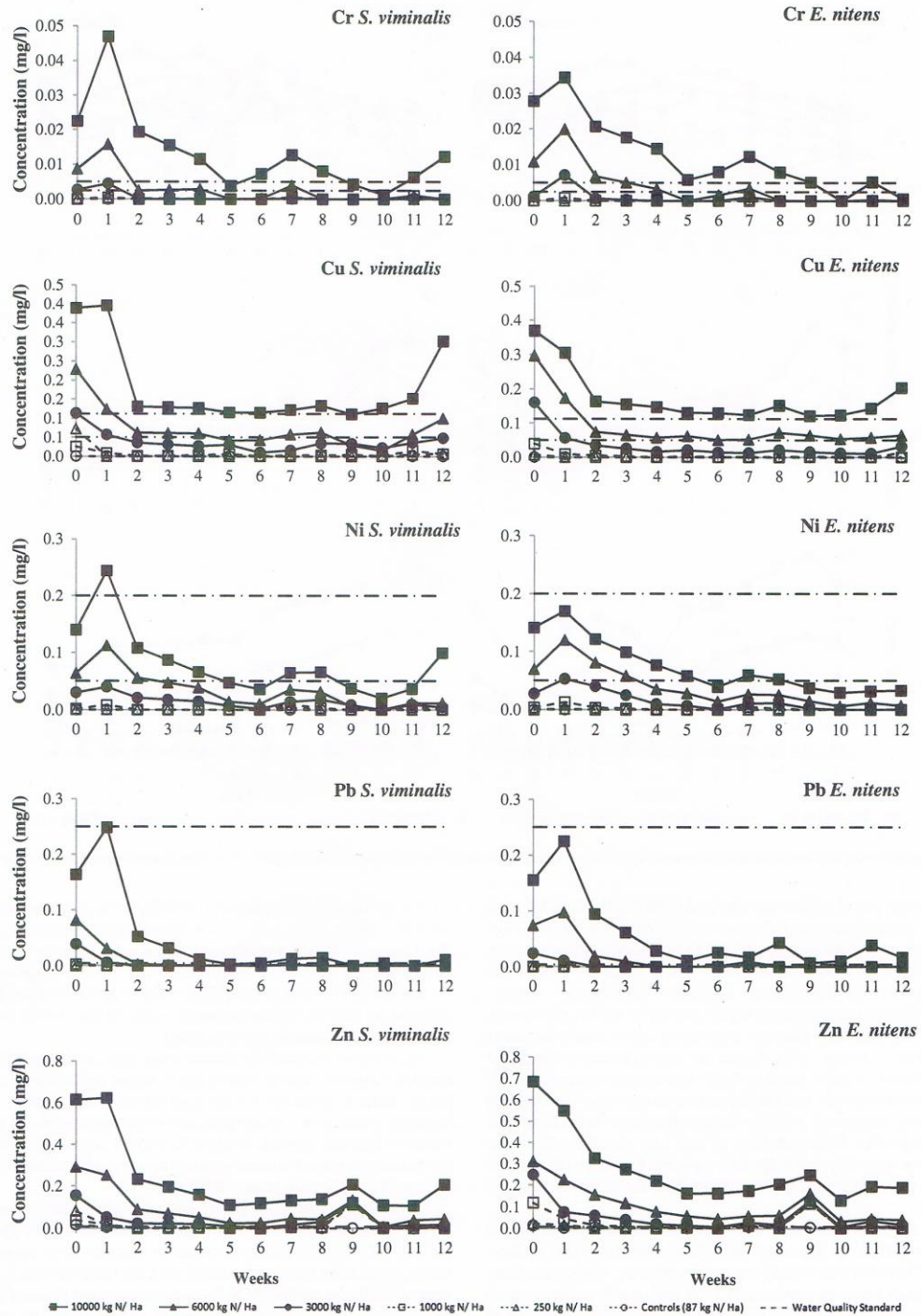
Fig. 3. Average heavy metal analysis of leachate. Water quality range for zinc 0.08–2 mg/l (not on scale).
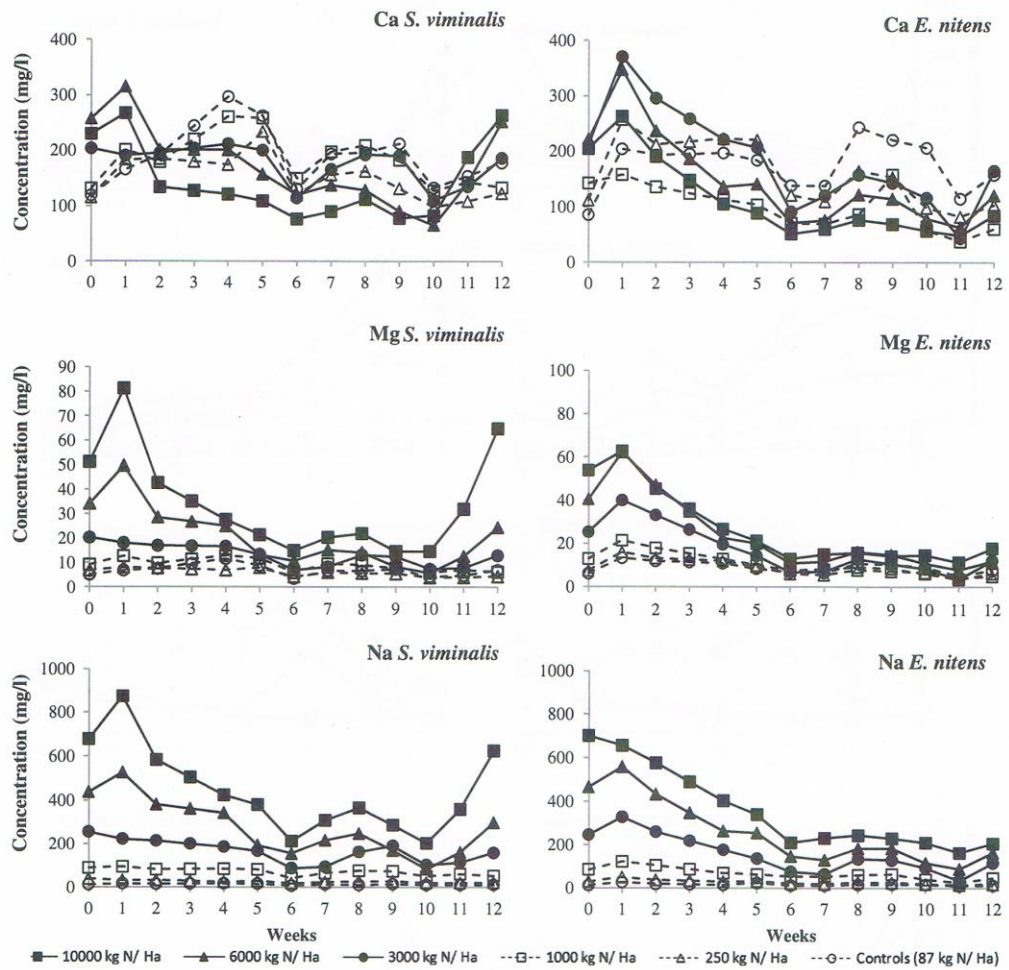
Fig. 4. Average weekly leachate analysis of soluble cations.

was negative correlation between nitrate concentrations and total nitrogen application rate. Specimens with application rates of less than 6000 kg N/Ha showed an increase in nitrate concentrations, peaking at week 4 and week 8. Those with higher applications rates stayed steady except for the 3000 kg N/Ha application rate peaking in week 4. *S. viminalis* and *E. nitens* showed nitrate concentration increasing in week 12 ($\geq$3000 kg N/Ha). Nitrite was below detection levels in all samples.

Initial ammonium concentrations in leachate samples were similar for both *S. viminalis* and *E. nitens* before decreasing rapidly between weeks 0 and week 4. Concentrations were less than 10 mg/l for all application rates after week 4 before slight increases in the 10,000 kg N/Ha application rate pots in week 12.

3.3. Heavy metal accumulation

Total heavy metal (Cr, Cu, Ni, Pb, Zn) accumulation within the root, stem and leaf material for *E. nitens* and *S. viminalis* at week 3 and week 12 are shown in Figs. 6 and 7. Overall *S. viminalis* accumulated greater concentrations of heavy metals compared to *E. nitens*. Zinc was present in all plant material for both tree species; copper was present

in the majority of *E. nitens* and *S. viminalis* organs however zinc and copper are both essential nutrients for plant growth. Chromium was present in all plant organs and lead was present in all root samples of both tree species, however nickel was only present in *S. viminalis* leaf samples in week 3 samples.

At week 3 *E. nitens* showed greatest heavy metal accumulation within the root followed by stem and leaf, whereas *S. viminalis* showed high accumulation within leaf material followed by root and stem material. After 12 weeks of growth, there was evidence in changes in heavy metal accumulation with plant organs. *E. nitens* showed a decrease in heavy metal accumulation within root material from lower application rates ($\leq$3000 kg N/Ha) whereas the root accumulation increased within the higher application rates ($\geq$6000 kg N/Ha). Stem and leaf heavy metal accumulation also decreased by the end of the 12 weeks growth trial. There was strong correlation between the application rate and the total heavy metal uptake evident in week 12 ($R^2 = 0.91$). The 6000 and 10,000 kg N/Ha application rate samples had the highest accumulation, with similar levels of metal accumulation in all other application rates including the controls samples. Greater metal accumulation within the roots was generally evident in week 3 and week 12.
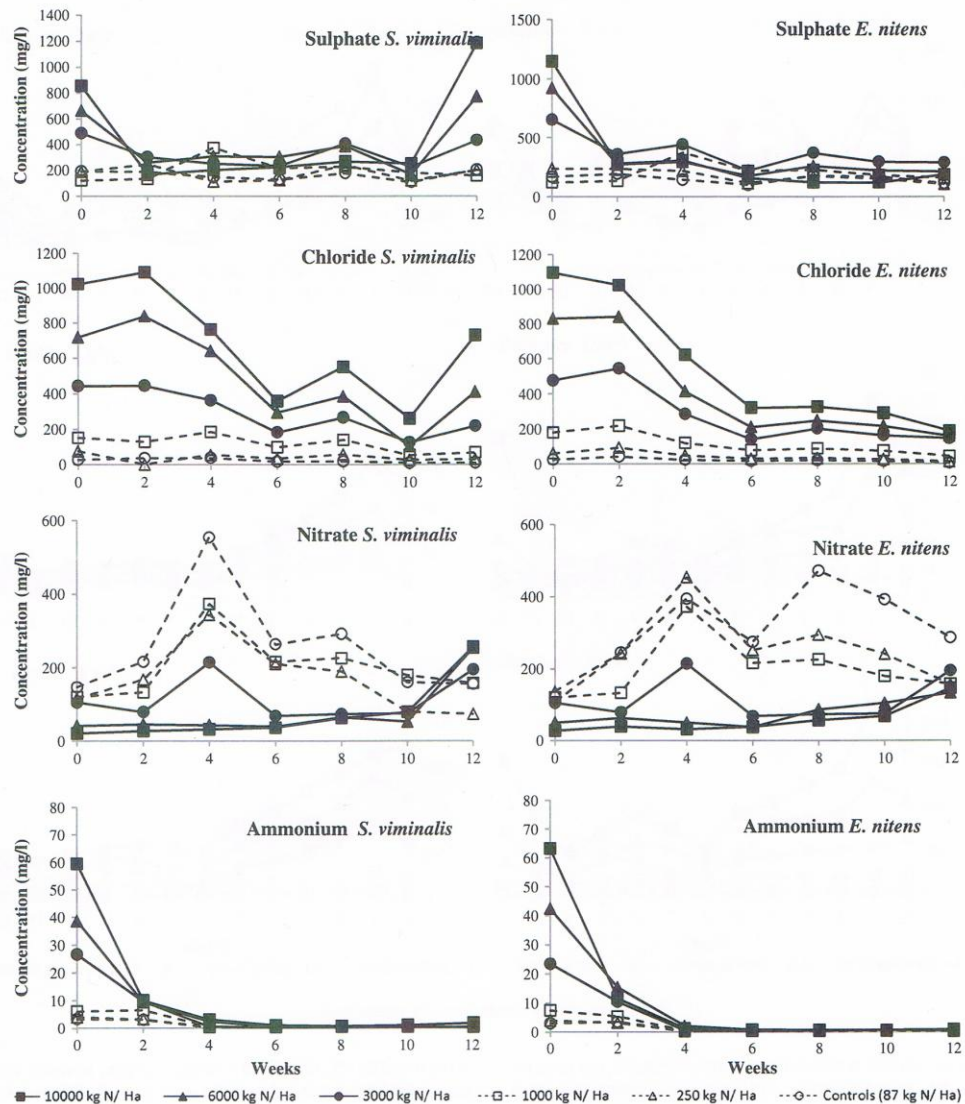
Fig. 5. Ion chromatography (soluble anions) and soluble ammonium analysis.

S. viminalis showed a decrease in heavy metal accumulation within the leaf material by the end of the growth trial, with root material containing highest total concentrations. There was strong correlation ($R^2 = 0.94$) between the higher application rates and the total heavy metal concentrations. The control plants showed greater average uptake than those in the 250 kg N/Ha application rate pots. Statistical MANOVA analysis of week 12 results indicated the tree species and application rate both had a significant effect on the total heavy metal content (Pillai's Trace p = 0.001). Results were split by tree species and re-analysed using a 1-way ANOVA to identify the influence of application rate on species heavy metal accumulation within roots, stems and leaves.

E. nitens showed a significant difference in heavy metal accumulation within the root and leaf material at application rates 6000 and 10,000 kg N/Ha ($p < 0.05$) compared to the lower application rates,

whereas there was no significance in the heavy metal accumulation in stem material at any application rate. Heavy metal accumulation in *S. viminalis* root material was significantly different ($p < 0.05$) at application rates 6000 and 10000 kg N/Ha, leaf material showed significant differences ($p < 0.05$) at application rates of greater than 1000 kg N/Ha, and stem material showed significant differences ($p < 0.05$) at application rates of 3000, 6000 and 10,000 kg N/Ha.

4. Discussion

4.1. Soil material characterisation

The characteristics of CLO and Kettering Loam showed the pH of both materials were within the recommended range for soil amendments of 6.0–8.0 (Edwards et al., 2011). The EC readings varied greatly
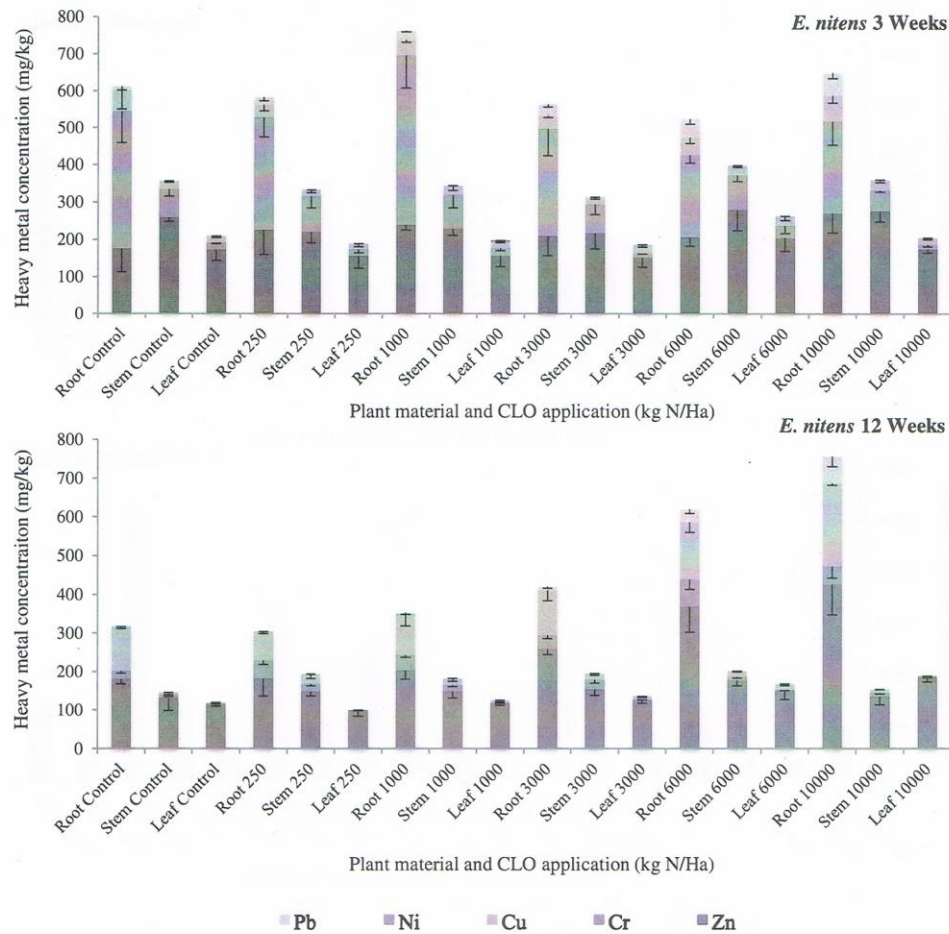
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Fig. 6. Heavy metal accumulation within *E. nitens* after 3 weeks and 12 weeks growth.

between CLO and Kettering Loam materials, with the CLO being classified as 'slightly saline' where EC levels of greater than 1.4 mS/cm can be detrimental to root development affecting plant growth and yield production (Cameron et al., 2009). The low C: N ratio of CLO (<20) suggests suitable stabilisation and maturity had occurred during and since aerobic composting (Edwards et al., 2011).

Total heavy metal concentrations in the CLO exceeded the PAS 100 limitations for copper, nickel, lead and zinc as a result of the mixed waste stream. The composition and total heavy metal concentrations of CLO can vary greatly with household type, sorting and pre-treatment methods; regional and seasonal changes can also affect CLO characteristics (Veeken and Hamelers, 2002).

4.2. Growth trials

Based on the average percentage change in dry biomass the optimum application rate for *S. viminalis* and *E. nitens* were identified as 6000 and 1000 kg N/Ha respectively after 12 weeks of growth. However when assessing the individual data greater ranges were identified at these application rates and greater consistencies between replicates were identified at 3000 kg N/Ha for both species. Both these levels

exceed 1500 kg N/Ha recommended by Bending et al. (1999, cited by Kilbride, 2006) on brownfield sites for woodland establishment.

4.3. Leachate analysis

The pH values of weekly leachate stayed above 6.5 for the 12 weeks even though the pots were watered with deionised water with an average pH of 5.64 suggesting a high buffering capacity of the CLO. The buffering capacity is attributed to high humic-like content of compost generally (Garcia-Gil et al., 2004). The higher pH values were associated with the higher application rates; this correlation has been attributed in the literature to the mineralization of carbon and the production of OH^- ions by ligand exchange and the introduction of large amounts of basic cations such as calcium and magnesium (Mkhabela and Warman, 2005).

Analysis of samples with application rates of ≥ 3000 kg N/Ha showed evidence of higher soluble ion concentration and EC, the latter exceeding the 1.4 mS/cm level that is thought to be detrimental to plant health due to a reduction in osmotic pressure limiting water availability (Cameron et al., 2009). Initial decreases in EC indicate a leaching of the soluble ions; this hypothesis is also supported by the SAR, cation and anion results shown in Figs. 2, 3, 4 and 5 respectively. These results also corroborate the increase in EC from week 10 to week 12 for
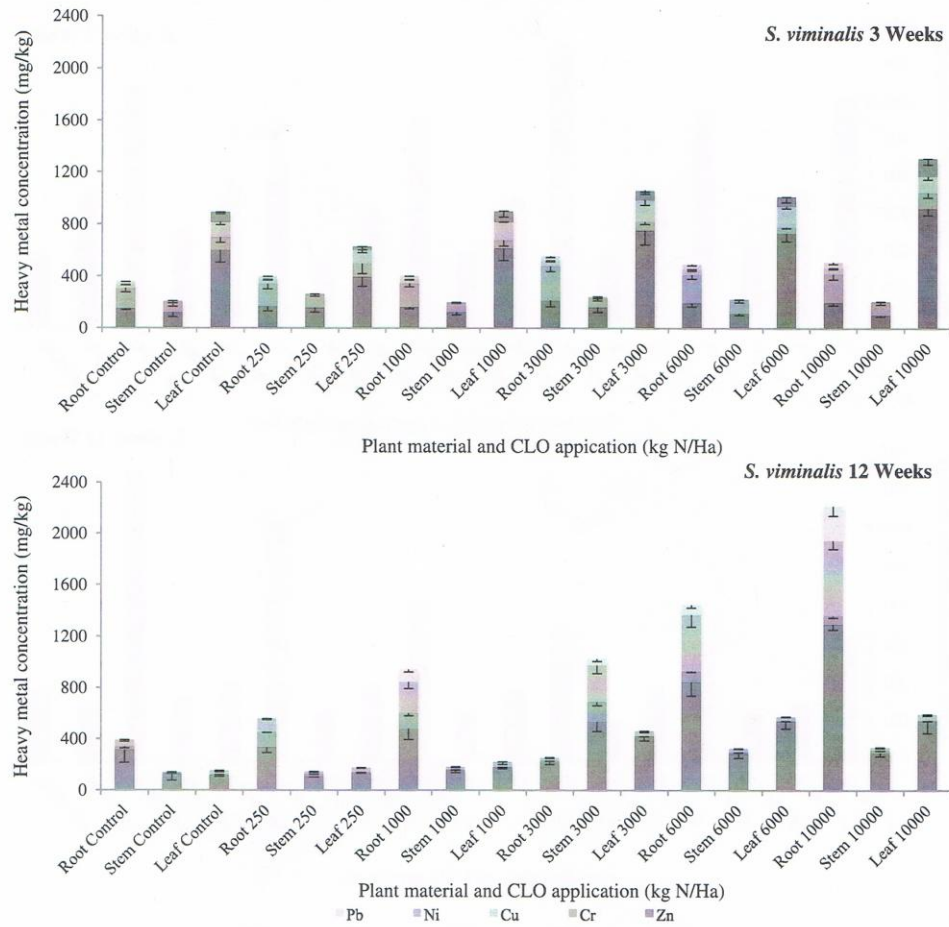
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Fig. 7. Heavy metal accumulation within *S. viminalis* after 3 weeks and 12 weeks growth.

S. viminalis pots with applications of ≥ 3000 kg N/Ha, suggesting a change in the soil systems causing the release of soluble cations and anions.

The weekly heavy metal analysis showed overall decreases in the initial concentrations consistent with the EC and soluble anions suggesting weakly bound forms or the soluble organic matter bound metals were leached from the pots, particularly at the higher application rates. Application rates of ≥ 3000 kg N/Ha exceeded the Environment Agency (2013) water quality levels for aquatic life limits for chromium, copper and nickel and would therefore have the potential to pollute ground and surface water on uncontained sites.

The lower application rate specimens (<3000 kg N/Ha) had lower heavy metal concentrations in the leachate. The leachable concentrations are likely to have been reduced due to the lower volumes of CLO used and the higher volumes of Kettering Loam present, with greater number of potential available binding sites on the Kettering Loam. A previous study by Page et al. (2012) investigated the effect of CLO when grown with perlite as an inert mixing medium at application rates equivalent to 1500 and 3000 kg N/Ha. The CLO had a detrimental effect on the growth and development of *S. viminalis* and *E. nitens*, all species died at a 3000 kg N/Ha application rate as a result of high soluble salts and limited binding sites available on perlite. Compared to this

study, when grown with Kettering Loam *S. viminalis* and *E. nitens* could survive at 10,000 kg N/Ha and 3000 kg N/Ha respectively. It was concluded the inert medium perlite was not realistic in representing the effect of CLO in soil systems due to the reduced quantity of binding sites (Page et al., 2012).

A notable increase in sulphate ions in *S. viminalis* pots of application rates of greater than 3000 kg N/Ha between weeks 10 and 12 was observed. It is possible that the release of sulphate ions into solution may be the result of heavy metal sulphides being oxidised, releasing metal ions, hydrogen ions and sulphate (Bozkurt et al., 2000). Metal sulphide oxidation commonly results in the release of H^+ ions and can lower pH levels, however, the buffering capacity of the CLO and Kettering Loam mixes used could account for the lack of pH decreases seen. Increases in nitrate concentration in application rates ≥ 3000 kg N/Ha in both species in week 12 also support the theory of oxidation processes occurring.

Flyhammar and Håkansson (1999) investigated the release of heavy metals in stabilized MSW under aerated and anaerobic conditions. Oxidation of the MSW resulted in increases in Ca, Mg, Mn and SO_4 concentrations, with a drop in pH from 9.0 to 8.0. The increased leaching of sulphate was attributed to the oxidation of sulphide phases and increased concentrations of Ca, Mg and Mn indicated the dissolution of carbonate solids and buffering against proton addition to the solution.

The neutral to alkaline pH limited the heavy metal concentrations in the leachate (Zn and Cd) however under acidic conditions 30% of the total heavy metals contents could be dissolved (Flyhammar and Håkansson, 1999). The increased solubility of Ca and Mg was also evident in this study as well as increased concentrations in sodium, chloride and nitrate, all associated with electrical conductivity of a solution.

Increased metal and sulphate leaching, possibly due to sulphide oxidation, was only evident in the S.viminalis pot trials. This could be a result of the poor health or premature death of E. nitens at applications rates of greater than 6000 kg N/Ha limiting root activity or a Salix spp. specific influence. Vervaeke et al. (2004) investigated the effects of S. viminalis on metal extractability in anoxic contaminated dredged sediment. The extractability of Cd, Zn, and Cu increased in the root zone of S. viminalis compared to the bulk soil as a result of increased oxidation at the root zone by active oxygen transfer and transpiration by roots. Long term field trials indicated lower metal concentrations in the root zone suggesting metal leaching over 2 years. This was linked to the increased mobility of metals, as well as a general improved soil structure from the application of an organic amendment, reducing compaction, increasing soil flow pathways, increasing leaching vertically though the profile (Vervaeke et al., 2004). The ability for plant roots to release oxygen supports the oxidation processes and changes in the solubility of cations and anions over time.

The rate of mineralisation, nitrification and denitrification can influence the water-soluble concentrations of inorganic nitrogen forms. Control pots watered with 25% Hoagland solution and application rates of ≤ 3000 kg N/Ha showed higher nitrate concentrations and lower ammonium concentrations suggesting sufficient nitrification. Nitrate peaks in week 4 for both species indicate excess nitrate concentrations that may be attributed to the removal of the double planting resulting in reducing nitrate uptake, thereby leading to an increase in nitrate leaching. Control pots for E. nitens also peaked in week 8; this cannot be attributed to the removal of double planting however may simply be excess nitrate not required by the individual plant. Ammonium concentrations were constantly low (<10 mg/l) suggesting total lower concentrations and sufficient binding capacity.

Whereas nitrate concentrations at higher application rates (>3000 kg N/Ha) were steady between weeks 0 and 10, ammonium was readily leached within the first 4 weeks before reaching steady concentrations. Ammonium leaching suggests limited binding sites (Cameron et al., 2009) and low nitrification within the compost samples, after windrow and bag storage. As previously mentioned, nitrate concentrations increased in week 12 for application rates ≥ 3000 kg N/Ha for both species, with greater concentrations associated with S. viminalis. This may be attributed to nitrogen initially being immobilised in the higher application rates pots, limiting nitrification and nitrate concentrations in leachate compared to lower application rates and control pots. Increased nitrate concentrations (week 12) at the higher applications suggest stabilisation, and nitrification, which is consistent with oxidation evidence present from the increased sulphate concentrations previously discussed. Similar patterns were seen Burgos et al. (2006) during incubation trials where nitrate was initially immobilised after MSW compost amendment, after 16 weeks nitrate leaching concentrations increased once stabilisation had occurred. The high solubility of nitrate poses a potential risk to the wider environment at all application rates as concentrations of > 40 mg/l are considered high by the Environment Agency's nutrient classification system (Environment Agency, 2013).

The leachate collected from the pot trials indicated the effect of different application rates on the quality of leachate. The water-soluble fraction of heavy metals decreased after the initial application of soil amendment. The application of organic amendments has previously been shown to reduce metal mobility by adsorption, complexation and precipitation processes (Farrell et al., 2010; Park et al., 2011), however changes in the soil chemistry can influence the solubility of salts and increase the heavy metal mobility. Although the heavy metal

concentrations in the leachate were greater in the higher application rates, the nitrate concentrations pose greater risk in the lower application rates. The overall quality of the leachate impacts the use and application rate of CLO as a soil amendment to ensure a diffuse pollution source is not generated.

4.4. Heavy metal accumulation

The concentrations of heavy metals in the S. viminalis and E. nitens roots, stems and leaves were analysed using XRF. After 12 weeks of growth, both species accumulated highest total heavy metal concentrations in the root material, with lowest concentrations found in the leaves for E. nitens and stem material for S. viminalis.

S. viminalis exhibited higher concentrations of heavy metals in leaf material in week 3 samples including chromium and nickel which are non-essential nutrients. However, heavy metal concentrations were lower in leaf material after 12 weeks growth. This indicates there was some translocation of heavy metals from the root to the above-ground organs initially, which may have been a method of compartmentalising phytotoxic metals (Pulford and Watson, 2003). S. viminalis regularly dropped older leaves, and later leaf growth may have contained lower heavy metal concentrations compared to the root material. Considering that the application rate did not have a significant effect on the biomass production of both species, and the correlation between application rate and heavy metal uptake, this suggests heavy metal uptake did not have a negative impact upon biomass production. Previous studies have investigated the use of S. viminalis and E. nitens for phytoremediation. Zacchini et al. (2009) investigated the tolerance, accumulation and translocation of Cd by Salix and Poplar clones grown hydroponically. Salix clones showed greater tolerance and translocation capabilities whereas poplar clones showed high bioaccumulation in root material. This study identified high metal accumulation in the root material with little translocation, although the movement of individual metal species may show specific compartmentalisation of some metals as seen by Pulford and Watson, 2003. Assareh et al. (2008) investigated the response of Eucalyptus spp. to Cu and Zn. It was found the heavy metal uptake and accumulation correlated with the metal concentrations in soils as shown in this study with highest total accumulation associated with the highest application rates in both S. viminalis and E. nitens.

Although both S. viminalis and E. nitens have shown evidence of heavy metal phytoremediation of soil the concentrations accumulated are not as high as specific hyperaccumulating species. There is potential to remove similar quantities of pollutants as with hyperaccumulators as a result of the high biomass production and repeated harvesting (Mughini et al., 2013).

5. Conclusion

The use of CLO as nutrient source for the growth of SRC energy crops was investigated to identify the risk to the environment from heavy metal leaching and the impact of different application rates on the biomass yield. The initial characteristics of CLO were investigated and compared to British Standard PAS 100 thresholds. As a result of the mixed waste source the heavy metal concentrations exceeded these limits and therefore the use of CLO is limited to brownfield sites and excluded from agricultural land.

Growth trials conducted at different application rates identified the effect on leachate quality, the tolerance limits for both S. viminalis and E. nitens and the effect on biomass growth over 12 weeks. Weekly leachate collection identified high leaching of soluble cations and anions within the first 2–4 weeks before levelling off. Changes in the soil chemistry and potential metal sulphide releases are thought to have increased the solubility of some anions and cations. Nitrate leaching at lower application rates could be a limiting factor in the use and management of CLO as a nutrient source.

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Heavy metal accumulation within plant organs identified the ability of both species to uptake available forms of heavy metals into the roots, stems and leaves. *S. viminalis* had a greater ability to accumulate metals than *E. nitens*, however long term accumulation in roots over time may affect biomass production in later coppicing seasons.

The use of CLO will ensure sufficient nutrients are available for rapid growth of SRC crops which naturally remediate the heavy metals present. However there is potential for heavy metals and other soluble compounds to be leached from the site if the optimum application rates for biomass production are used (3000 kg N/Ha). The use of CLO at application rates of greater than 250 kg N/Ha may be limited to sites with leachate collection and containment systems to reduce the risk to surrounding agricultural land, ground water and water courses. Alternatively a lower application rate is required to reduce leachate concentrations but will also limit biomass production. The soil pH is commonly used to assess the mobility of heavy metals within soil systems however, as shown by this study, neutral and slightly alkaline soils can still leach heavy metals, therefore pH cannot be used to solely assess the risk of heavy metal leaching from soils.

Acknowledgements

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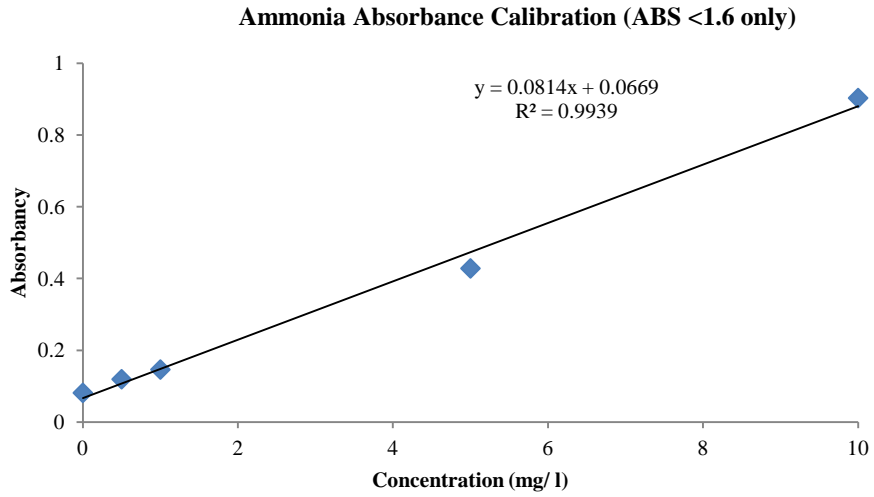
APPENDIX 2: XRF LIMITS OF DETECTION.

Limits of detection for 3- Beam Soil using the X-5000 Portable XRF Analyser.
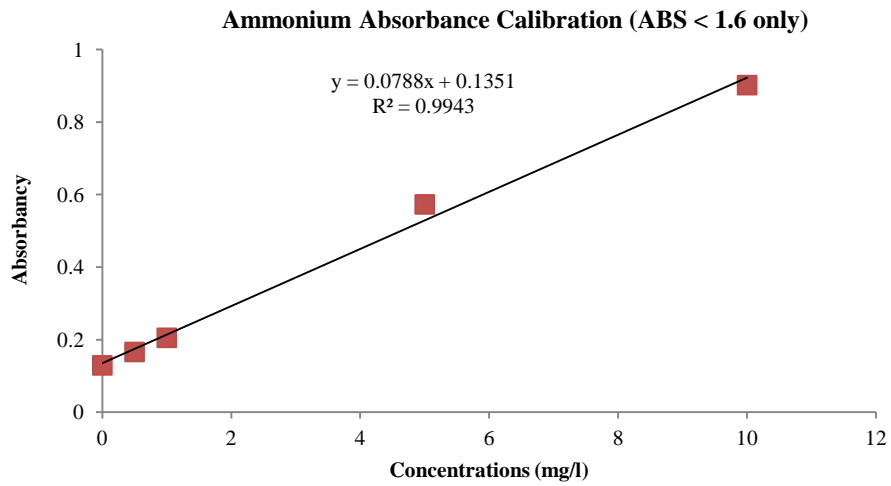Limits are in ppm unless otherwise stated.

Element	Limit of Detection	Element	Limit of Detection
Mg	Not Available	U	2
Al	1.4%	Sr	1
Si	0.5%	Zr	1
P	380	Th	2
S	90	Mo	1
Cl	33	Ag	1
K	18	Cd	2
Ca	17	Sn	3
Ti	2	Sb	4
Cr	2	Ba	20
V	1	Hg	3
Mn	2	Pb	2
Fe	4	La	30
Ni	4	Ce	45
Cu	3	Pr	75
Zn	2	Nd	90
As	2	Pm	130
Se	1	Sm	150
Rb	1		

APPENDIX 3: AMMONIUM CALIBRATION CURVES.

Optimum application rate growth trials ammonium calibration

Ammonia Absorbance Calibration (ABS <1.6 only)

$y = 0.0814x + 0.0669$
$R^2 = 0.9939$

Column growth trials ammonium calibration.

Ammonium Absorbance Calibration (ABS < 1.6 only)

$y = 0.0788x + 0.1351$
$R^2 = 0.9943$

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APPENDIX 4: PLANT IMAGE ANALYSIS MATLAB CODE.

Plant Image Analysis program written by Craig Morgan as part of the CUROP Research Placement, 2011.

The Use of Image Analysis in Matlab to Measure Plant Growth
CUROP Research Placement, summer 2011

INTRODUCTION

The following document outlines a plant analysis program created in Matlab. The aim of the program is to provide a quick, simple and non-intrusive method of measuring plant growth over time. The system takes images as inputs, outputting values of height, volume, envelope volume and density factor. The calculated data is exported to Microsoft Excel.

The program is designed to be run from run from the Graphical User Interface (GUI) shown in Figure 1. The explanations contained within the remainder of this document are described in relation to this GUI. Note that in Figure 1, as when the program is run, the GUI contains various gaps. These are filled with images and buttons etc. as the user follows the data entry procedure.

The various stages of the program are listed below. Each of these points is explained in detail within this document. The code for the program is also included as an appendix.

- System Start-up
- System calibration
- Data Input
- Image Preparation
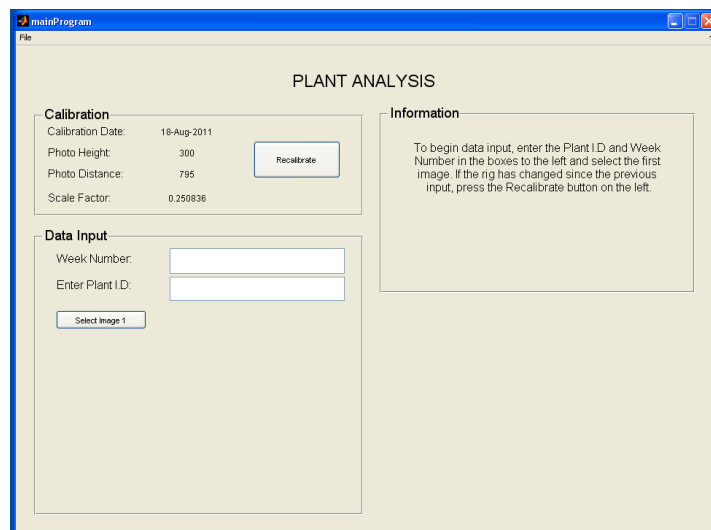- Size Calculation
- Data Storage

Figure 1: Main GUI on start-up

SYSTEM START-UP

The following steps are required to open the program.

1) Open Matlab

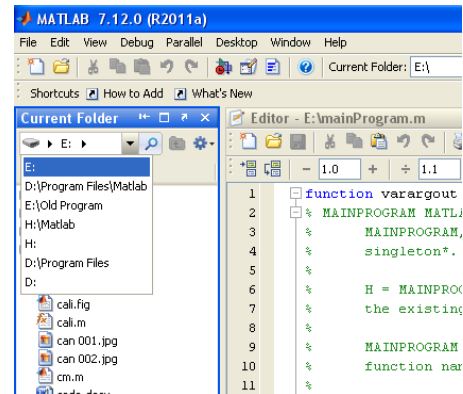2) Select the correct drive from the 'Current Folder' panel in the Matlab workspace as shown in Figure 2.

Figure 2: Select Folder

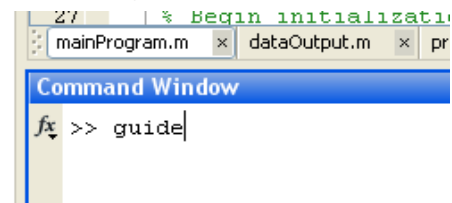3) Enter "guide" in the 'Command Window' as shown in Figure 3.

Figure 3: Command Window

4) The 'GUIDE Quick Start' dialog box then appears, shown in Figure 4. Select the file called 'mainProgram.fig' and click open.
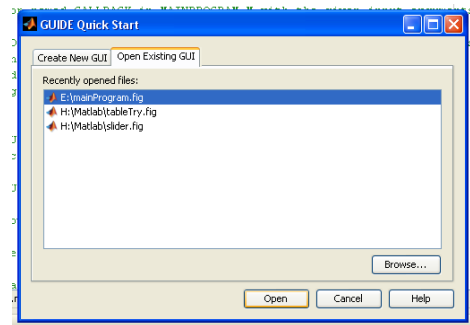

Figure 4: GUIDE menu

5) The editable version of the GUI then appears. To actually run the program, click the green 'play' symbol. The GUI as shown in Figure 1 is then opened.

Figure 5: Figure screen

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SYSTEM CALIBRATION

The program relies on the conversion of distances measured in pixels to their equivalents in millimetres. To allow these conversions, the system must first be calibrated as, outlined below. This calibration is not required every time the system is run, but only when the photography rig is altered. When cropping the image during calibration, it is important to leave sufficient space around the crop for plant growth. When the plants begin to approach the known height, the system needs to be recalibrated with a greater known height used.

1) The user selects the 'Recalibrate' button from the main GUI.

2) The calibration screen is opened by the system.

3) The user enters the known height to which the manual crop will be applied, as well as the distance from the camera stand to the rig setup.

4) The user then selects an image to use for calibration and crops from the top of the pot to the known height, using the ruler shown within the image. This is shown in Figure 6. The coordinates of this user crop are stored by the system and automatically applied to images uploaded later. By cropping the images, the pot etc.

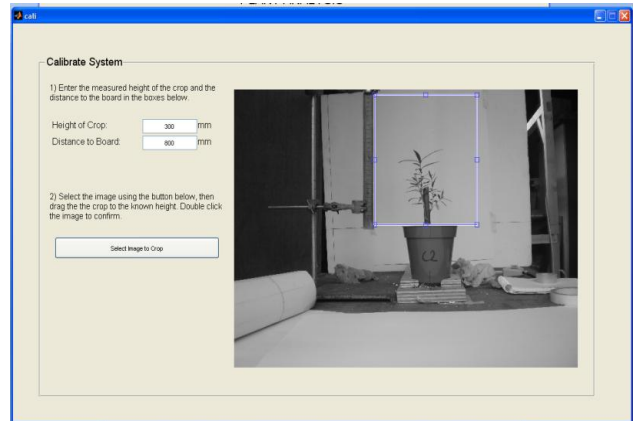are eliminated from the analysis process later.

Figure 6: Calibration Screen
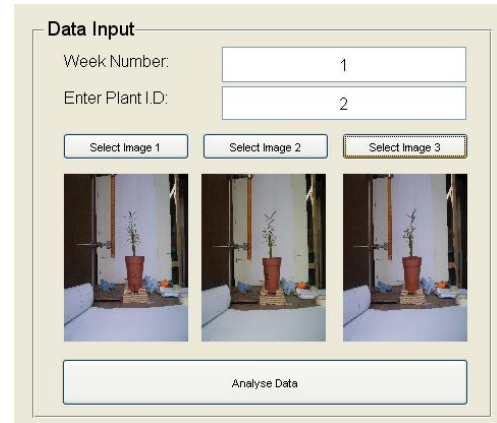
Table 1: Calibration Variables

INPUTS	
Image Crop, Known height	
OUTPUTS	
Scaling factor	= Known height (mm) / Image height (pixels)
Crop coordinates	The coordinates of the user crop to be used later.

DATA INPUT

The data to be analysed by the system is selected by the user within the data input panel of the main GUI, shown in Figure 7. This requires the input of the week number and the plant's individual I.D number. The three photographs to be analysed are selected from file using the 'Select Image' buttons.

Figure 7: Data Input Panel

Table 2: Data Input Variables

OUTPUTS
ID, Week number, Image1, Image2, Image3

IMAGE PREPARATION

Before the size of the plant can be assessed, the images must first be adjusted to allow the measurement process to be undertaken. This process is initiated by the 'Analyse Data' button in the data input panel. These steps are totally automated and hidden from the user, but described here for illustrative purposes.

1) The image is cropped using the coordinates from the calibration, removing the pot etc. from the analysis.

2) The contrast is adjusted to ensure enough differentiation between the plant and the background.

3) The image is converted to binary.

4) The image is further cropped to the extremities of the plant, as shown in Figure 8.

Figure 8: Image after being prepared for analysis

Table 3: Image Preparation Variables

INPUTS	
Crop coordinates, Image 1, Image 2, Image 3	
OUTPUTS	
ImageA, ImageB, ImageC	The images adjusted as described above

IMAGE ANALYSIS

Having prepared the images for analysis as outlined above, calculations are then carried out to obtain height, volume, envelope volume and density factor. This process is repeated for each of the three images and averaged for greater accuracy.

Height
Since the image has been cropped to the plant extents, the height can be obtained by multiplying the height of the image in pixels by the earlier calculated scaling factor.

Volume
The system calculates the 2-dimensional area of the image in pixels. Since the height is also known, the average width can be calculated by simple division. This average width is then used as the diameter of an idealised cylindrical representation of volume, along with height.

Envelope Volume
The envelope volume is defined here as the space that the plant takes up when idealised as a solid entity. To calculate the envelope volume, the gaps within the binary image as shown in Figure 8 are filled, shown in Figure 9. The volume of this new image is then calculated in an identical manner to the volume as described above.

Density Factor
The density factor is a percentage comparison between the volume and envelope volume described above. This parameter is intended to give an indication of the leaf density; those plants with more dense leaves will have less variation between volume and envelope volume and therefore a higher density factor.
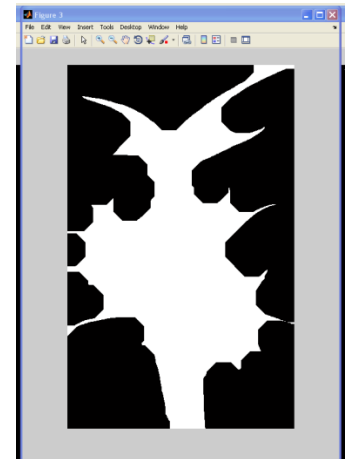

Figure 9: Filled image to calculate envelope volume

Table 4: Image Analysis Variables

INPUTS	
Scaling factor	Converts pixel values to millimetre equivalents
ImageA, ImageB, ImageC	The images adjusted as described above
OUTPUTS	
Height (mm)	= Image height (pixels) * Scaling factor
Volume (mm^3)	2D area(mm) = plant area (pixels)*Scaling factor^2 Avg. width (mm) = 2D area/height Radius = Avg. width/2 = height*π*radius^2
Envelope Volume (mm^3)	= Calculated as above, using filled image
Density Factor (%)	= (Volume/Envelope Volume)*100

DATA STORAGE

Having calculated the size parameters as described above for all three images, the program averages the results and displays the data within the GUI as shown in Figure 10. The user then has the option to save using the "Save Data" button.

The program exports the calculated data to a Microsoft Excel spreadsheet. Each plant has its own sheet within the Excel file, with a separate row for each data upload. The newly calculated information is automatically saved in the correct place using the I.D number and Week number entered earlier. When the data has been saved, the user is given the option to either enter further data or exit the system.
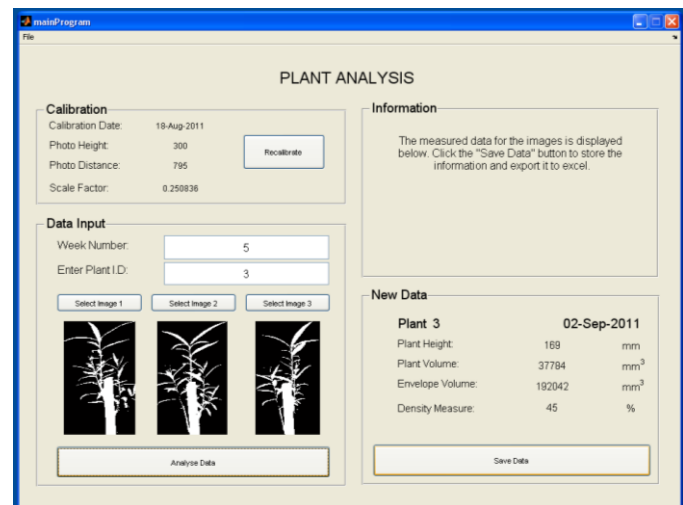

Figure 10: Plant Image analysis results and export to excel GUI.

Matlab Code developed by Craig Morgan as part of the CUROP, 2011 funding programme is available from the author.

APPENDIX 5: STATISTICAL OUTPUTS FOR ANALYSIS.

Electronic copy, available from author.

APPENDIX 6: COMPLETE DATA SETS.

Electronic copy, available from author.