



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



K. Page^a, M.J. Harbottle^{a,*}, P.J. Cleall^a, T.R. Hutchings^b

^a Cardiff University, School of Engineering, Queens Building, The Parade, Cardiff, CF24 3AA, United Kingdom

^b Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, United Kingdom

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.

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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.

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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

* Corresponding author. Tel.: +44 2920 875759.

E-mail addresses: pageke@cardiff.ac.uk (K. Page), harbottlem@cardiff.ac.uk (M.J. Harbottle), cleall@cardiff.ac.uk (P.J. Cleall), Tony.hutchings@forestry.gsi.gov.uk (T.R. Hutchings).

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 release 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 nutrient 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 limitations 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 investigated 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 potential to phytoremediate contaminated water and soils (McKendry, 2002; Weih and Nordh, 2002; Aronsson et al., 2010). Previous studies 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 indicated 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 benefits, there is potential to limit the release of heavy metals into the environment by taking up bioavailable forms and accumulating them in plant tissue that is then harvested as biomass. However the incineration 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 characteristics 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 production, 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 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. 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 conductivity (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 fraction. 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 supplied with well-established root systems in commercial compost. For replanting in CLO/Kettering Loam, the root systems were washed to remove 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 application rates 250, 1000, 3000, 6000 and 10000 kg N/ha. The increasing 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).

$$\text{SAR} = \frac{[\text{Na}^+]}{\sqrt{\frac{1}{2}([\text{Ca}^{2+}] + [\text{Mg}^{2+}])}} \quad (1)$$

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 identify 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

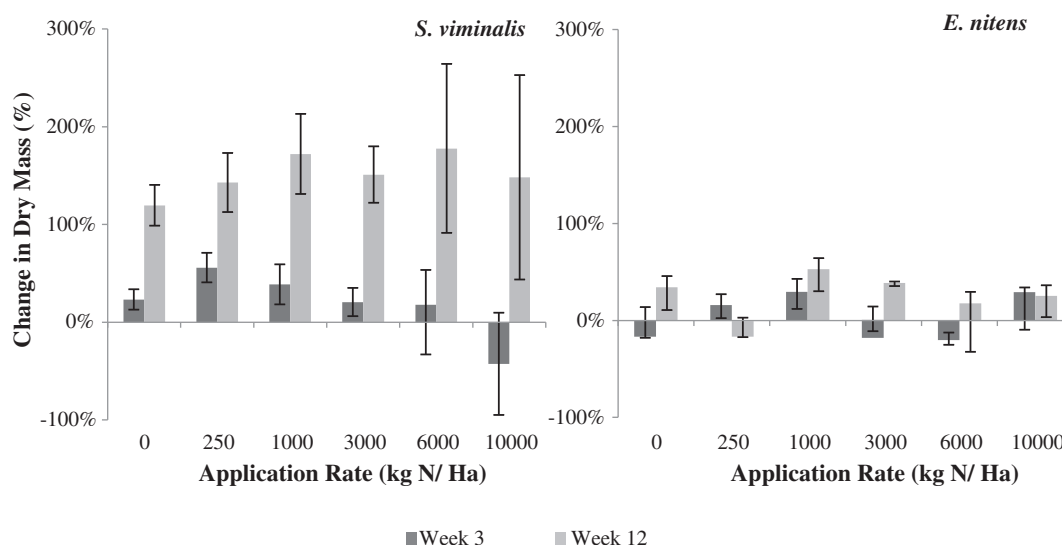


Fig. 1. Average dry percentage change in mass after 3 weeks and 12 weeks growth at different CLO application rates.

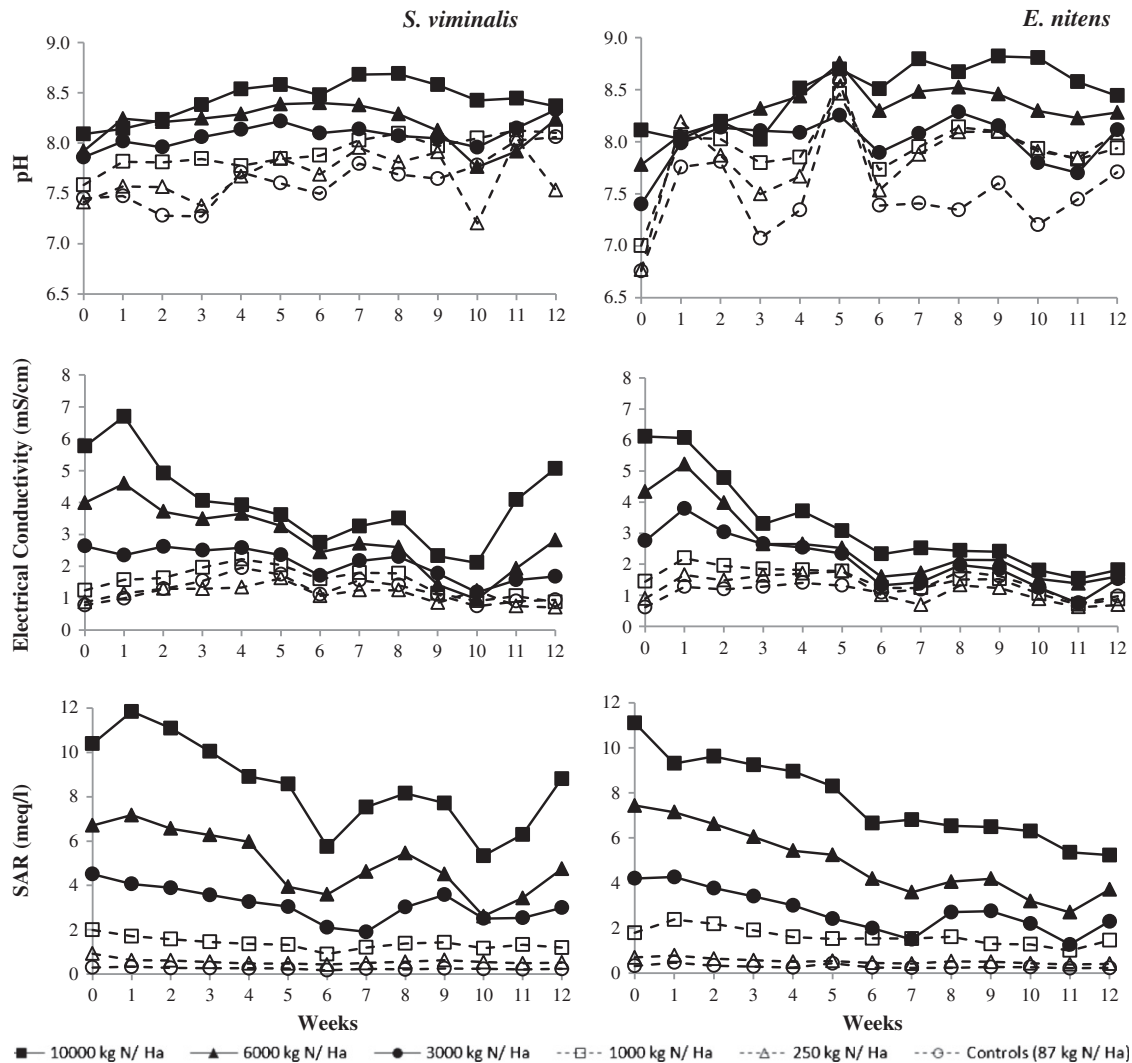


Fig. 2. Average weekly leachate pH, electrical conductivity and sodium adsorption ratio for *S. viminialis* and *E. nitens* at different CLO total nitrogen application rates (kg N/Ha).

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 CaCO₃/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. viminialis* 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. viminialis*) 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. viminialis* for all application rates \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. viminialis*, 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. viminialis* 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. viminialis* pots at application rates were greater than 3000 kg N/Ha.

In general *S. viminialis* and *E. nitens* pots showed similar nitrate patterns during the 12 week growth trials for all CLO loading rates. There

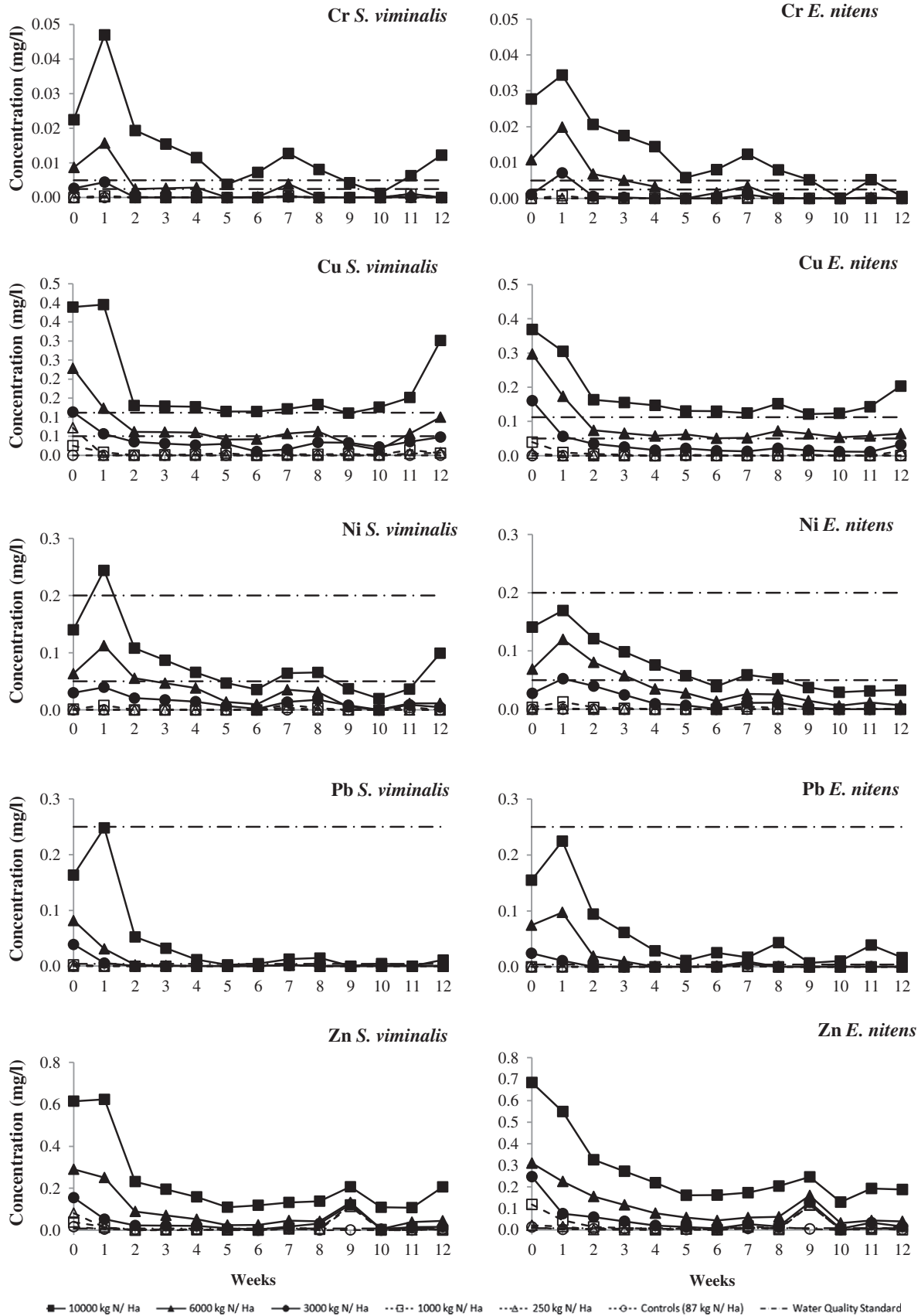


Fig. 3. Average heavy metal analysis of leachate. Water quality range for zinc 0.08–2 mg/l (not on scale).

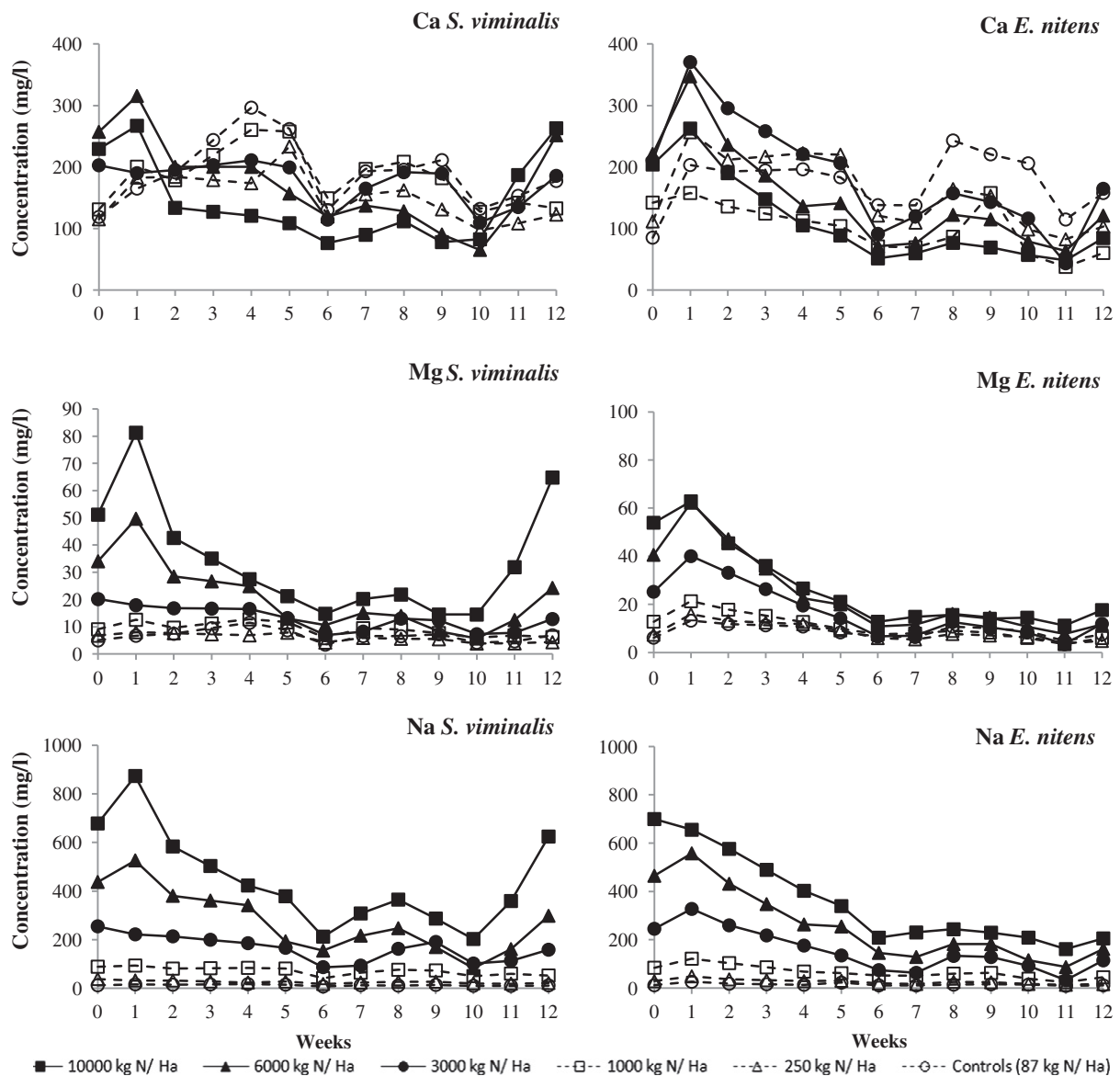


Fig. 4. Average weekly leachate analysis of soluble cations.

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 application 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 (≥ 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 (≤ 3000 kg N/Ha) whereas the root accumulation increased within the higher application rates (≥ 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. Greater metal accumulation within the roots was generally evident in week 3 and week 12.

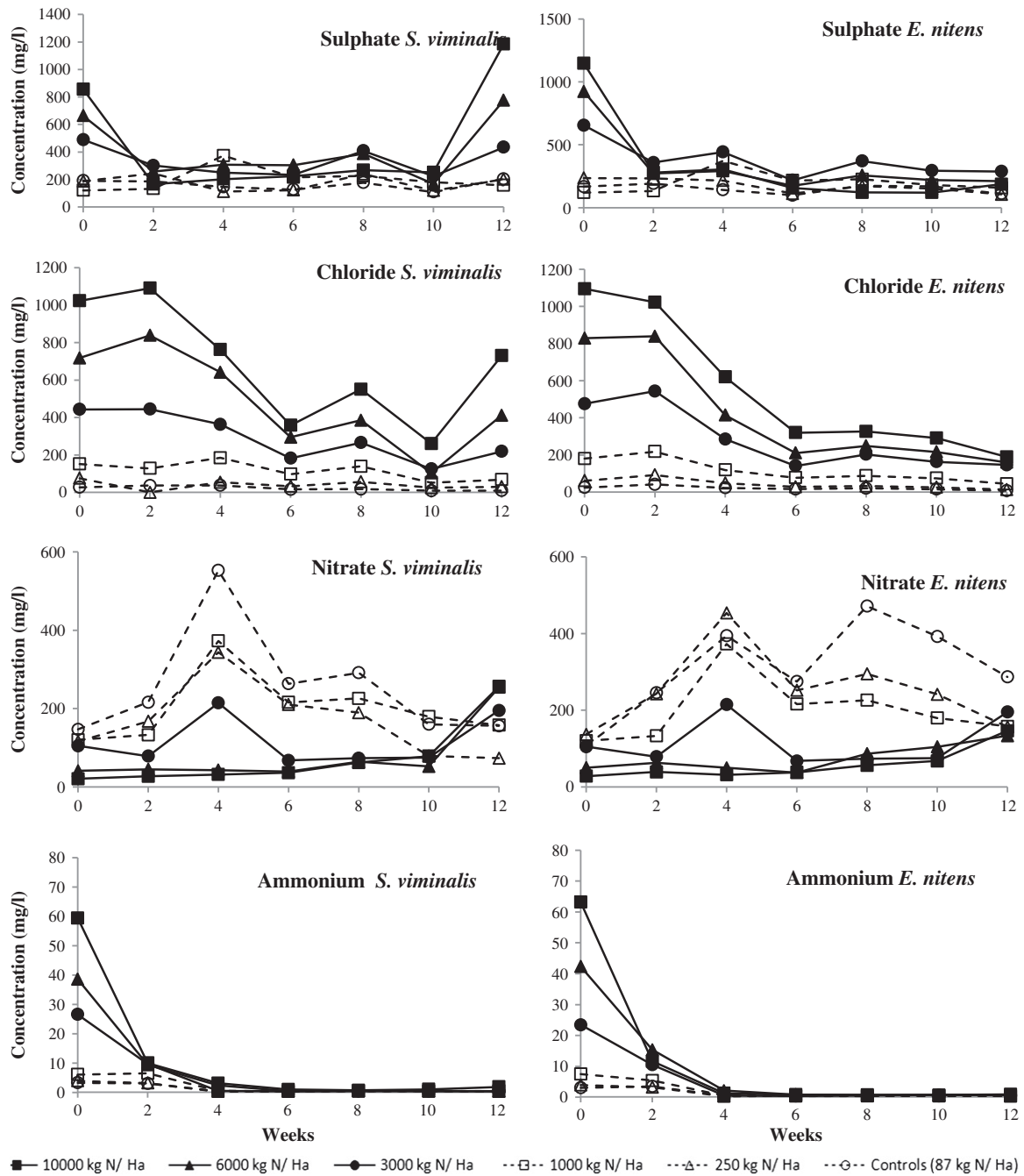


Fig. 5. Ion chromatography (soluble anions) and soluble ammonium analysis.

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

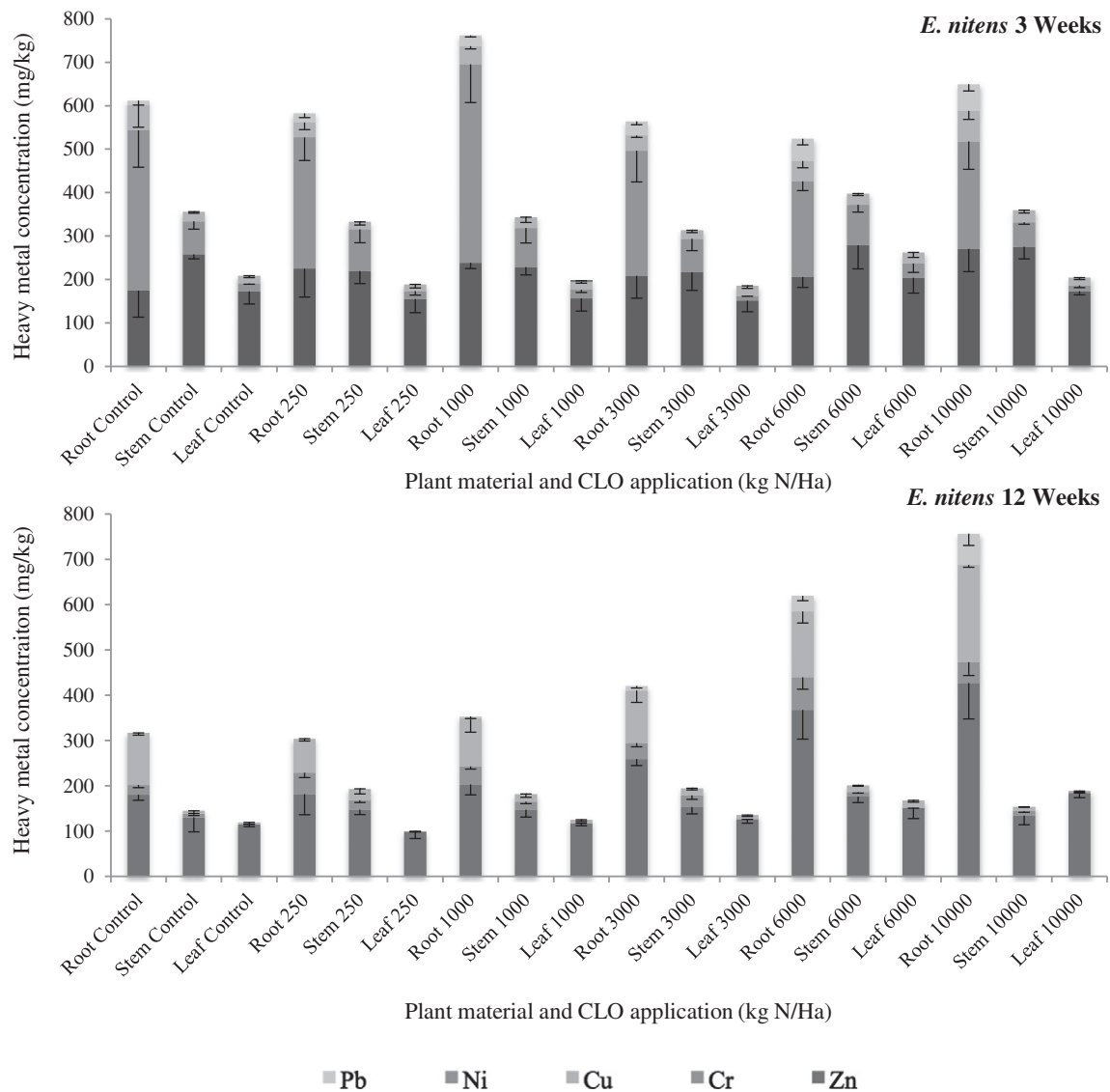


Fig. 6. Heavy metal accumulation within *E. nitens* after 3 weeks and 12 weeks growth.

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

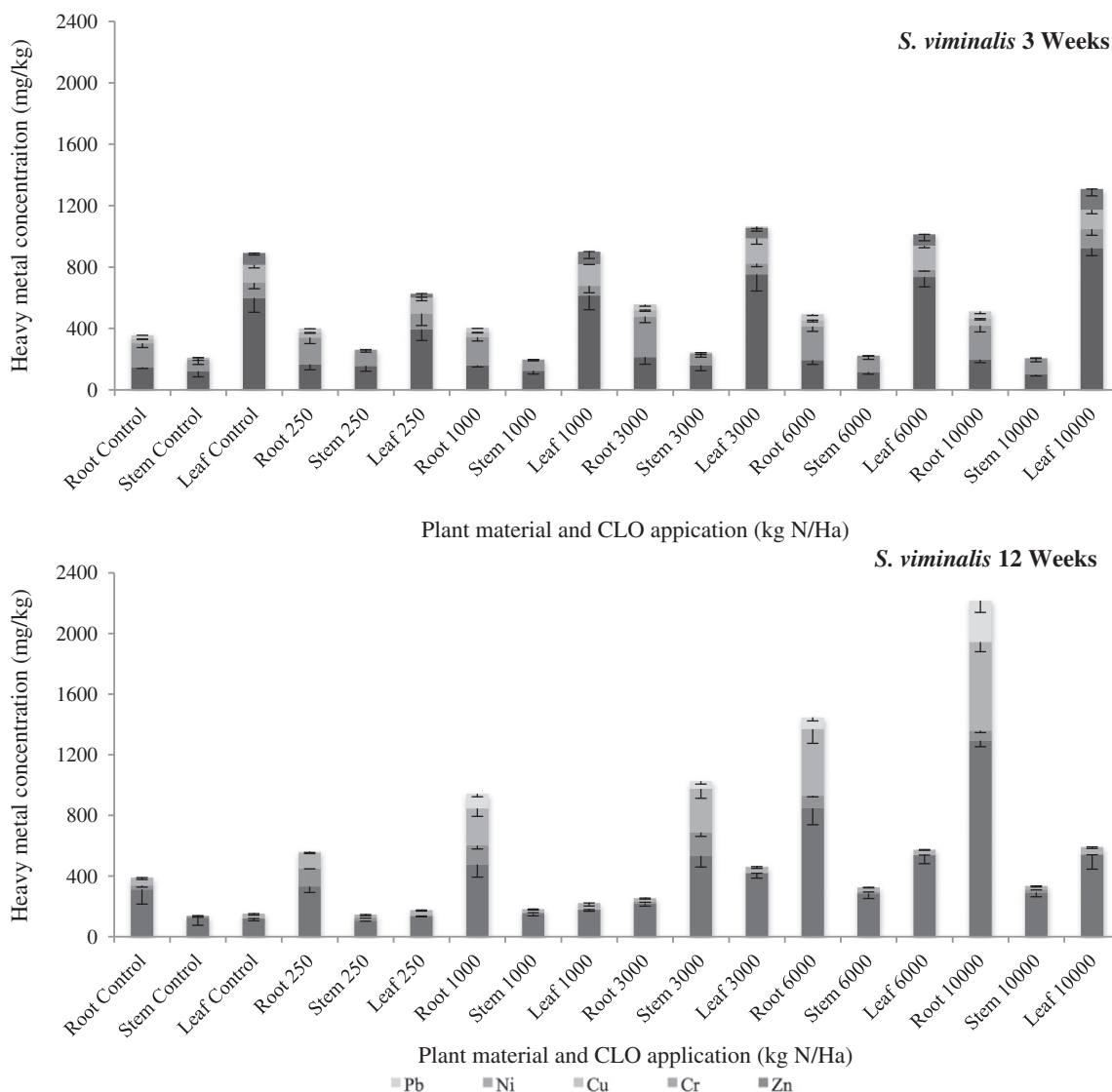


Fig. 7. Heavy metal accumulation within *S. viminialis* after 3 weeks and 12 weeks growth.

S. viminialis 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 uncontaminated 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. viminialis* 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. viminialis* 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. viminialis* 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 application 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 through 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.

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.

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