Alternative waste treatment research program

Project 4: Effects of Mixed Waste Organic Outputs on NSW Soils

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Executive Summary

1. Introduction

Considerable interest is currently focused on the use of *mixed waste organic outputs* (MWOO) from alternative waste technology facilities as a soil amendment. These products have been promoted not only as a means to reduce waste going to landfill but also as a material with the potential to improve soil physical, chemical and biological properties. However, concerns exist regarding possible negative environmental impacts of MWOO from chemical and physical contamination and the fate and behaviour of these constituents once applied to land.

In 2010, the NSW Department of Environment, Climate Change and Water (now EPA) gazetted "*The organic outputs derived from mixed waste exemption 2010*". This was amended to 2011 and allows for the application of MWOO products, at defined rates, for soil improvement or site rehabilitation at mine sites; plantation forestry use; non-contact agricultural; or broadacre agricultural use. The exemption aims to facilitate beneficial re-use and avoid harmful effects of MWOO application to land and it is intended that trials to examine the benefits and the environmental and human health impacts of contaminants in the outputs be conducted and completed prior to a review and proposed extension of the exemption.

Work completed to date relating to MWOO in NSW is limited and has focused principally on: source, composition and screening of MWOO; preliminary chemical characterization of chemical contaminant concentrations, agronomic effects on plant growth or limitations and regulation of MWOO use.

This project was initiated with the aim of addressing a series of key knowledge needs with respect to MWOO application to land and soils in NSW and aimed to evaluate:

i) The effect of MWOO application on soil condition/health including physical, chemical and biological indicators of soil health (organic carbon, bulk density, nutrient status)

ii) Contamination effects of MWOO application on soils: metals and metalloids and their availability, mobility and concentrations in soil water and leachate

iii) The potential plant uptake of contaminants following amendment with MWOO

iv) The impact of MWOO application rate and method of application (surface application or incorporation) on the nature and magnitude of these effects

The work was undertaken through a series of discrete but related experiments over 18 months; i) a glasshouse soil column experiment, ii) biological activity, iii) soil properties and plant growth, iv) metal accumulation and plant uptake, iv) runoff and leaching under simulated rainfall and v) leaching of metals and metalloids. For each individual experiment, subsamples of the same ten soils selected from across NSW were utilised along with sub-samples from the same batch of MWOO sourced from the Global Renewables UR-3R facility in Sydney, New South Wales. MWOO was collected from the production plant and stored in a cool room at <5°C prior to use. Despite the inherent limitations with glasshouse and laboratory experiments, this work provides the first comprehensive exploration of the effects of MWOO on NSW soils and lays a sound foundation for any future review of the NSW General Exemption.

2. Effects of MWOO application to soils in NSW: Glasshouse Soil Column Experiment

2.1 Glasshouse soil column experiment

A large-scale glasshouse soil column experiment was established in February 2013 at the University of New England glasshouse facility. This soil column experiment was intended to test the effects of MWOO applications on a range of NSW soils under controlled conditions. The glasshouse soil column experiment was undertaken using a series of custom built soil columns constructed from PVC pipe of 0.8m length x 0.15m internal diameter. Ten soils from across NSW were selected for use in the soil column experiment that had a range of texture, pH, inherent fertility and organic matter/carbon content. These soils represented a range of soil conditions typical of the State and soils to which MWOO is likely to be applied. Mixed waste organic output material was collected from the Global Renewables (UR3R) processing plant in November 2014

MWOO was applied at rates of 0 t ha^{-1}, 20 t ha^{-1} and 50 t ha^{-1} on soils for agricultural uses and at a higher rate of 140 t ha^{-1} for soils derived from mine sites. Two methods of MWOO application are commonly used in NSW, namely: surface application and incorporation using farm machinery to combine the applied MWOO with the underlying soil to a depth of typically 15 cm. In the glasshouse soil column experiment, MWOO was therefore both i) surface-applied and ii) incorporated to 15 cm depth. These application methods conform to current regulation and agricultural practice in NSW. For each application rate, Soils were collected and analysed from each treatment at 6 months (t_1), 12 months (t_2) and 18 months (t_3) from 0-5, 5-10, 10-15, 15-20, 25-30, 45-50 and 65-70 cm depth increments.

2.2 Glasshouse Soil Column Experiment: Effects of MWOO on soil physical and chemical properties

No significant change in bulk density (as a measure of soil physical condition) could be detected across the entire dataset in response to MWOO. This result suggests that the rates and methods of MWOO application and the inherent variability in the dataset were such that change in this parameter could not be detected. It is possible that the addition of MWOO and the carbon and nitrogen associated with it did have some effect on soil physical aggregation and structure but this analysis was beyond the scope of the current study but would benefit from further study.

The MWOO applied had an initial carbon and nitrogen concentration of 26.4 and 1.45% respectively. Following MWOO application, soil organic carbon and nitrogen increased relative to control soils for almost all soil types (the exception being OM1). The response to MWOO application was proportionally larger for sandy soils with lower initial organic carbon and nitrogen contents. For all soils, the enrichment with carbon and nitrogen was larger at the higher application rates. Significant effects were only found in the layers to which MWOO was applied (i.e. 0-5 cm surface applied and 0-15 cm incorporated) and were typically of a larger magnitude for the surface applied treatment. Carbon and nitrogen decreased steadily in all soils through the 18 months of the experiment although at the end of that period still typically had larger carbon and nitrogen concentrations than control soils. There was no evidence of translocation of either carbon or nitrogen into deeper soil layers below those treated through the experimental period in any of the soils examined.

The MWOO applied to this experiment had pH8.5. Following MWOO application, soil pH increased (by as much as 2 pH units) in soils with inherently low pH. For these soils, increases were found in the layers of application only and increased with increasing application rate. Where initial (Control) pH was higher, the effect on soil pH became progressively smaller. The response of soil pH in all clay soils was limited to the high application rates and surface application presumably due to the buffering effect of the clay itself. In soils with inherently high pH (>pH8.0) there was an apparent (although not significant) drop in pH following MWOO application. These results suggest that pH will be modified following MWOO application relative to the inherent soil pH and the pH of the MWOO material. For soils where a significant change in pH was observed through the period of the experiment, the pH changed on a trajectory of declining pH toward the initial/inherent pH of the soil itself.

Soil electrical conductivity increased in response to MWOO application in all the soils examined but this effect was again restricted to the application soil depths and typically at the higher application rates. For sandy soils, electrical conductivity diminished progressively through the experimental period following this initial increase. The change was less obvious in loam soils but for clay soils, a progressive increase in electrical conductivity was observed through time in the layers affected by MWOO application. Although a number of significant effects were determined associated with MWOO application, at no stage did any of the electrical conductivity values exceed 2.5 dSm^{-1} which does not represent a significant concern since electrical conductivity of >4 dSm^{-1} is normally considered the threshold beyond which negative effects on plants and soil begin to take place. However, under some climatic conditions (e.g. low precipitation, high evaporation) it is possible that the liberation of salts from a single application of MWOO might approach or exceed this 4.0dSm^{-1} threshold. Repeat applications of MWOO might also have the potential to exceed this threshold, especially on clay rich soils. Examination of repeat application scenarios and of climatic conditions and soil types that might promote increased EC would be a valuable area for further research.

A subset of four of the ten NSW soils (S1, SL1, SL2, C1) were analysed for the effect of MWOO on soil phosphorus concentration. In sandy and loam soils with inherent soil phosphorus concentrations (<20 mg kg^{-1}) there was a significant increase in soil phosphorus concentration. This was particularly so for the higher application rates and at the 140 t ha^{-1} application rate on the Minesite soil, phosphorus concentration was increased to almost 170 mg kg^{-1}. In the clay soils however, which had inherently high (>100 mg kg^{-1}) phosphorus concentration, no significant change in concentration was detected following MWOO application. MWOO therefore undoubtedly has a fertiliser value but its effectiveness will be determined by soil type with the most significant gains being found on soil with low clay content where inherent phosphorus concentration is low and the long-term impact of MWOO as a fertiliser remains uncertain.

2.3 Glasshouse Soil Column Experiment: Effects of MWOO application on metal and metalloid accumulation and distribution

Application of MWOO significantly affected the concentration and distribution of seven priority targeted metals and metalloids in ten different NSW soils. The impact was influenced by a range of the factors including the element initial concentration in MWOO, the background soil concentration, application method and rate. Generally, the most important factor was the concentration of the metals and metalloids in MWOO compared to background soil concentrations. For some of the soils used in this work, background metal and metalloid concentrations exceeded the current maximum allowable values for soil in the NSW General Exemption and this was reflected in changes in soil concentrations following MWOO application.

Increases in soil concentrations in the MWOO contacts depths (0-5 cm for surface applied treatments and 0-15 cm for incorporated treatments) were consistently observed in all soils (regardless of soil properties) for many of the elements examined, but were most significant for the metals with higher concentrations in MWOO (Cu, Pb and Zn) and in soils with lower background concentrations. Increase in soil concentrations were, at times, observed at the lower rate of application (20 t ha^{-1}) in soils with lower background values, but were more clear and significant at the higher application rate, 50 and 140 t ha^{-1}, for all the soils. Greater increases were observed at higher application rates for the same mode of MWOO application although increases were not always proportional to application rate. The greatest increase in soil concentrations was observed in the 0-5 cm depth of the surface applied treatments at the highest application rates. Increases in soil concentration remained in the MWOO contact depths over the 18 months of the experiment with no significant decrease or increase in concentration in any soil during this period. This demonstrated some limitation for MWOO repeat applications on a number of the NSW soils. Accumulation of some metals with the one MWOO application e.g. Pb for surface applied MWOO, resulted in soil concentrations close to 50% of the maximum allowable value for soil to which MWOO can be applied which would limit any repeat MWOO applications. Furthermore, some of the soil concentrations observed after MWOO application were close to the range considered phytotoxic for plants. Phytotoxicity and bioavailability are strongly influenced by soil characteristics and may well be important in some of the NSW soils here in light of the range in the soil characteristics documented. Therefore, it is vital that phytotoxicity and bioavailability are integrated in risk evaluation for MWOO amended NSW soils

Considering down profile movement, despite some artefacts associated with reconstructed soil cores, the results clearly showed for Cu, Pb and Zn, the metals present in MWOO at the highest concentrations, that over 18 months at the higher MWOO application rates (50 t ha^{-1} and higher), there was some movement to soil immediately (5 cm) below the MWOO contact zone and this was more extensive (up to 10 cm below the MWOO contact depth) for the more labile of these metals. It is possible that water addition to maintain 60% field capacity might have resulted in some movement of the naturally occurring elements down the soil in these disturbed profiles, possibly by enhanced colloidal movement. However, with the MWOO amendment this was not observed.

The experiment has illustrated the need for additional research to quantify the limitations for repeat MWOO applications in NSW soils, especially for surface MWOO application on soils with higher background metal and metalloid concentrations and the implications of these repeat applications for any down profile movement. It is also important concurrently to quantify the bioavailability and phytotoxicity of the metals and metalloids in the MWOO amended soils to soil organisms and plants for a better estimation of risk.

3. Soil Biological Activity

A respiration experiment was conducted over a 180 day period to assess the effects of MWOO application on soil microbial activity. Four of the NSW soils were used in this experiment (S1, SL1, SL2, C1) to provide a range of soil texture and pH. MWOO was applied at rates of 0 t ha^{-1}, 20 t ha^{-1}, 50 t ha^{-1} and 140 t ha^{-1} to all soils. The CO_2 evolved from soils is a measure of microbial activity in the soil and was monitored regularly over the experimental period and decomposition curves constructed.

All of the soils examined exhibited a similar response to MWOO application with a significant initial response of increased CO_2 evolution which we attribute to a supply of fresh carbon (and nitrogen) to the soil. For each soil type, this initial increase in respiration induced by the MWOO application was progressively larger at higher application rates. This initial increase in respiration was followed by a two phase decomposition trend with respiration rate diminishing rapidly to around 34 days at which point we believe the labile, “fast pool” became limited and more resistant (slow pool) carbon began to dominate. After 34 days we could detect no difference in the respiration rate between any of the treatments.

Addition of MWOO to soils can therefore significantly increase soil microbial activity (as measured by soil respiration). The magnitude of change was relative to the quantity of MWOO and presumably carbon, nitrogen and other nutrients that it provides. The benefit would however seem to be limited in duration having diminished to control levels after 34 days regardless of soil type and application rate. This we interpret to mean the rapid decomposition of labile “fast” pool carbon diminished after this time period leaving only the more resistant carbon fractions beyond this time. This “slow” pool will undoubtedly continue to decompose, albeit at a slower rate. Nevertheless, soils to which MWOO had been applied had higher soil carbon compared with controls at 18 month sampling time. It is possible that repeat applications of MWOO might continue to build this “slow” carbon pool and therefore long-term carbon storage. Further work is required to elucidate the effects of MWOO repeat application on soil biological activity.

4. Plant growth and metal uptake

A ten week glasshouse experiment with wheat (*Triticum aestivum)* was conducted to assess the accumulation and distribution of heavy metals in four NWS soils and the subsequent uptake by plants after adding MWOO to the soil. The MWOO was again applied at four application rates (0 t ha^{-1}, 20 t ha^{-1}, 50 t ha^{-1} and 140 t ha^{-1}) and again using two methods of application (surface and incorporated to 15 cm). Soils were analysed for Cu, Zn, Ni, Cr, Pb, Cd and As at three soil depths (0-5, 5-15 and 15-25 cm).

The application of MWOO once again significantly increased pH, EC, total C, total N, CEC and P ($P < 0.05$) of amended soils. These changes were observed to increase with increasing application rate and occurred mostly in the surface soil layers (0-5 cm). The 140 t ha^{-1} showed the largest increases. Wheat root growth and aboveground biomass were significantly increased ($P < 0.05$) by the application of MWOO at 20 and 50 t ha^{-1} application rates. A decline in the aboveground biomass was observed for the high application rate (140 t ha^{-1}) with average biomass below that of the control. Although this effect was not significant ($P > 0.05$) at the broad scale across the soils studied, individual soils – particularly Kirby Sand and CROA soils showed a significant decline in wheat growth at the high rates (140 t ha^{-1}) of MWOO application. The aboveground biomass was also observed to be significantly higher ($P<0.01$) where MWOO was incorporated compared with surface application. MWOO application would therefore appear to enhance wheat growth but over-application might result in plant growth problems perhaps due to toxicity to plants, nitrogen immobilisation or other undetermined problems, particularly in soils with a lower clay content.

MWOO application significantly increased ($P < 0.05$) Zn, Ni, Cr and Pb concentrations in the soil compared with controls, particularly in the surface soil layer (0-5 cm). Roots, stems, leaves and grains of wheat were analysed and concentrations of Cu, Ni and Cr were significantly higher in the roots ($P < 0.05$) compared with aboveground plant parts. Zn concentrations however, were higher in stems than roots. Concentrations of Pb, Cd and As in grains were below the limit of recovery of 0.1 mg kg^{-1} and while other metals were detected in the grain, concentrations were all within allowable concentrations. Translocation of the heavy metals to wheat grains was observed for Zn, Cr, Ni and Cu, but not Pb, As and Cd. However, the grain concentrations of Pb, As and Cd were below limits of recovery and maximum residue limits for foodstuffs in Australia. Should maximum residue limits be reduced or General Exemption criteria altered, then further assessment for Cd would be required because in this study the LOR for Cd was the same as the current maximum residue limit.

5. Leaching and Runoff following MWOO application

A small-scale, simulated rainfall experiment was undertaken to assess the effects of MWOO application, (0 t ha^{-1}, 20 t ha^{-1}, 50 t ha^{-1} and 100 t ha^{-1}; surface and incorporated) on the chemical composition and the metal and metalloid concentrations in runoff and leachate of two of the soils used previously (S1, C2). Properties assessed included: time to runoff, total runoff/leachate, runoff/leachate rate, sediment yield, pH, electrical conductivity, dissolved organic carbon, nitrate, ammonia and seven metals and metalloids; arsenic, cadmium, chromium, copper, nickel, lead and zinc. MWOO application increased trace metal and metalloid concentrations in surface water runoff and leachate from the two soils studied and sandy loam soils generally had higher concentrations than the clay soils. Metal and metalloid concentrations in runoff and leachate were generally higher when MWOO was surface applied and at higher application rates. Our results suggest some concern for water resources associated with application of this MWOO to soil, since metal concentrations in runoff and leachate were increased. Risk is apparently lowest with incorporation of this MWOO at a low application rate of 20 t ha^{-1} in a clay soil and higher at greater application rates especially for surface MWOO application which varies with soil type.

In order to examine the nature of leachate through the soil profile, a large scale column leaching experiment was subsequently undertaken with leachate being collected at two depths, 15 cm and 30 cm, from five of the NSW soils (S1, C2, SL1, SL2, SL3). The aim of this component of the work was to assess how consecutive rainfall events, influenced metal/metalloid concentrations and chemical constituents in leachate under the different MWOO application rates (0 t ha^{-1}, 20 t ha^{-1}, 50 t ha^{-1} and 140 t ha^{-1}) and application methods (surface application and incorporation to 15 cm). Quantification was undertaken of: total leachate, leachate rate, sediment yield, pH, electrical conductivity, dissolved organic carbon, nitrate, ammonia and seven metals and metalloids; arsenic, cadmium, chromium, copper, nickel, lead and zinc.

Metal and metalloid concentrations in leachate increased with increasing MWOO application rate but the influence of MWOO was evident even at 20 t ha^{-1} in some soils. The surface applied treatments typically showed higher metal and metalloid concentrations than the incorporated treatments and metal and metalloid concentrations varied depending on the leachate sampling depth. At 15 cm, metal and metalloid leachate concentrations were higher when MWOO was incorporated. At 30 cm, however, metal and metalloid leachate concentrations were higher when MWOO was surface applied.

Differences in metal and metalloid concentrations in leachate between soil types were also observed. In sampling event 1 metal and metalloid concentrations were generally higher in soils with high initial soil metal concentrations and the coarser textured soils. In sampling event 2 however, metal and metalloid concentrations in leachate were higher in soils with more DOC leaching. The total metal and metalloid lost as a percentage of metals added in MWOO was generally less than 1% for all metals and metalloids across all experiments. Nevertheless, in light of the influence of MWOO on soil water trace metal and metalloid concentrations leachate monitoring is recommended for MWOO application to NSW soils. Extension of this laboratory based research should include assessment at field scale and ecotoxicological considerations of concentrations detected for Australian soils.

6. Teaching and training

Throughout the program of research, student involvement and participation was promoted. A number of undergraduate Honours students *(name withheld)* along with one MSc student *(name withheld)* and one PhD student *(name withheld)* all contributed to the research. All have now successfully completed their work and have either submitted their work for examination or have been awarded their degree. All components of the work are now being prepared for publication.

1. Introduction

1.1 Mixed Waste Organic Outputs (MWOO) in Australia

In 2010-11 Australians generated approximately 48 million tonnes of waste (approximately 2.2 tonnes of waste per capita) of which 29% was from municipal sources (DSEWPC, 2014). This constitutes a 46% increase in the waste generated since 2002–03 (DEWHA, 2009; ABS, 2010; DEWHA, 2010). Australia is frequently described as a high producer of waste when compared with other countries (ABS, 2005; Hyder, 2009b; DSEWPC, 2014). With continued consumerism and Australia's population projected to increase, this continual increase in waste production is predicted to result in an estimated 81 million tonnes of waste being generated per annum by 2020 (DEWHA, 2010). The States responsible for the largest proportions of the country's waste are the three most populated: New South Wales (NSW), Victoria and Queensland (DSEWPC, 2014).

Australia has always had a high dependence on landfill for waste management and disposal. Concerns associated with landfill disposal of waste include greenhouse gas emissions and long-term pollution of the environment. Other issues such as associated traffic, noise, dust, and odours also result in strong community opposition to new landfills. Despite increasing recycling rates in recent years (DSEWPC, 2014), during 2010–11, nearly half (49%) of all municipal solid waste was disposed to landfill (DSEWPC, 2014) .

The overarching national policy framework for waste management and resource recovery was agreed in 1992 by the Council of Australian Governments (COAG) through its endorsement of the National Strategy for Ecologically Sustainable Development. This included agreement on a national approach to waste minimisation and management. Management of waste at both a National and State level is guided by a hierarchical approach that seeks to consider waste management options against the following priorities:

- **Avoidance**, including action to reduce the amount of waste generated by households, industry and all levels of government;
- **Resource Recovery,** including reuse, reprocessing, recycling and energy recovery, consistent with the most efficient use of the recovered resources; and,
- **Disposal,** including management of all disposal options in the most environmentally responsible manner (DEWHA, 2009).

In November 2009 the National Waste Policy (NWP) was agreed by all Australian State and Territory environment ministers and endorsed by COAG. The NWP established Australia's waste management and resource recovery direction to 2020 with aims to:

- avoid the generation of waste and reduce the amount of waste (including hazardous waste) for disposal;
- manage waste as a resource;
- ensure that waste treatment, disposal, recovery and re-use is undertaken in a safe, scientific and environmentally sound manner; and,
- contribute to the reduction in greenhouse gas emissions, energy conservation and production, water efficiency and the productivity of the land (DEWHA, 2009).

The NWP embeds the hierarchical approach to waste management in national policy and is now the main driver for reducing waste generation and turning waste into recoverable resources. It has resulted in a commitment at both a national and, through a multi-jurisdictional approach, a State level, to avoid high waste volumes being disposed to landfill and incorporates strategies to achieve this. One such strategy is to enhance biodegradable (organic) resource recovery and reduce greenhouse gas emissions through its diversion from landfill disposal (DEWHA, 2009).

In NSW, Resource NSW established the first NSW Waste Avoidance and Resource Recovery Strategy in 2003 (Resource NSW, 2003) that provided a framework in NSW for reducing waste and making better use of resources, aligning with the national framework. This was superseded by the Waste Avoidance and Resource Recovery Strategy 2007 (DECC, 2007) that has recently been reviewed with a new draft strategy published in 2013 (EPA, 2013). The 2013 draft strategy proposes long-term directions for waste management in NSW to 2021, builds on the objectives of the previous strategy, and includes new targets for waste minimisation and resource recovery in six key areas:

- avoiding and reducing waste generation;
- increasing recycling;
- diverting more waste from landfill;
- managing problem wastes better;
- reducing litter; and
- combating illegal dumping.

As a consequence of the need to meet targets and address these policy requirements, rapid growth in waste recovery and re-use technologies has occurred throughout NSW, and indeed Australia. One such technology is the processing of mixed solid waste to produce an organic output for land application as a fertiliser or soil amendment. This has been one of the key initiatives taken up by industry. With reference to NSW, this output is referred to throughout this report as mixed waste organic output (MWOO).

1.2 Background to MWOO production and use in NSW

For many years mechanical and biological processing technologies have been used to produce composts from source separated green waste streams. Adaptation to the treatment of other wastes, such as mixed solid waste, proved popular in the 1980s, particularly in northern Europe and the United States of America (USA), due to the reduction in waste volume going to landfill, environmental benefits, monetary savings and also reported soil improvements (He et al., 1992; Jakobsen, 1995; Otten, 2001; Hansen et al., 2006a; Zhang et al., 2006). However, market demand for the output as a soil amendment declined in the early 1990s due to concerns regarding contaminants (Harms and Sauerbeck, 1983) and the emergence of the organic agriculture market (Iglesias-Jimenez and Alvarez, 1993). Subsequent moves toward improvements in source separation and, in many jurisdictions, specific regulations for land application (EA, 2009a) have resulted in a resurgence in popularity of these materials as soil amendments (Hyder, 2006).

In Australia the growth of mixed waste processing facilities has been rapid, driven by policy requirements and mechanisms such as landfill levies used to promote the waste avoidance and resource recovery strategies (WMAA, 2010). Of the States and Territories, NSW has the highest number of facilities, that is four facilities currently commercially processing a mixed waste stream (usually municipal solid waste (MSW) with smaller volumes of certain other waste streams) to produce MWOO. These include:

- Coffs Coast Resource Recovery Facility (Biomass Solutions (Coffs Harbour) Pty Ltd);
- SITA Bedminster (SITA Australia Pty Ltd, Raymond Terrace);
- Eastern Creek UR-3R (Global Renewables, Eastern Creek); and,
- SITA Advanced Waste Treatment (SITA Australia Pty Ltd, Kemps Creek).

Detailed information on these facilities and their waste streams is included in chapter 2. The processing operations essentially comprise three main stages:

- Mechanical pre-processing of the waste streams to remove contaminants and concentrate the organic output;

- Biological pasteurisation to microbiologically treat the mechanically processed waste material and reduce plant and animal pathogens. The biological treatment can be aerobic, anaerobic or a combination of both (hybrid) but aerobic treatment is used in the operational NSW facilities; and,

- Stabilisation to mature the final organic output.

Throughout this report the mechanical and biological processing of a mixed waste stream to produce an organic output will be referred to as mechanical and biological treatment (MBT). This review focuses on aerobic MBT as relevant to NSW facilities and the scientific data available. Anaerobic MBT is not currently used in NSW for processing mixed waste to MWOO and information on anaerobic MBT MWOO is comparatively scarce.

Land application of MWOO can provide a number of benefits (DEWHA, 2010). With the rapid increase in MBT facilities and MWOO production, however, ensuring the quality of MWOO for land application and protection of the environment and human health is critical. In NSW this is controlled through State regulation and guidelines.

1.3 Regulation of MWOO and its land application

Waste regulation in Australia is mainly the responsibility of State, Territory and local governments. Control of waste processing facilities and land application of waste and waste derived material is captured within the State waste regulation system. To date, only NSW has produced standards specific to MWOO quality and its land application (WMAA, 2010). In other States, guidelines for biosolids or composts are applied to MWOO (e.g. Australian Standard for composts, soil conditioners and mulches (AS4454, 2012); the NSW Environmental Guidelines: Use and disposal of biosolids products (EPA, 1997)) although it is not clear that these are appropriate.

In NSW, the disposal, processing and recovery of waste is managed under the *Protection of the Environment Operations Act 1997* and the *Protection of the Environment Operations (Waste) Regulation 2005*. Land application of waste, including waste derived material, can trigger various regulatory requirements such as the need to hold an environment protection license (EPL). Under Clause 51 and 51A of the *Protection of the Environment Operations (Waste) Regulation 2005* the Environment Protection Authority (EPA) has the power to exempt a person from certain regulatory requirements that would normally apply to the land application of a waste material, such as applying for an EPL. The mechanism by which this occurs is the Resource Recovery Exemption. Resource recovery exemptions are granted by the EPA where the land application of the waste material is a “bona-fide, fit for purpose, reuse opportunity rather than a means of waste disposal” (DECCW, 2011; OEH, 2011). The EPA may issue either general or specific exemptions. General exemptions are publicly available and are relevant to commonly recovered, high volume and well characterised waste materials. A general exemption for MWOO was first gazetted on 5th March 2010. This was revoked and the current exemption, the *organic outputs derived from mixed waste exemption 2014* was gazetted on 6 June 2014 (EPA, 2014). Throughout this report this exemption will be referred to as the NSW General Exemption.

1.4 The NSW General Exemption

The NSW General Exemption is reproduced in Appendix A. Mixed waste that can be accepted for MWOO production is defined in the exemption. The exemption also includes a range of criteria for MWOO and conditions for its soil application in NSW.

The general conditions of the exemption include:

1. The chemical concentration or other attributes of the organic outputs must not exceed absolute maximum concentrations specified in the exemption for specific contaminants.
2. The organic outputs can only be applied to land as a soil amendment for:
   - soil improvement or site rehabilitation at mine sites (no more than **140 t ha^{-1} dry weight (dw)** in total at any given location);
   - plantation forestry use (no more than **50 t ha^{-1} (dw)** in total at any given location);
   - non-contact agricultural use (no more than **50 t ha^{-1} (dw**) in total at any given location); or,
   - broad acre agricultural use (no more than **10 t ha^{-1} (dw)** in total at any given location).
3. The organic output must not be used:
   - in urban landscaping;
   - at public contact sites;
   - on or in home lawns and gardens;
   - in potting mix; or,
   - in turf production.

The exemption includes a range of processor and consumer responsibilities including application rates, buffer zones, land use limitations and maximum allowable soil and organic output contaminant concentrations.

The exemption is scheduled for periodic review. For this reason, and due to the continually evolving and growing nature of the MWOO production industry, it has become important in NSW to understand current MWOO characteristics and effects when MWOO is applied to NSW soils. This will enable any review of the NSW General Exemption to be underpinned by current and sound scientific knowledge appropriate for NSW.

1.5 Application of MWOO to soils

MWOO products have been promoted not only as a means to reduce waste going to landfill but also as a material with the potential to improve soil physical, chemical and biological properties. Research undertaken to date regarding the impacts of MWOO materials on soils has been undertaken internationally and the results of these studies were extensively reviewed in Wilson et al. (2014). Very few studies (e.g. Dorahy et al. 2006) have however been undertaken in NSW or Australia and many uncertainties remain with regard to the effects of MWOO on soils in Australia. Much of the international work has considered the effects of MWOO on soil physical, chemical and biological parameters (e.g. bulk density, C, N, pH, nutrient status etc.) and plant growth response while others have focused on the potential contamination issues associated with MWOO application.

A number of changes in soil physical, chemical and biological properties that contribute to soil condition, fertility and plant growth have been reported following MWOO land application. For example, changes in soil physical properties that have been reported are largely beneficial and include reduced bulk density, improved porosity and structure with associated improvements in water holding capacity. MWOO application is also typically reported to result in increased soil organic carbon and nitrogen in proportion to the organic carbon content of the MWOO material and its application rate. This suggests a potential for MWOO to be used to enhance soil carbon content and soil condition. Increases have also been observed in soil pH, cation exchange capacity and a range of key plant nutrients (including phosphorous, potassium, sulfur and magnesium) are typically proportional to the rate of MWOO application although usually only significant at higher rates of MWOO application (>40 t ha^{-1}).

The effects of MWOO application on soil microbial activity are also usually reported to be positive at moderate application rates. High application rates can result in reduced microbial activity although causal factors (e.g. heavy metal toxicity, nutrient dynamics or other variables) often remain unclear. Effects on plant and crop yield can vary but are usually positive and typically become significant at moderate application rates (usually >40 t ha^{-1}). However, the potential for over-application of MWOO to suppress plant growth has also been reported although, again, the causes are rarely clear but might relate to plant toxicity or nutrient (e.g. nitrogen) immobilisation.

Contamination effects have however, also been associated with MWOO application elsewhere. For example, heavy metals can be contributed to the input waste stream from a variety of sources with copper (Cu), lead

(Pb), zinc (Zn), chromium (Cr) and cadmium (Cd) common. Heavy metal concentrations for Australian MWOO (2006-2010 samples) have a similar order of magnitude to those reported elsewhere with the order of concentration of the most commonly occurring elements being: Zn > manganese (Mn) > Pb > Cu > Cr > Ni. Risks for MWOO land application include heavy metal accumulation in soils, leaching and runoff and plant uptake and phytotoxicity. When applied to soil in MWOO, heavy metal concentrations will often accumulate in soil, usually in proportion to the MWOO application rate, although this increase is moderated by a range of soil properties that are also moderated by the MWOO itself, including pH, clay content and organic matter content. Most elements, when applied to neutral or alkaline soils are transformed into insoluble or partially soluble forms with lower extractability and mobility than observed in acidic and sandy soils.

Soil to plant bioconcentration factors for heavy metals are typically reduced in MWOO amended soils compared with unamended controls. Nevertheless, increases in heavy metal concentrations in plants grown in soil amended with MWOO have been observed, including into edible plant parts. These increases have tended to be more significant at higher MWOO application rates (>80 t ha^{-1}), higher MWOO concentrations and with repeat applications. Heavy metal concentrations in plants are seldom phytotoxic at moderate rates of application (typically <80 t ha^{-1}). Where MWOO is applied at higher rates (e.g. 140 t ha^{-1}, as allowed in the NSW General Exemption for soil improvement or rehabilitation at mine sites), heavy metal concentrations in some plant species can reach plant critical toxicity concentrations and maximum residue limits for foods, especially for Zn, Cu and Pb.

Leaching of Zn, Cu, Cd, Ni and Pb, mainly as soluble complexes, has been observed in soil columns, although mobilisation by other mechanisms, such as preferential flow pathways and runoff, remains unquantified. The mobile heavy metal fraction is usually <10% of the total heavy metal concentration in soil. However, heavy metal concentrations in leachate are nevertheless potentially of concern at higher MWOO application rates and using MWOO with higher heavy metal concentrations. Risks of ecosystem impacts such as ecotoxicity and foodchain transfers are considered to be low based on research in compost and biosolids amended soils, unless MWOO application occurs at high and/or repeat applications.

Although these investigations have taken place in many parts of the world, the effects of MWOO on soils in NSW (and Australia) are very few. The work reported here is the first major investigation of the effects of MWOO application to NSW (and Australian) soils and will provide the first substantive information relating to the effects of MWOO on soil physical, chemical and biological parameters, the effects on plant growth and metal uptake and the risk of contamination through metal accumulation in soils, leaching and runoff.

1.6 Aims of Project 4

Considerable interest has been focused on the use of *mixed waste organic outputs* (MWOO) from alternative waste technology facilities as a soil amendment. However, concerns remain regarding possible negative environmental impacts of MWOO from chemical and physical contamination and the fate and behaviour of these constituents once applied to land. Work completed to date relating to MWOO in NSW is limited and has focused principally on: source, composition and screening of MWOO; preliminary chemical characterization of chemical contaminant concentrations; agronomic effects on plant growth; or limitations and regulation of MWOO use (see Wilson et al. 2014).

This work has only partially met EPA needs in that it has not fully considered i) the likely inputs and chemicals of concern in AWT or ii) the environmental effects of AWT following land application. The authors of these previous reports have typically concluded that further work is required to improve our understanding of the fate and behaviour of AWT products in soil following MWOO amendment.

This project was initiated with the aim of addressing a series of key knowledge needs with respect to MWOO application to land and soils in NSW and aims to evaluate:

v) The effect of MWOO application on soil condition/health including physical, chemical and biological indicators of soil health (organic carbon, bulk density, nutrient status)
vi) Contamination effects of MWOO application on soils: metals and metalloids and their availability, mobility and concentrations in soil water and leachate
vii) The effects of MWOO application on plant growth and potential plant uptake of contaminants
viii) The impact of MWOO application rate and method of application (surface application or incorporation) on the nature and magnitude of these effects

The work was undertaken through a series of discrete but related experiments; i) a glasshouse soil column experiment, ii) biological activity, iii) soil properties and plant growth, iv) metal accumulation and plant uptake, iv) runoff and leaching and v) leaching of metals and metalloids. For each individual experiment, subsamples of the same ten soils selected from across NSW were utilised along with sub-samples from the same batch of MWOO sourced from the Global Renewables UR-3R facility in Sydney, New South Wales. MWOO was collected from the production process and stored in a cool room at <5°C prior to use.

2. Effects of MWOO application to soils in NSW: Glasshouse Soil Column Experiment

2.1 Glasshouse Soil Column Experiment

A glasshouse soil column experiment was established in February 2013 at the University of New England glasshouse facility. This soil column experiment was intended to test the effects of MWOO applications on a range of NSW soils under controlled conditions. The column experiment, further allowed the exploration of change in soil through a whole soil column.

2.1.1 Soil selection and preparation

Ten soils from across NSW were selected for use in the soil column experiment, to represent a range of soil conditions typical of the State and soils to which MWOO is likely to be applied. These soils had a range of texture, pH, inherent fertility and organic matter/carbon content (as agreed with NSW EPA).

Soils were collected from the upper 15 cm of the soil profile from a range of locations indicated in Table 2.1. The soils used were largely agricultural (cropping or pasture) with the exception of the Minesite soil, which was derived from overburden topsoil from a coal mine and the highly organic soil which was taken from the Mother of Ducks Lagoon Nature Reserve near Guyra in NSW. All soils were collected between January and February 2013. Each soil was transported to University of New England soil preparation facility immediately following sampling. Soils were then air dried at ambient temperature and humidity in the open, sieved to <4mm and stored briefly in cool conditions until they were used to pack the soil columns. Triplicate subsamples, sieved to 2 mm, were used for soil characterisation.

2.1.2 Experimental Setup

The glasshouse soil column experiment was undertaken using a series of polyethylene (0.85m length × 0.15 m internal diameter) soil columns. Soil was packed into the columns to a uniform height of 82 cm. The soil columns were completely randomised and the experiment was conducted under controlled climate conditions in the University of New England glasshouse facility with average temperature 26°C and 60% humidity. Soils in each pot were initially watered slowly to achieve wetting through the full column profile and to achieve their 60% field moisture capacity. Wetting was carried out for all soils prior to MWOO application. Soil columns were subsequently watered regularly to maintain constant moisture content of 60% field capacity uniformly for each soil. Pots were regularly weeded to remove any germinating plants. At 6, 12 and 18 months following MWOO amendment, soil columns were destructively sampled and samples analysed for metals and metalloids included the 0-5, 5-10, 10-15, 15-20, 25-30 cm depths for all the soils in addition to the 65-70 cm depth sample for the three sandy soils, S1, S2, S3.

2.1.3 MWOO collection, application rates and methods

The MWOO was obtained from the Global Renewables UR-3R facility servicing the Sydney metropolitan area. This was collected in bulk, air-dried and well homogenised with triplicate subsamples used for characterisation purposes.

The MWOO was applied to pots at 20 t ha^{-1} and 50 t ha^{-1} to all agricultural soils and at 140 t ha^{-1} for soils derived from mine sites. All MWOO application rates were based on "dry-weight" corrected for MWOO moisture content. An un-amended, "control" soil was used through the duration of the experiment against which change in soil properties could be measured.

Two methods of MWOO application are commonly used in NSW, namely: surface application and incorporation using farm machinery to combine the applied MWOO with the underlying soil to a depth of typically 15 cm. In the glasshouse soil column experiment, MWOO was both i) surface-applied and ii) incorporated to 15 cm depth. These application methods conform to current regulation and agricultural practice in NSW.

Table 2.1. Characteristics of soils and MWOO used in Glasshouse Soil Column Experiment

Soil↕	S1 (n=3) Kirby Sand	S2 (n=3) Tingha Sand	S3 (n=3) Gunnedah River Loam	SL1 (n=3) CROA	SL2 (n=3) Minesite	SL3 (n=3) Warialda Loam	OM1 (n=3) Guyra Organic	C1 (n=3) Kirby Clay	C2 (n=3) Inverell Clay	C3 (n=3) Gunnedah Black Clay	MWOO (n=4)	MAV in soil	MAV in mine site	MAV in MWOO
pH ($CaCl_2$)	4.44 ± 0.09	6.00 ± 0.03	7.42 ± 0.04	4.64 ± 0.05	5.62 ± 0.04	7.93 ± 0.04	5.94 ± 0.02	5.28 ± 0.07	6.79 ± 0.06	7.78 ± 0.05	7.64 ± 0.07	-	-	-
pH (H2O)	6.28 ± 0.03	7.50 ± 0.68	8.26 ± 0.04	5.91 ± 0.02	6.41 ± 0.22	8.88 ± 0.06	6.68 ± 0.15	6.13 ± 0.03	7.43 ± 0.26	9.11 ± 0.01	8.55 ± 0.05	-	-	-
EC (µS cm^{-1})	16.53 ± 0.03	12.2 ± 0.83	210.16 ± 0.35	57.56 ± 1.10	186.03 ± 0.99	76.56 ± 0.81	146.33 ± 4.14	88.33 ± 3.51	193.16 ± 6.57	197.66 ± 0.42	6250. ± 1.67	-	-	-
Field capacity (g/g dry soil)	0.31 ± 0.01	0.21 ± 0.02	0.33 ± 0.01	0.42 ± 0.05	0.29 ± 0.04	0.33 ± 0.01	0.86 ± 0.09	0.58 ± 0.05	0.70 ± 0.07	0.64 ± 0.04	-	-	-	-
Clay %	13.2	5.0	13.7	27.6	14.3	15	16.9	55.0	66.0	63.8	-	-	-	-
Silt %	10.5	5.0	12.0	41.4	29.2	32.4	51.7	26.0	16.0	16.6	-	-	-	-
Sand %	76.3	90.0	74.3	30.0	56.5	52.6	31.4	19.0	18.0	19.6	-	-	-	-
Soil texture	Sandy loam	Sand	Sandy loam	Silty loam	Silty loam	Silty loam	Silty loam	Clay	Clay	Clay	-	-	-	-
Classification	Chromosol	Tenosol	Rudosol	Chromosol	Chromosol	Chromosol/ Calcarosol	Hydrosol	Dermosol	Vertosol	Vertosol	-	-	-	-
Site location	371479E, 6632389S	323665E, 6683658S	256384E, 6572244S	288827E, 62224525S	320729E, 6406907S	247122E, 6728341S	371479E, 6656210S	368782E, 6632154S	314913E, 6704502S	239702E, 6566946S	-	-	-	-
Soil mass per pot (kg)	17.7	20.0	20.9	16.1	20.5	18.5	10.2	16.1	16.3	17.2	-	-	-	-
Nitrogen %	0.03 ± 0.01	BDL	0.06 ± 0.01	0.18 ± 0.01	0.09 ± 0.01	0.03 ± 0.01	0.92 ± 0.01	0.41 ± 0.01	0.13 ± 0.01	0.15 ± 0.0	1.45 ± 0.42	-	-	-
Organic Carbon %	0.34 ± 0.02	0.04 ± 0.01	0.70 ± 0.01	2.20 ± 0.02	1.48 ± 0.06	0.47 ± 0.02	9.76 ± 0.02	4.67 ± 0.1	3.34 ± 0.01	2.49 ± 0.02	26.4 ± 1.55	-	-	-
Phosphorus (mg kg^{-1})	5.5 ± 0.07	2.8 ± 0.24	38.2 ± 2.19	21.1 ± 1.04	12.8 ± 0.57	5.2 ± 0.09	17.1 ± 0.52	132.5 ± 3.72	107.3 ± 0.95	16.9 ± 0.49	765.10 ± 16.5	-	-	-
Soil↕	S1 (n=3)	S2 (n=3)	S3 (n=3)	SL1 (n=3)	SL2 (Mine- site)	SL3 (n=3)	OM1 (n=3)	C1 (n=3)	C2 (n=3)	C3 (n=3)	MWOO (n=4)	MAV in	MAV in	MAV

					(n=3)							soil	mine site	in MWOO
Total As (mg kg^{-1})	59.7 ± 6.2*	4.1 ± 0.6	3.6 ± 0.2	8.1 ± 0.8	5.6 ± 0.5	4.2 ± 0.1	3.1 ± 0.4	1.9 ± 0.5	0.9 ± 0.6	1.6 ± 0.3	5.3 ± 0.5	20	20	20
Total Cd (mg kg^{-1})	BDL	BDL	0.7 ± 0.1	0.6 ± 0.2	0.4 ± 0.5	0.2 ± 0.0	4.3 ± 0.4	3.6 ± 0.2	2.8 ± 0.2	1.2 ± 0.1	2.2 ± 2.14	1	5	3
Total Cr (mg kg^{-1})	18.0 ± 0.1*	15.3 ± 3.9*	34.2 ± 2.5*	47.6 ± 1.9*	27.4 ± 3.2*	20.0 ± 0.5*	293.7 ± 7.4*	233.6 ± 11.4*	227 ± 2.7*	113.2 ± 6.1°	85.4 ± 12.28	100	250	100
Total Cu (mg kg^{-1})	13.6 ± 0.3*	6.7 ± 1.4*	20.7 ± 1.3*	18.3 ± 0.5*	8.3 ± 0.1*	23.8 ± 0.5*	52.8 ± 0.0*	52.0 ± 0.9*	59.5 ± 0.6*	46.9 ± 0.7*	180.8 ± 40.46	100	375	375
Total Ni (mg kg^{-1})	7.1 ± 0.4*	6.3 ± 1.0*	23.6 ± 2.5*	15.6 ± 0.6*	9.0 ± 0.3*	10.3 ± 0.2*	165.6 ±3.8*	137.5 ± 0.5*	158.7 ± 1.1*	86.6 ± 1.8*	40.2 ± 10.84	60	125	60
Total Pb (mg kg^{-1})	7 ± 0.3*	4.5 ± 0.7*	5.6 ± 0.2°	22.7 ± 1.9*	11.4 ± 1.4*	4.3 ± 0.7*	16.0 ± 1.3*	5.1 ± 0.3*	5.2 ± 0.9*	4.9 ± 2.5*	275.1 ± 35.55	150	150	250
Total Zn (mg kg^{-1})	11.0 ± 0.7*	20.6 ± 18.9*	51.5 ± 1.0*	34.9 ± 1.6*	36.5 ± 1.9*	46.7 ± 0.9*	82.1 ± 1.4*	109.3 ± 2.4*	106.1 ± 0.5*	71.3 ± 0.3*	411.1 ± 16.34	200	700	700

EC = electric conductivity, BDL = below detection limit, all values are mean ± standard error, results are expressed on dry weight basis, MAV = Maximum Allowable Values based on NSW General Exemption, Values with * superscript are significantly different compared to MWOO at ($P < 0.001$), Values with ° superscript are significantly different compared to MWOO at ($P < 0.05$)

The treatments for each soil were set up in triplicate together with unamended controls, and appropriate column numbers were established for destructive sampling of triplicate columns at 6, 12 and 18 months, giving a total of 459 soil columns.

2.1.4 Glasshouse column layout

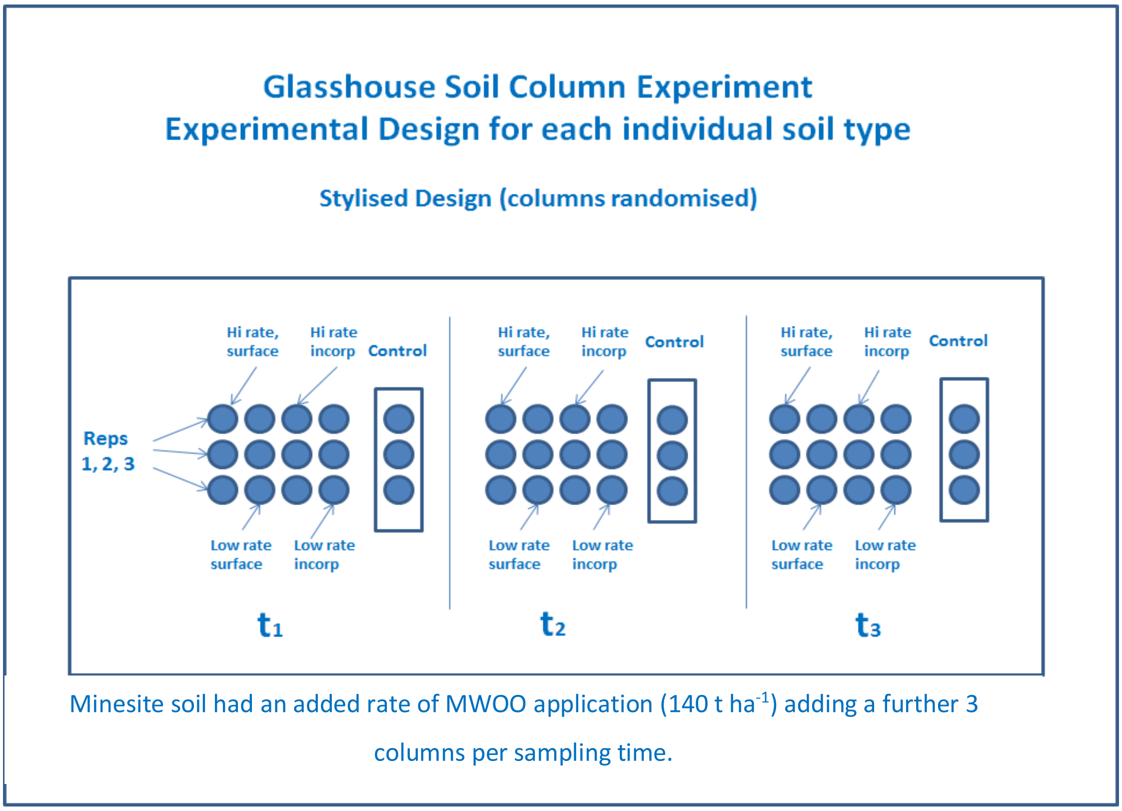
For each soil type assessed, the soil column experiment was arranged using the following design (Figure 2.1). For each soil type, a total of 45 pots/soil columns were established. This pot array comprised 2 x application rate, 2 x application method, 3 x replication plus 3 x control with 3 x sampling times (=450 pots). For the Minesite soil, the 140 tha^{-1} application rate added a further 3 replicates at each sampling time (giving 459 pots in total). Each soil column contained approximately 20kg soil in excess of 780kg of each soil type to be collected and processed for each soil type.

2.1.5 Soil Sampling

The glasshouse soil column experiment was initiated in February 2013. Soil sampling was undertaken at three, pre-determined times after the initiation of the experiment: 6 months (August 2013), 12 months (February 2014) and 18 months (August 2014). At the initiation of the experiment, columns for each soil type, application rate and method were identified for sampling at each time (t_1, t_2, t_3).

At each sampling all soil columns identified for that sampling period were selected and destructively sampled. Sampling was undertaken using an 80mm stainless steel coring tube driven down the centre of each column and extracted undisturbed. Each soil core was then sub-divided into a number of depth increments: 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 25-30 cm, 45-50 cm, 65-70 cm. All sampling was undertaken with great care to avoid any cross-contamination.

To preserve sample depth and integrity, sampling was achieved using stainless steel corers (0.85m × 0.08m ID). The core was extruded onto silver foil lined poly pipe and cut into the seven sample depths. All equipment was cleaned between each core with phosphate free detergent and deionized water. For the clay soils and the organic soil, because coring was not possible for the extent of the column, the column was sampled incrementally as the column pipe was split open. All samples were air-dried (40°C), sieved to 2 mm, bulk density determined, and stored in polyethylene sealed sample vials prior to analysis.



Minesite soil had an added rate of MWOO application (140 t ha^{-1}) adding a further 3 columns per sampling time.

Figure 2.1: Glasshouse soil column experiment design

2.1.6 Soil sample processing

Fresh samples were weighed at the point of sampling and a 10g sub-sample dried at 105°C to determine oven dry moisture. A further 10g was collected in glass for subsequent analysis of organic compounds. The remaining sample was dried at 40°C and sieved to <2mm. At this point a further sub-sample dried at 105°C. The <2mm, 40°C dried sample was then split for routine analysis and analysis of metals.

2.1.7 Soil Analysis

The undisturbed sampling method allowed for a known volume of soil to be determined from each core and depth increment. From this and the 105°C corrected dry mass of the soil, bulk density was calculated.

Soil condition parameters were determined at the NSW State Government Soils Laboratory at Yanco, NSW. For each soil sample, pH, EC, soil organic carbon and soil organic nitrogen were determined (Rayment and Higginson 1992). For a subset of the samples, Extractable Phosphorus was determined.

Metals concentrations of each soil sample were determined at the University of New England using ultrawave microwave digestion (aqua regia) with subsequent analysis using ICP-OES. The metals and metalloids examined included those identified in the NSW General Exemption and considered to be elements of concern for MWOO amended soils which are As, Cd, Cr, Cu, Ni, Pb and Zn.

2.2 Effects of MWOO application on soil physical and chemical properties

2.2.1 Summary

MWOO was applied to soil columns at rates of 0, 20 and 50 t ha^{-1} (and 140 t ha^{-1} for Minesite soils) and using two application methods – surface application and incorporation (to 15 cm). Soils were sampled and analysed for a range of properties at 6 months (t_1), 12 months (t_2) and 18 months (t_3).

No significant change in bulk density (as a measure of soil physical condition) could be detected across the entire dataset in response to MWOO. This result suggests that the rates and methods of MWOO application and the inherent variability in the dataset were such that change in this parameter could not be detected. It is possible that the addition of MWOO and the carbon and nitrogen associated with it did have some effect on soil physical aggregation and structure but this analysis was beyond the scope of the current study but would benefit from further study.

Soil organic carbon and nitrogen increased relative to the controls soils for almost all soil types (the exception being OM1). The response to MWOO application was proportionally larger for sandy soils with lower initial organic carbon and nitrogen contents. For all soils the enrichment with carbon and nitrogen was larger at the higher application rates. Significant effects were only found in the layers to which MWOO was applied (i.e. 0-5 cm surface applied and 0-15 cm incorporated) and were typically of a larger magnitude for the surface applied treatment. Carbon and nitrogen decreased steadily in all soils through the 18 months of the experiment although at the end of that period still typically had larger carbon and nitrogen concentrations than control soils. There was no evidence of translocation of either carbon or nitrogen into deeper soil layers below those treated through the experimental period in any of the soils examined.

Soil pH also responded to the application of MWOO with pH8.5. Soil pH increased (by as much as 2 pH units) in soils with inherently low pH. For these soils increases were found in the layers of application only and increased with increasing application rate. Where initial (Control) pH was higher, the effect on soil pH became progressively smaller. The response of soil pH in clay soils was limited to the high application rates and surface application presumably due to the buffering effect of the clay itself. In soils with inherently high pH (>pH8.0) there was an apparent (but not significant drop in pH following MWOO application. These results suggest that pH will be modified following MWOO application relative to the inherent soil pH and the pH of the MWOO material. For all soils where a significant change was observed, the pH altered through the experimental period on a trajectory toward its initial/inherent soil pH.

Soil electrical conductivity increased in all the soils examined but again restricted to the application soil depths and typically at the higher application rates. For sandy soils, electrical conductivity diminished progressively through the experimental period. The change was less obvious in loam soils but for clay soils, a progressive increase in electrical conductivity was observed through time in the layers affected by MWOO application. Although a number of significant effects were determined associated with MWOO application, at no stage did any of the electrical conductivity values exceed 2.5 dSm^{-1} and would therefore not appear to represent any concern to plant growth following one application. However, if a cumulative effect were to take place following repeat applications, or indeed as a result of particular precipitation/evaporation climatic conditions, this soil property might be of concern although this effect remains untested.

A subset of four of the ten NSW soils (S1, SL1, SL2, C1) were analysed for the effect of MWOO on soil phosphorus concentration. In sandy and loam soils with inherent soil phosphorus concentrations (<20 mg kg^{-1}) there was a significant increase in soil phosphorus concentration. This was particularly so for the higher application rates and at the 140 t ha^{-1} application rate on the Minesite soil, phosphorus concentration was increased to almost 170 mg kg^{-1}. In the clay soils however, which had inherently high (>100 mg kg-1) phosphorus concentration, no significant change in concentration was detected following MWOO application. MWOO therefore undoubtedly has a fertiliser value but its effectiveness will be determined by soil type with the most significant gains being found on soil with low clay content where inherent phosphorus concentration is low and the long-term impact of MWOO as a fertiliser remains uncertain.

2.2.2 Introduction

A variety of effects on soil properties have been reported in the literature resulting from the application of MWOO to soils including both physical and chemical properties. Bulk density is a good measure of a soil's physical condition and is expressed as mass per unit volume. Bulk density is influenced by soil organic matter content, porosity and structure. A soil that has a high organic matter status and has good structure and porosity will typically have a lower bulk density which implies a soil in which water and nutrient movement is not inhibited and therefore has good condition. A soil with high bulk density typically has poor structure and porosity and probably also low organic matter status. Soil bulk density is commonly reported to decrease significantly after organic amendment application. For example, Hemmat et al. (2010) reported significant decreases in bulk density in the 0-20 cm layer of soils under irrigated wheat-corn in Central Iran to which mixed solid waste (MSW) compost had been applied at a range of rates (10-100 t ha^{-1}). These workers concluded that the reduction in bulk density was inversely proportional to the rate of application, a result that conforms with those of Kluge and Bolduan (2001), Aggelides and Londra (2000) and Eden et al. (2014). It might therefore be expected that MWOO applications will result in improvements in soil physical structure and therefore reduced bulk density.

Organic matter (OM) is a key component of a healthy soil and plays a critical role in a range of soil functions. Soil organic matter enhances soil aggregation and structure and moderates soil moisture holding characteristics and temperature. Organic matter also influences soil chemical characteristics. For example, it has been estimated that as much as 95% of soil nitrogen (N) and sulfur (S) and as much as 25% of phosphorus (P) are in organic forms in non-agricultural soils (Amlinger et al., 2007). The biological component of soil is also largely dependent on the quantity and nature of soil organic matter inputs. Soils in Australia are, by global standards, low in OM due to limitations imposed by climate and a relatively impoverished soil resource. It has been estimated that many Australian soils have also lost up to half their original organic matter content as a result of agricultural practices (Dalal and Chan, 2001). Strategies that promote soil carbon storage through OM incorporation have the potential to greatly improve soil condition and resilience in the face of continued demand for food and fibre in production landscapes.

Mixed waste organic output products are commonly promoted on the basis that they have a significant organic matter component and therefore their application to soils has the capacity to increase soil organic matter content. A number of studies in Australia and internationally have reported an increase in soil organic matter and soil organic carbon following MWOO application (Civeira and Lavado, 2006; Civeira, 2010; Eden et al., 2014). For example, increased soil organic matter after MWOO application was reported by Dorahy et al. (2006) in Australia (NSW); Aggelides and Londra (2000) in Greece; Kluge and Bolduan (2001) in Austria; Civeira (2010) in Argentina, Hemmat et al. (2010) in Iran and Eden et al. (2014) in France. Mays et al. (1973), Hortensine and Rothwell (1973) and Mays and Giordano (1989) reported increases in soil organic matter between 0.001 and 0.008% for every tonne of MSW compost applied to soils. Madrid et al. (1998) applied MSW compost to sandy soils in Spain at a rate of 30 t ha^{-1}, and reported increases in organic matter content of up to 0.59% during four growing seasons while Lillywhite et al. (2009) reported that soil organic matter increased following MSW compost application to soils in the United Kingdom (UK) and that the increase was proportional to the rate of application, although principally at the higher rates of application.

Soil organic carbon is now a widely used parameter to assess changes in soil organic matter. On sandy soils in Spain, Walter et al. (2006) reported significant increases (up to 0.7%) in soil organic carbon (SOC) following applications of 40, 80 and 100 t ha^{-1} MSW compost, and in south-west Poland, Weber et al. (2007) reported increases of as much as 1.1 to 2.2 g kg^{-1} SOC following MSW compost application (18, 36 and 72 t ha^{-1} dry matter). This work also demonstrated that increases in SOC were directly related to MWOO application rate, a result echoed by Pedra et al. (2007), but also that significant results were typically only associated with medium and high rates of application. In Thailand, Prabpai et al. (2009) reported increases in soil organic matter of between 0.4 to 2.7% with increases in proportion to MSW application rates of 20, 40, 60 and 80% MSW:soil.

Albaladejo et al. (2009) incorporated both source separated and non-source-separated MSW compost into the top 20 cm of soils in Spain at rates of 15 and 25 kg m^{-2} (150 and 250 t ha^{-1}) and detected significant increases in soil carbon content 12 months after application. This carbon enrichment was particularly large (+2.5%) for source-separated composts and at the higher rate of application. Barral et al. (2009) concluded that application rates in excess of between 4.0 and 7.2 t ha^{-1} (depending on soil type) MSW compost would be required simply to maintain soil organic carbon concentration. Fagnano et al. (2011) applied and incorporated 10, 30 and 60 t ha^{-1} of urban waste compost to a field trial site near Naples in Italy. They also found SOC increased and that this increase was proportional to rate of application, but again, mainly at the highest application rate. Poulsen et al. (2013) applied municipal waste compost to soils in Denmark and after 4 years reported a significant increase in soil carbon, although the absolute value of this increase was not specified. While in a 15 year experiment in France, Eden et al. (2014) reported a progressive increase in soil organic carbon throughout the experimental period.

A broad measure of soil salt content is electrical conductivity (EC) which indicates the ability of an aqueous solution to carry an electric current. Where the concentration of soluble salts increases in a soil, EC tends to increase. Plants can be detrimentally affected, both physically and chemically, by excess salts in soils. A typical EC range for agricultural soil is 0-4 dS m^{-1} whereas 3.69-7.49 dS m^{-1} has been reported for MWOO (Brady and Weil, 1998). Application of MWOO to soil typically increases soil EC and soil EC values have been reported to increase in proportion to MSW compost application at rates of between 40-120 t ha^{-1} (Iglesias-Jimenez and Alvarez, 1993; Walter et al., 2006). He et al. (1995), in a USA study, reported that at high MSW compost application rates EC could prevent seed germination, although nutrient uptake by plants and leaching can modify the increased EC (Zhang et al., 2006). Reduced plant growth has not been reported, although reductions in soil biological activity has been attributed to increased EC (Iglesias-Jimenez and Alvarez, 1993) .

Most soils have pH values (in water) in the range 4-9 but many soils in NSW are prone to natural or induced acidity where the soil pH is less than 7. Soil pH is strongly correlated with nutrient and contaminant availability.

Mixed waste organic output materials are commonly alkaline (e.g. Dorahy et al., 2006) and, where soils are acidic, the effect of MWOO application is typically to increase soil pH (Mkhabela and Warman, 2005). The relative magnitude of the pH change however differs as a consequence of soil type, inherent acidity and local environment. Increases in soil pH have been reported by a number of authors and is usually proportional to the application rate. Such an effect is often referred to as avoided acidity or a “liming effect’ and can be very beneficial to soils prone to natural or induced acidity. Prabpai et al. (2009) in Thailand reported increased soil pH from 6.0 to 7.8 proportional to MSW compost application rate, while in Denmark, Poulsen et al. (2013) applied municipal waste compost at the equivalent of 100kg N ha^{-1} yr^{-1} to sandy loam soils and reported a pH change from 7.16 to 7.78.

Crecchio et al. (2001), however, reported only a limited effect on soil pH resulting from MSW compost application. This work was carried out on clay rich soils in Italy, and these workers concluded that the buffer capacity of the soils outweighed the effects of the alkalinity added. The buffer capacity of a soil is essentially the ability of a soil to resist pH change and this typically increases with soil clay and organic matter content. Lillywhite et al. (2009) reported little significant difference in soil pH resulting from low and high rates of MSW compost application to soils that were already neutral (pH 7.0), while Albaladejo et al. (2009), who incorporated MSW compost to calcareous soils in Spain with an initial alkaline pH (7.7), reported a significant pH decline after 12 months. These results indicate that change in soil pH will be determined to a large degree by the existing pH and buffer capacity of the soil prior to application.

Among the soil extractable plant nutrients, Phosphorus (P) is critical for plant nutrition, particularly for fruit and seed development and MWOO would seem to have considerable potential as a P fertiliser. Not all of the P present in MWOO will be immediately available in the soil for plant nutrition. For example, Iglesias-Jimenez and Alvarez (1993) reported very low P mineralisation immediately following MSW compost application, but after 3 months, plant P uptake was significant and equal in magnitude to quantities contributed by inorganic fertilisers. This implies that P supply from MWOO will require an initial period of mineralisation before significant quantities become available for plant growth. For instance, De Haan (1981) and Soumare et al. (2003) reported that 2 years after application, 10-50% of total P in MSW compost was available to plants; a “fertiliser efficiency” that is comparable to that of inorganic fertilisers (e.g Amlinger et al., 2007). Evidence therefore suggests that MWOO application to soil will generally increase extractable P concentrations and that these nutrient gains will be proportional to application rates.

In this component of the work we sought to assess the effects of MWOO application at the various application rates and application methods on soil organic carbon, total nitrogen, electrical conductivity, and pH (both H_2O and $CaCl_2$).

2.2.3 Methods

All soils analysis was undertaken at the NSW Office of Environment and Heritage Soils Laboratory at Yanco, NSW following standard Australian procedures. Soil organic carbon and nitrogen were determined using dry combustion (LECO) with carbon corrected for carbonate content and all quantities based on oven dry mass. Soil pH and electrical conductivity were measured using a 1:5 soil:water suspension while extractable phosphorus was undertaken using standard Colwell P analysis, extracting in a bicarbonate solution and measuring P concentration using UV-Vis spectrometry. All methods conform to Rayment and Higginson (1992).

Statistical analysis was undertaken using analysis of variance (ANOVA) with MWOO application rate, application method and soil depth as fixed factors. Analysis of each soil type was undertaken separately since differences clearly existed between the soil types. Homogeneity of variance in each dataset was determined using Q-Q plots of observed and predicted values and transformation (Log, Log10) undertaken where required to achieve normal distribution of the dataset. Transformation modified the magnitude but did not affect the direction or significance of probability values generated. Analysis of Variance was also undertaken to determine the change in soil properties between the sampling times t_1 (6 months), t_2 (12 months) and t_3 (18 months).

2.2.4 Results

2.2.4.1 Effects on Soil Properties

Bulk Density

No significant and meaningful change in soil bulk density could be detected across the entire dataset in response to MWOO application. Neither the application rate, nor the application method resulted in any detectable change in bulk density at any depth in the soil profiles examined.

Organic Carbon

For all the sandy soils (S1, S2, S3) the application of MWOO had resulted in a significant increases in soil organic carbon content (Figures 2.2, 2.3, 2.4). These increases were significant ($P<0.001$) at all application rates compared to controls and the magnitude of the change was proportional to the application rate for each soil. Each application method resulted in significant ($P<0.001$) increases in soil organic carbon. An Application

Method x Depth interaction was found for each of these sandy soils indicating that this effect was significant only in the surface (0-5 cm) layer for the surface application but in the three surface depth increments (0-5 cm, 5-10 cm and 10-15 cm) for the incorporated application method. Throughout, the relative increase in organic carbon was largest for the surface application rates compared with the incorporated application method.

In the loam soils (SL1, SL2, SL3) a similar pattern of significant increase in soil organic carbon was found in response to the MWOO application (Figures 2.5, 2.6, 2.7). For SL2 and SL3, this increase was significant for each application rate with Control < 20 t ha^{-1} < 50 t ha^{-1}. In SL1 however, this effect was significant only for the 50 t ha^{-1} application rate. For the Minesite soil (SL3), where an MWOO application rate of 140 t ha^{-1} had been included, the largest increase in soil organic carbon was recorded at this rate of application. Each application method resulted in significant ($P>0.02$) increases in soil organic carbon. An Application Method x Depth interaction was found for each of these soils indicating that increased organic carbon was significant only in the surface (0-5 cm) layer for the surface application but in the three surface depth increments (0-5 cm, 5-10 cm and 10-15 cm) for the incorporated application method.

For each of the Clay soils (C1, C2, C3) a significant MWOO application was again found on the soil organic carbon content (Figures 2.8, 2.9. 2.10). For each of these clay rich soils, organic carbon again increased significantly in the order Control < 20 t ha^{-1} < 50 t ha^{-1}. For the Inverell clay (S2), each application method resulted in a significant ($P=0.047$) change in soil organic carbon. However, in the other two clay soils (S1, S3) no application method effect could be found. Once again, an Application Method x Depth interaction was found for each of these clay rich soils but indicated that where an increase in organic carbon had occurred, this effect was significant only in the surface (0-5 cm) layer.

In the organic soil (OM1), although there was an apparent increase on organic carbon content at the 50 t ha^{-1} application rate for the surface application, no significant increase in organic carbon was in fact detected for any of the application rates or methods.

Nitrogen

Change in the nitrogen content of the various soils largely followed the pattern of organic carbon. However, the significant changes detected were generally fewer than for organic carbon. For all sandy soils (S1, S2, S3) the application of MWOO resulted in significant ($P>0.01$) increases in nitrogen concentration compared with control soils. In soils S2 and S3, the nitrogen concentration was increased significantly following both 20 and 50 t ha^{-1} application rates with the highest concentrations following the higher application rate. For these soils, concentrations were significantly increased under both surface application (0-5 cm) and incorporated application methods (0-5, 5-10 cm). However, for S1, nitrogen concentration was increased only at the 50 application rate and at the surface (0-5 cm) depth.

In the loam soils (SL1, SL2, SL3) nitrogen concentration was again increased significantly ($P<0.000$) by all application rates with a progressive increase with increasing application rates (20 < 50 < 140 t ha^{-1}). Throughout, a significant depth interaction ($P<0.001$) indicated that the two application method differed in their effects with surface applications increasing nitrogen concentration only in the surface (0-5 cm) layer while incorporated applications increased nitrogen concentration in the top three depth increments (0-5 cm, 5-10 cm, 10-15 cm).

For the clay soils (C1, C2, C3) there was a small but significant ($P>0.05$) increase in nitrogen concentration compared with the control soils for both application rates although both rates typically had a statistically similar effect. Only for the S3 was the nitrogen concentration significantly higher ($P=0.004$) under the 50 t ha^{-1} application rate.

In the organic soil (OM1) no statistically significant increase in nitrogen concentration could be detected at either application rate nor under any specific application method.

Electrical Conductivity

Electrical conductivity was significantly affected by the application of MWOO. For the sandy soil (S1), there appeared to be an increase (0.06 to 0.19 dSm^{-1}) in soil EC following the application of MWOO for both application rates compared with the control. However, this effect was only significant ($P>0.04$) for the 50 t ha^{-1} application rate. For soils S2 and S3 however, both application rates (20 and 50 t ha-1) resulted in a significant ($P<0.001$) increase in electrical conductivity compared with the control soil [0.01 to 0.12 (20 t ha^{-1}), 0.38 (50 t ha^{-1}) and 0.03 to 0.10 (20 t ha^{-1}), 0.24 (50 t ha^{-1}) respectively]. A significant ($P>0.05$) application method x depth interaction for both of these latter soils indicated that the depth to which this effect occurred differed between the application methods. For the surface application, only the top (0-5 cm) layer was affected significantly by MWOO application while for the incorporated method, the top two depths (0-5, 5-10 cm) were affected.

In all the loam soils (SL1, SL2, SL3), a significant increase in electrical conductivity resulted from the application of MWOO (up to 0.7 dSm^{-1} for S1). For S1, the magnitude of change was similar ($P=0.153$) between the application rates while for SL2 the effect was in the order Control < 20 t ha^{-1} < 50 t ha^{-1}. For S3, only the 50 t ha^{-1} application had resulted in an electrical conductivity increase. For SL1 and SL2, differences were found between the surface and incorporated application methods. In these soils, the magnitude of change in any given soil layer was larger for the surface application method. However, surface application significantly

(P<0.001) affected only the top (0-5 cm) layer while for incorporated applications, the top three layers (0-5, 5-10, 10-15 cm) were affected. In SL3, no significant (P=0.613) effect of application method could be detected.

For the clay soils (C1, C2) no significant (P=0.153, P=0.305 respectively) difference existed between the application rates (20 and 50 t ha^{-1}) in their effect on electrical conductivity although both were significantly higher than the control soils. It was observed in the Control soil C1 that electrical conductivity also appeared to increase significantly in the surface (0-5 cm) layer compared with the deeper control soil layers during the experiment. Both application rate (P<0.001) and application method (P<0.001) affected electrical conductivity in the clay soil (C3). Here the electrical conductivity increased with increasing application rate and effects were seen in the top (0-5 cm) layer following surface application and in the top three layers (0-5, 5-10, 10-15 cm) when MWOO was incorporated. In C1 no significant (P=0.191) difference could be found between the application rates in their effect on electrical conductivity and all effects were restricted to the top (0-5 cm) layer.

In the organic soil (OM1), no significant application rate or method effect could be detected although, as with soil C1, the EC of the control soil also increased significantly (P<0.001) in the 0-5 cm layer compared with underlying control soils.

Although a number of significant effects were determined associated with MWOO application, at no stage did any of the electrical conductivity values exceed 2.5 dSm^{-1}.

Soil pH

Patterns in soil pH change were similar for both pH (H_2O) and pH ($CaCl_2$).

For all sand soils (S1, S2, S3) a significant increase in soil pH resulted from each application rate (control < 20 < 50 t ha^{-1}). For each soil, the surface application increased the pH only in the surface layer while the incorporated application increased soil pH in the top three depth increments (0-5, 5-10, 10-15 cm). The largest relative increase in pH resulting from MWOO application occurred in the soil with the lowest initial pH (S1) with a difference of almost two pH units (pH 4.50 to 6.42 – $CaCl_2$) between control and MWOO applied soil . For the soils with higher initial pH (S2, S3) the increase was proportionally smaller (pH 6.06 to 7.27 and 7.71 to 8.13 respectively in $CaCl_2$).

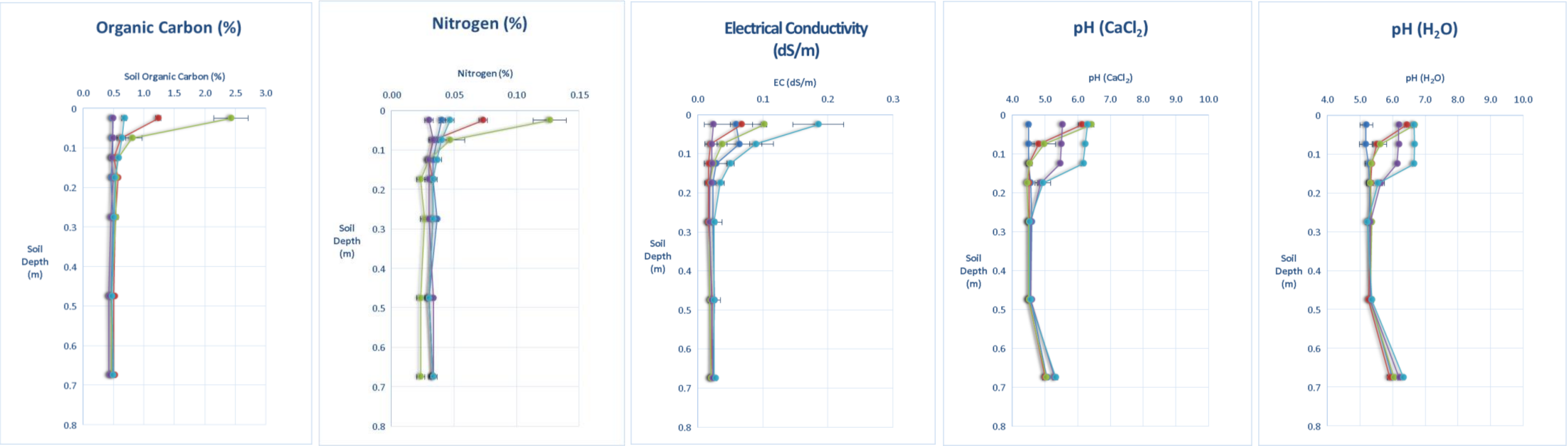
For the Loam soils, different effects on pH were found in the different soils. In soils SL1 and SL3, no significant change in pH could be detected even though both soils had quite different initial pH. In the soil S3 with initially high pH (~8.0) the MWOO application actually appeared to induce a reduction in soil pH of up to 0.4 pH units for the high (50 t ha^{-1}) application rate although again, this result was not significant. In soil SL2, a significant (P<0.001) increase in soil pH was detected but only at the 50 and 140 t ha^{-1} application rates. A significant

($P<0.001$) application method x depth interaction indicated that the effect on the soil differed between methods. For the surface application at 50 t ha^{-1}, pH was increased in the surface (0-5 cm) soil layer only, while for the incorporated method, the increase affected all three surface layers (0-5, 5-10, 10-20 cm).

In the two clay soils (C1, C2) with the lower initial soil pH, a significantly ($P>0.06$) higher pH was found only at the 50 t ha^{-1} application rate (pH 4.79 to 5.35; pH 6.00 to 6.52 respectively) but again only for the surface application. For the high pH clay soil (S3) no significant pH change could be detected at any depth under either application rate or application method.

Figure 2.2 Soil Properties S1 (Kirby Sand): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months). [Error Bars =1SE]

S1 - Kirby Sand Time 1



Organic Carbon (%)

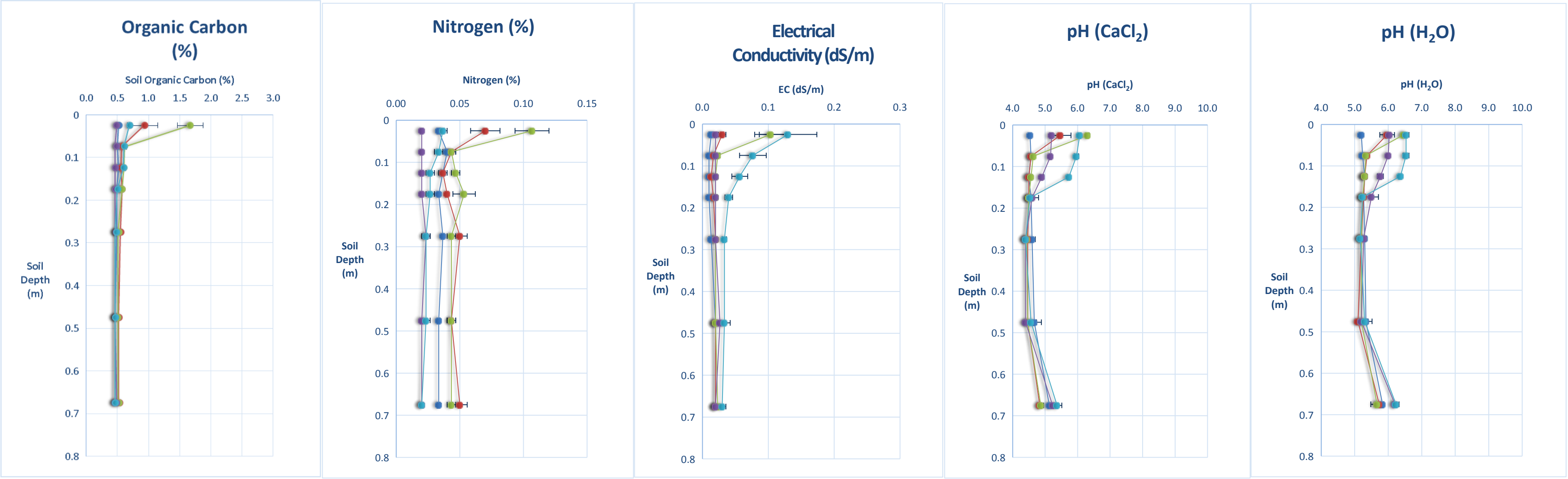
Nitrogen (%)

Electrical Conductivity (dS/m)

pH ($CaCl_2$)

pH (H_2O)

S1 - Kirby Sand Time 2



Organic Carbon (%)

Nitrogen (%)

Electrical Conductivity (dS/m)

pH ($CaCl_2$)

pH (H_2O)

S1 - Kirby Sand Time 3

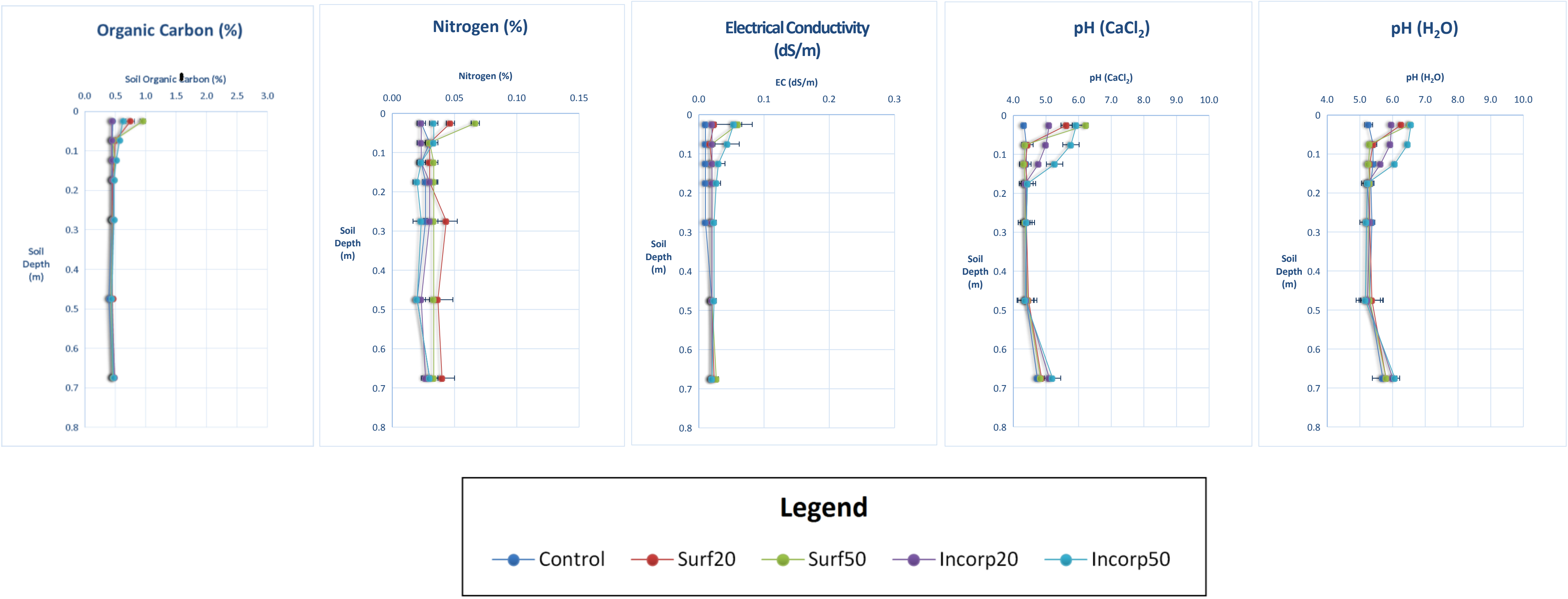
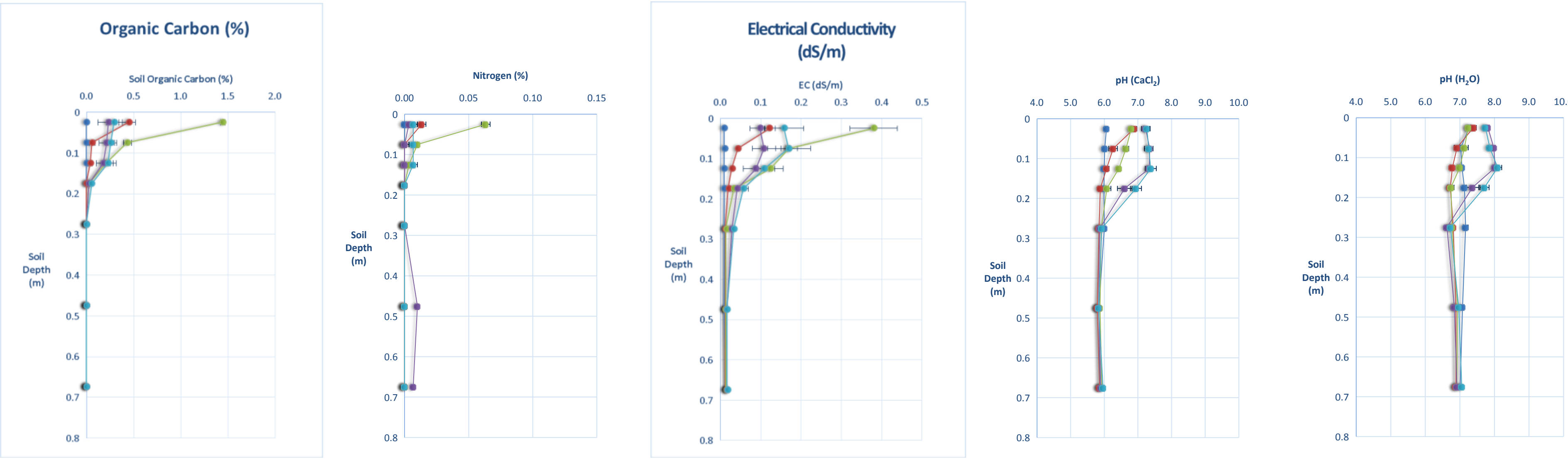
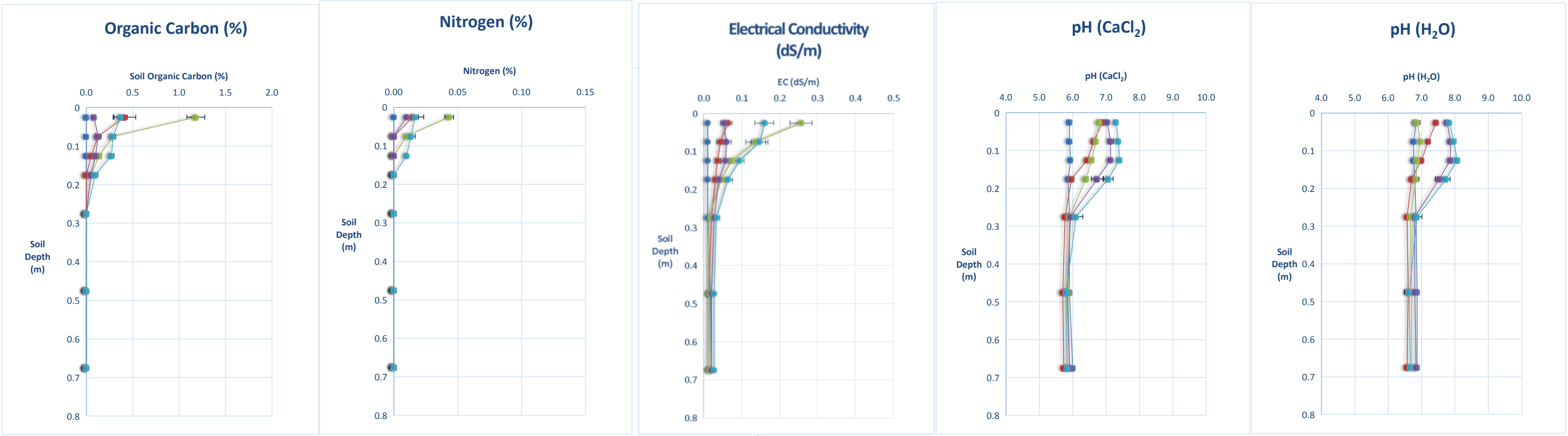

Organic Carbon (%)
Soil Organic Carbon (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Soil Depth (m)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Nitrogen (%)
Nitrogen (%)
0.00
0.05
0.10
0.15
Soil Depth (m)
Electrical Conductivity (dS/m)
EC (dS/m)
0.0
0.1
0.2
0.3
Soil Depth (m)
pH (CaCl$_2$)
pH (CaCl$_2$)
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Soil Depth (m)
pH (H$_2$O)
pH (H$_2$O)
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Soil Depth (m)
Legend
Control
Surf20
Surf50
Incorp20
Incorp50

Figure 2.3 Soil Properties S2 (Tingha Sand): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

S2 - Tingha Sand Time 1



S2 - Tingha Sand Time 2



S2 - Tingha Sand Time 3

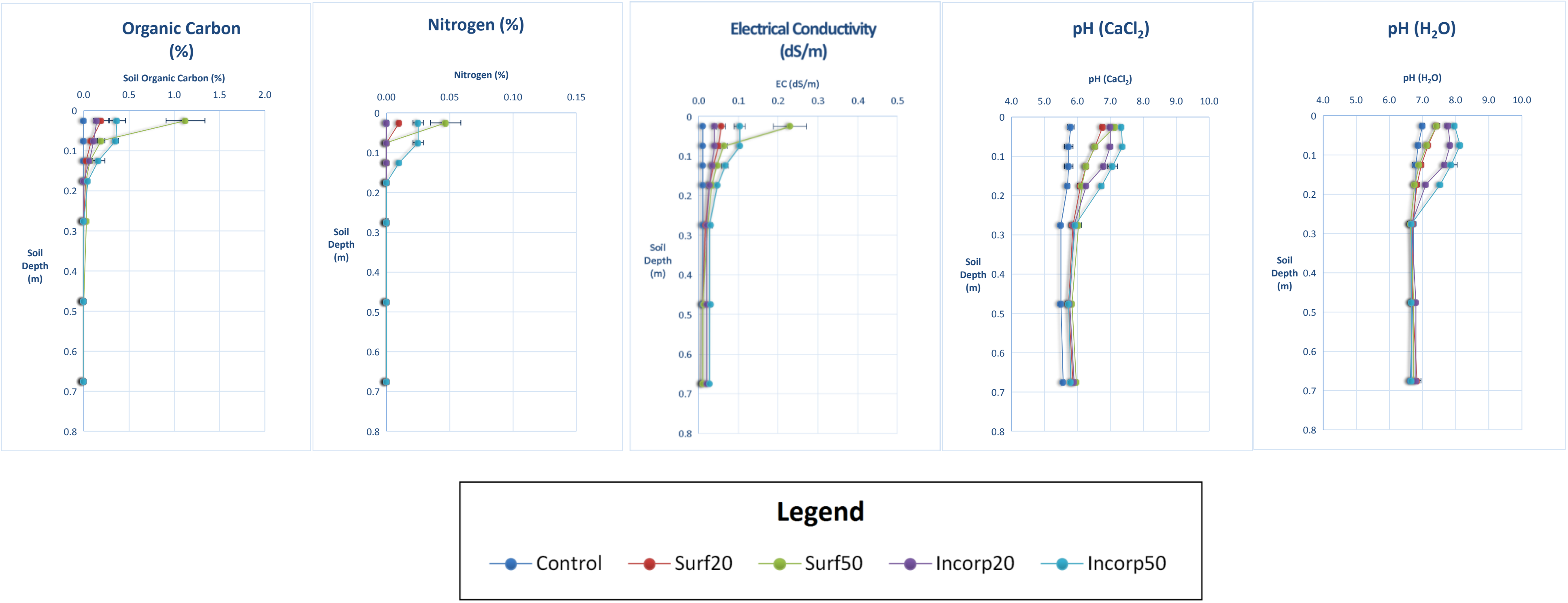
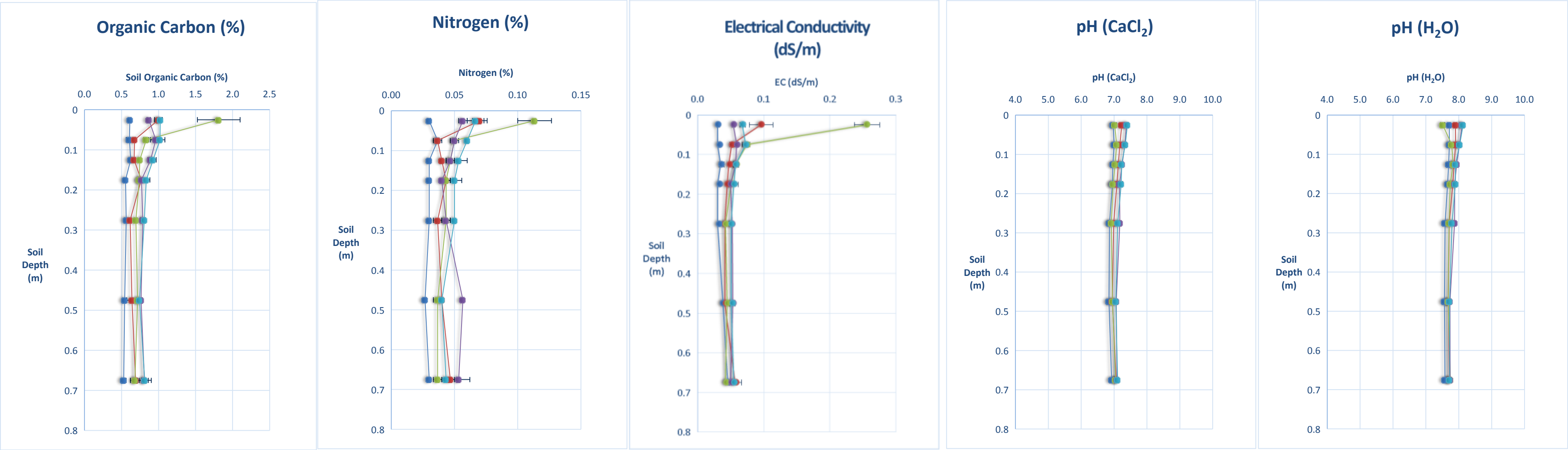
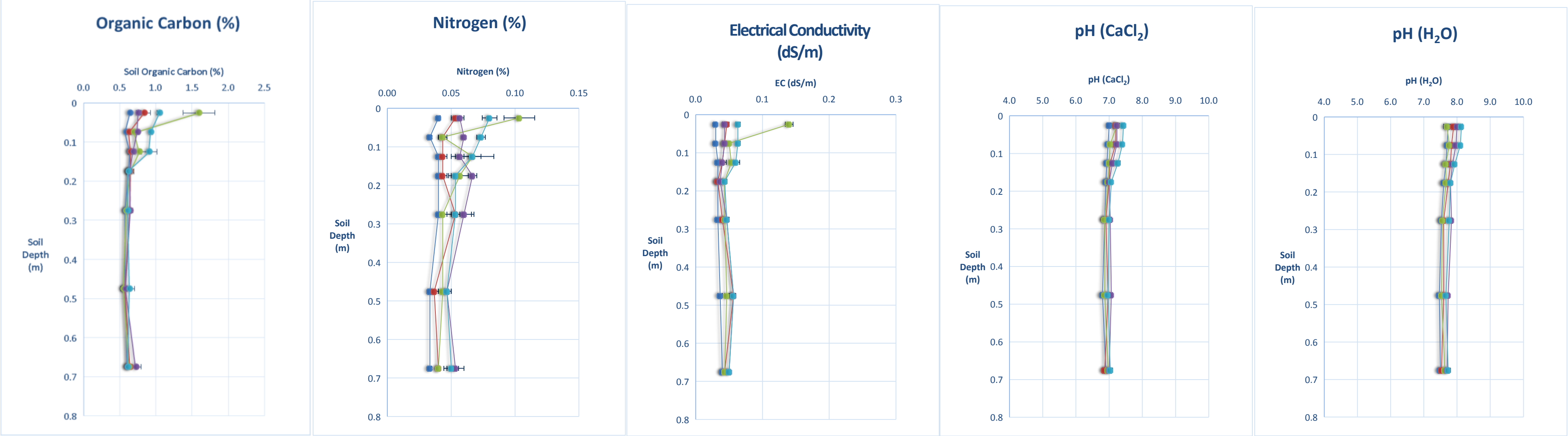


Figure 2.4 Soil Properties S3 (Gunnedah River Loam): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

S3 – Gunnedah River Loam Time 1



S3 - Gunnedah River Loam Time 2



S3 - Gunnedah River Loam Time 3

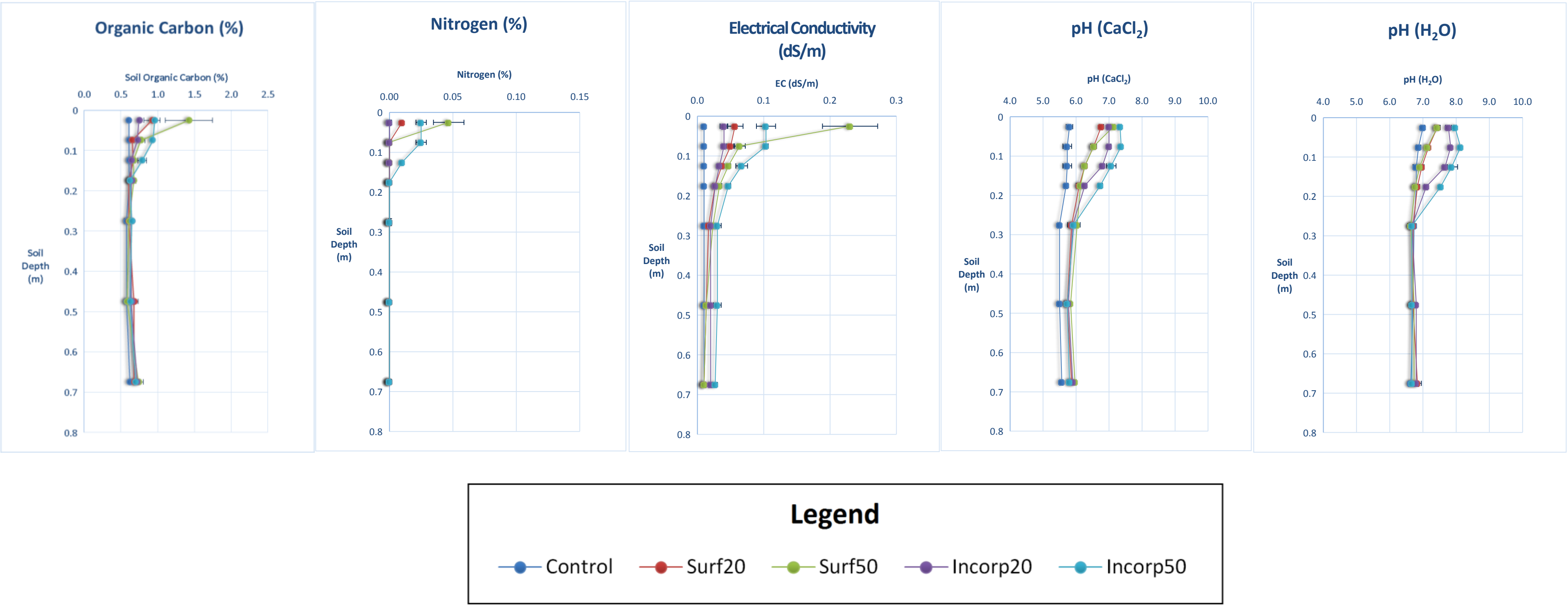
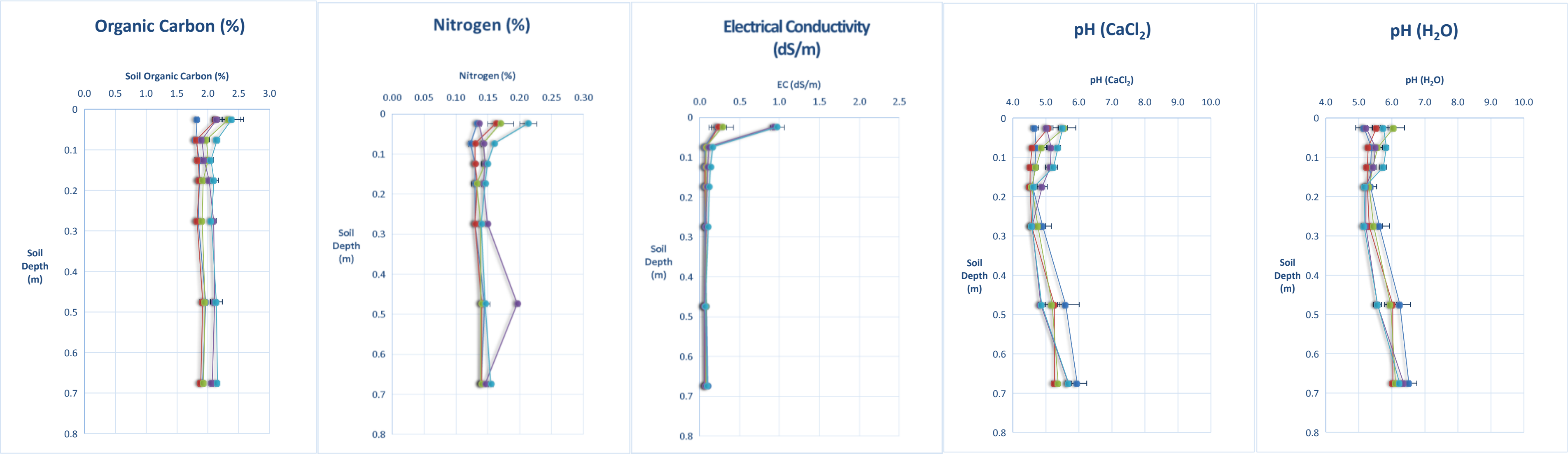
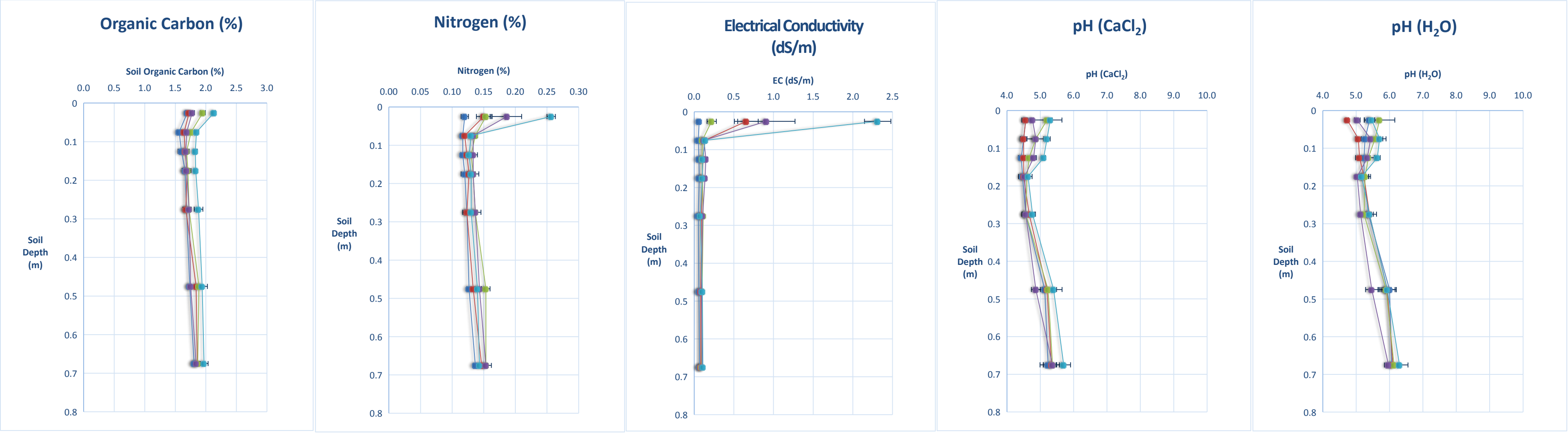


Figure 2.5 Soil Properties SL1 (CROA): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

SL1 - CROA Time 1



SL1 - CROA Time 2



SL1 - CROA Time 3

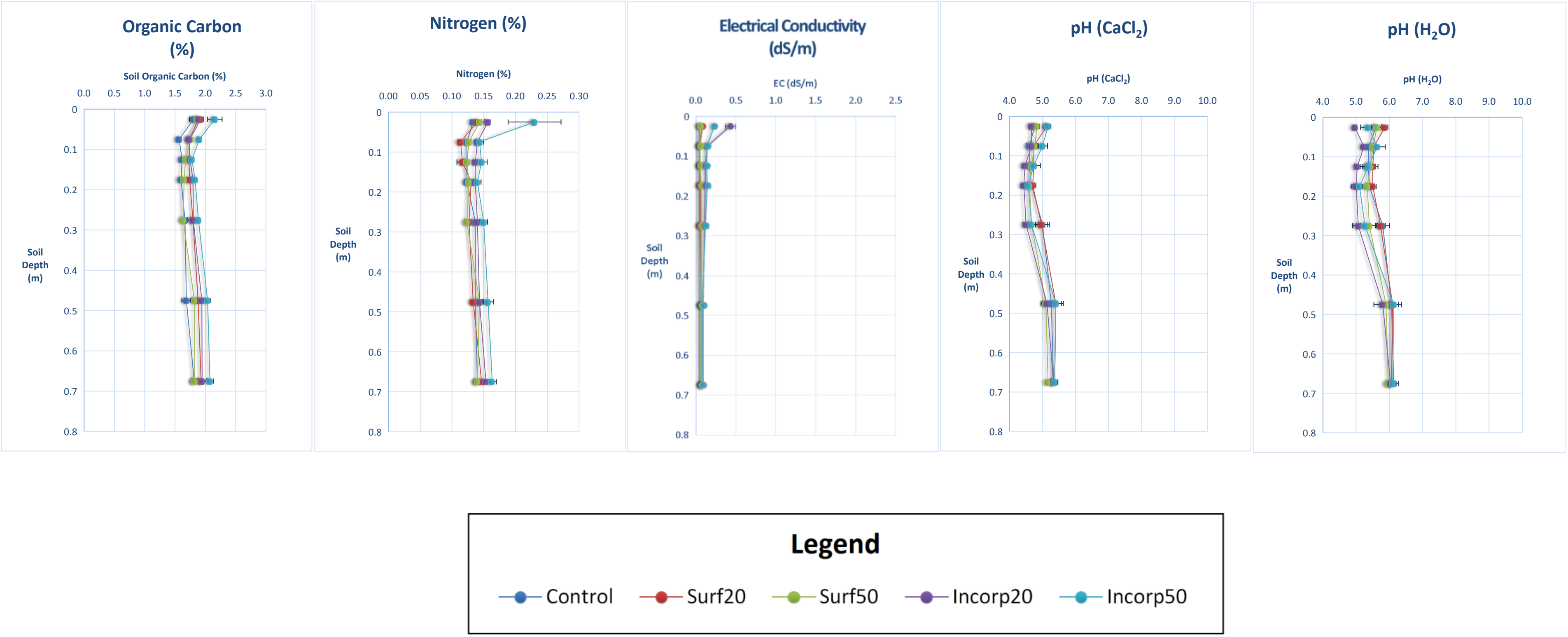
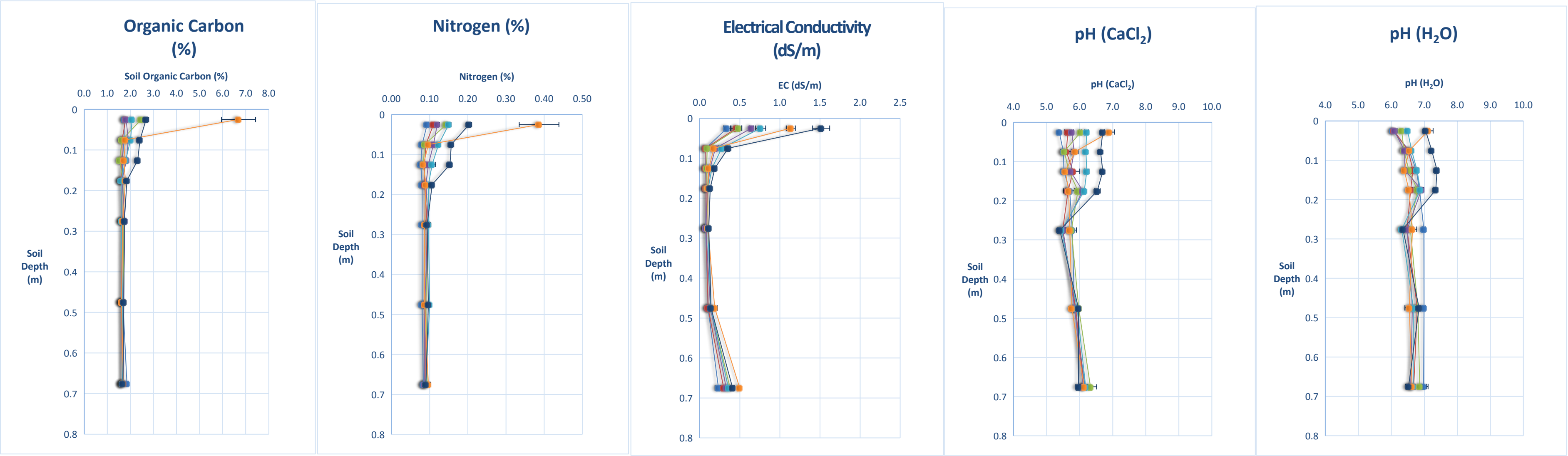
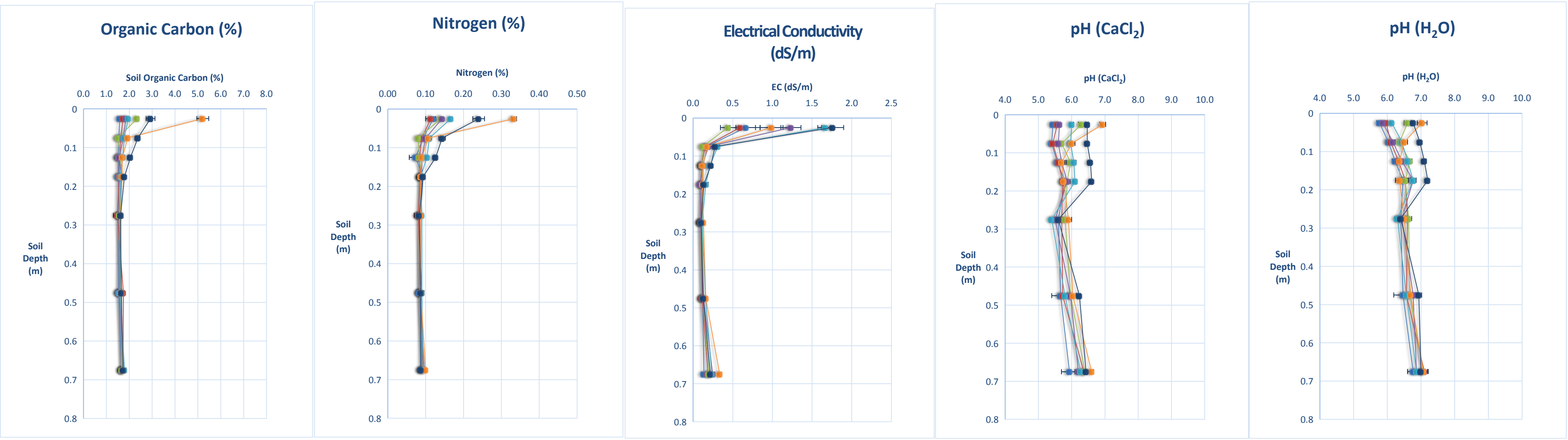


Figure 2.6 Soil Properties SL2 (Minesite): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

SL2 - MINESITE Time 1



SL2 - MINESITE Time 2



SL2 - MINESITE Time 3

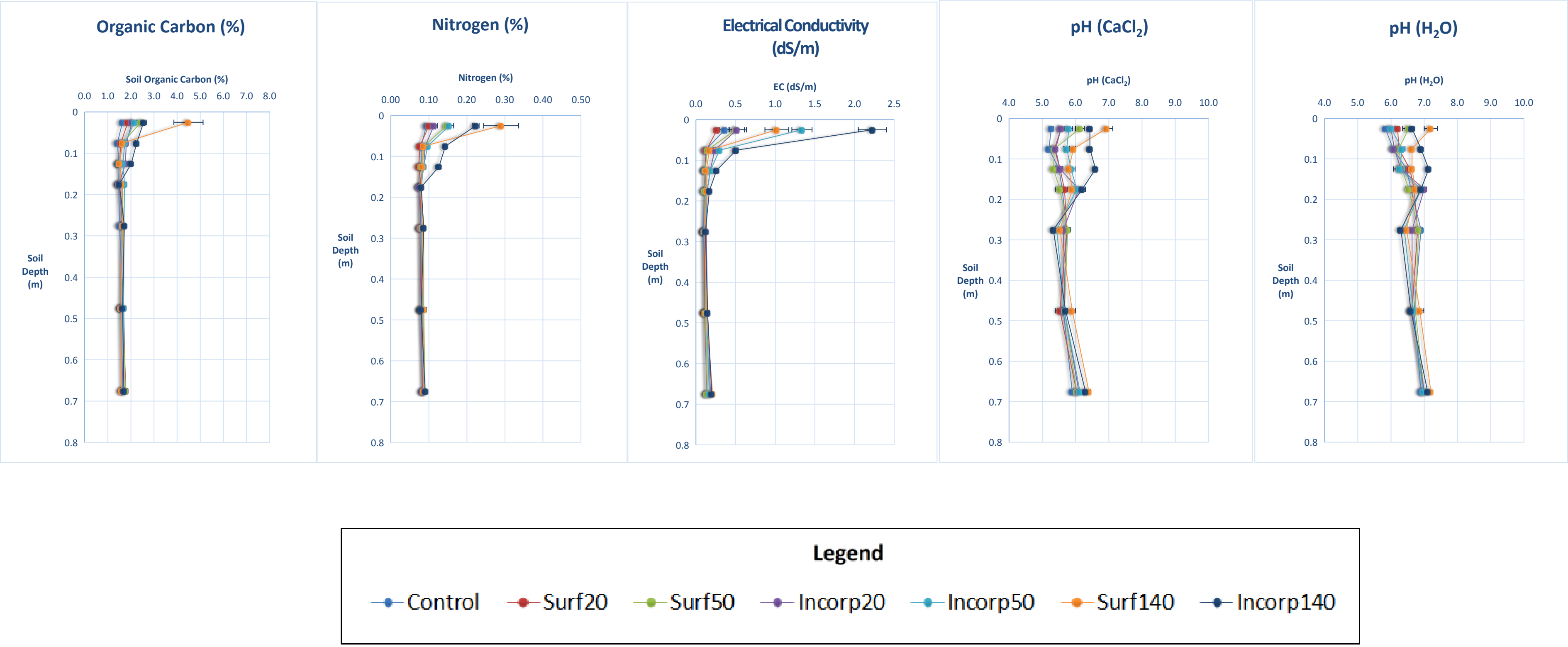
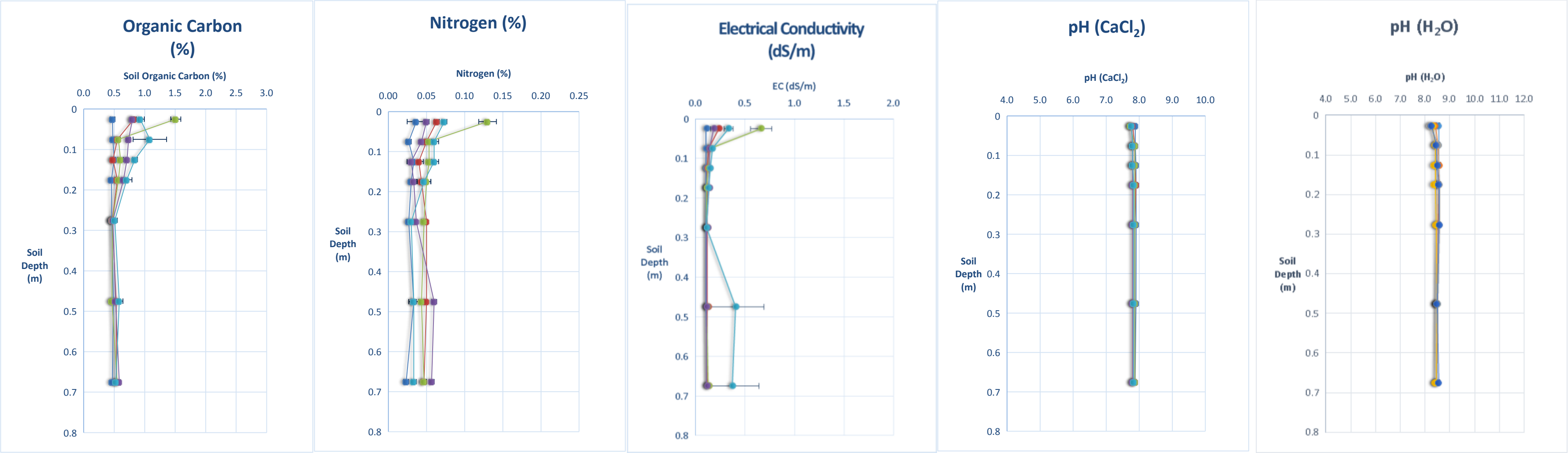
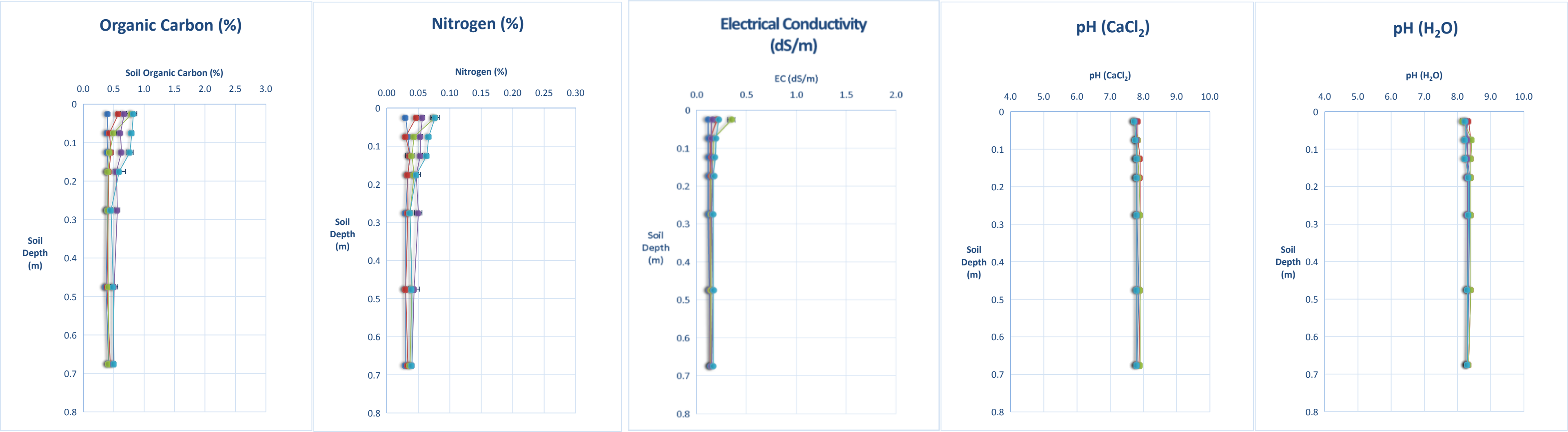


Figure 2.7 Soil Properties SL3 (Warialda Loam): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

SL3 – Warialda Loam Time 1



SL3 – Warialda Loam Time 2



SL3 – Warialda Loam Time 3

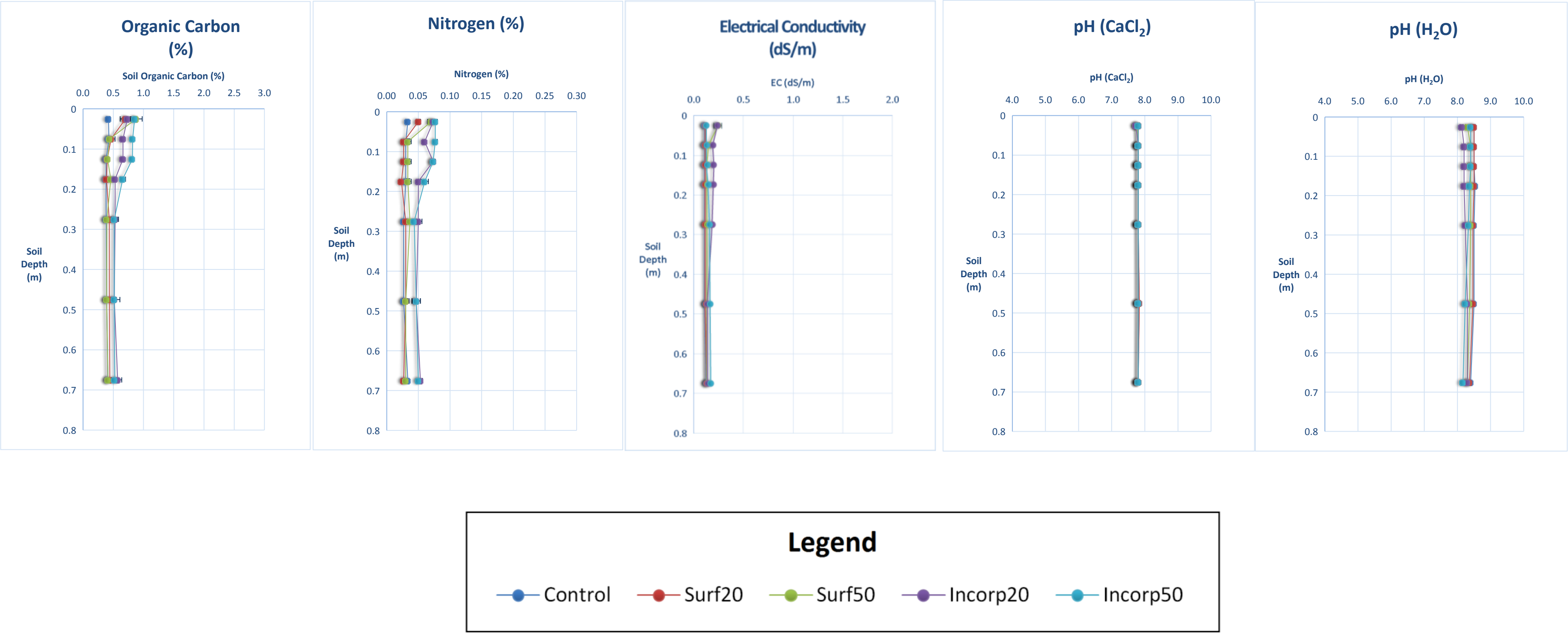
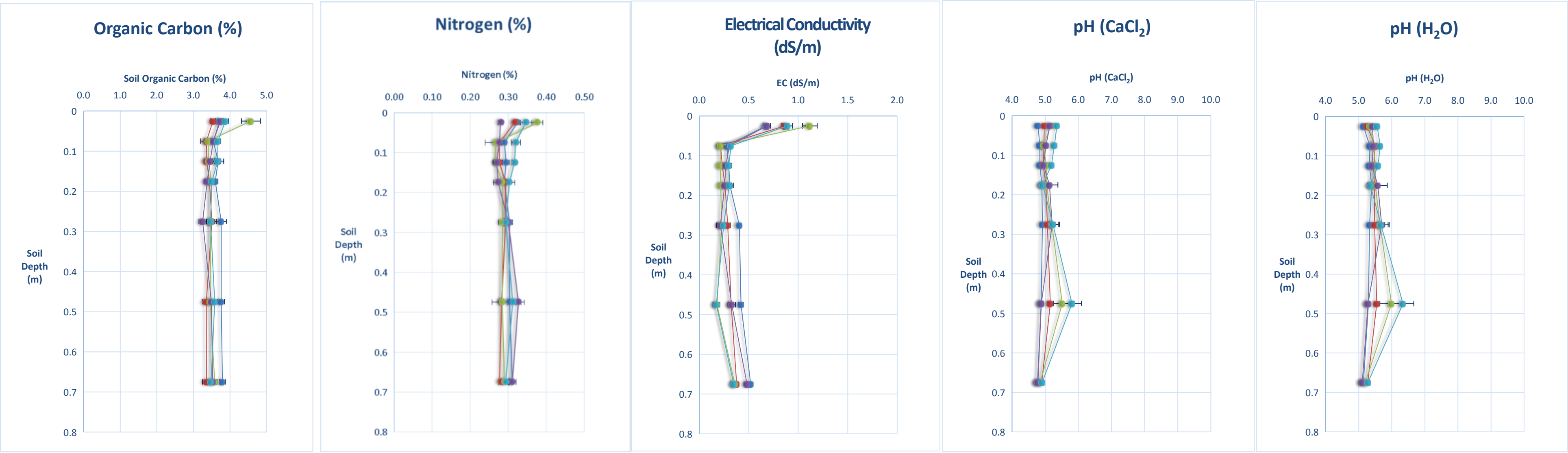
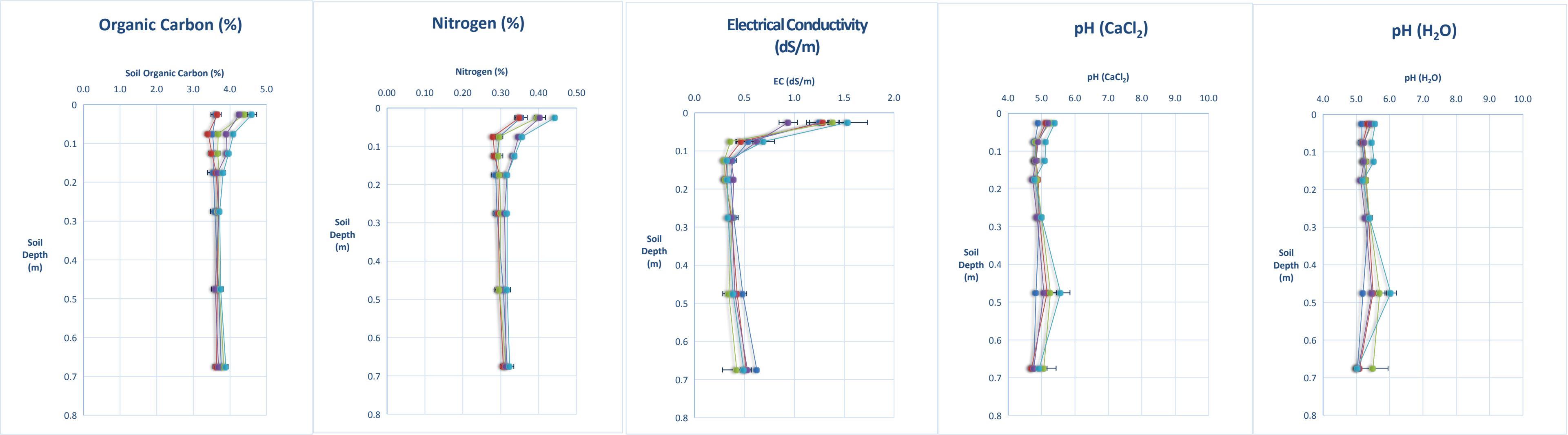


Figure 2.8 Soil Properties C1 (Kirby Clay): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

C1 – Kirby Clay Time 1



C1 - Kirby Clay Time 2



C3 - Kirby Clay Time 3

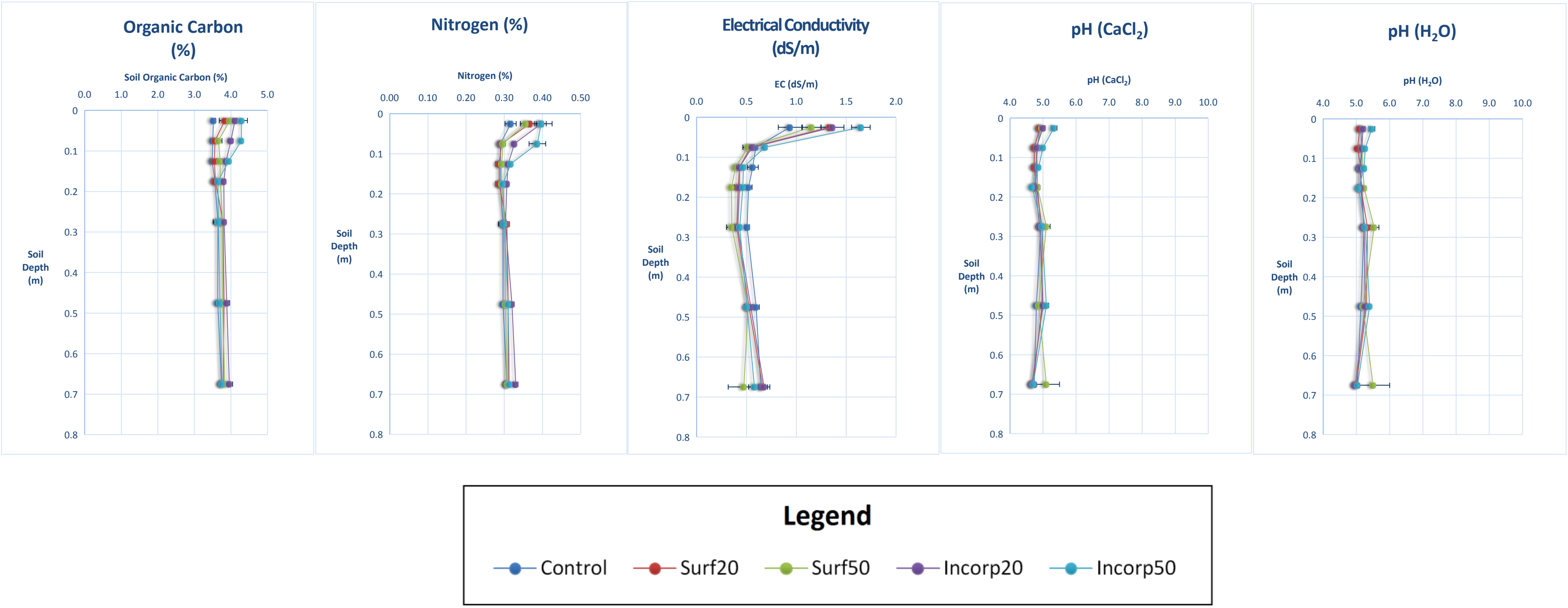
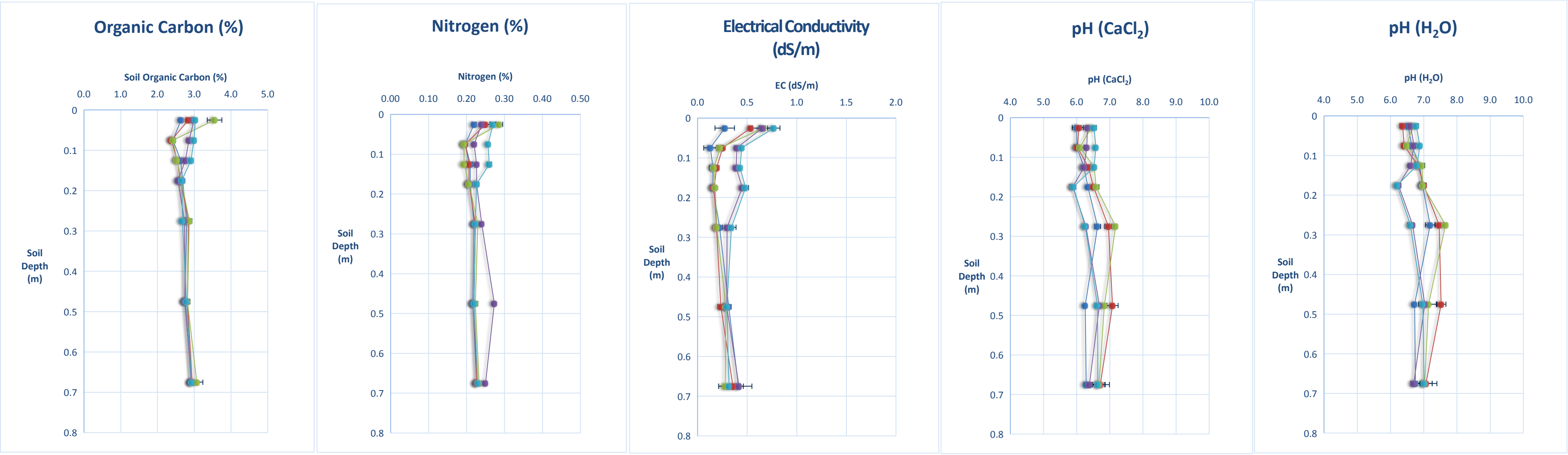
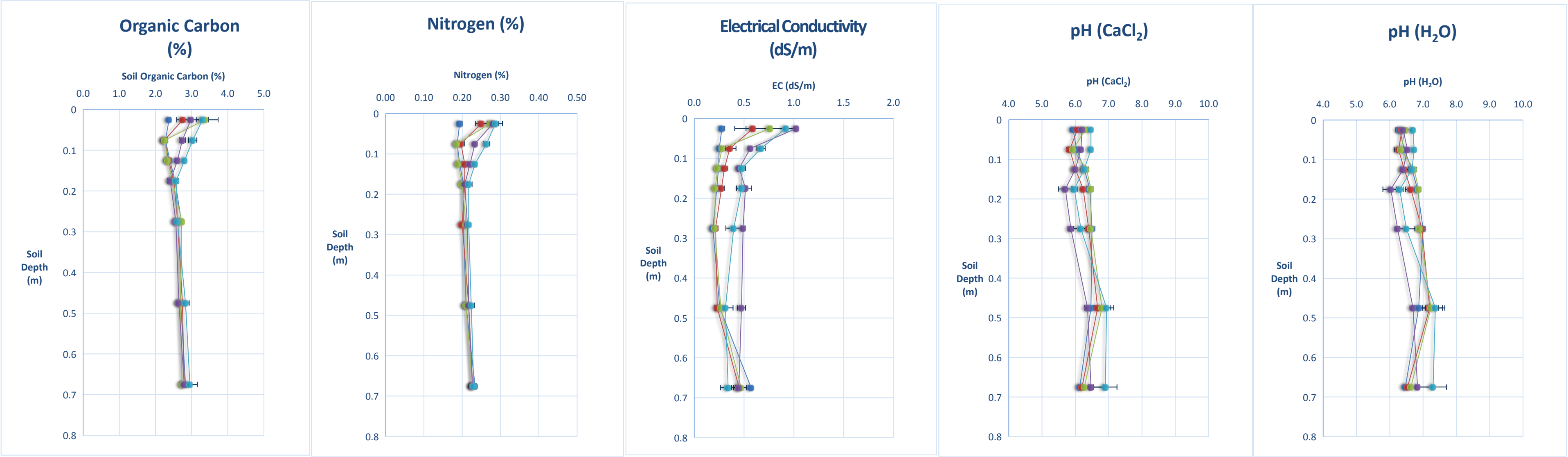


Figure 2.9 Soil Properties C2 (Inverell Clay): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

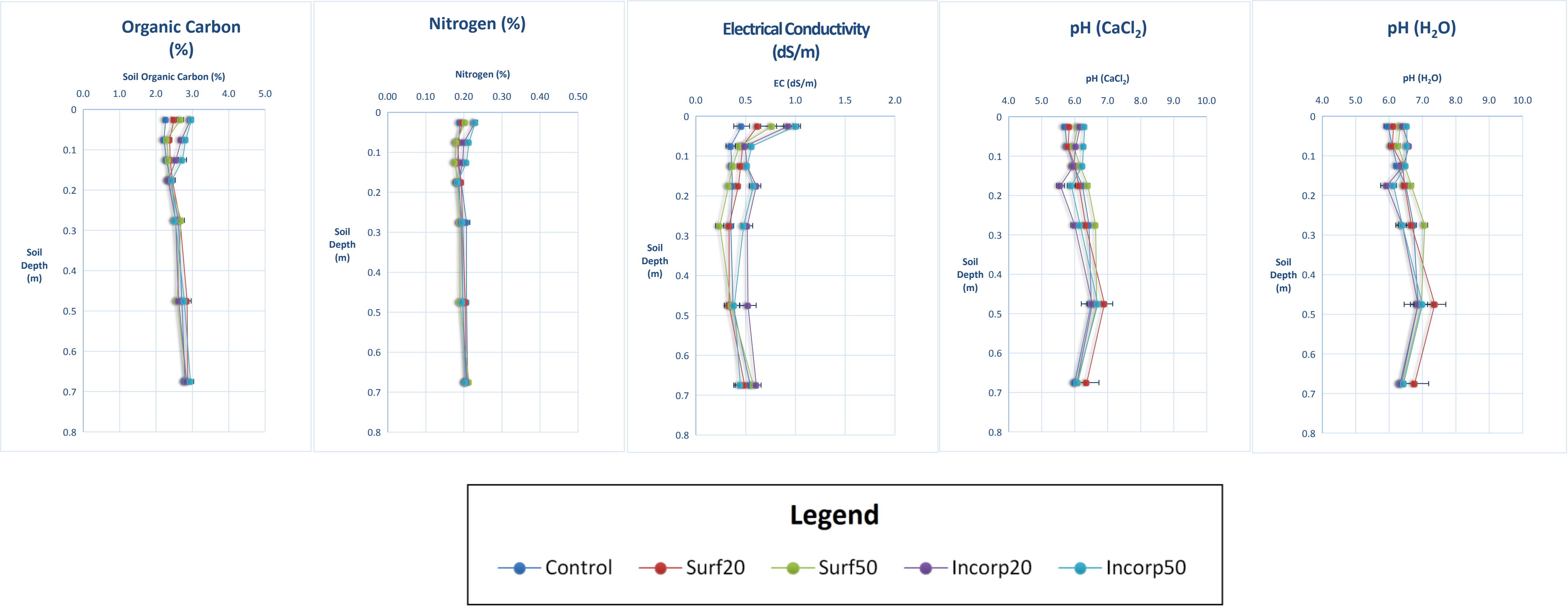
C2 – Inverell Clay Time 1



C2 - Inverell Clay Time 2



C2 - Inverell Clay Time 3



Organic Carbon (%)

Nitrogen (%)

Electrical Conductivity (dS/m)

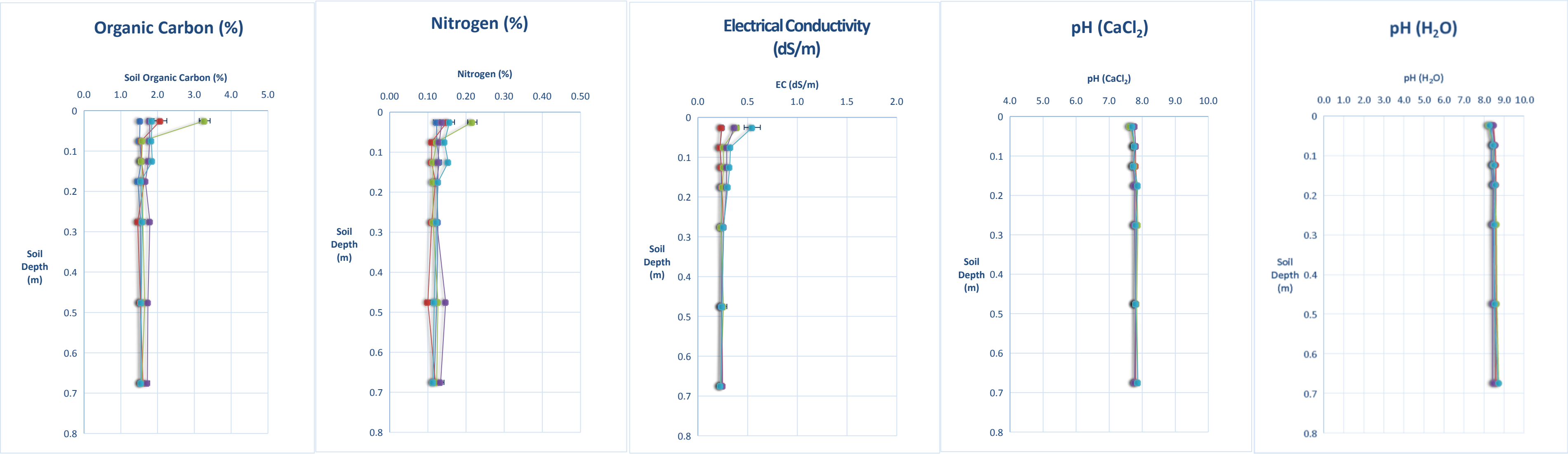
pH ($CaCl_2$)

pH (H_2O)

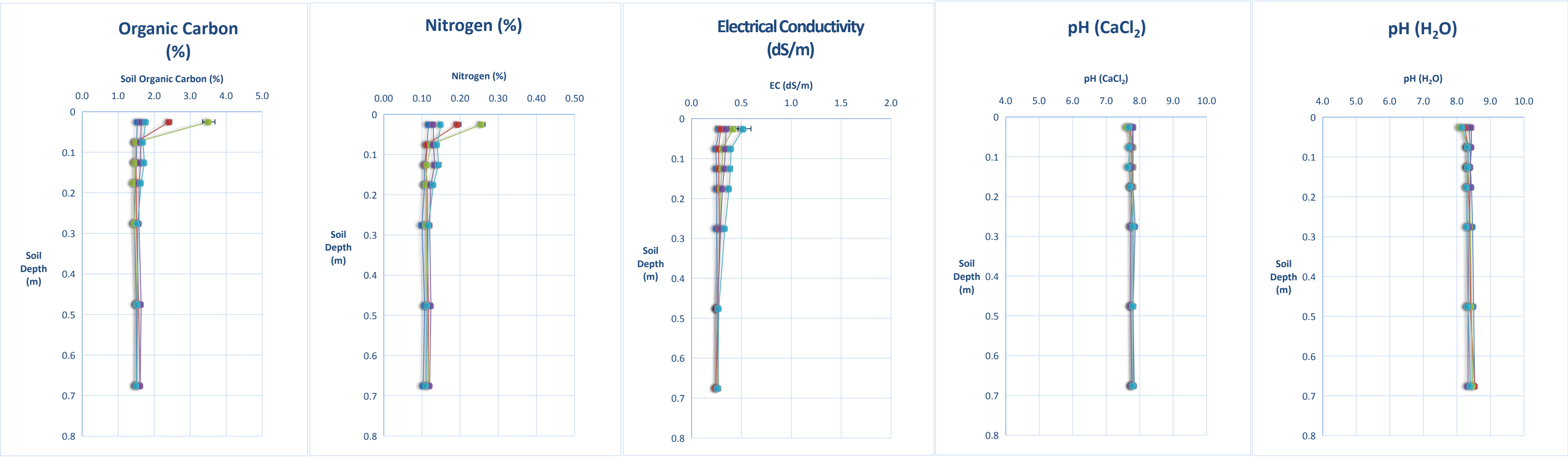
Legend

Figure 2.10 Soil Properties C3 (Gunnedah Black Clay): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

C3 - Gunnedah Black Clay Time 1



C3 - Gunnedah Black Clay Time 2



C3 – Gunnedah Black Clay Time 3

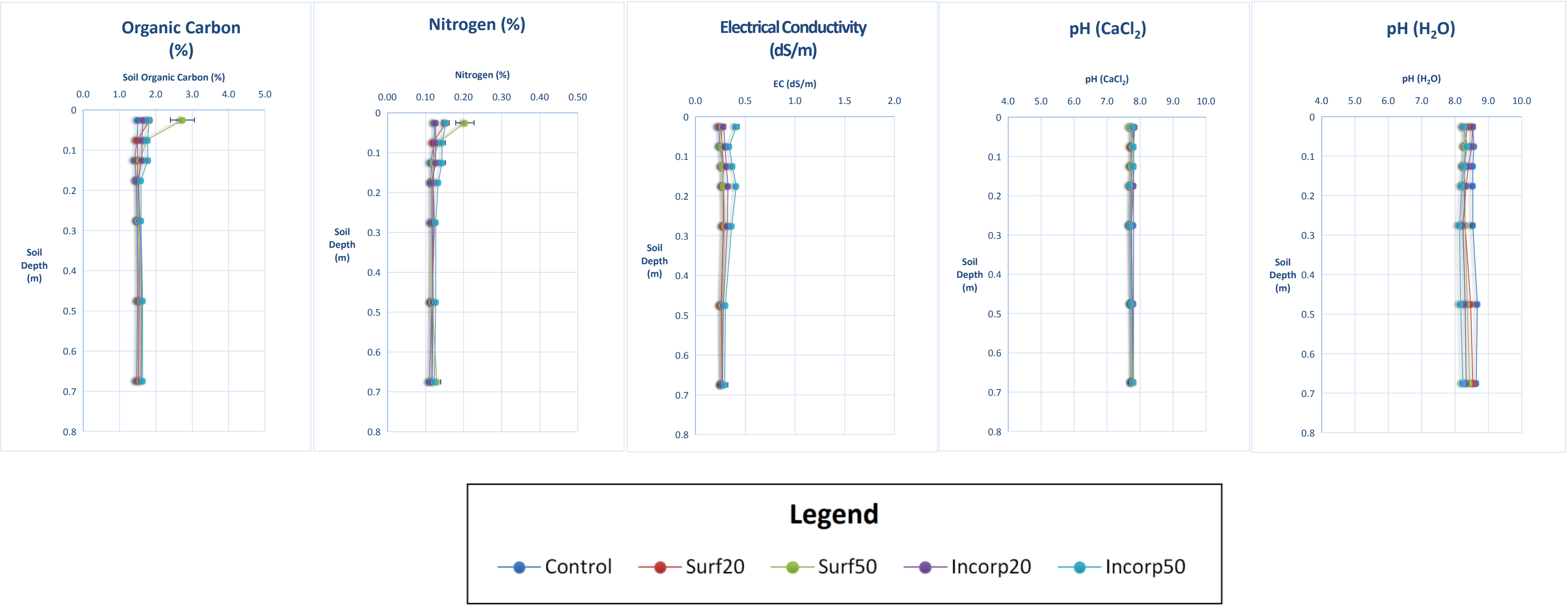
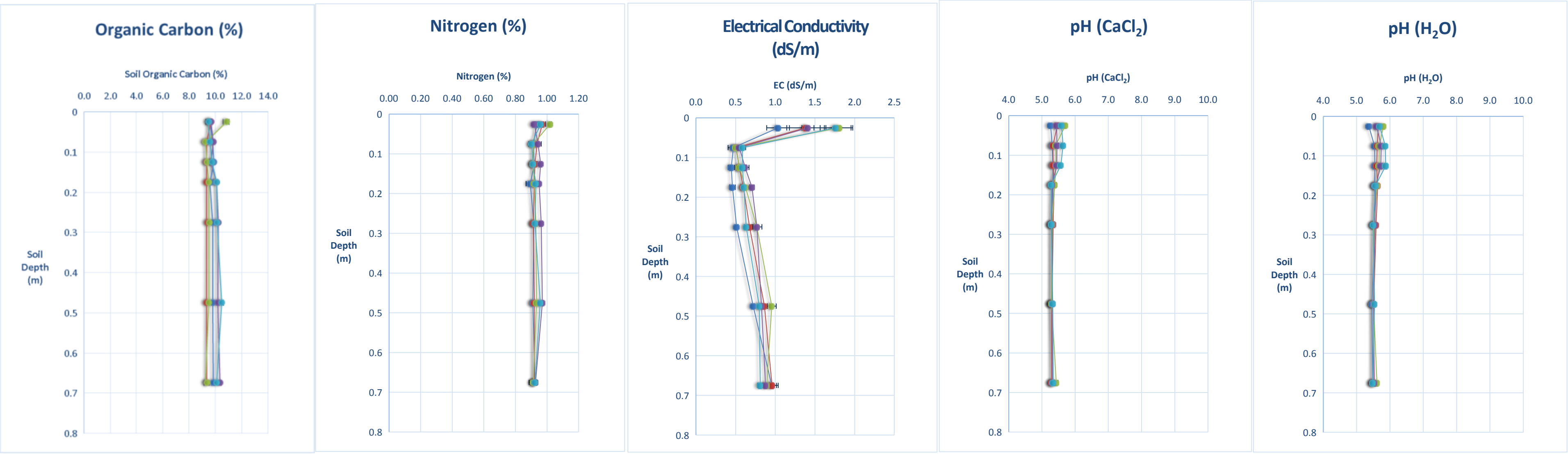
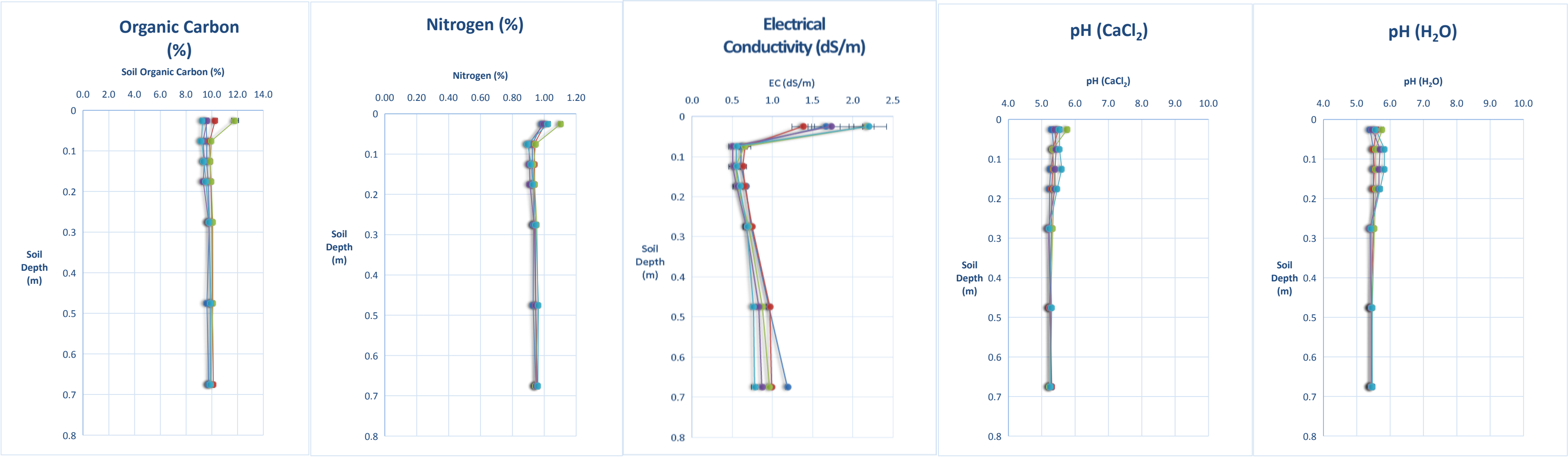


Figure 2.11 Soil Properties OM1 (Guyra Organic): Time 1 (6 months), Time 2 (12 months), Time 3 (18 months).

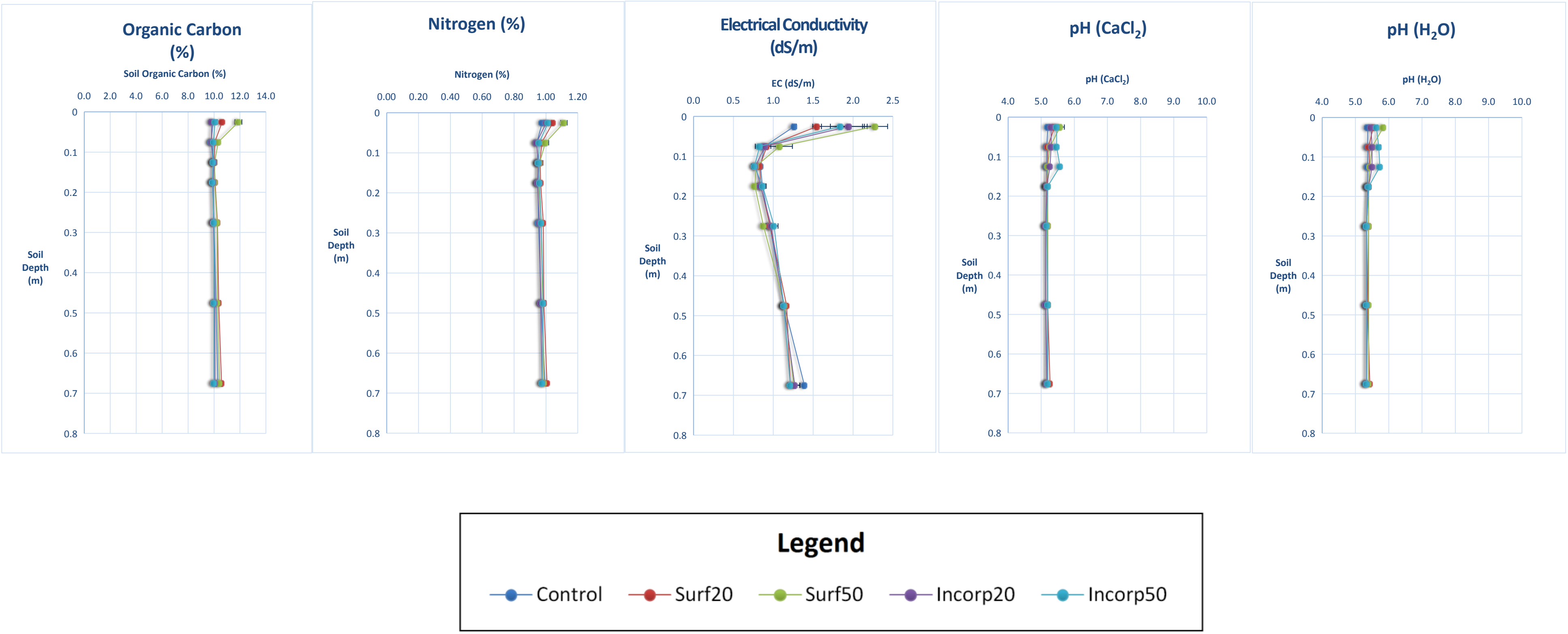
OM1 - Guyra Organic Time 1



OM1 - Guyra Organic Time 2



OM1 - Guyra Organic Time 3



2.2.4.2 Change in soil properties through time

Soil Carbon and Nitrogen

For all the sandy soils (S1, S2, S3), Loam Soils (SL1, SL2, SL3) and clay soils (C1, C2, C3) significant ($P<0.001$) changes in both organic carbon and nitrogen were found between the sampling times. Examination of Figure 2.2, 2.3 and 2.4 indicates that these changes were largely attributable to a progressive decline in these properties in the soil columns through time. Although this decline occurred in all application rates and methods, a significant Time x Depth interaction throughout ($P<0.003$) indicated that the effect was significant only at the higher applications and in the near surface (0-5, 5-10 cm) layers. For example, in the surface (0-5 cm) layer of SL2 at the 140 t ha^{-1} application rate, the organic carbon concentration decreased by almost 2% over the 18 month experimental period.

For the organic soil (OM1) a similar response through time was observed. However, for this soil, a significant ($P<0.001$) result was found only at the highest surface application (50 t ha^{-1}) and in the surface (0-5 cm) soil layer.

Electrical conductivity

All the sandy soils showed a significant change in EC through time. For each soil the initially strong impact of MWOO application on EC (at 50 t ha^{-1} for S1 and both 20 and 50 t ha^{-1} for S2 and S3) diminished through time. No significant Time x Depth interaction could be found indicating that those layers affected by MWOO applications (0-5 cm surface applied; 0-5, 501- cm incorporated) diminished in a similar manner through time.

For the loam soils (SL1, SL2, SL3) no significant change in electrical conductivity was observed through time. The values determined for electrical conductivity were however very variable, with apparent increases in Time 2 for both S1 and S2. For S3 and apparent decline in electrical conductivity from the initial MWOO effect but again, this effect was not statistically significant.

Both clay soils C1 and C2, the initial MWOO effect on electrical conductivity changed significantly through time. Electrical conductivity increased steadily in the affected layers in these soils from Time 1 to Time 3. Although a slight decrease in electrical conductivity appeared to occur through time in C3, this effect was not significant ($P>0.05$).

In the organic soils (OM1) electrical conductivity increased significantly through each successive sampling time. This was true of all depths where an MWOO effect was recorded and particularly ($P>0.003$) for the 50 t ha^{-1} surface application rate at the soil surface (0-5 cm).

Soil pH

As was the case for results relating to MWOO application effects, pH measured in both water and $CaCl_2$ generated overall similar patterns in the way they changed over time. In the sand soils, a significant ($P>0.022$) pH change was recorded for each sampling time. This was accounted for by a significant decline in soil pH following the initial MWOO effects. No significant Time x Depth interaction suggesting that all soil depths affected by MWOO application changed in the same way through time.

In the loam soils (SL1, SL2, SL3) again, a significant ($P<0.027$) pH change was observed for each soil in those layers where an MWOO effect was observed. This was accounted by a decline in pH from Time 1 to Time 3. This was change was particularly strong at the higher rates of application.

Of the clay soils only C2 and C3 recorded a significant ($P<0.05$) change in pH through the three sampling times. This was largely accounted for by the reduction in pH, particularly in the surface layers and at the higher application rates, through time. The organic soil (OM1) a significant decline in soil pH occurred in the soils where an MWOO effect was recorded such that pH values became progressively closer to control values at t_3. For example, pH for the surface applied, 50 t ha^{-1} was t_1= 5.59 $CaCl_2$ and t_3=5.47 $CaCl_2$.

2.2.4.3 Soil Phosphorus Concentration

Phosphorus concentration responded significantly to MWOO application in each of the four selected soils. In S1, all application rates resulted in a significant increase in phosphorus concentration compared with control soils. The magnitude of change was considerably larger for the surface applications (5, 30 and 53 mg kg^{-1} in control, 20 t ha^{-1} and 50 t ha^{-1} application rates respectively) and although the 0-5 cm layer accounted for most of the increase for this application method, the 5-10 cm layer nevertheless also had a significant increase. For the incorporated application method, all three surface layers (0-5, 5-10, 10-15 cm) all showed a significant increase in phosphorus content compared with control soils and again the magnitude of this change increased with increasing rate of application.

In soil SL1, a similar overall pattern was found. Surface applications at both 20 and 50 t ha^{-1} resulted in significant increases in phosphorus concentration in the 0-5 cm layer (16, 26 and 38 mg kg^{-1} in control, 20 t ha^{-1} and 50 t ha^{-1} application rates respectively). Incorporation of the MWOO resulted in significant increases in phosphorus concentration in the 0-5, 5-10 and 10-15 cm layers compared with the control.

In the Minesite soil (SL2), only the higher application rates (50 t ha^{-1} and 140 t ha^{-1}) resulted in a significant increase in phosphorus concentration. This was especially true of the 140 t ha^{-1} surface application which increased the P concentration from the control soil (22 mg kg^{-1}) to a maximum of 170 mg kg^{-1}. The application of MWOO at 140 t ha^{-1} incorporated resulted in an increased P concentration in all three surface layers (0-5, 5-10, 10-15 cm). In the clay soil C1, no significant increase in phosphorus concentration was found at any depth or for any application rate or method.

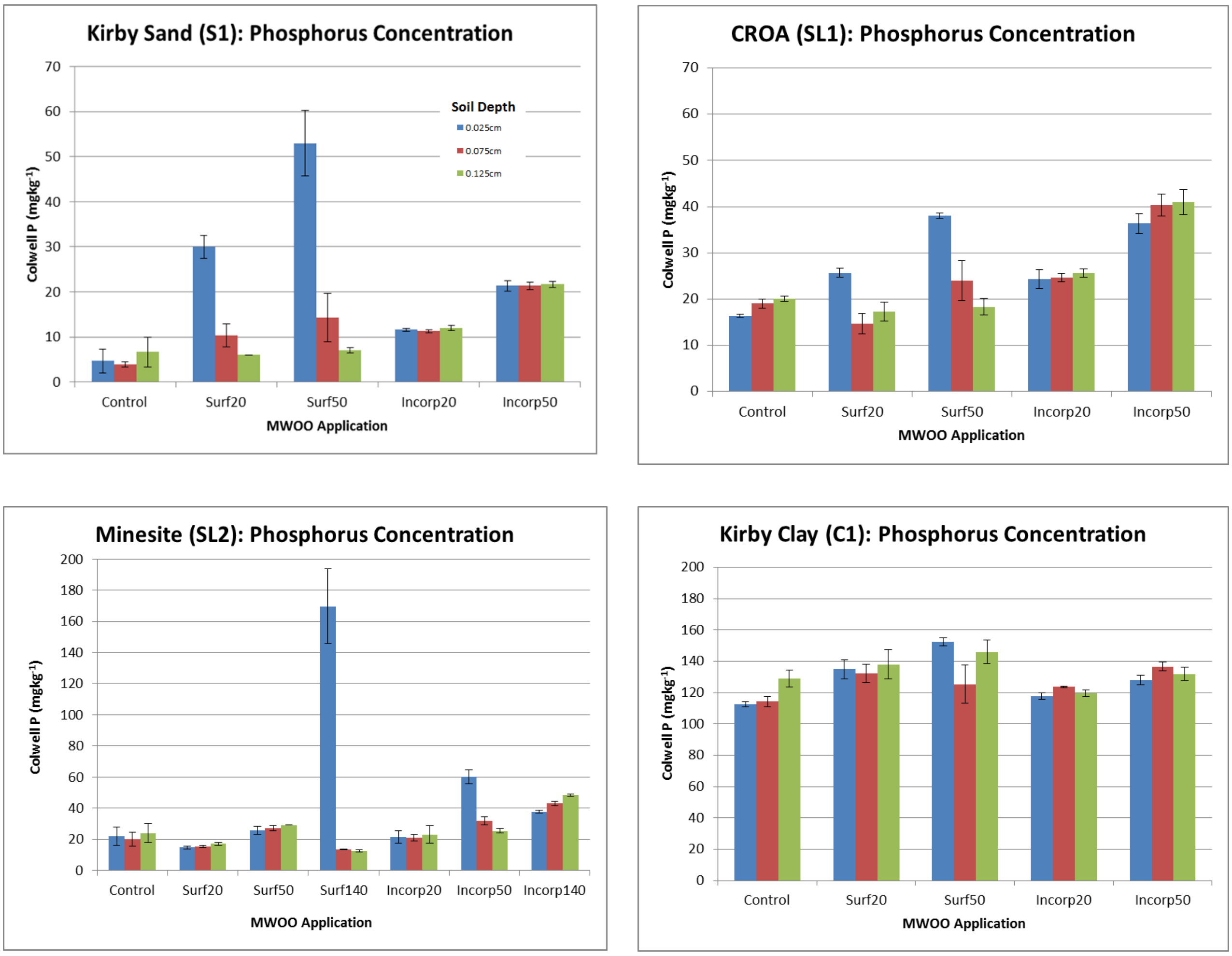


Figure 2.12 Soil Phosphorus Concentration: S1, SL1, SL2, C1

2.2.5 Discussion

2.2.5.1 MWOO effects on soil properties

Many research results relating to the application of MWOO to soils have reported significant decrease in soil bulk density in response to this soil amendment (e.g. Hemmat et al. 2010, Kluge and Bolduan, 2001, Aggelides and Londra, 2000 and Eden et al. 2014). These changes have typically been associated with large rates of application (up to 100 t ha^{-1}) and are usually inversely proportional to the rate of application. Such changes have usually also been restricted to the surface soil layers. Our results however, demonstrated no significant and meaningful change in bulk density across the entire dataset. Soil bulk density is however, notoriously difficult to assess reliably from field samples and there was some considerable variability in our dataset which might have limited our statistical capacity to detect any change. However, our results are unequivocal and we can only conclude that no discernible change in soil bulk density will be imposed by the addition of MWOO in the soils and at the application rates and methods studied. Further work relating to aggregate stability, water holding capacity or other measures of soil structure might be valuable to determine the effects of MWOO on soil structural condition but such analysis was beyond the scope of our study.

A number of research investigations internationally have reported increased soil organic carbon and total nitrogen concentrations following the application of MWOO to soils (e.g. Civeira and Lavado, 2006; Lillywhite et al. 2009; Civeira, 2010; Eden et al., 2014). In most instances, these investigations have identified a significant increase in C and N proportional to the application rate. Our results largely conform with research findings elsewhere. For sandy and loam soils, organic carbon and nitrogen concentrations were elevated in soils at all application rates relative to controls, generally in the order Control < 20 t ha^{-1} < 50 t ha^{-1}< 140 t ha^{-1}. The effects of MWOO on C and N content differed depending upon initial C and N contents. In the sandy soils, with low initial carbon content, C concentration was increased by as much as 2.0% in the surface layer under surface MWOO application. In loam soils however, where initial soil carbon content was higher (although still only ~1.7%) the relative increase in response to MWOO was smaller (0.75%). For all the sandy and loam soils studied, there was a progressive decline in organic carbon and nitrogen content following MWOO application through the three sampling times. Although these declines offset the initial gains to some degree, even at t_3 (after 18 months) the MWOO effect where it was significant at t_1, was still observable in the soils and depths affected.

We acknowledge that the 18 month period of our experiment is limited and that ongoing decomposition of organic carbon and N mineralisation is likely to occur over longer time periods. Attempts have been made to model organic matter (C and to a lesser extent N) contents in soils over time following MWOO application. For example, Peltre et al.(2012) examined a range of organic amendments (with only one MWOO material among them) across a range of sites in northern Europe and attempted to predict their long-term behaviour using RothC. The success of such modelling efforts is however, dependent upon the range of factors and the limitations of the calibration samples used. For example, the nature and composition of the MWOO materials is known to be diverse and variable in space and time. There is also the nature of the soils to which the material is applied; clay content, pH etc. Reliable prediction of the long-term behaviour of MWOO in a range of environments using RothC, or other soil process models, would therefore require an extensive and large scale modelling exercise. We believe that our results could contribute to such a modelling approach but there is a real and current need for more extensive work to model MWOO behaviour long-term across Australian soils and environments.

Few previous studies have examined the effects of different application methods of MWOO on soils but our findings suggest that MWOO effects on C and N were restricted to the layers to which MWOO was applied (i.e. surface applied: 0-5 cm; incorporated: 0-5 cm, 5-10 cm and 10-15 cm). This result suggests that no downward migration of the MWOO effect was present in any of these soils regardless of texture and initial C and N content.

Soil electrical conductivity increased in response to MWOO applications in almost all soils, with the exception of the organic soil (OM1). This increase is probably associated with salts associated with the MWOO and corresponds to changes in soil pH. This conforms with results elsewhere (e.g. Iglesias-Jimenez and Alvarez, 1993; Walter et al., 2006). However, at no stage did any of the soils exceed 2.5 dSm^{-1}. Some workers (e.g. He et al. 1995) have identified the potential for elevated soil EC to inhibit seed germination and/or plant growth. However, such results are generally only observed where EC values exceed 4.0 dSm^{-1}.

For sandy soils (S1, S2, S3) and loam soils (SL1, SL2, SL3) in our study, the elevated soil electrical conductivity progressively diminished through the sequential sample periods of the glasshouse soil column experiment. However, for the clay (C1, C2) and organic (OM1) soils, there was evidence of a progressive increase in electrical conductivity through the experiment. This we believe results from the liberation of salts (and possibly NO_3) from the decomposing MWOO material elevating electrical conductivity progressively through the experimental period. This effect was the result of just one MWOO application. Our work was of course undertaken in glasshouse conditions with a controlled environment, regular watering of soil columns etc. It is possible that under some climatic conditions (e.g. low precipitation, high evaporation) that the liberation of salts from a single application of MWOO might approach or exceed this 4.0dSm^{-1} threshold. However, the assessment of this behaviour would require additional work, examining particular climatic conditions and soil types in order to confidently predict where such effects might take place. Equally, if the effect were to compound following repeat applications of MWOO, the cumulative effects might have the potential to increase soil electrical conductivity to a point (>4.0 dSm^{-1}) where plant growth is affected but such repeat applications remain untested. Throughout the experiment, there was no evidence of change in electrical conductivity with depth in the soils beyond the application depths.

Soil pH has been found to respond to MWOO applications, but the direction and magnitude of these effects are typically dependent on the initial soil pH and the nature of the MWOO material applied. As with MWOO products reported elsewhere, our MWOO material was alkaline, with a pH in of 8.5. In our soils with a low initial pH, soil pH was modified upward. This increase was especially significant at the higher rates of application and was significant only in the surface layers (0-5 cm surface applied; 0-5, 5-10, 10-15 cm incorporated). These results conform to those of (Mkhabela and Warman (2005), Prabpai et al. (2009) and Poulsen et al. (2013) who similarly observed pH increase following MWOO application to acid soils. The pH change imposed did not however last and there was evidence in most soils of a progressive reduction in pH through the three sampling periods, approaching more closely the initial (or control) value. This effect we attribute to a liming effect associated with the alkalinity of the MWOO.

Soils with initially higher pH (>pH7.0), the effects were less clear and generally insignificant. Soils with initially high pH there was some evidence (although not significant) of a decline in pH following MWOO application. Only one soil showed evidence of a different pattern. This soil (SL3), with an initial pH of 7.88 ($CaCl_2$), appeared to show a small (although non-significant) reduction in soil pH following MWOO application in the surface layers. The pH then appeared to increase through time toward the initial (or control) value. It is likely that with continued decomposition and particularly N mineralisation, soil pH would continue to change with additional time and most probably continue on a trajectory of declining pH toward that of the original or control soil pH. However, our experiment was limited to 18 months and assessment and prediction of the rate and magnitude of pH change in the range of soils analysed would require additional, long-term work.

Throughout, no soils showed any evidence of a pH effect in response to MWOO application below the layers of MWOO application (0-5 cm surface applied; 0-5, 5-10, 10-15 cm incorporated).

Soil nutrient status, as indicated by extractable phosphorus concentration, was significantly affected by the application of MWOO. In all soils examined, soil phosphorus concentration was increased compared with the control soils and the magnitude of this change increased with increasing application rate. This conforms with the work of De Haan (1981), Stevenson (1986), Soumare et al. (2003) Crecchio et al. (2004) and Amlinger et al. (2007) who reported increased phosphorus concentration in soils following MWOO applications. The MWOO material we used in our experiment had an initial extractable phosphorus concentration of 760 μgg^{-1} (or 0.076%) which although considerable, is a good deal lower than the concentrations found in commercial fertilizers (which often exceed 40% P). However, it would seem appear to have a fertiliser value. In sandy and loam soils with low phosphorus concentrations in control soils, the extractable phosphorus concentration was increased by as much as 50 mg kg^{-1} at the high (50 t ha^{-1}) application rate, when surface applied. The increase was as much as 150 mg kg^{-1} following the surface, 140 t ha^{-1} application. The magnitude of change was lower (typically ~20 mg kg^{-1}) in sandy and loam soils following the incorporated application method but was found to be significant through all three surface soil layers (0-5, 5-10, 10-15 cm). The limited effect on the clay soil probably results from the inherently larger phosphorus concentration (largely due to their basalt parent material) and to their high clay content which is likely to rapidly immobilize phosphorus applied.

Giusquiani et al. (1988) and Iglesias-Jimenez et al. (1993) similarly observed that the application of MSW compost to soil improved P availability. However, these latter studies suggested that soil P retention decreased progressively after MSW application as a result of phosphor-humic complexes, adding further to the complexity relating to the processes by which P in MWOO add to soil P nutrition. It is likely that the fertiliser effect of MWOO applied to soils will diminish through time as phosphorus becomes progressively immobilised in the soil.

2.2.6 Conclusions

Soil bulk density is unlikely to be significantly affected by MWOO applications for the soil types, application rates and application methods we studied. Significant increases in soil carbon and nitrogen can be imposed by MWOO application but the magnitude of change is largely determined by the MWOO organic matter content and by the initial soil values, being proportionally larger in soils with low C and N content. Increases will mainly be restricted to the surface layers (i.e. surface applied: 0-5 cm; incorporated: 0-5 cm, 5-10 cm and 10-15 cm) and largely at the high application rates (50 t ha^{-1} or more). Through time however, progressive decline in soil carbon and nitrogen concentration would seem likely as decomposition of the organic materials progresses. Soil electrical conductivity was elevated in almost all soil types studied but at no stage exceeded 2.5 dSm^{-1} and only at the high application rates (50 t ha^{-1}) while a threshold of 4.0 dSm^{-1} is recognised beyond which plant growth is inhibited. Electrical conductivity change would therefore not appear to represent any concern to plant growth following the one MWOO application studied here. However, under episodic hot, dry conditions that are common in Australia, higher EC might prove to be of potential concern where evaporation results in salt concentration in the MWOO applied layer. Similarly, if a cumulative effect were to take place following repeat applications, this soil property might be of concern although this effect remains untested. Soil pH change is induced by MWOO application but will be determined by the MWOO pH and initial soil pH. Following application soil pH responded by moving in the trajectory of the MWOO pH (8.5 in this instance). For most soil parameters where change was imposed, initial modification of soil properties diminishes with increasing time following the soil amendment.

Soil nutrient status (phosphorus concentration) was significantly increased in soils with inherently low nutrient status and lo clay content. However, higher fertility, clay soils showed a non-significant response. MWOO undoubtedly has a fertiliser value but its effectiveness will be determined by soil type and its long-term effectiveness is uncertain.

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2.3 Effects of MWOO application on metal and metalloid accumulation and distribution

2.3.1 Summary

Mixed waste organic output (MWOO) has been used as a soil amendment for many years. However, concern has been expressed with regard the introduction of contaminants to soil ecosystems and there is a need to fully understand the risk associated with utilising this potentially beneficial resource. The glasshouse soil column experiment was utilised out to evaluate and compare the accumulation and distribution of As, Cd, Cr, Cu, Ni, Pb and Zn with MWOO amendment in the ten different NSW soils under the various application rates and methods.

Total concentration of some the elements studied showed a significant increase in the MWOO contact depths (0-5 cm surface applied and 0-15 cm depth incorporated). The significance of this, however varied depending on the element, soil type, initial concentration in MWOO and in background soils, application method and rate. Generally, Cu, Pb and Zn, with the highest concentration in MWOO, (180.8, 275.1 and 411.1 mg kg^{-1}, respectively), showed the greatest increases in MWOO contact application depths in most of the soils. Significant increases were typically observed at the higher application rates only (50 and 140 t ha^{-1}). Larger increases were observed in MWOO contact depths for surface application treatments compared to incorporated treatments at the same application rate. In all soils and treatments the increase initially observed 6 months after MWOO application in MWOO contact depths remained similar to 18 months with little evidence of any significant loss. The elements As, Cd, Ni and Cr were present at lower concentrations (5.3, 2.2, 40.2 and 85.4 mg kg^{-1}, respectively) in MWOO and slight but significant increases were only observed in MWOO contact depths in the sandy and loam soils.

Significant increases in Pb, Cu and Zn concentration were observed over the 18 months of the experiment in some of the sandy soils, loams and one of the clays, usually at the depth immediately below the MWOO contact zone. This was also observed for As, Cd, Cr in some sandy soils and for Ni in the loams. In the high organic matter content soil, however, no change in metal or metalloid concentration was observed below MWOO contact depth for any of the elements, nor for As, Cd, Cr in the loams and clay soils. Overall, for As, Cd, and Ni, the factor which showed the least impact on metal distribution and accumulation was application method, whilst for Cu, Pb and Zn the time period showed the least effect and for Cr application rate followed by application method showed lower importance compared to other factors.

2.3.2 Introduction

Mechanical and biological treatment of organic waste produces a stable complex that can be used as a soil amendment and fertilizer. This product, known as municipal solid waste (MSW) compost or more recently mixed waste organic output (MWOO), is largely derived from municipal waste, food waste and garden organics. It has become popular because it can reduce the volume of waste storage needed, has a relatively low cost and can be suitable for agricultural application (Eriksen, Coale & Bollero 1999; Wolkowski 2003), providing plant nutrients and many improvements in soil physical, chemical and biological properties (Iglesias-Jimenez & Alvarez 1993).

Despite the beneficial aspects of MWOO, it can contain high concentrations of metals and metalloids, originating from items such as batteries, plastics, paints, inks, body care products, medicines and household pesticides which may be present in the source waste streams (NHHWF 2000; Bardos 2004). The metals and metalloids of greatest concern for the application of MWOO to soil include As, Cd, Cr, Cu, Ni, Pb, Se and Zn based (Wilson et al. (2014) because of their toxicity and effects on humans and the environment. Risks include long-term accumulation due to persistence in the soil and r negative impacts with ecosystem exposure through the food chain, on soil organisms, plants, animals and humans (Smith 1996, 2009).

Considerable research has been reported on the fate and behaviour of metals and metalloids originating from composted waste in the soil. In many studies the MWOO application has led to an increase in concentrations of metals and metalloids in the soil (Richard & Woodbury 1992; He, Logan & Traina 1995; Tisdell & Breslin 1995; Amlinger & Ludwig-Boltzmann 1996; Petruzzelli 1996; Vogtman, Bours & Fuchshofen 1996; Illera *et al.* 2000). For example, Ayari et al. (2010) studied the long-term accumulation of metals and metalloids in the top 20 cm of a Tunisian clay loam amended with MSW compost. The concentrations of all measured metals (Cd, Cr, Cu, Ni, Pb, Zn) elevated in MSW compost amended soils compared to untreated soils, especially for the higher 80 t ha^{-1} year^{-1} application rate. Achiba *et al*. (2009) reported that the application of MSW compost increased the metal and metalloid content of the superficial soil layer in proportion to the application rate. Madrid *et al*. (2007) reported that a moderate application rate (21 t ha^{-1}) of MWOO increased total Zn and Pb after the second application, and increased total Cu and Ni concentration after the third MWOO application (18 t ha^{-1}) in the 0-25 cm depth of soil. Increased total Zn, Pb, Cu and Ni concentration was also reported by Walter *et al*. (2006) in MWOO amended calcareous soils compared to controls. Dorahy et al. (2006) examined the impact of MWOO application on an Australian acidic dermosol and reported a significant increase in total concentration of Cu, Pb and Zn in soil at 100 and 200 t ha^{-1} MWOO application rates and that the total concentration of Zn in soils at the 200 t h^{-1} MWOO application rate exceeded 200 mg kg^{-1}, the maximum allowable concentration (NSW EPA 1997). The studies show that the increase in contaminant concentrations depends on MWOO application rate and might exceed regulatory allowable concentrations.

Weber et al. (2007) examined the effects of repeat MWOO applications and reported a significant increase in total soil Zn and Cu concentration at the three rates of application, 30, 60 and 120 t ha^{-1}, after the first year of application, but observed an increase for Ni, Cr and Cd only at 120 t ha^{-1}. Warman (2009) reported a significant increase in total concentration of Cu and Zn in 0-15 cm soil layer after 3 successive years MWOO application in acidic sandy loam soil.

These two studies illustrate that repeated MWOO application can result in higher increase in metal and metalloid accumulation in the soils.

Overall studies indicate that MWOO application to soil can increase total soil concentration of metals and metalloids, and the increase is higher with higher MWOO concentrations and also with repeating application. Despite this, the influence of a range of different soil factors such as soil texture, soil pH and soil organic matter content on metal accumulation and distribution has been little studied and when reported is usually examined in only one or two soils, not always considering long term effects. Furthermore, study of metal and metalloid accumulation and distribution following MWOO application in Australian soils is scarce with only one study documented to date (Dorahy *et al.* 2006).

This controlled glasshouse, soil column experiment is the first to comprehensively evaluate the concentrations, accumulation and distribution of metals and metalloids (As, Cd, Cu, Cr, Ni, Pb and Zn) over an 18 month time period following MWOO application in different NSW soils. The soils encompassed a range of different soil types and characteristics (10 soils included) with MWOO application method (surface or incorporation) and rate of application also considered.

2.3.3 Materials and Methods

Soils were sampled from the glasshouse soil column experiment as described in Section 2.1. Physico-chemical analysis was undertaken on air-dried, 2 mm sieved triplicate samples for soils and MWOO amended soils. The MWOO was air-dried, homogenized and ground (< 0.5 mm). Moisture content was determined by drying at 105°C overnight. The pH (TPS 901-CP pH meter) and electrical conductivity (EC) (TPS labCHEM Conductivity- TDS- Temperature meter) were determined after mixing with water (1: 5 v/v slurry) for 1 hour. Particle size analysis of soils was carried out using the hydrometer method (Bouyoucos 1962). The total carbon and nitrogen content were determined on ground samples (< 0.5 mm) by an automated elemental TruSpec series Carbon and Nitrogen analyser, (LECO corporation). Colwell phosphorus was determined by the malachite green method (Motomizu, Wakimoto & Toei 1983) using 0.5M NaHCO3 and a ratio of 1:100 (w/v) fresh sample:extractant and shaken for 16 hours, filtering the solution through the Whatman No.42 filter papers. Final solutions were analysed by a Libra S11 single beam visible spectrophotometer.

Total metals and metalloids were extracted using aqua regia digestion (HCl/HNO_3, v/v, 3:1). Briefly, 4.5 ml of concentrated HCl (36% Crown Scientific, Australia) and 1.5 ml of concentrated HNO_3 (70%, Crown Scientific, Australia) were added to 0.5 g samples, sonicated, then digesting by ultrawave microwave digestion (Ultra Wave model with T660 Terminal, 50 Hz frequency supplied by Milestone Srl, Via Fatebenefratelli, Italy) with temperature programme: 10 minutes to 175°C, 10 minutes at 175°C, 10 minutes venting, 40 minutes releasing pressure and cooling down),. This was an adaptation of ANZECC (1996) and NEPM (2013) which is explained in detail in Chapter 3. The digested samples were filtered through Whatman No. 42 filter paper and made up to 25 ml with deionised water. Final solution metal and metalloid concentrations were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (supplied by Agilent, Australia with a 5 channel sample introduction pump; echelle optics; VistaChip CCD; OneNeb nebulizer; inert spray chamber; radial torch; SPS 3 Auto sampler and PolyScience Model 3370 water re-circulator). A certified reference material NIST SRM 4711a (Montana standard) was included in the analysis to ensure internal quality assurance/quality control (QA/QC). Acceptable recovery was between 80% and 120% and the precision based on relative standard deviation (% RSD) was below 20%.

Statistical analyses were carried out using R-software version 3.0.0 (R Core Team 2013). Analysis of variance was used to evaluate the effect of application rate, method, soil type and depth on concentration of metals and metalloids in treatments. Tests of normality and variance homogeneity were carried out and square root transformation was applied to concentration data to normalise the variances. Significantly different means were separated using 95% confidence intervals (standard error × 1.96), i.e. overlapping errors are not significant (Day & Quinn 1989). Spearman's Rho (*r*) correlations were carried out allowing the determination of correlations between metals and pH, EC and TOC content of the soils. All nutrient and metal and metalloid values are expressed on a dry weight basis.

2.3.4 Results

2.3.4.1 Soil and MWOO characterisation

Physical and chemical characteristics of the MWOO and soil samples used in the pot trial experiment are listed in Table 2.1. The soils included three soils with high sand content (>70% sand) with pH $_{(H_2O)}$ of 6.3 (S1), 7.5 (S2) to 8.3 (S3), three loams with pH $_{(H2O)}$ at 5.9 (SL1), 6.4 (SL2) and 8.9 (SL3), three soils with high (> 50%) clay content with pH $_{(H2O)}$ at 6.1 (C1), 7.4 (C2) and 9.1 (C3) and one slightly acidic (pH $_{(H_2O)}$ 6.7) high organic matter soil (OM1) with total organic carbon (OC) of 9.8%. The soil with highest percentage sand (S2) (90% sand) showed the lowest EC value whereas, the higher EC values were observed in the clay soils, OM1, and the soils with higher pH values. The total nitrogen, organic carbon and phosphorus (ranged from 0.0 - 0.92%, 0.04 - 9.76% and 2.8 - 132.5 mg kg^{-1} respectively), with the lowest values recorded again in the soil with the highest sand content, S2. The higher carbon concentrations were observed in the organic soil, OM1, and the clays (C1, C2, C3) whilst the highest nitrogen concentration was recorded for the high organic soil, OM1, and the highest P concentration in the clays, C1 and C2.

Considering the metals and metalloids in the soils, mean As concentrations ranged from 0.9-8.1 mg kg^{-1}), except in S1 where a mean value of 59.7 mg kg^{-1} was detected. The mean Cd concentration ranged from (< detection limit to 4.3 mg kg^{-1}). Mean Chromium in soils ranged from 15.3 to 293.7 mg kg^{-1} with concentrations in OM1 and the clay soils > 100 mg kg^{-1}. The Pb concentrations in all soils were less than 23 mg kg^{-1}, Cu, Ni and Zn concentration ranged from 6.7-56.5 mean mg kg^{-1}), (range 6.3-165.6 mean mg kg^{-1}), (range 11.0-109 mean mg kg^{-1}), respectively, with the higher concentrations detected in the clay soils and OM1.

The MWOO was alkaline (mean pH $_{(H2O)}$ = 8.55), higher than all the soils except for SL3 and C3 which were the more basic loam and clay. Significantly higher EC (6950 mean µS m^{-1}) and N, C and P (1.45, 26.4 mean % and 765 mean mg kg^{-1}), respectively, was found in MWOO compared to all the soils studied. The lead concentration in this MWOO exceeded the maximum allowable value for MWOO as listed in the NSW General Exemption. All other trace elements were within the maximum concentration allowed.

2.3.4.2 MWOO application effects on soil metal and metalloid concentrations and distribution

Total soil metal analysis showed acceptable recovery from the NIST Montana Standard of between 80% and 120% for the seven elements assessed over the three sampling time points and the precision based on relative standard deviation (% RSD) was below 20%. Figures 2.13 to 2.82 illustrate soil metal and metalloid concentrations and distribution for each MWOO treatment and control in each soil for all sampling periods. The figures illustrate mean element concentration with 95% confidence intervals on the replicates.

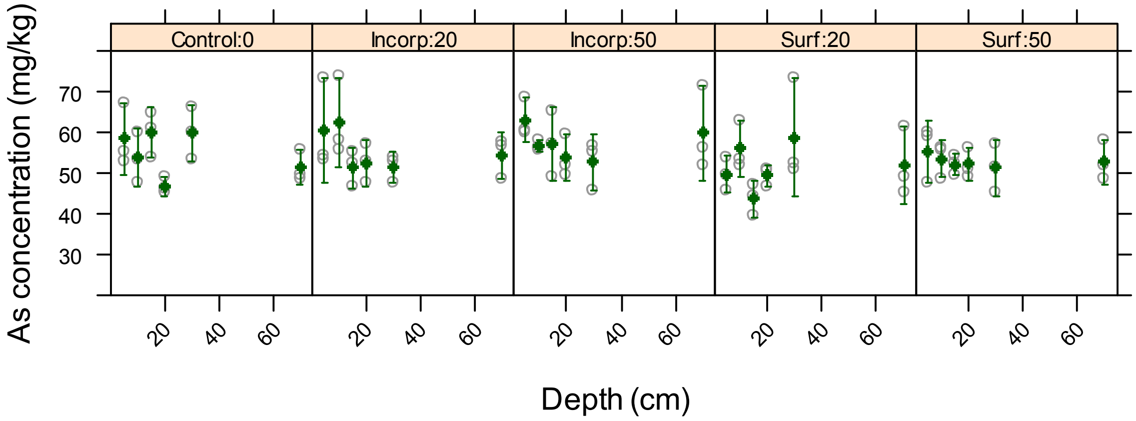
Changes in the seven metal and metalloid concentrations and distribution are described in the following sections for similarly textured soils followed by a consideration of inter soil differences. Metal and metalloid concentrations in

MWOO are inherently highly variable and this is evidenced in MWOO amended treatments compared to the controls for many of the elements analysed (e.g. Pb in S2, Zn in S1). Low MWOO and soil concentrations for some elements also contributed to high variability in some data (e.g. for As). As a consequence, in the following sections, changes in metal and metalloid concentrations are assessed with regard to the control samples considering the variability on all samples within the control that were not significantly different from each other. This is considered the soil "inherent variability". In addition MWOO contact depths referred to indicate the 0–5 cm depth for the surface applied treatments and 0-15 cm depth for the incorporated treatments.

Sandy soils – S1, S2, S3

All MWOO treatments and controls for the sandy soils were analysed at 0-5, 5-10, 10-15, 15-20, 25-30 and 65 - 70 cm depths. The MWOO was applied to these soils at rates of 20 and 50 t ha^{-1}, both surface applied and incorporated. An examination of the distribution of metals and metalloids in the control columns of these sandy soils after 6 months of maintaining moisture at 60% field capacity shows that for all metals detected there was no significant difference in concentration compared to all other samples in the control except for Ni and Zn at 65 – 70 cm in S1, the most acidic sandy soil, where significant increase in Ni and Zn was observed compared to all other depths (from a mean of approximately 7.5 to 11.5 mg kg^{-1} and 13.3 to 22.7 mg kg^{-1}, respectively), and also for As, Cr, Cu, Ni and Pb at 65-70 cm in S2, the more neutral sandy soil. At 12 and 18 months, however, there was no significant change in any of the metal and metalloid concentrations over the depth profile of the control for these sampling periods in all three sandy soils.

The MWOO As concentration was significantly ($P < 0.001$) lower (for S1) or similar to the As concentration in these soils. At the 6 month sampling period, with MWOO application there was no significant change observed in As concentration or distribution in S1, the soil with the high background As. In S2, however, the only significant change compared to the inherent soil variability with MWOO addition was detected at 65 – 70 cm depth in surface applied MWOO treatments, where As significantly increased similar to the control, In S3 As significantly increased at 0-5 and 10–15 depth in the 50 t ha^{-1} incorporated MWOO treatment. At the 12 and 18 month sampling periods, arsenic distribution in different depths was not significantly different



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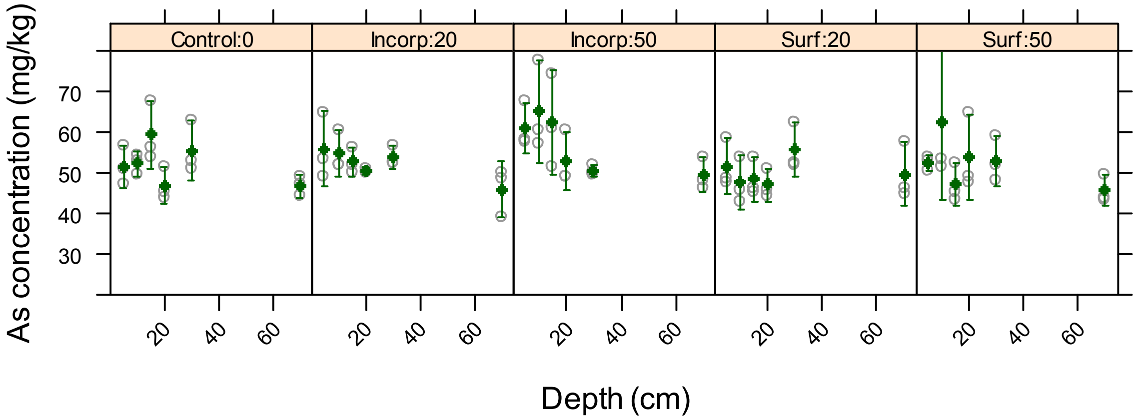

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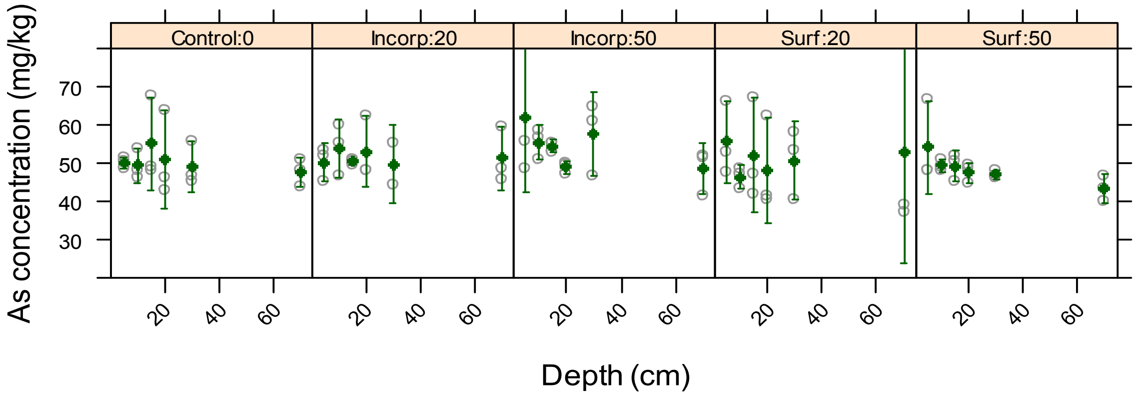
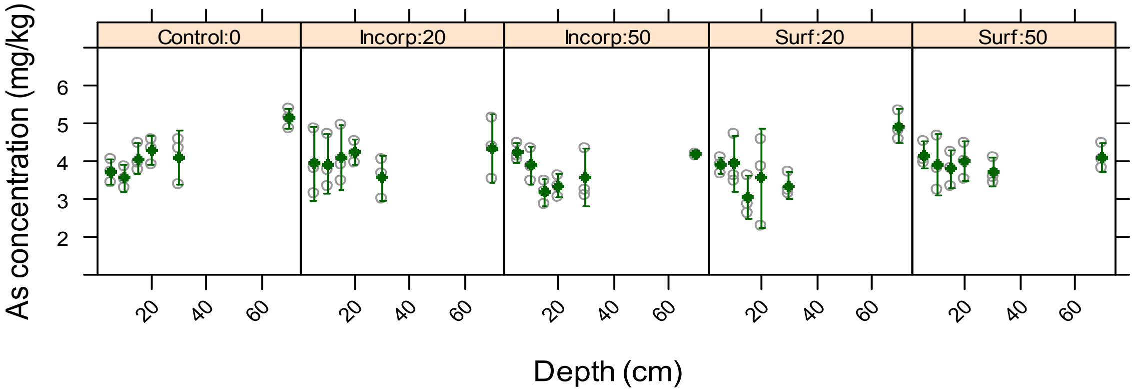


Figure 2.13. As concentration in S1 soil following MWOO application during three period of time (6, 12 and 18 month), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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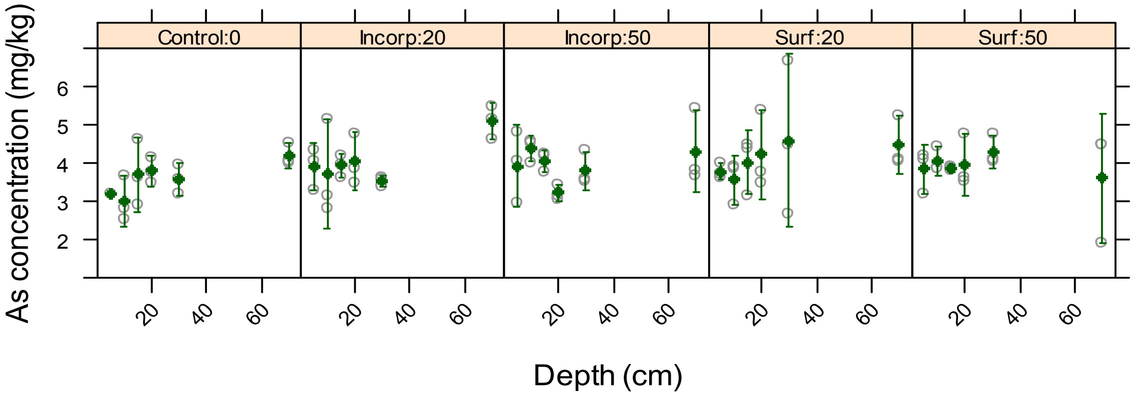

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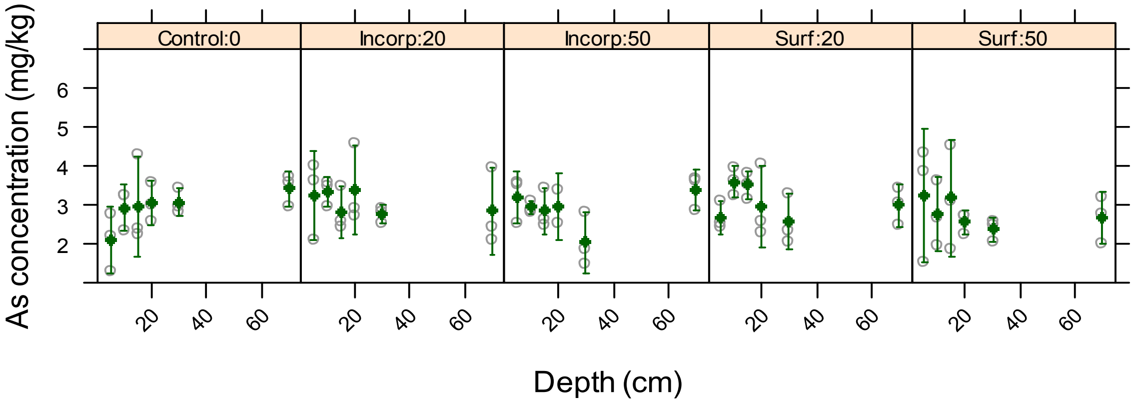

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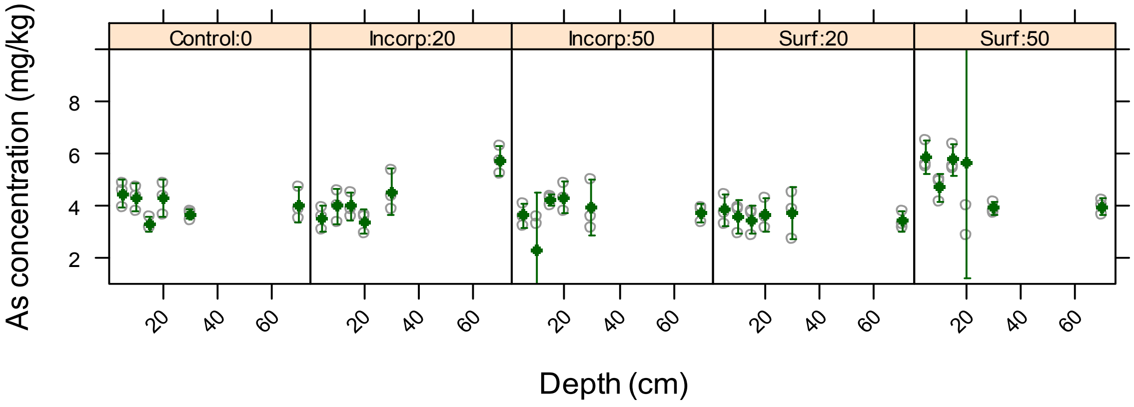
Figure 2.14. As concentration in S2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals,



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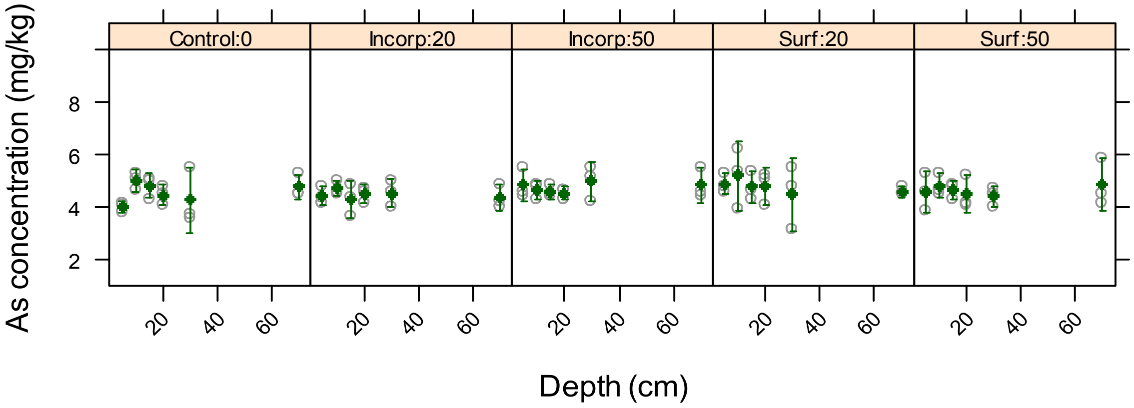

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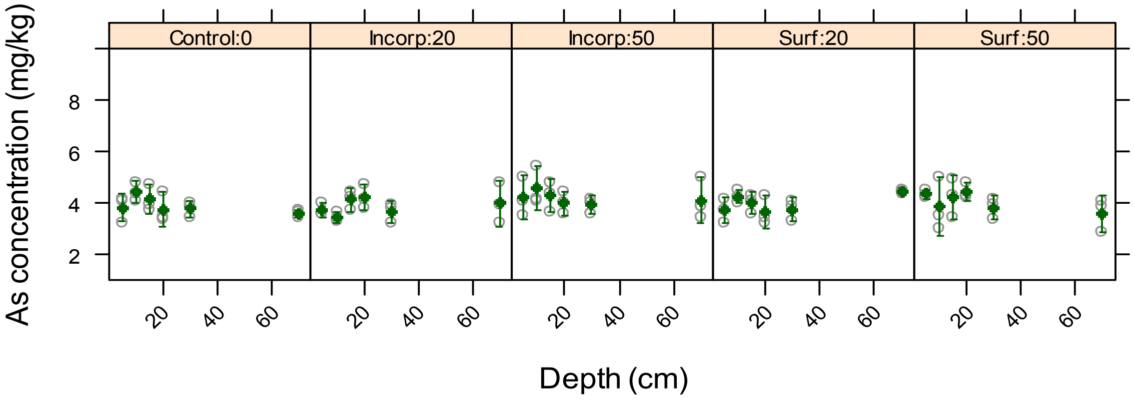
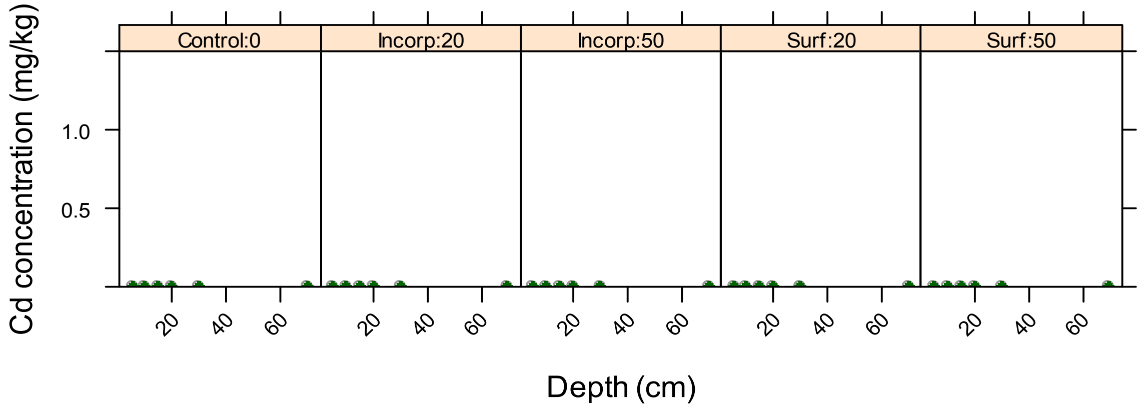


12 month

Figure 2.15. As concentration in S3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals,



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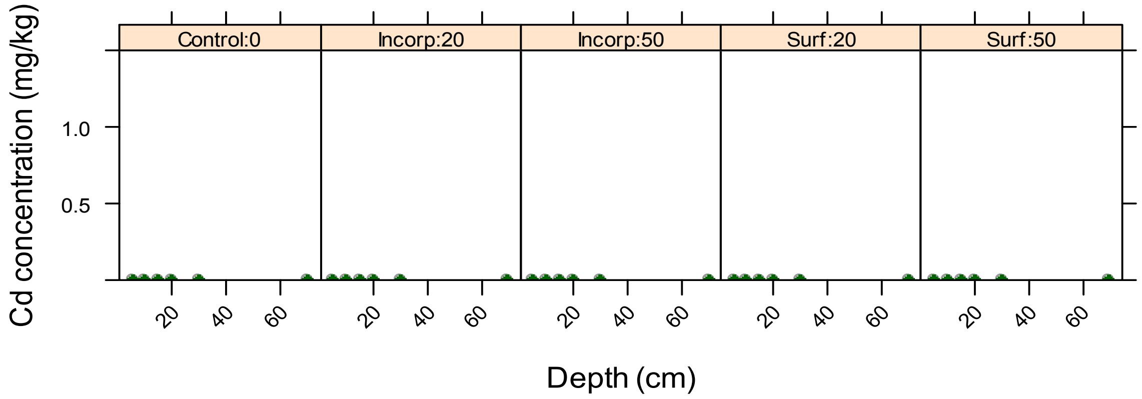

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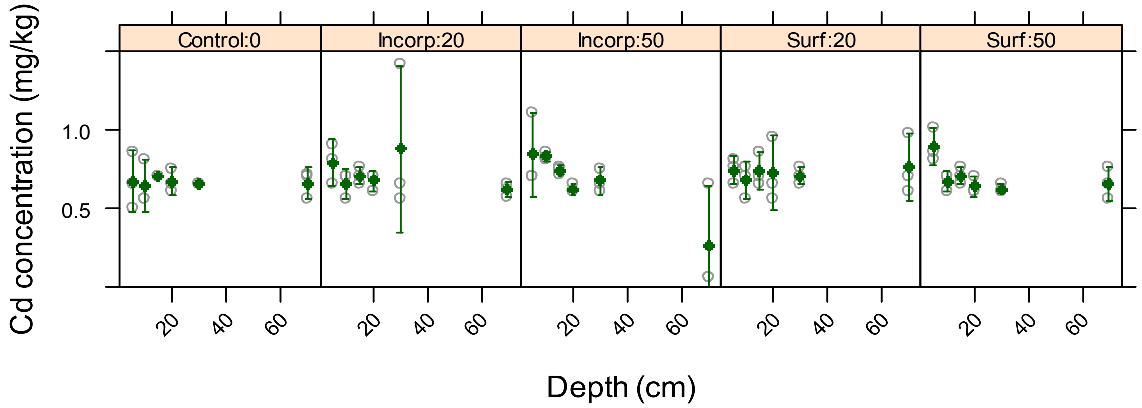
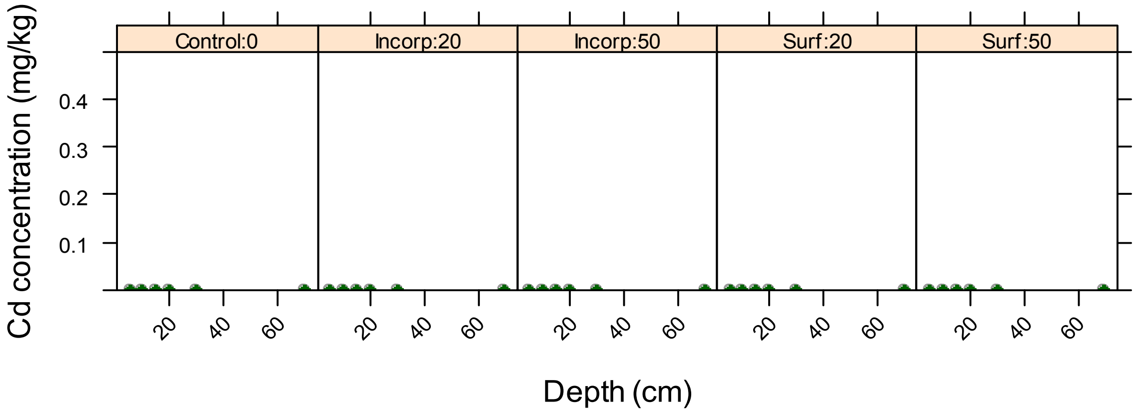


18 month

Figure 2.16. Cd concentration in S1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals,



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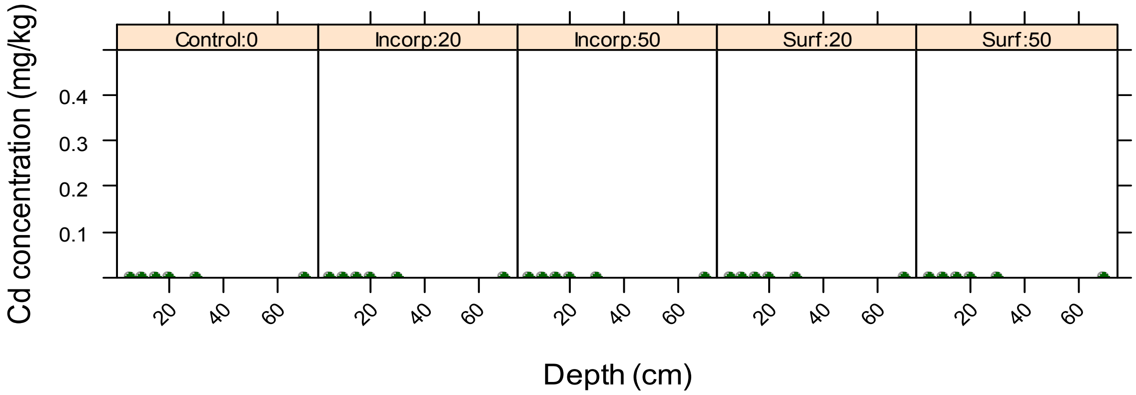

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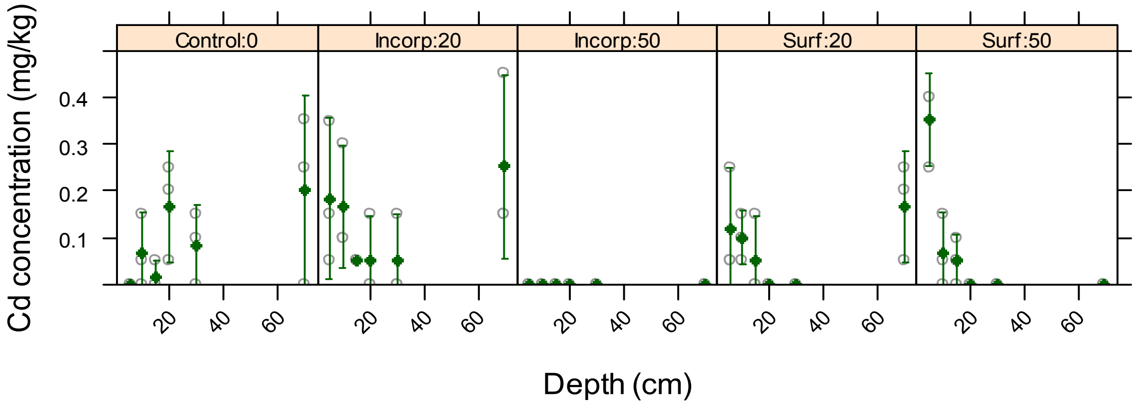
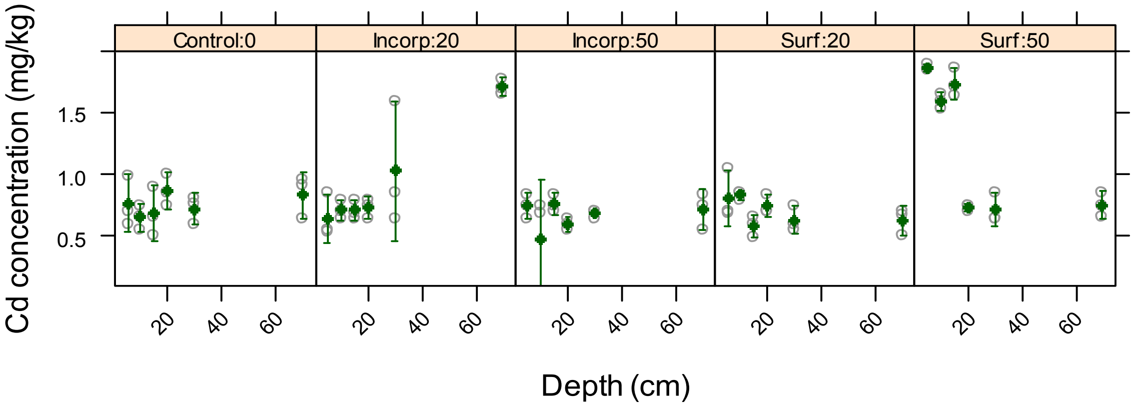


16 month

Figure 2.17. Cd concentration in S2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals,



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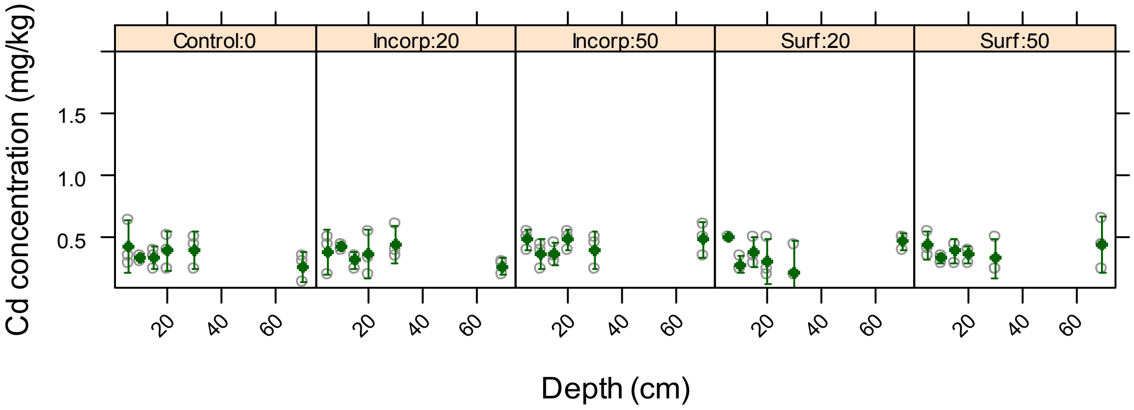

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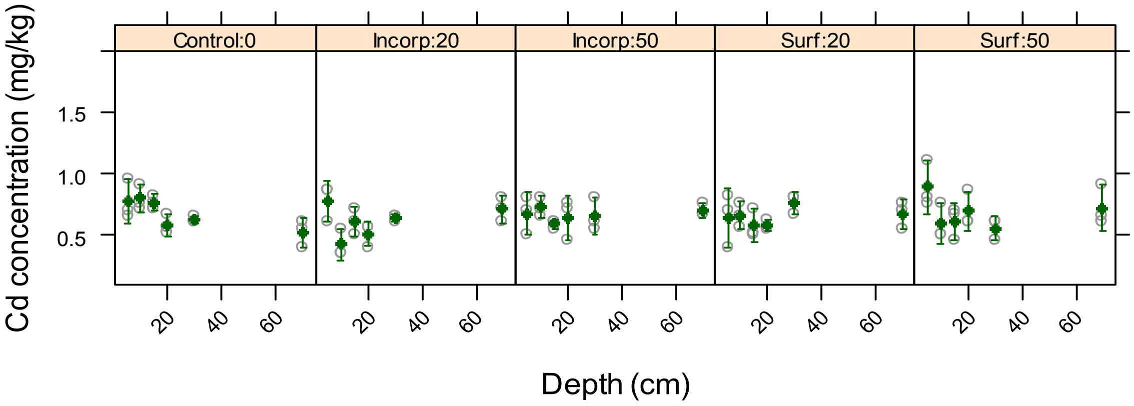
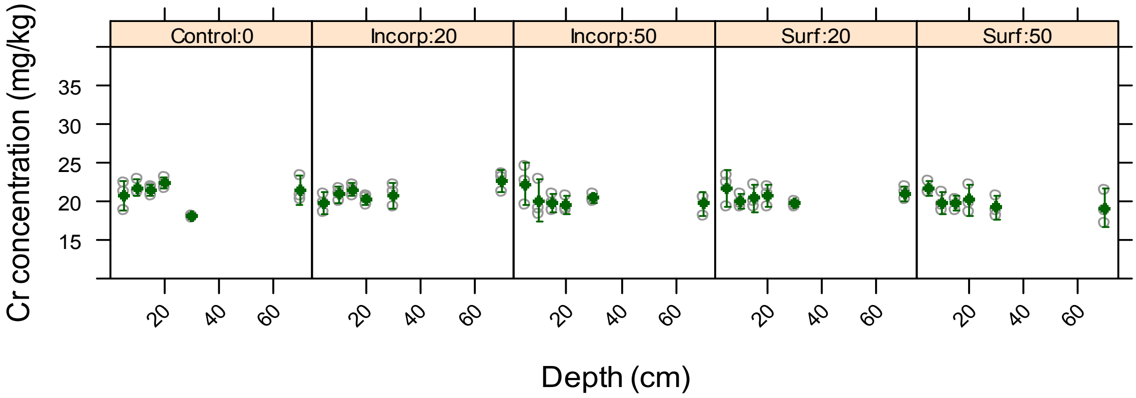


18 month

Figure 2.18. Cd concentration in S3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals,



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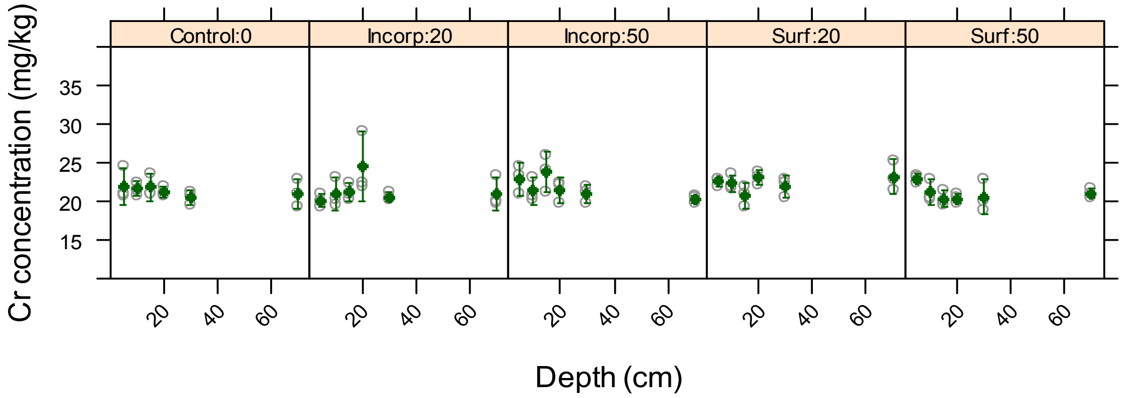

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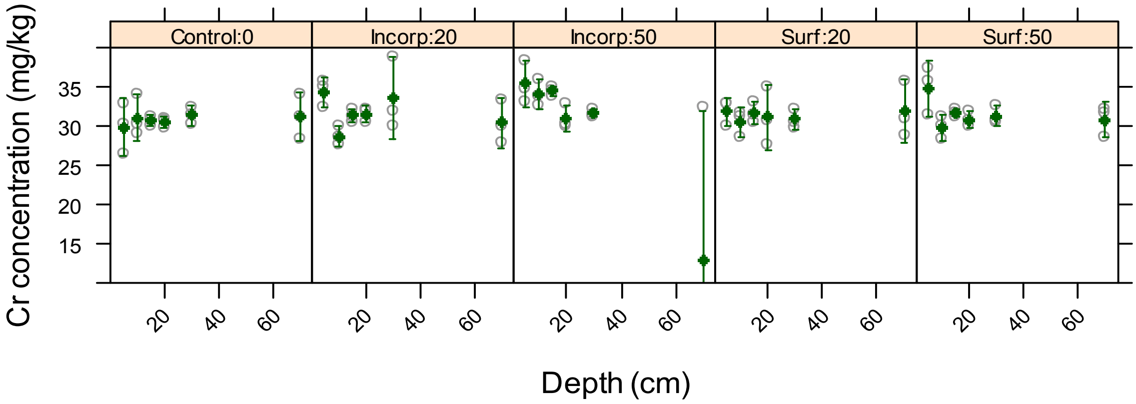
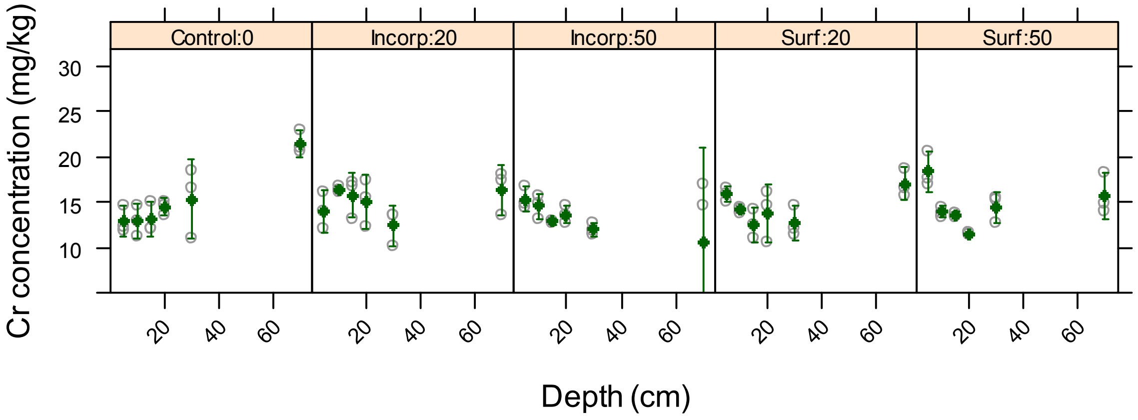
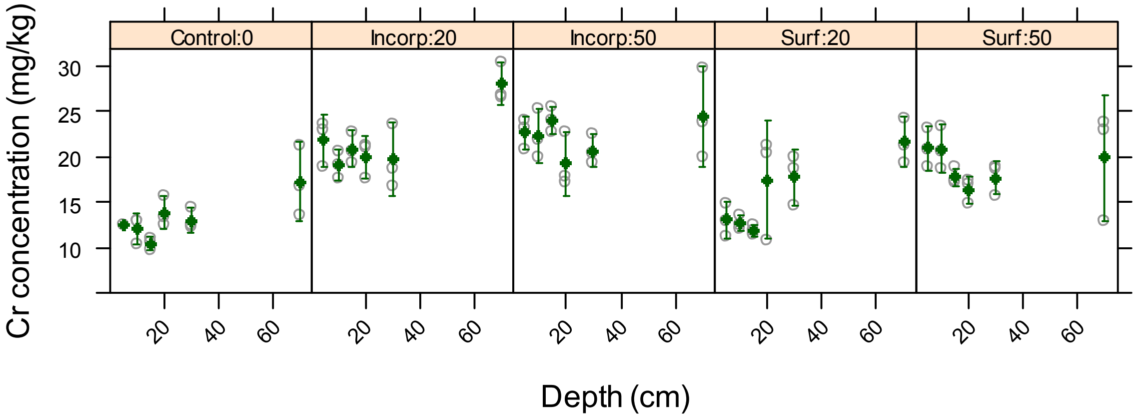


18 month

Figure 2.19. Cr concentration in S1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals





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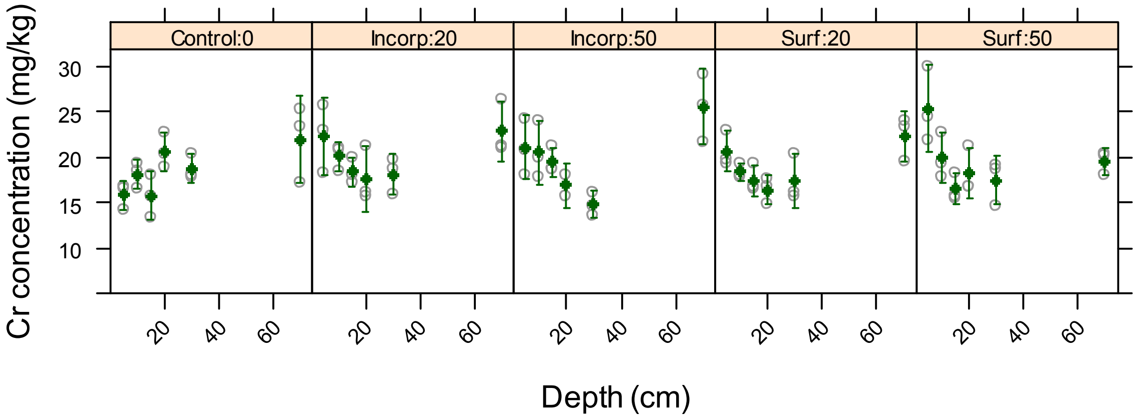

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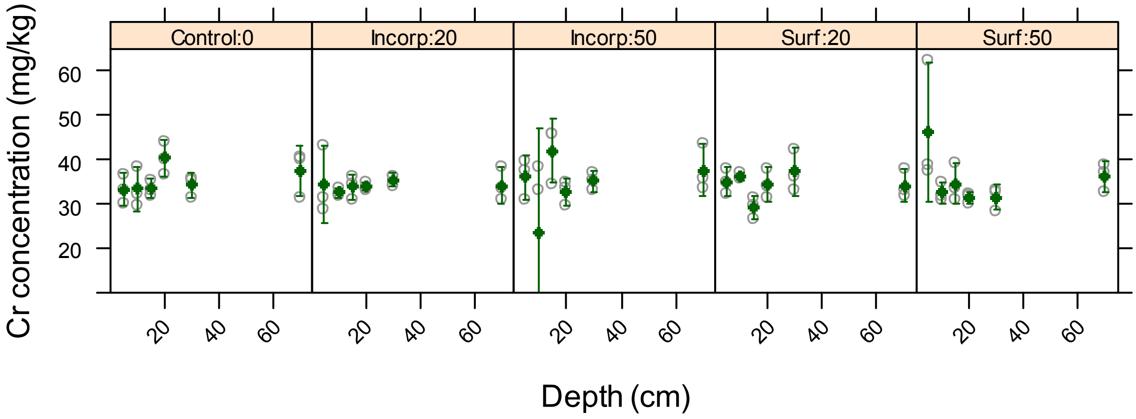
Figure 2.20. Cr concentration in S2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (O) with means and 95% confidence intervals



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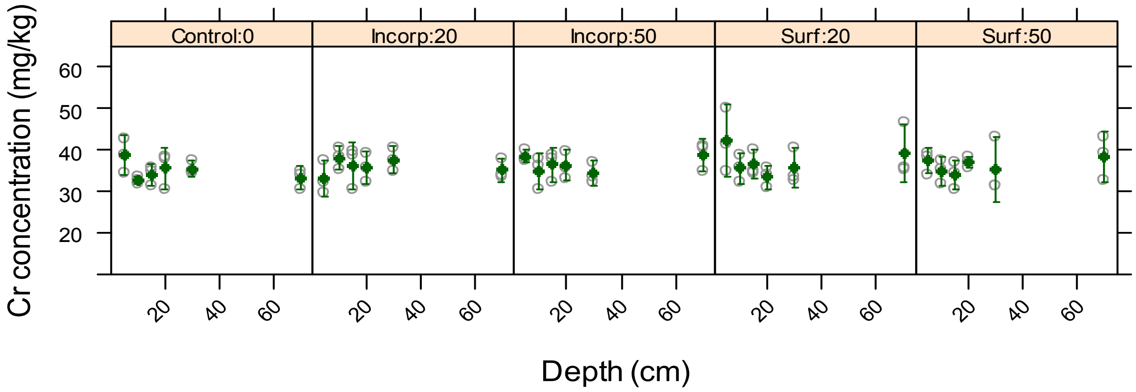

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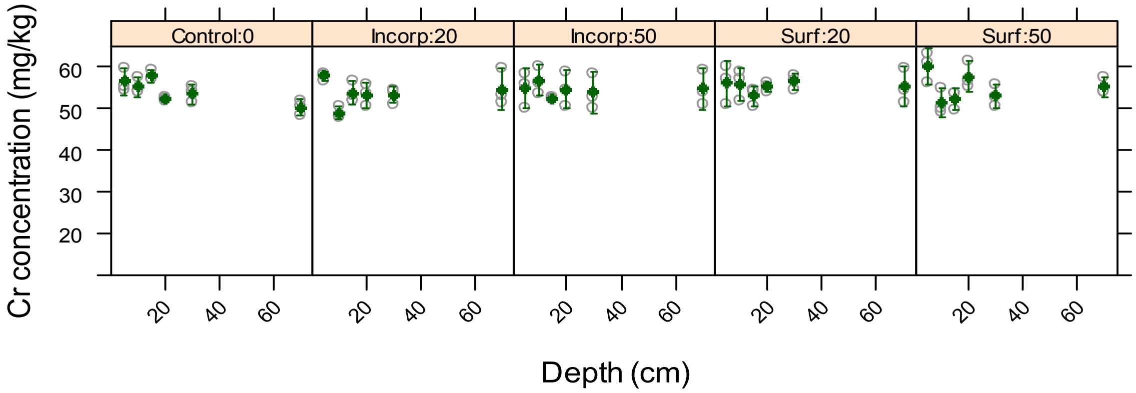
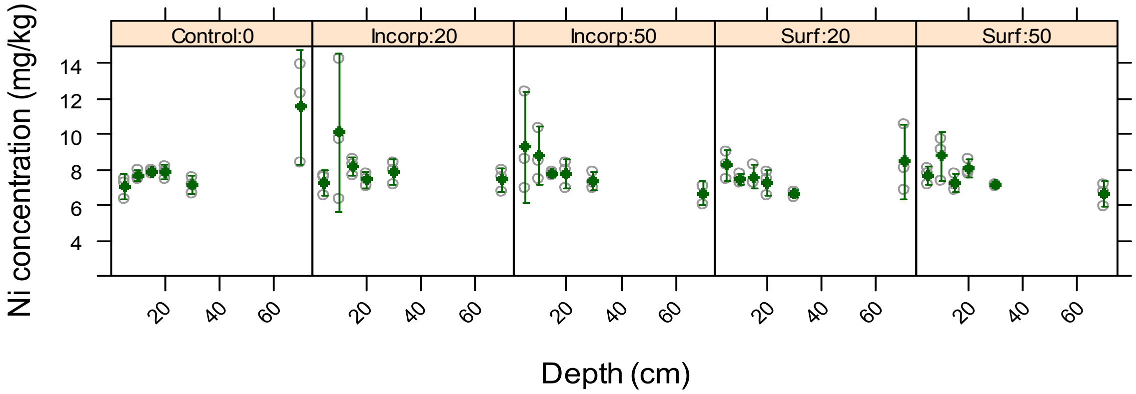


18 month

Figure 2.21. Cr concentration in S3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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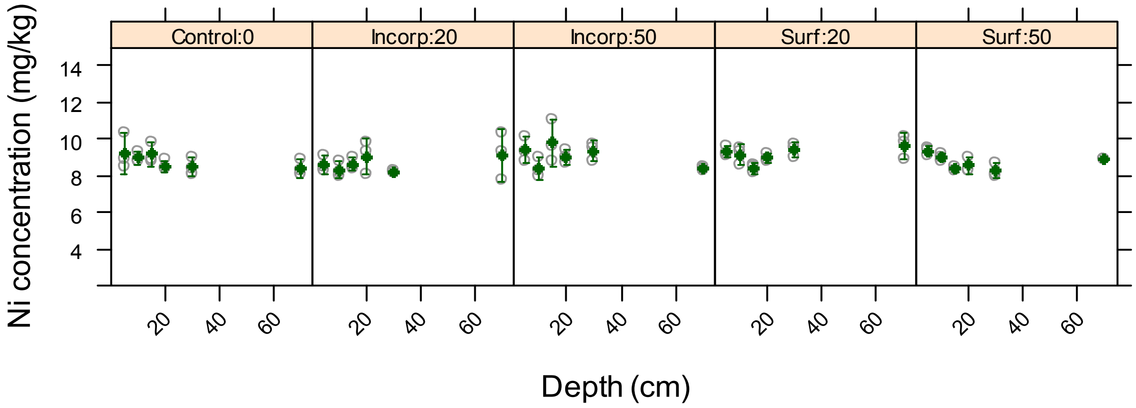

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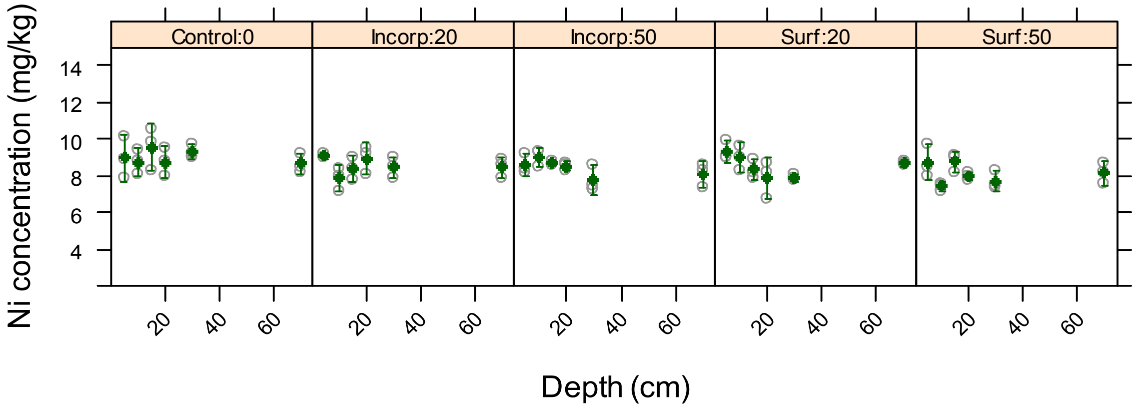
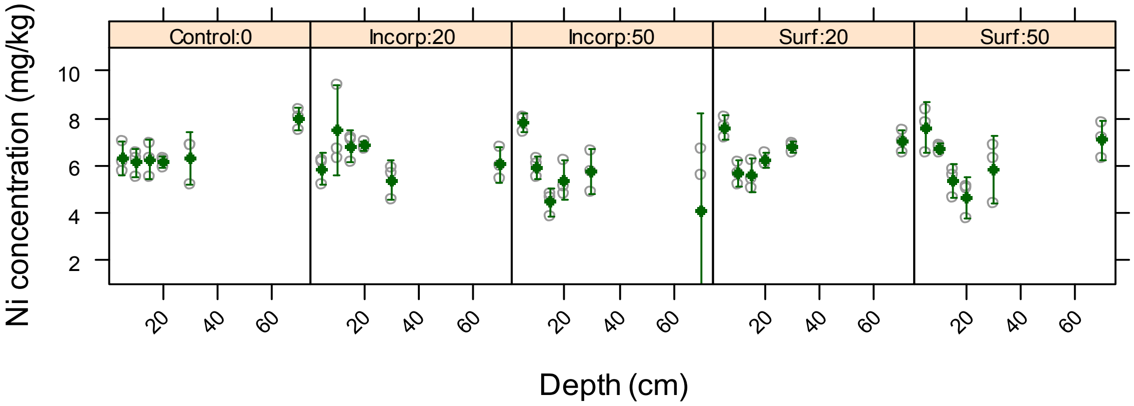


18 month

Figure 2.22. Ni concentration in S1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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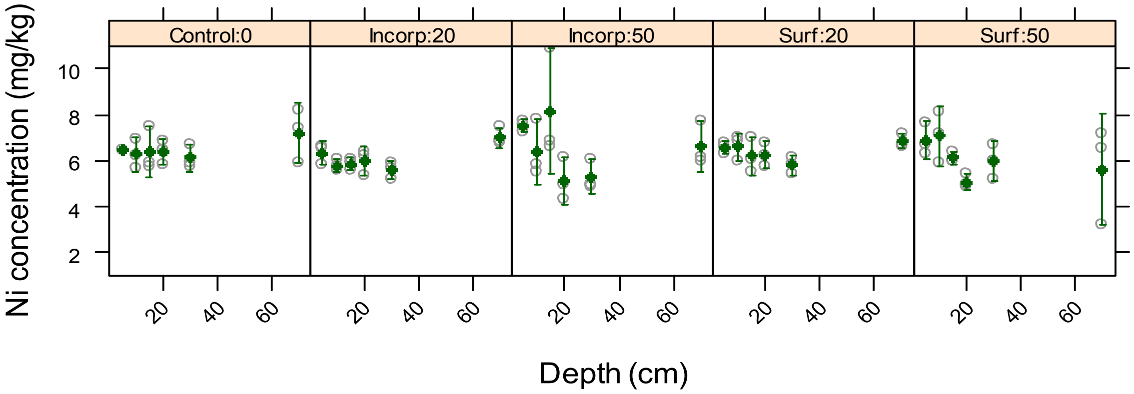

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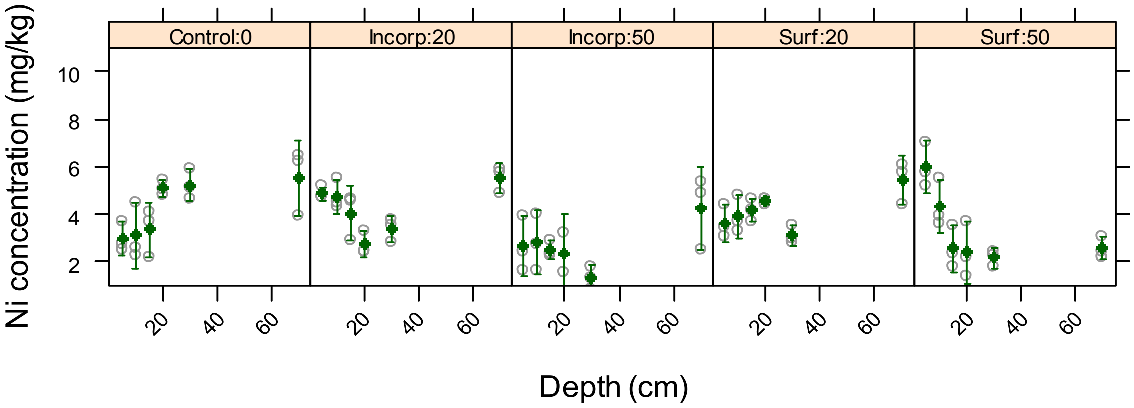
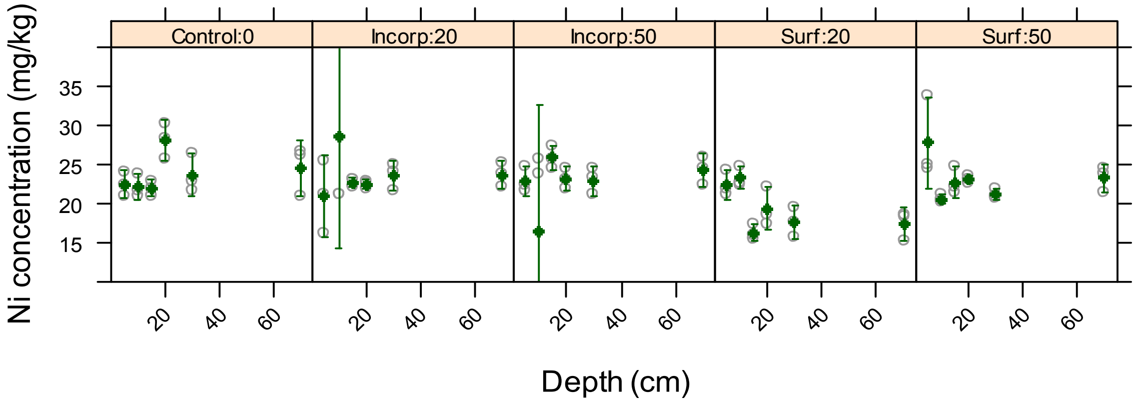


18 month

Figure 2.23. Ni concentration in S2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (o) with means and 95% confidence intervals



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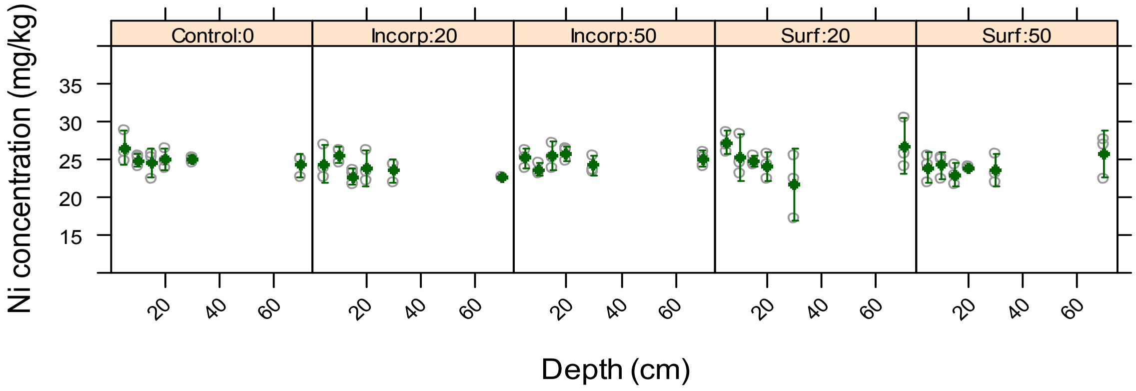

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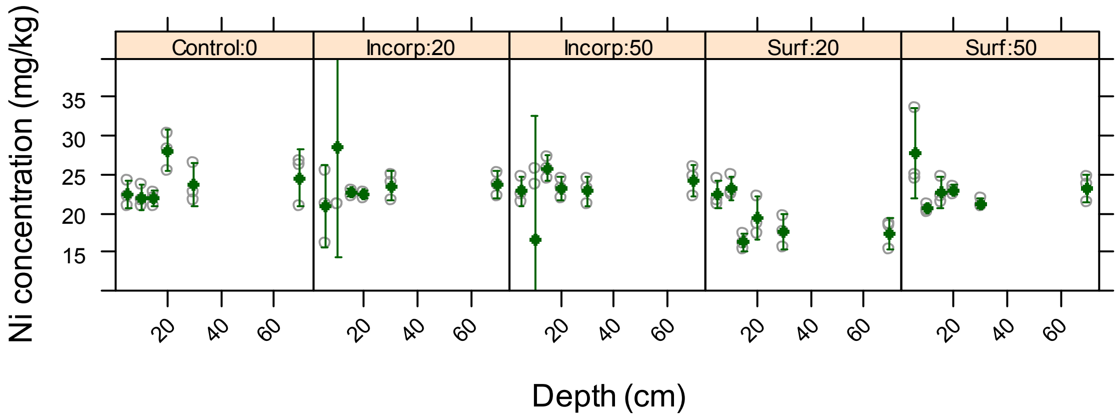
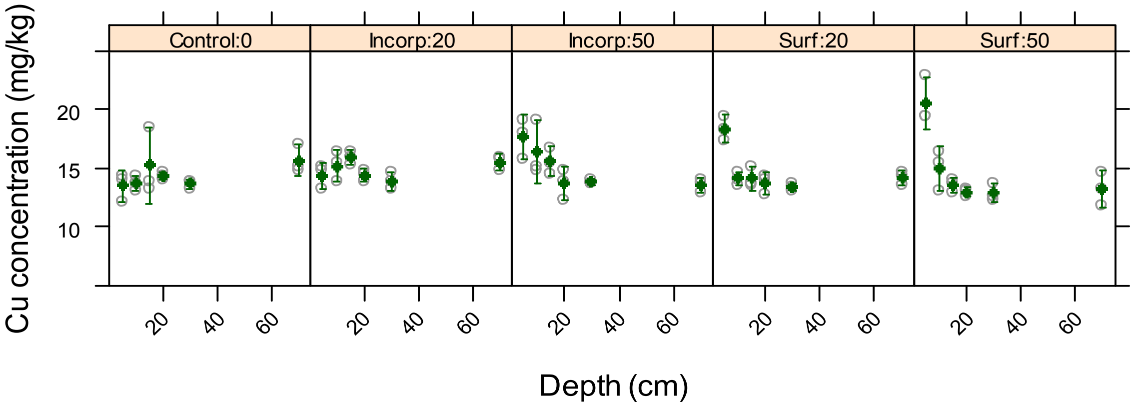


18 month

Figure 2.24. Ni concentration in S3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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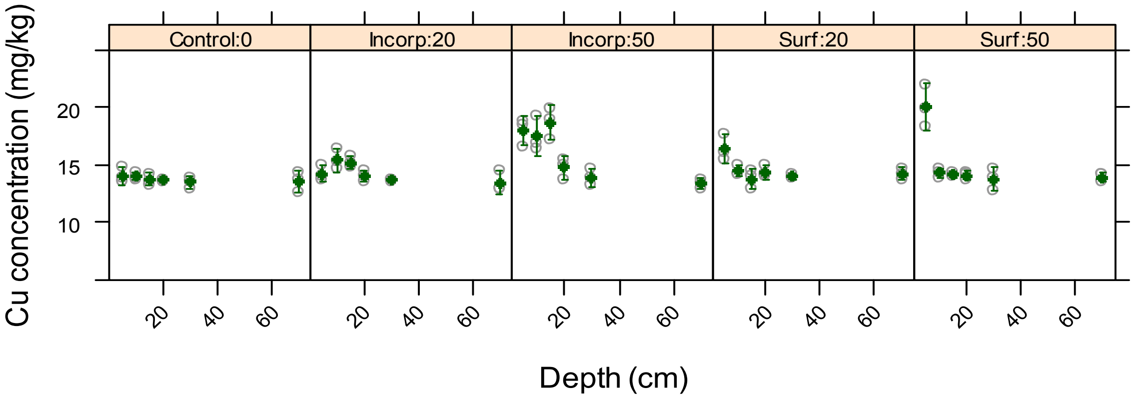

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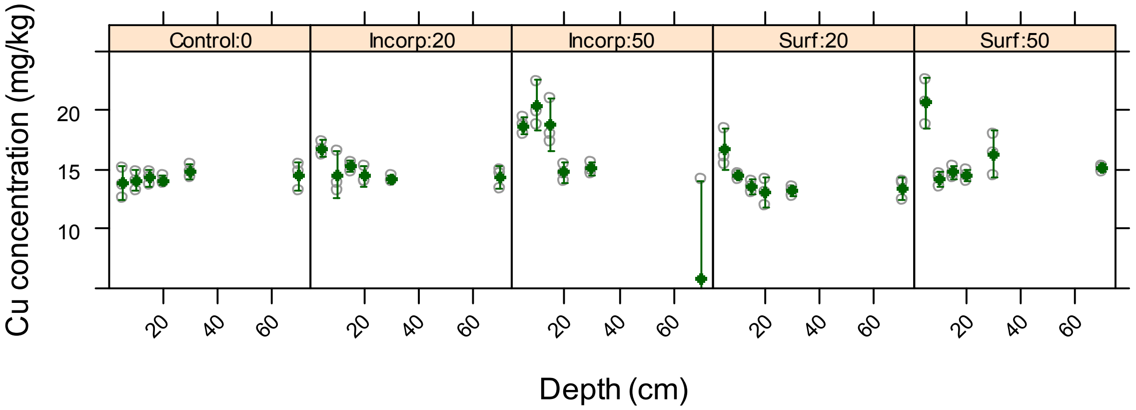
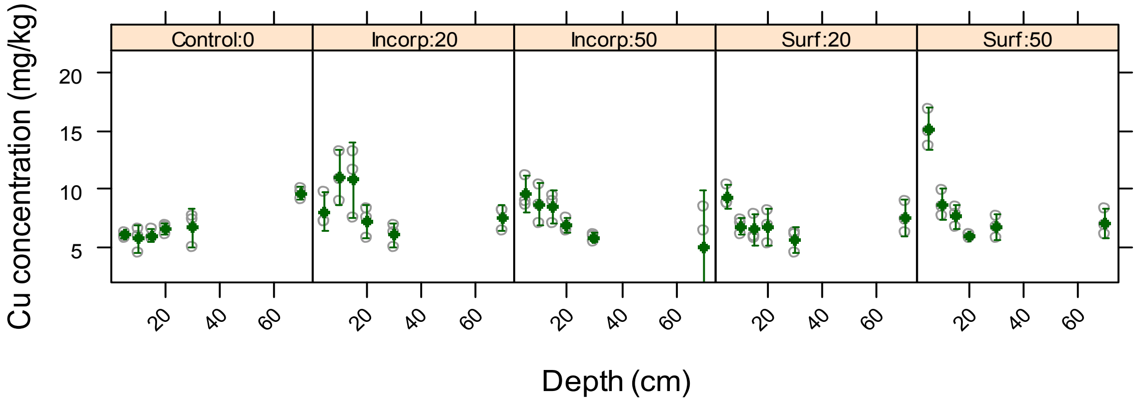
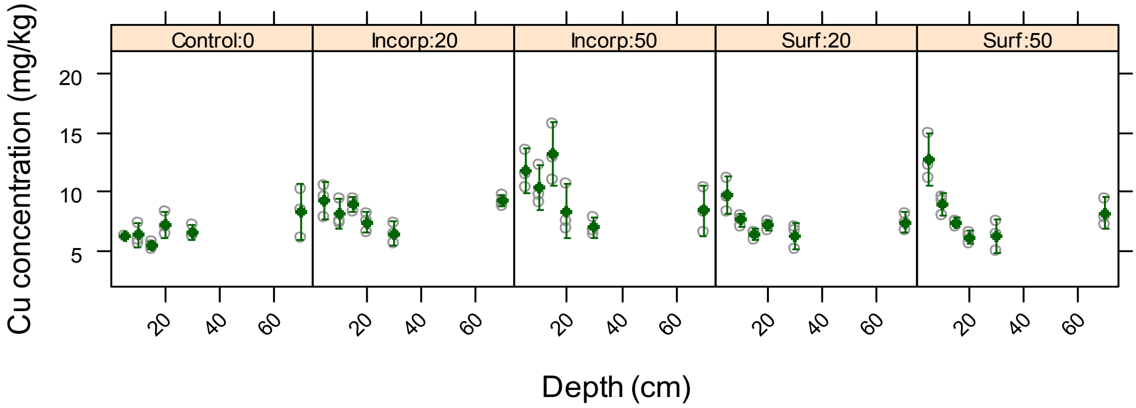


18 month

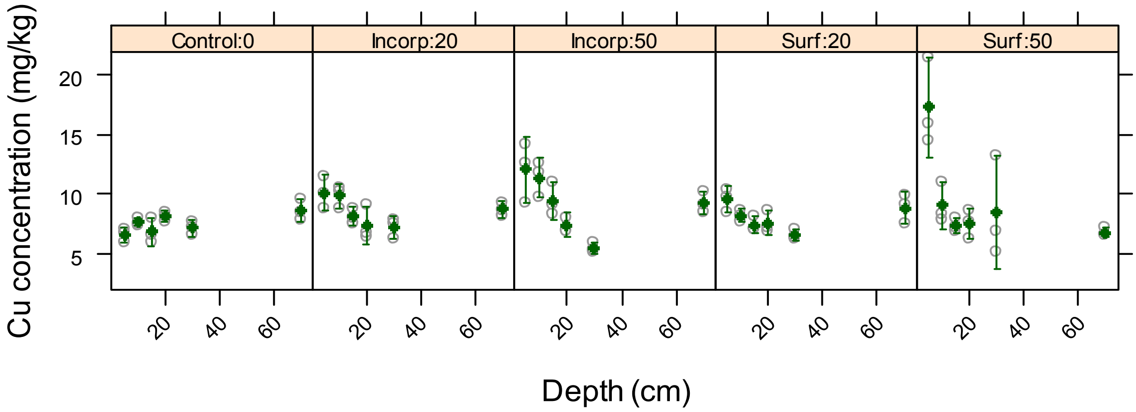
Figure 2.25. Cu concentration in S1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

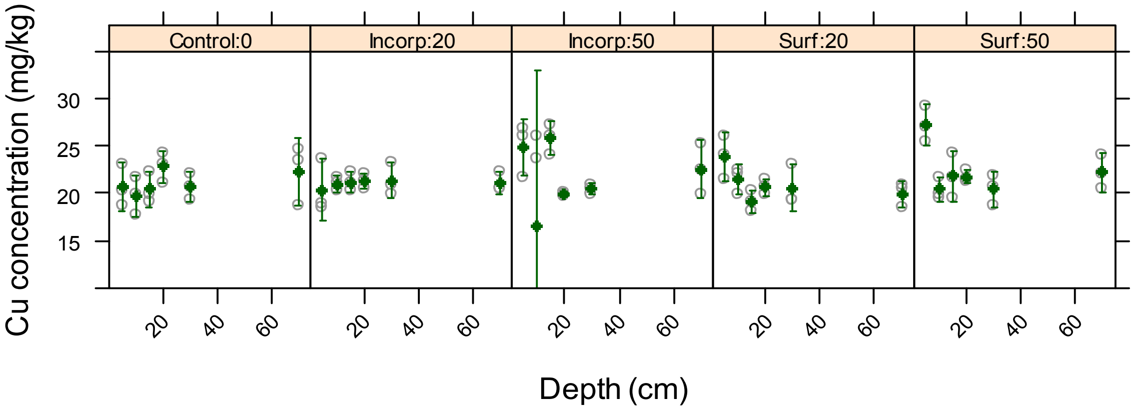


12 month



18 month

Figure 2.26. Cu concentration in S2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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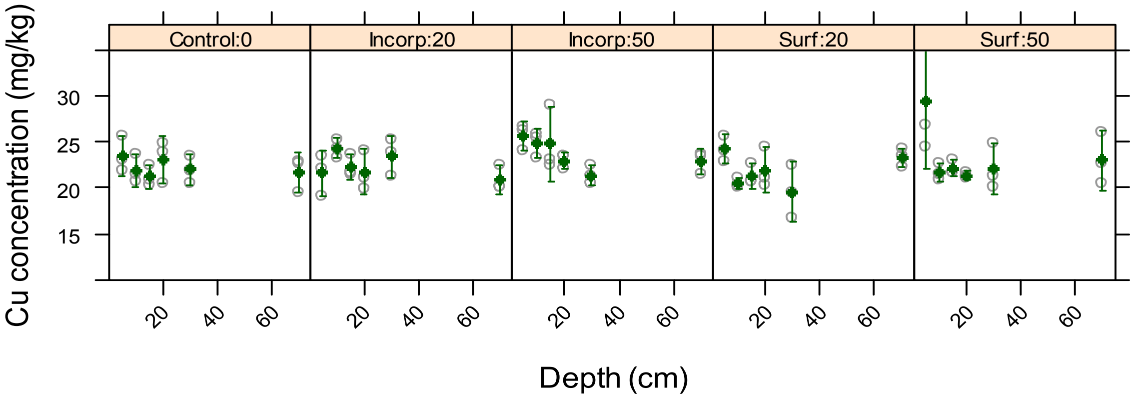

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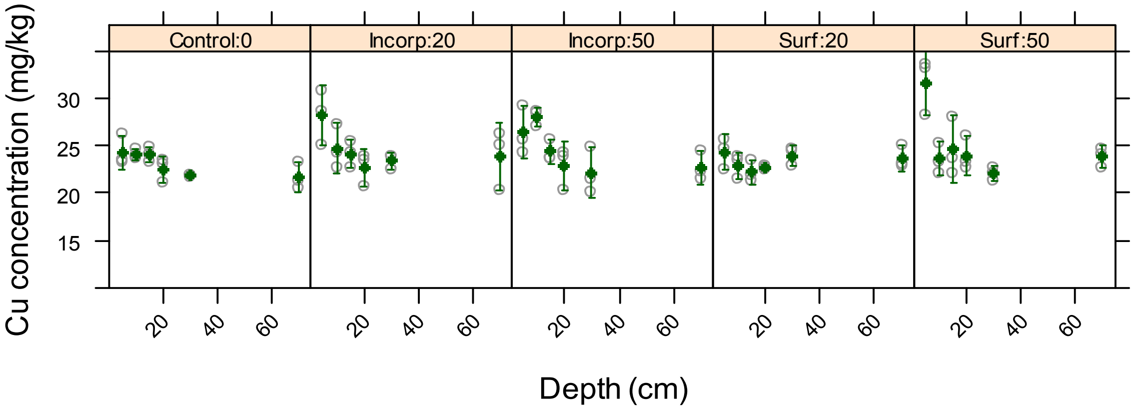
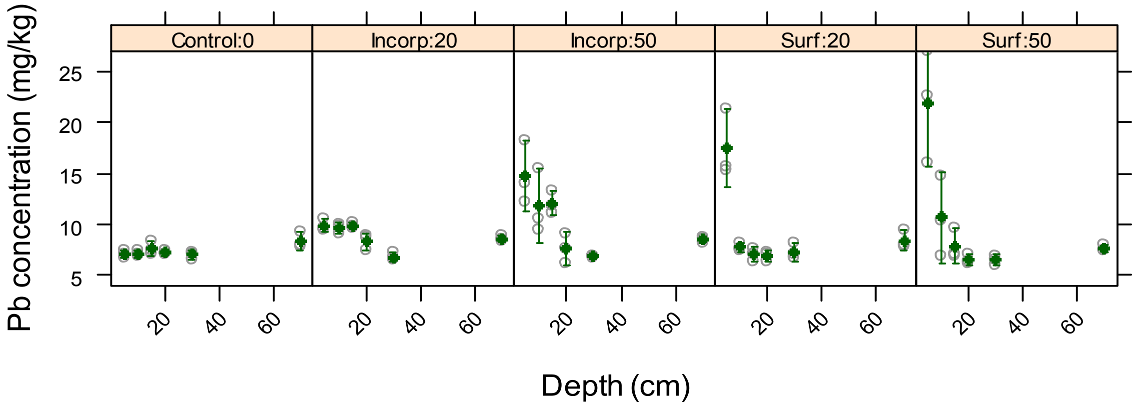


18 month

Figure 2.27. Cu concentration in S3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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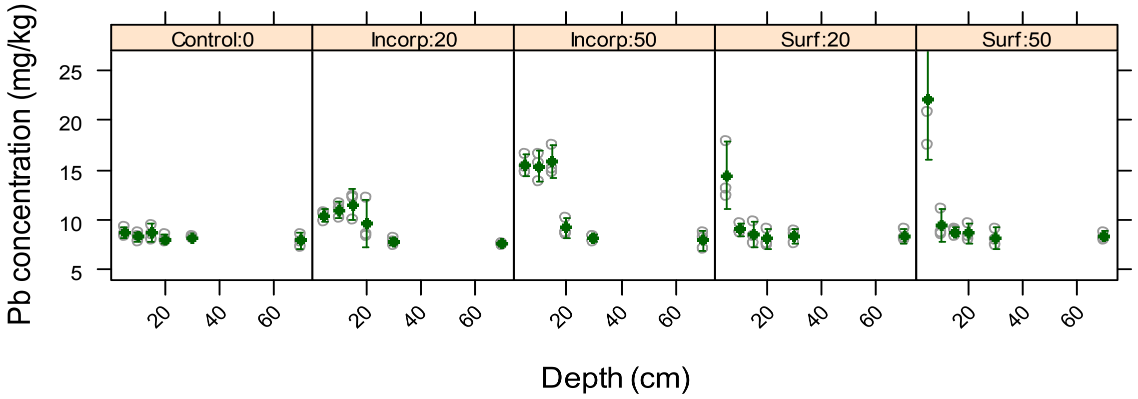

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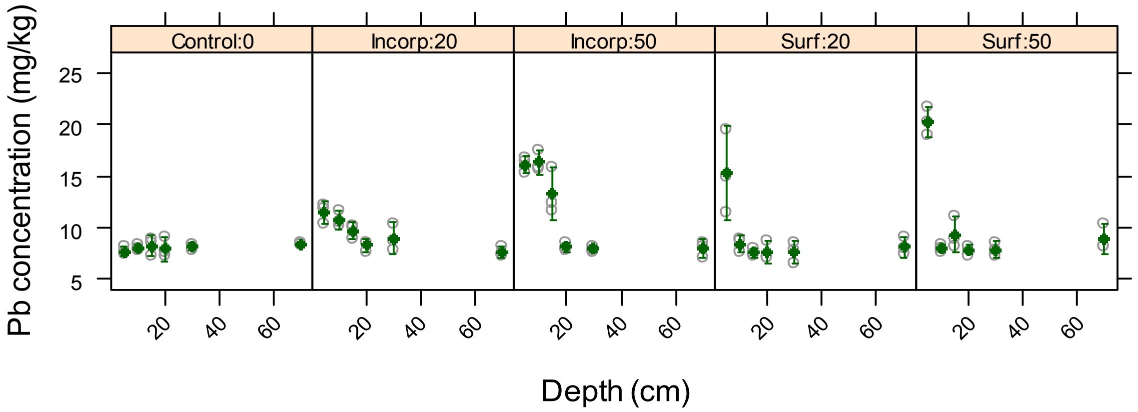
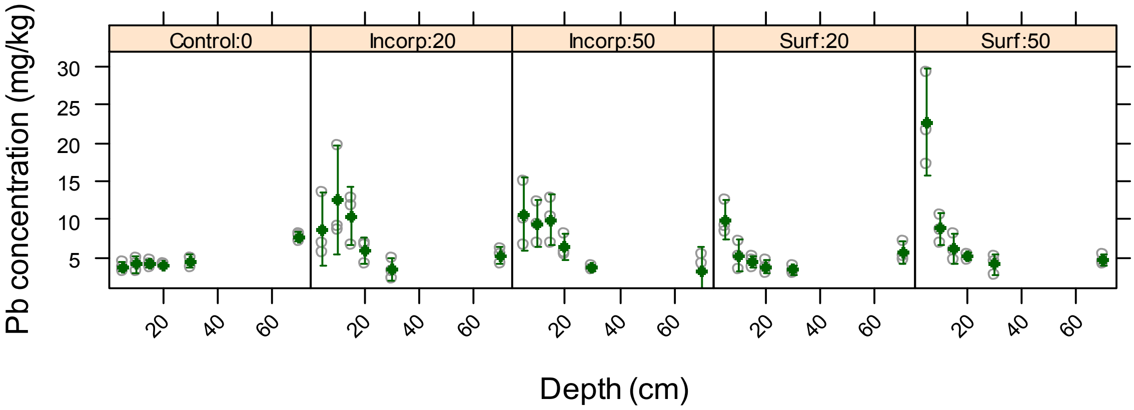


18 month

Figure 2.28. Pb concentration in S1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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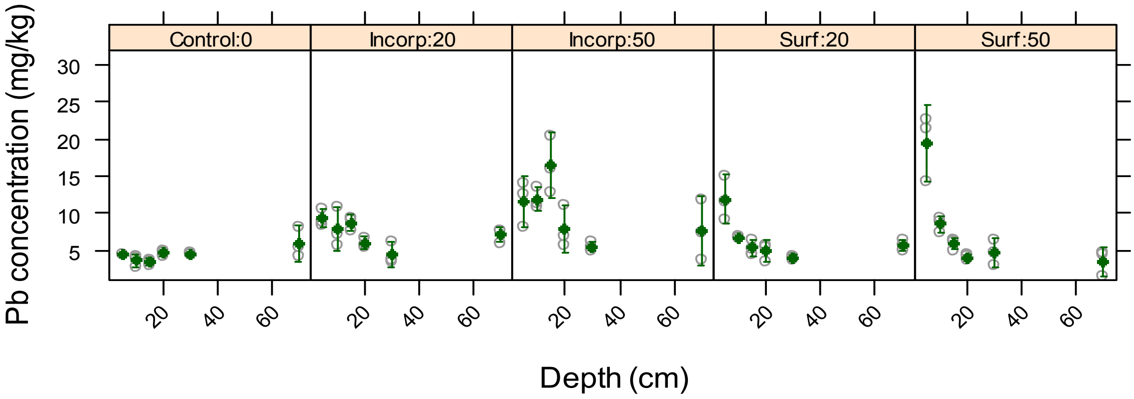

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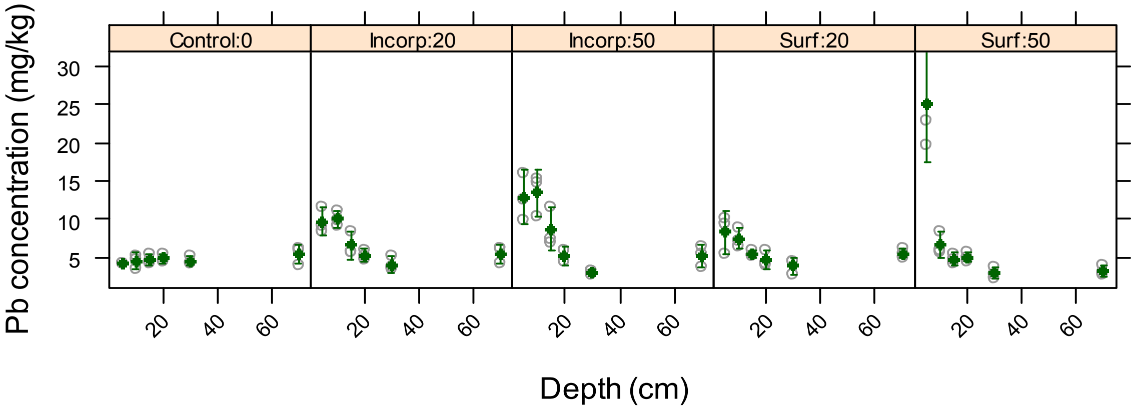
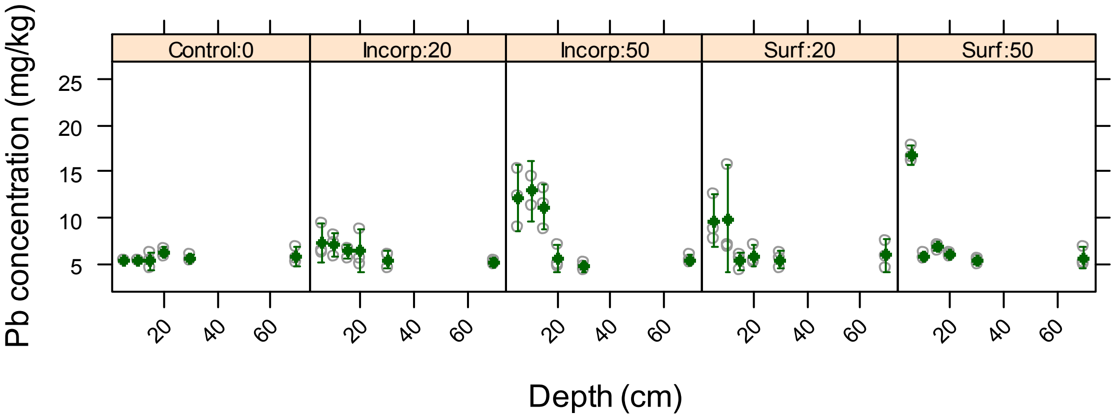


18 month

Figure 2.29. Pb concentration in S2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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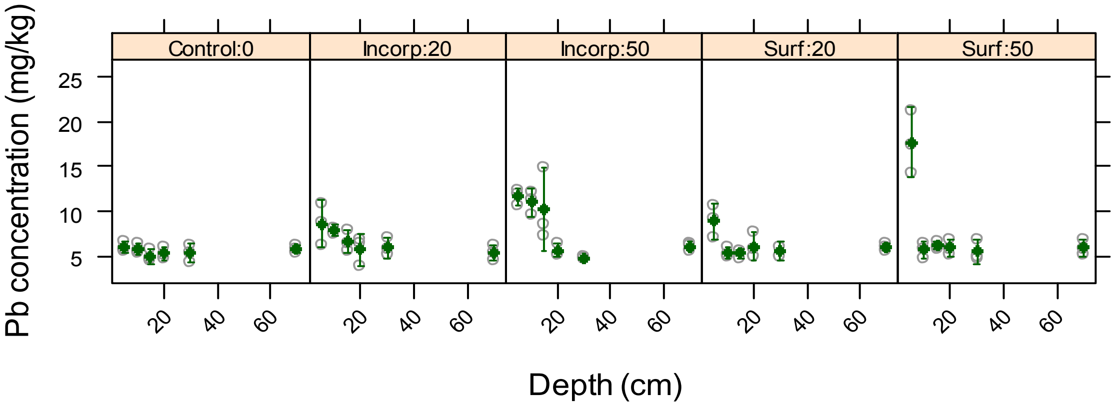

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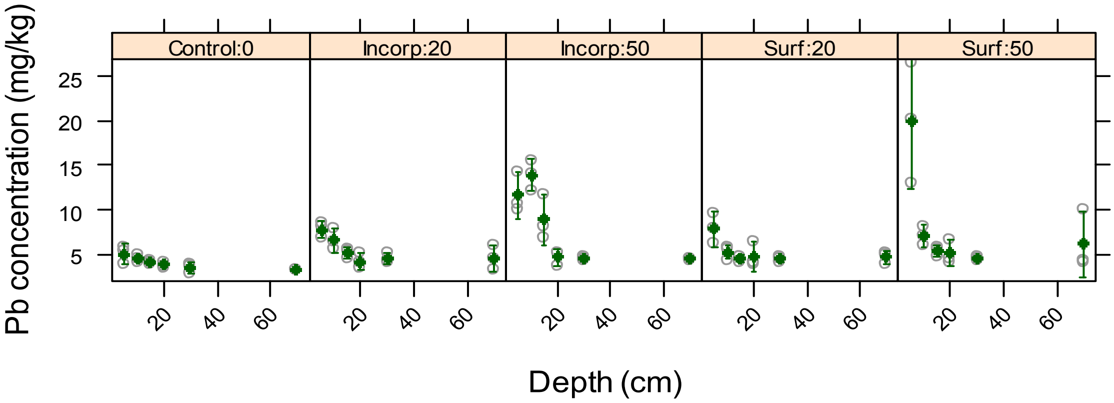
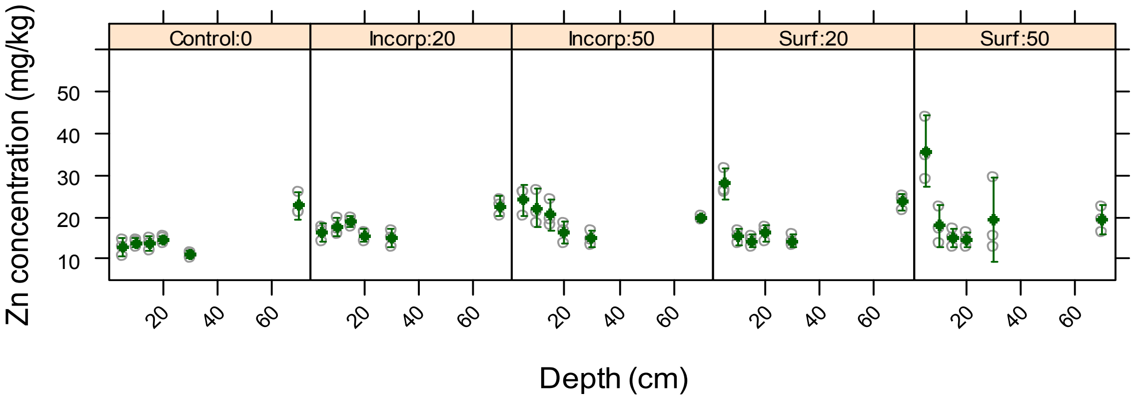


18 month

Figure 2.30. Pb concentration in S3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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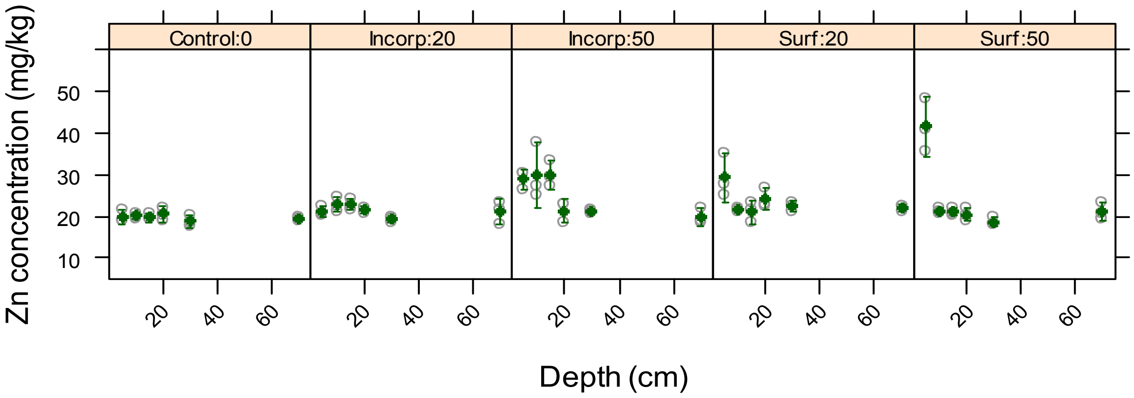

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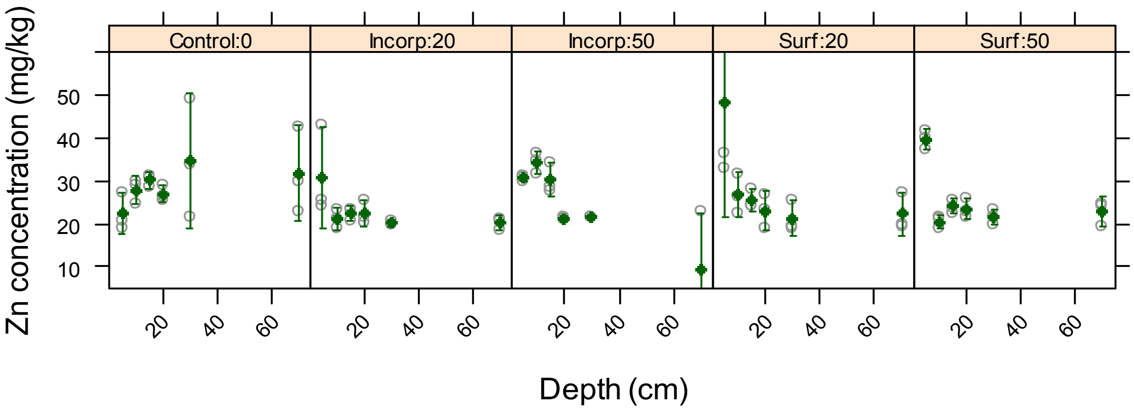
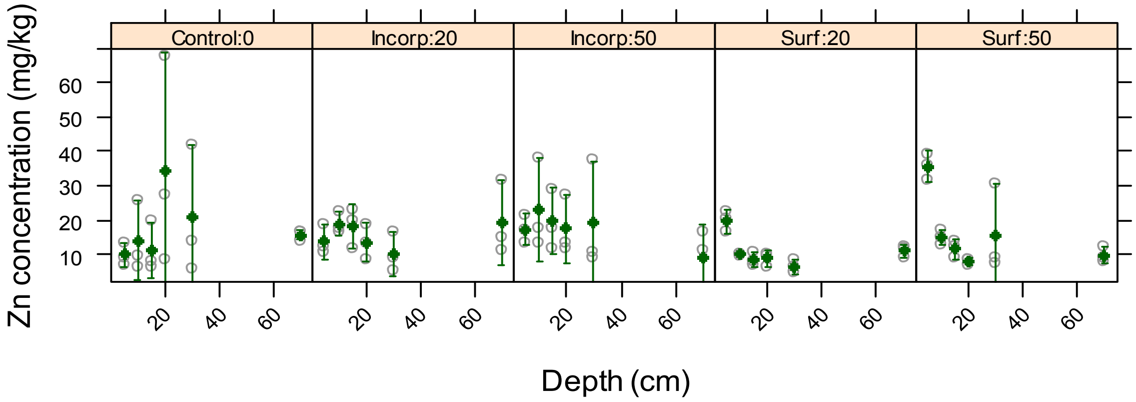


18 month

Figure 2.31. Zn concentration in S1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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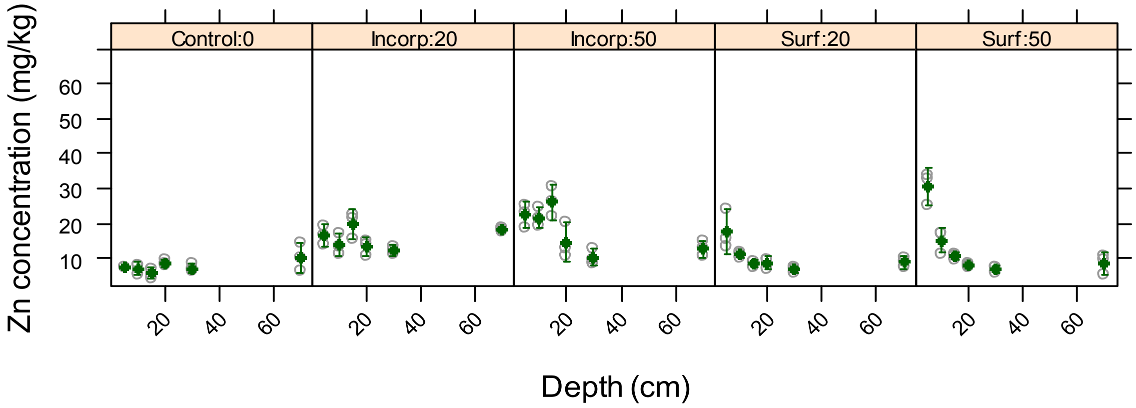

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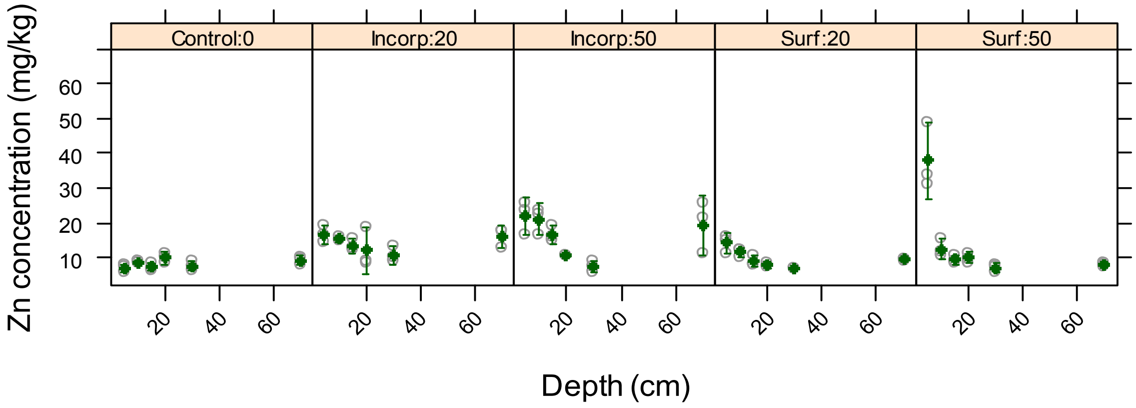
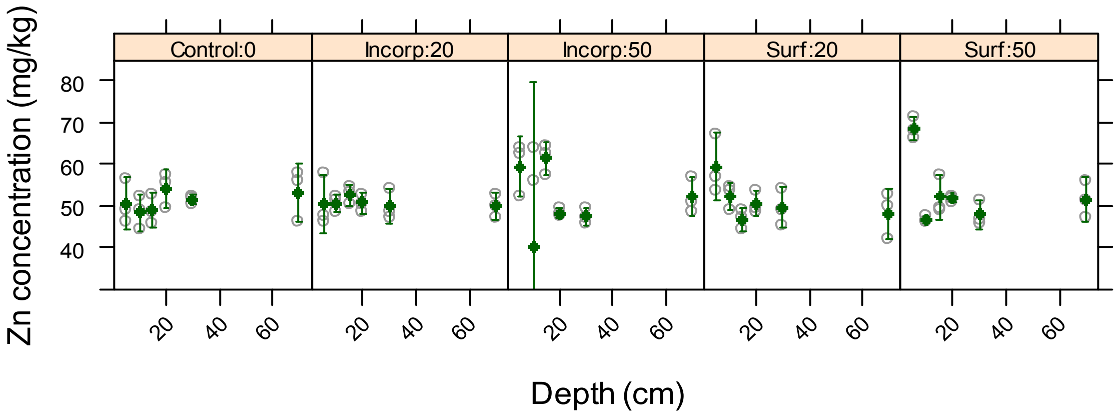


18 month

Figure 2.32. Zn concentration in S2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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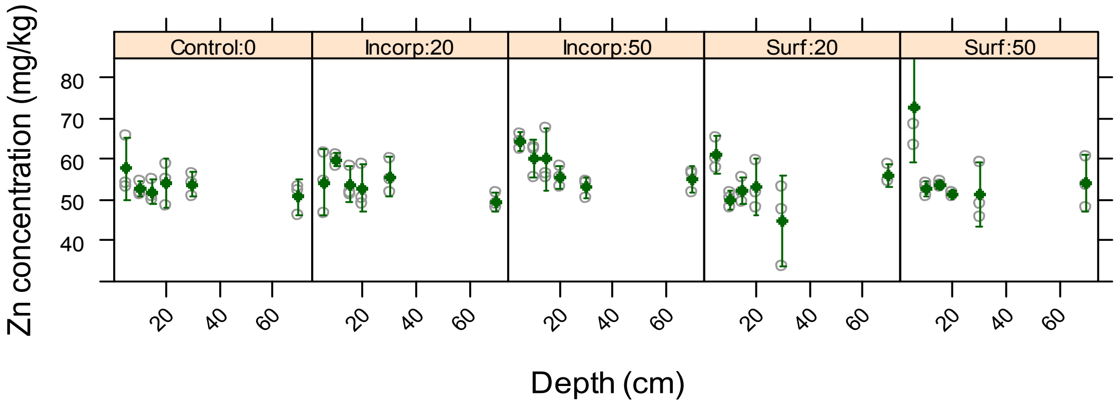

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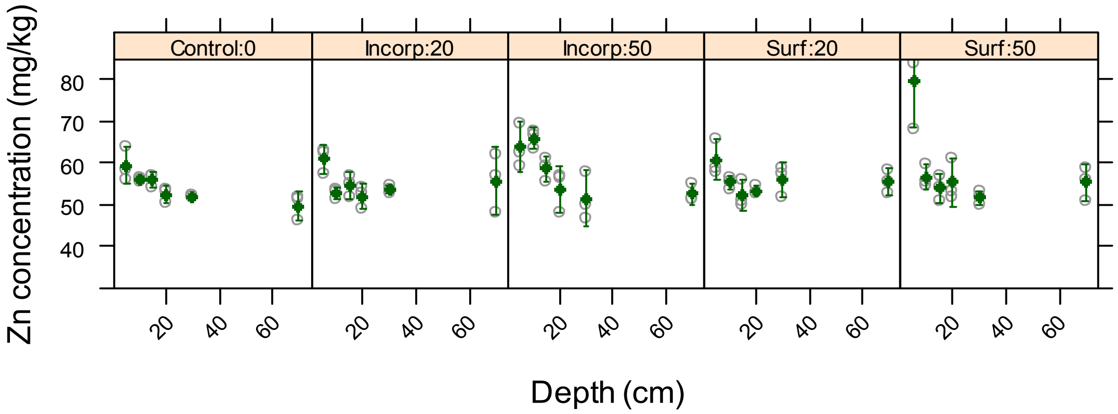
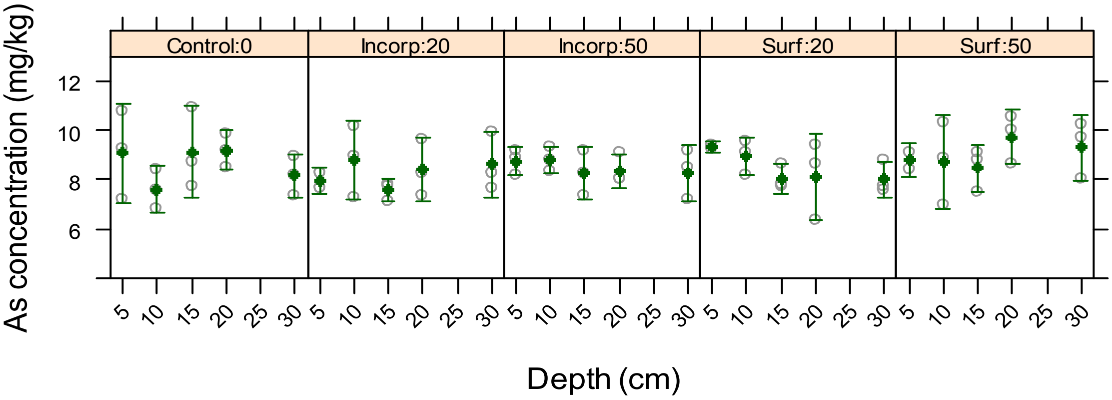


18 month

Figure 2.33. Zn concentration in S3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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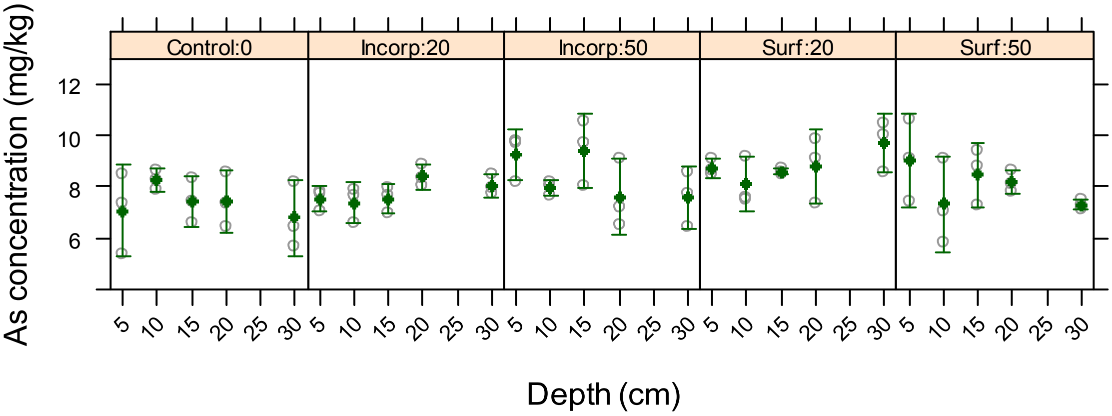

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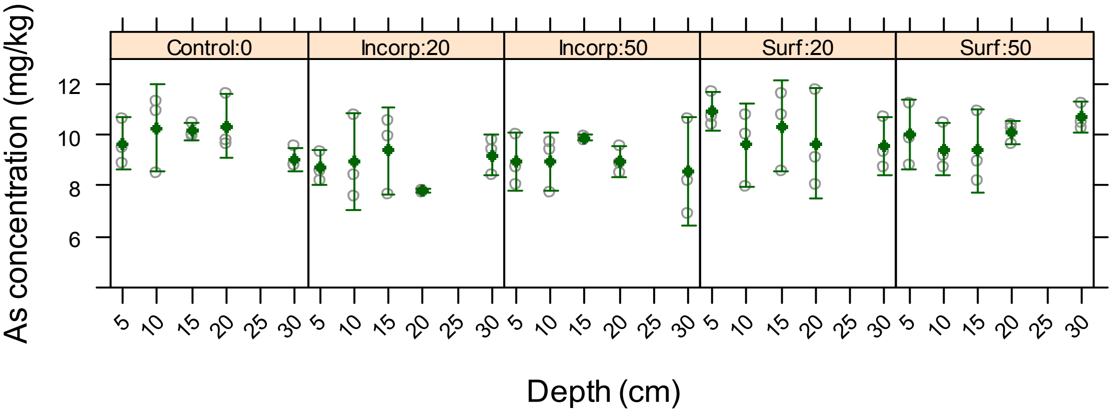
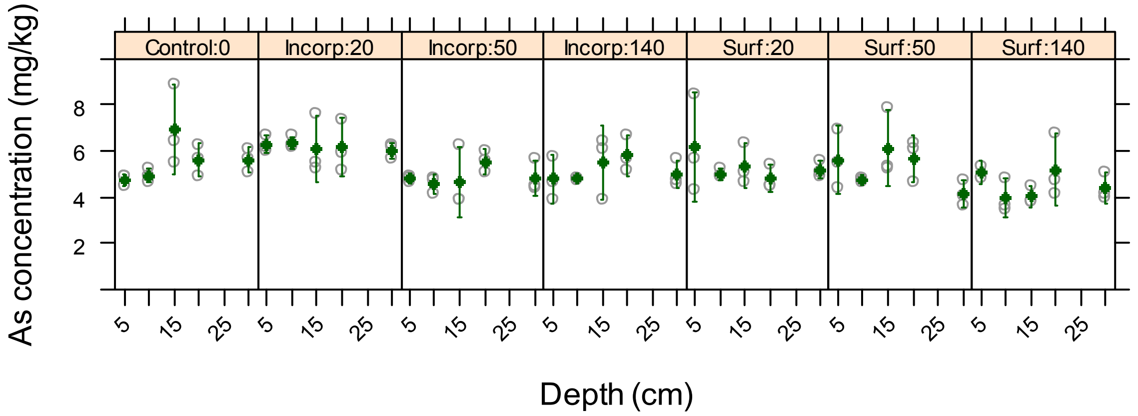


18 month

Figure 2.34. As concentration in SL1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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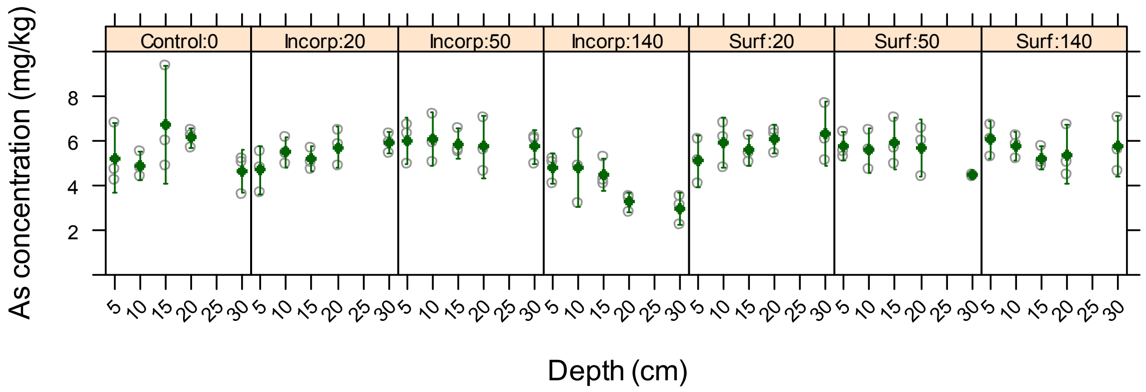

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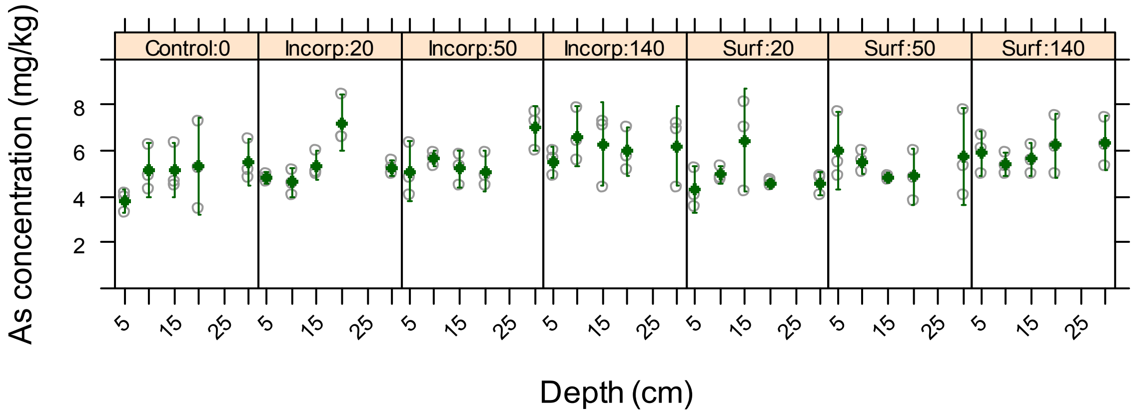
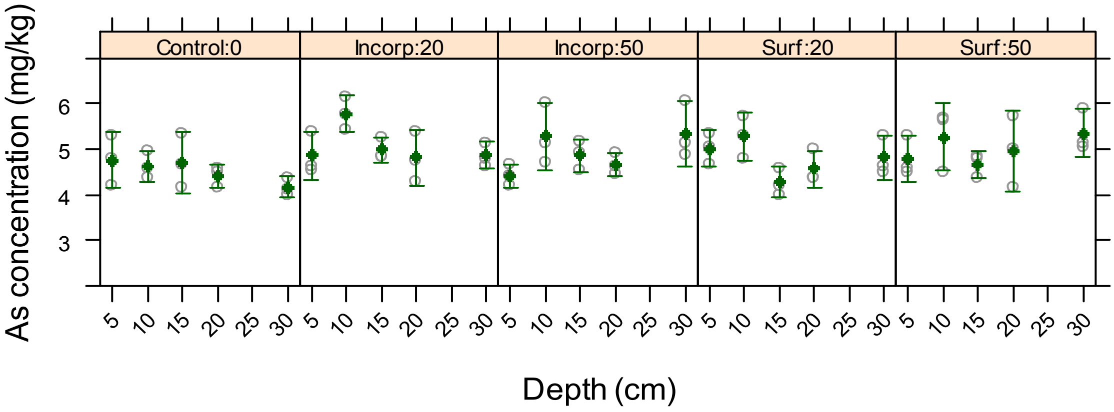


18 month

Figure 2.35. As concentration in SL2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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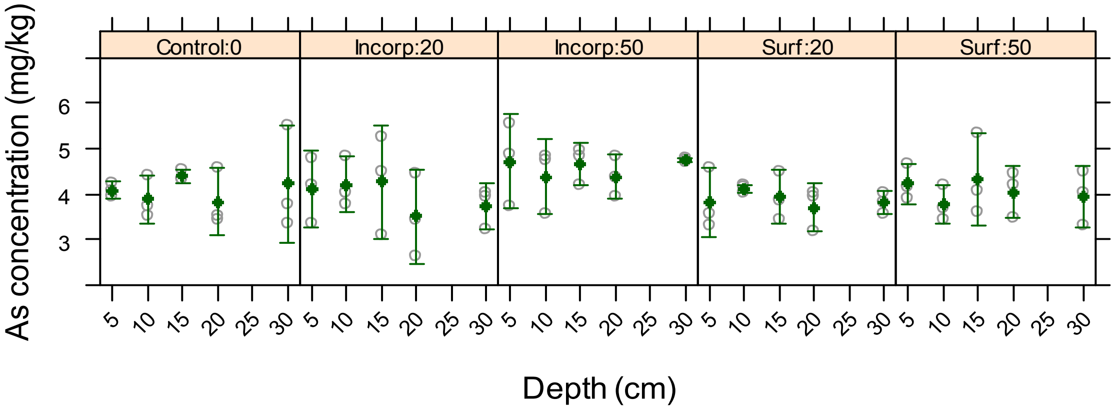

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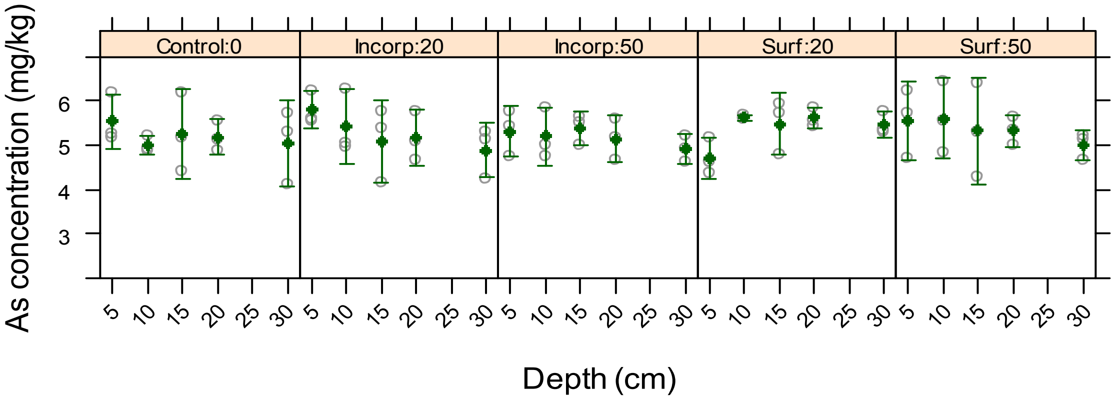
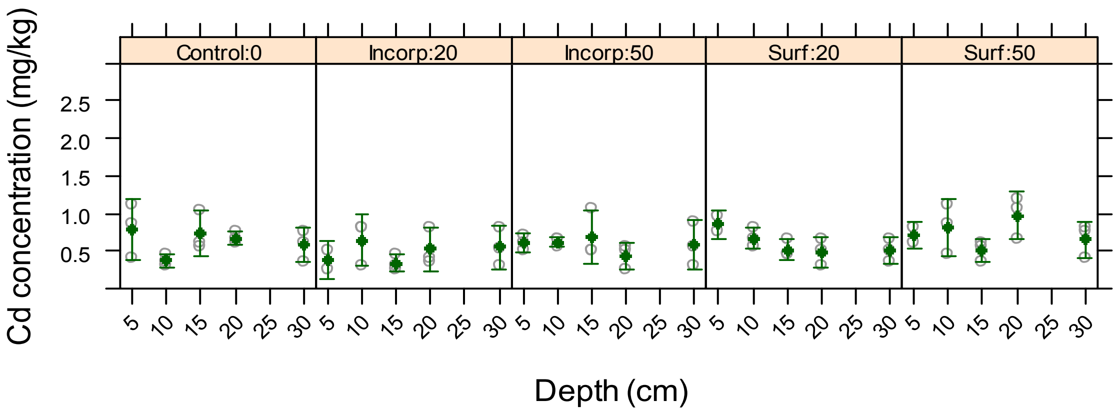


18 month

Figure 2.36. As concentration in SL3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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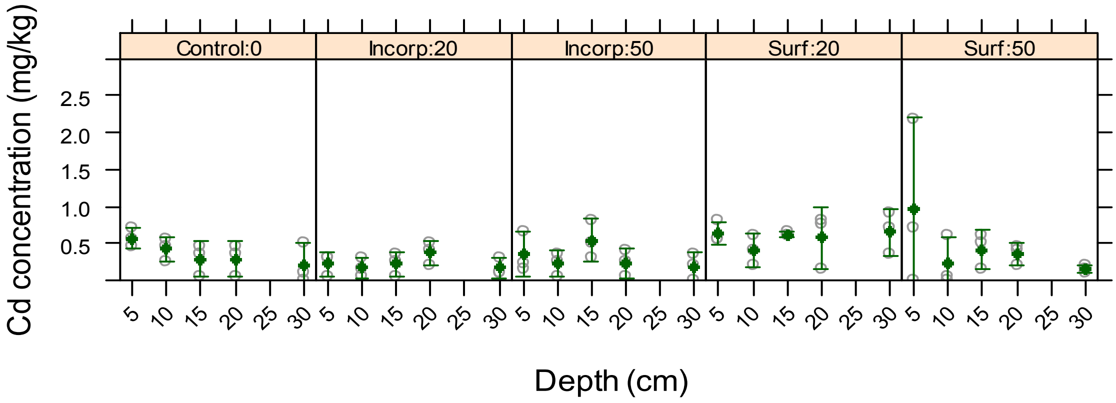

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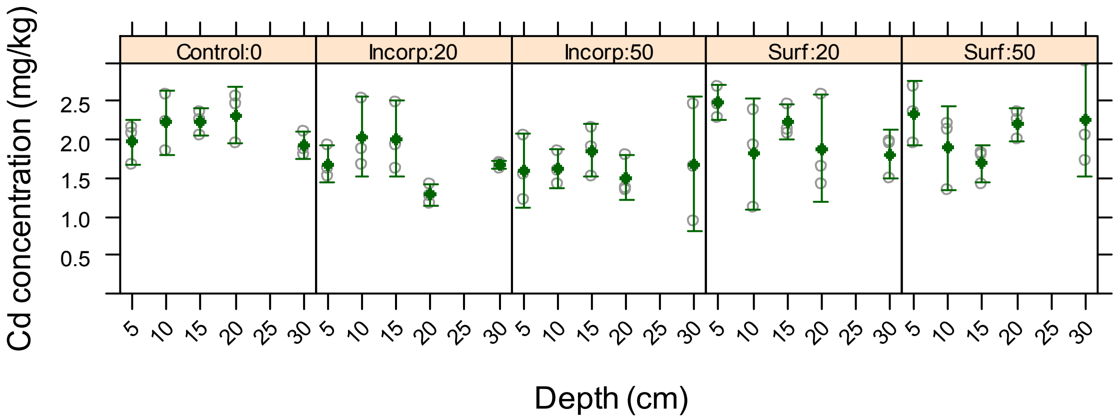
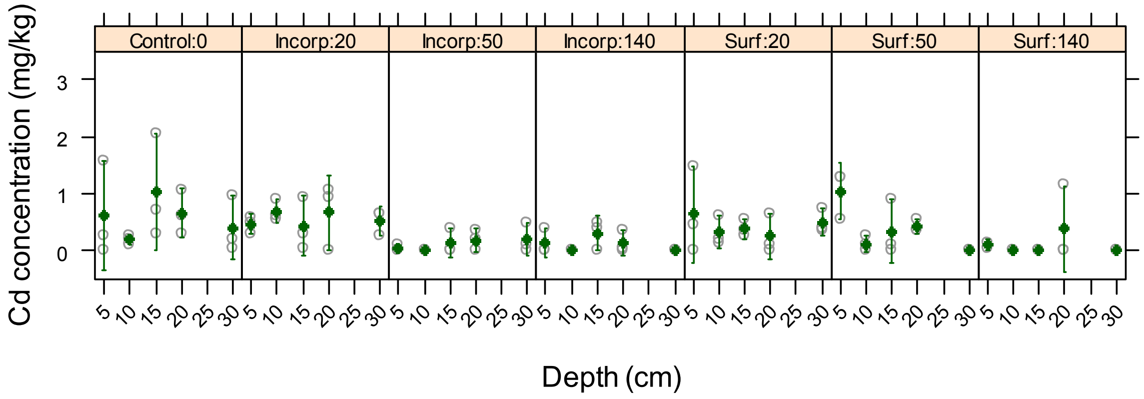


18 month

Figure 2.37. Cd concentration in SL1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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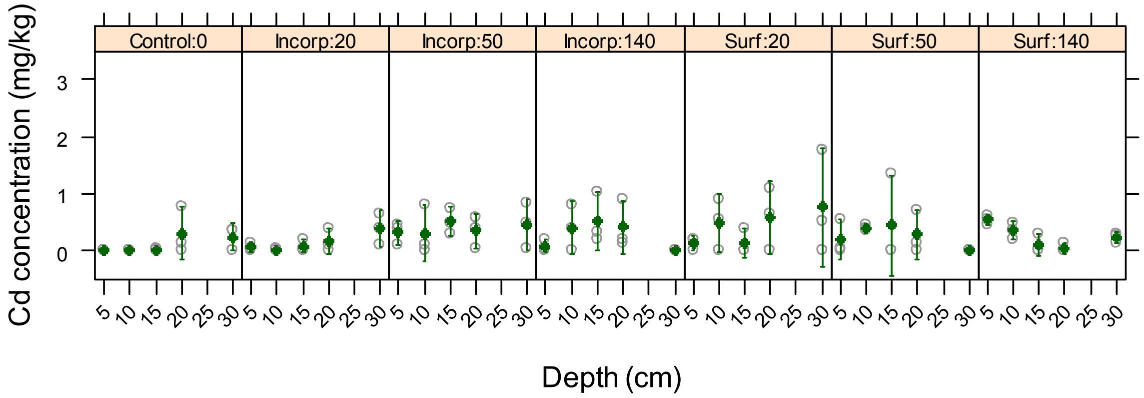

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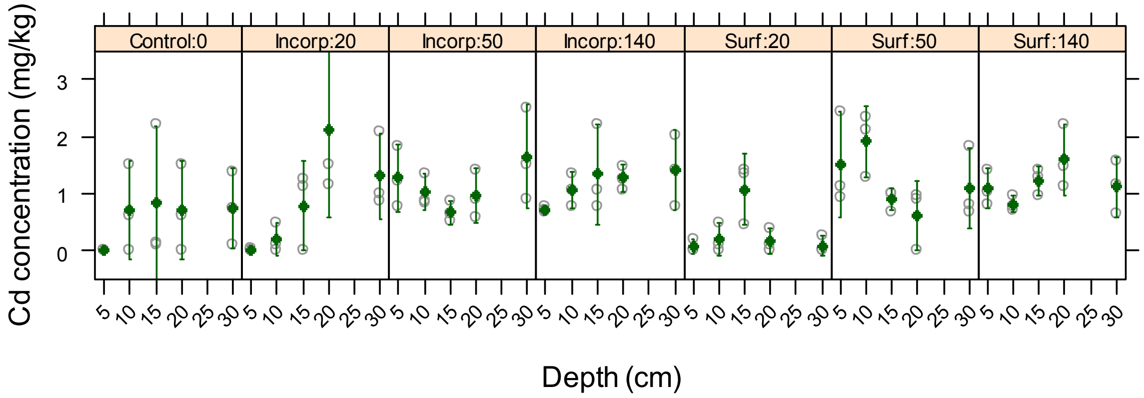
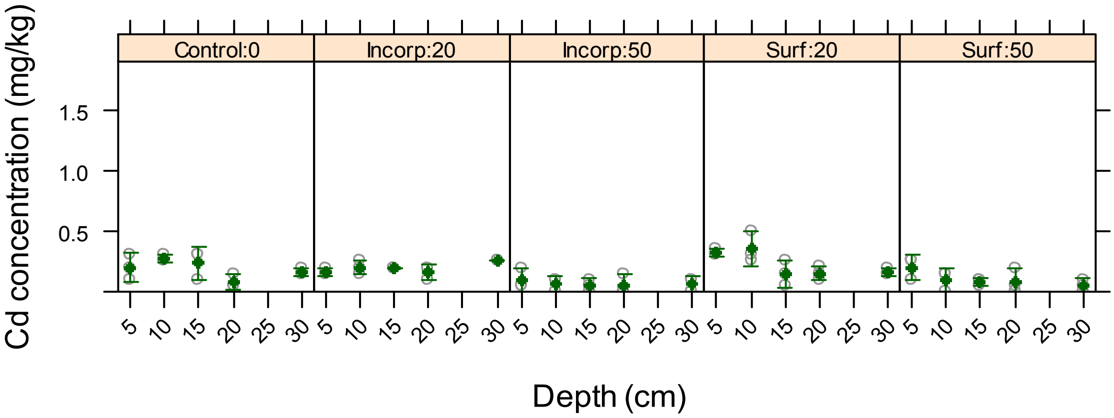


18 month

Figure 2.38. Cd concentration in SL2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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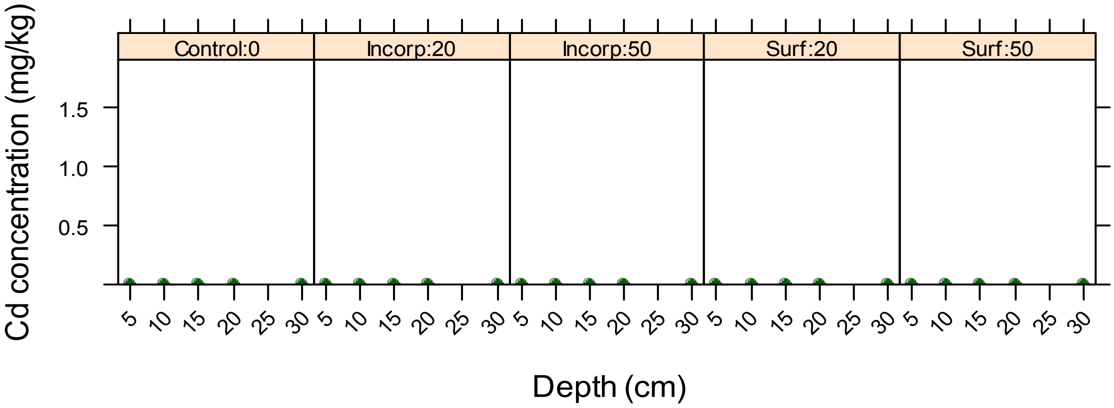

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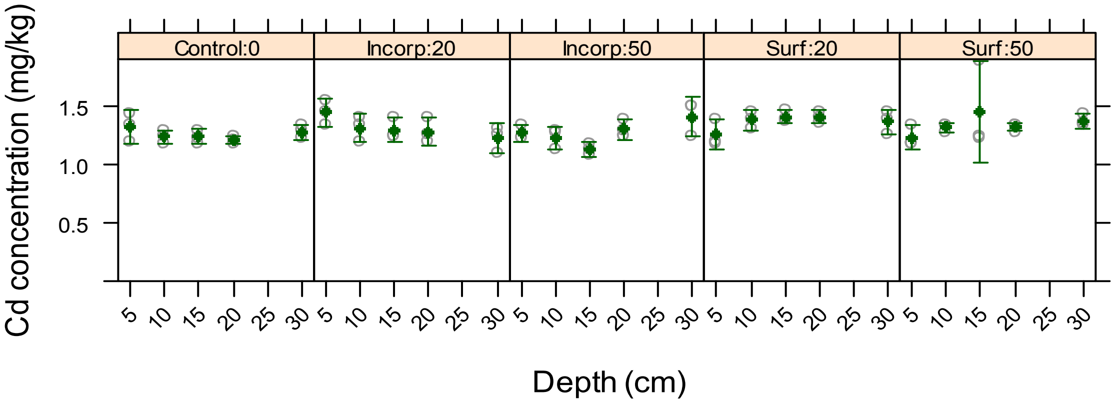
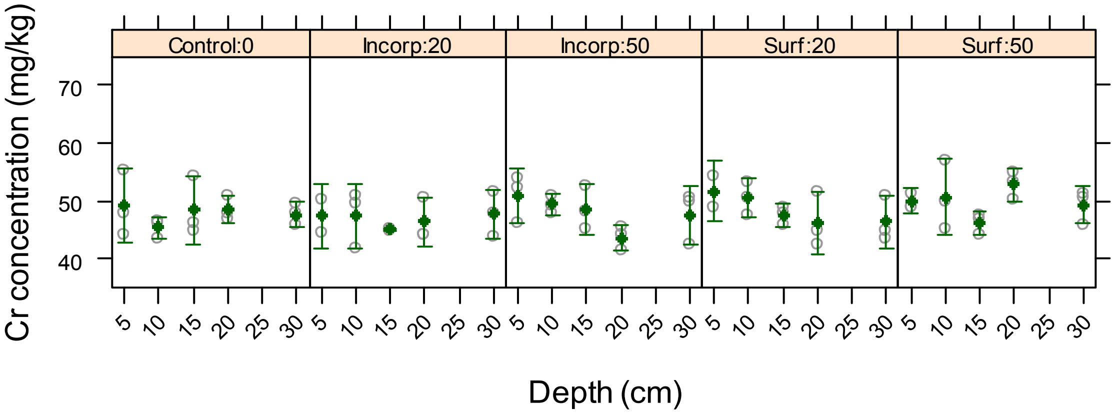


18 month

Figure 2.39. Cd concentration in SL3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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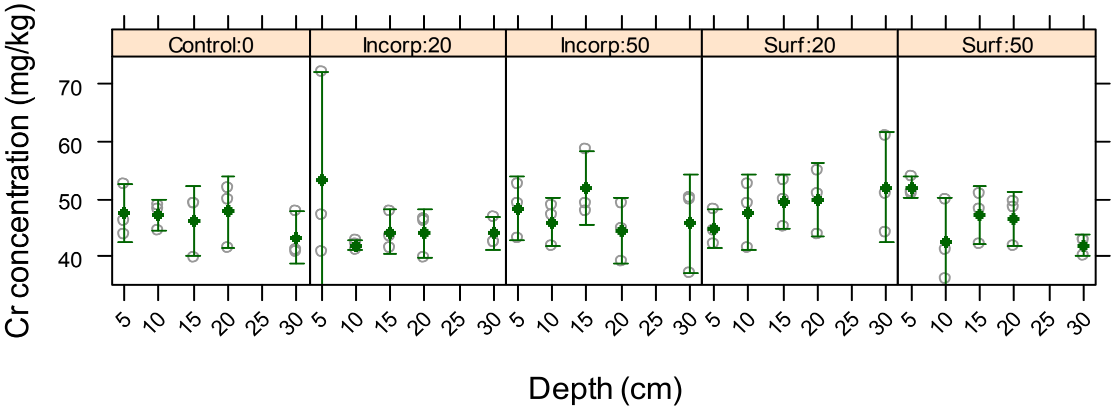

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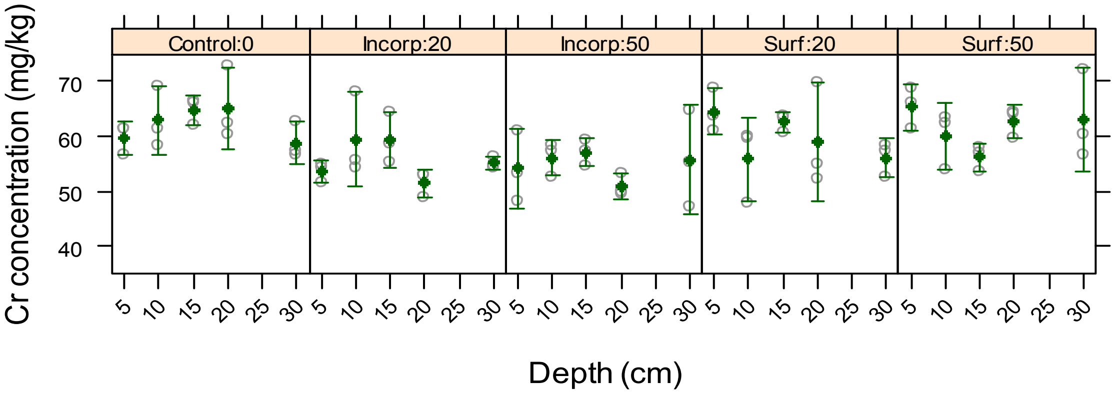
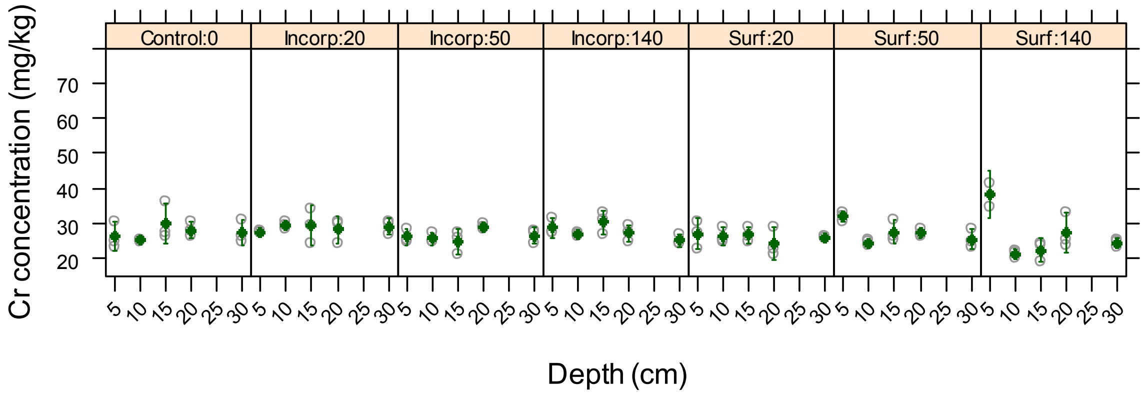


18 month

Figure 2.40. Cr concentration in SL1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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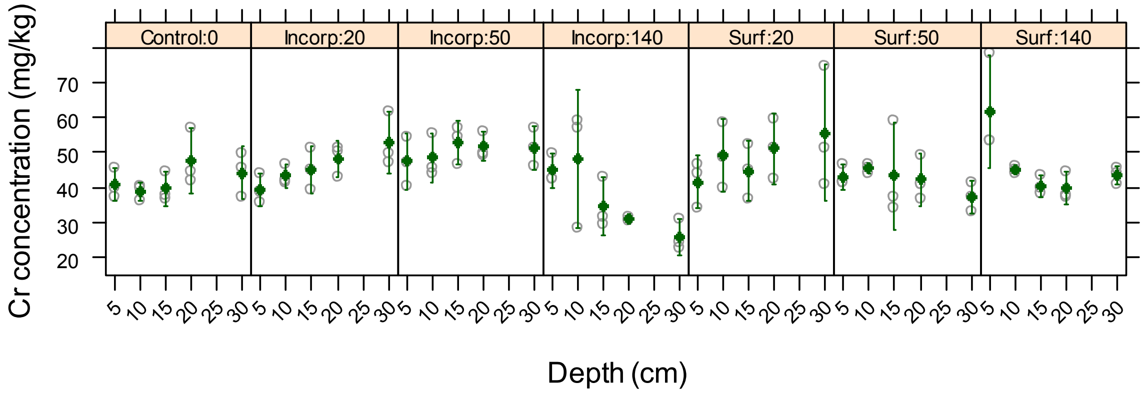

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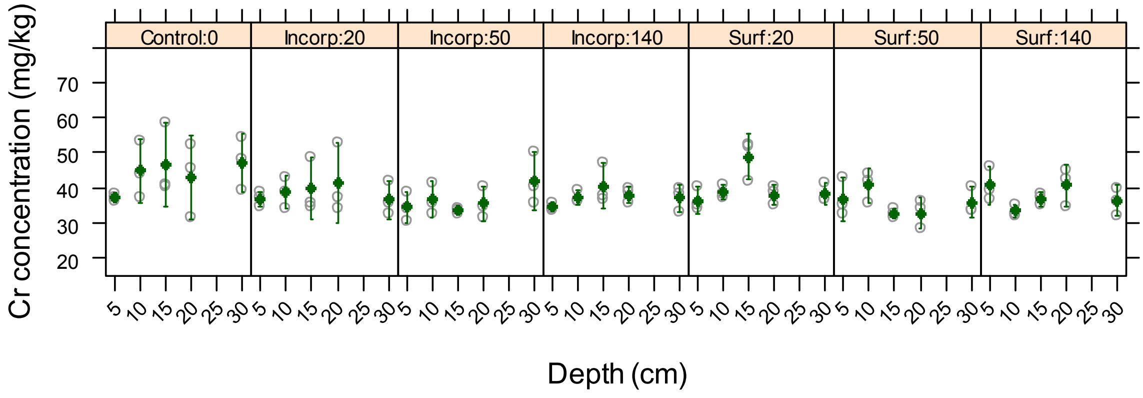
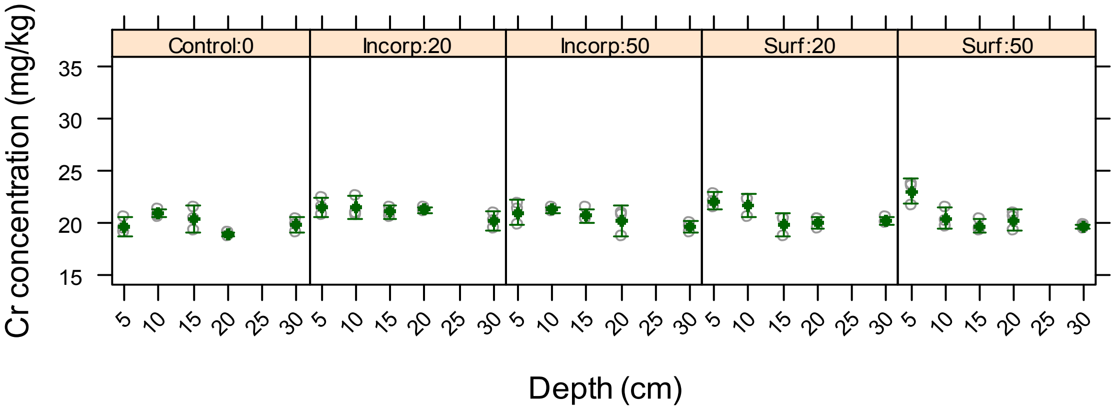
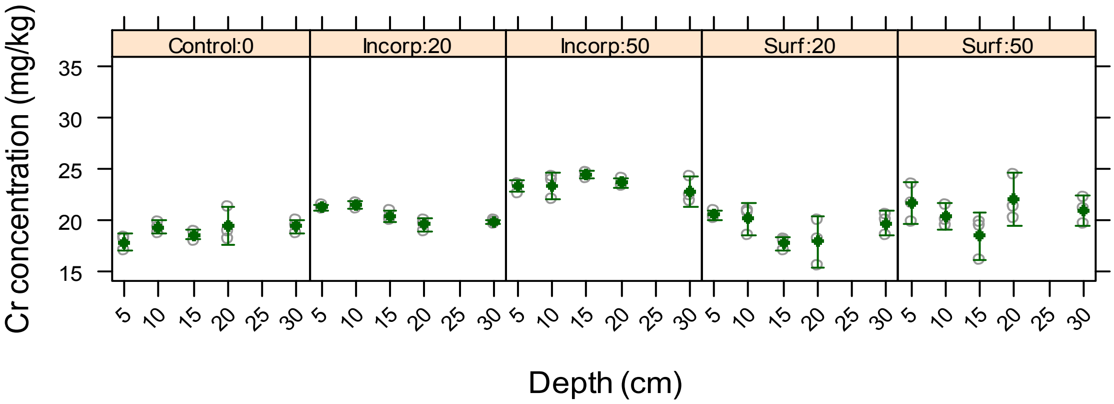


18 month

Figure 2.41. Cr concentration in SL2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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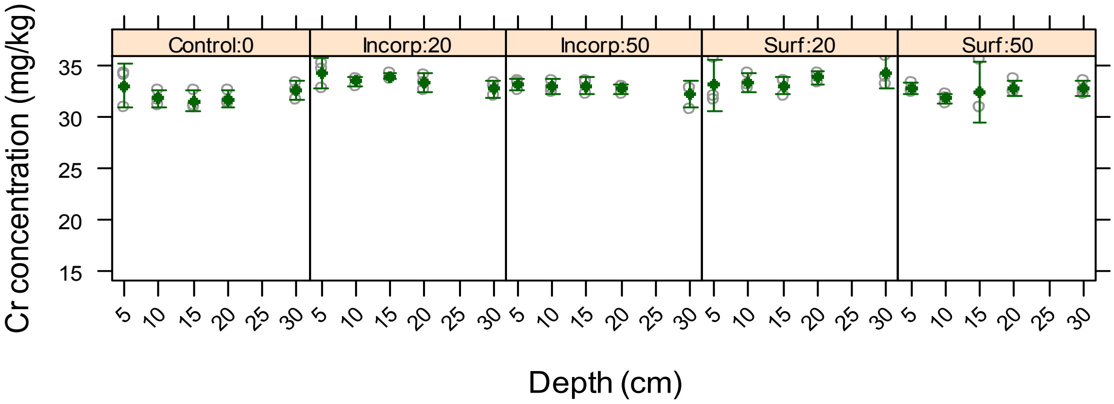
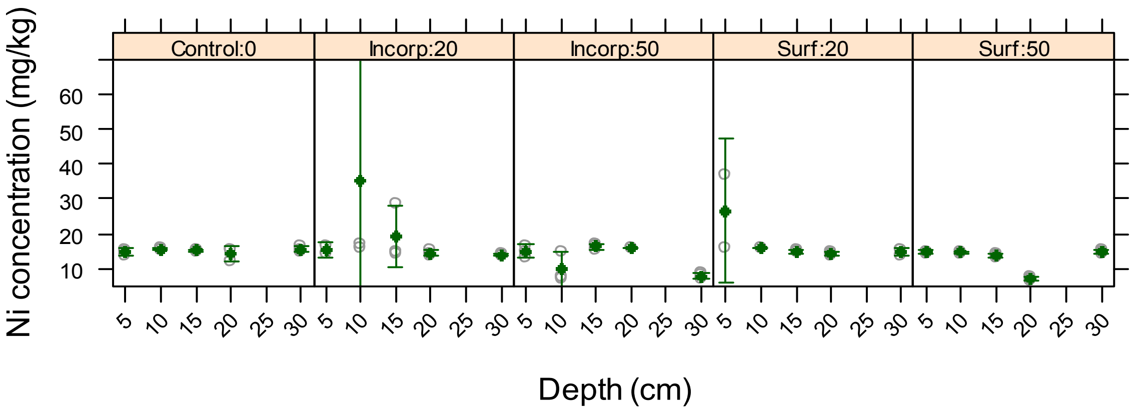


18 month

Figure 2.42. Cr concentration in SL3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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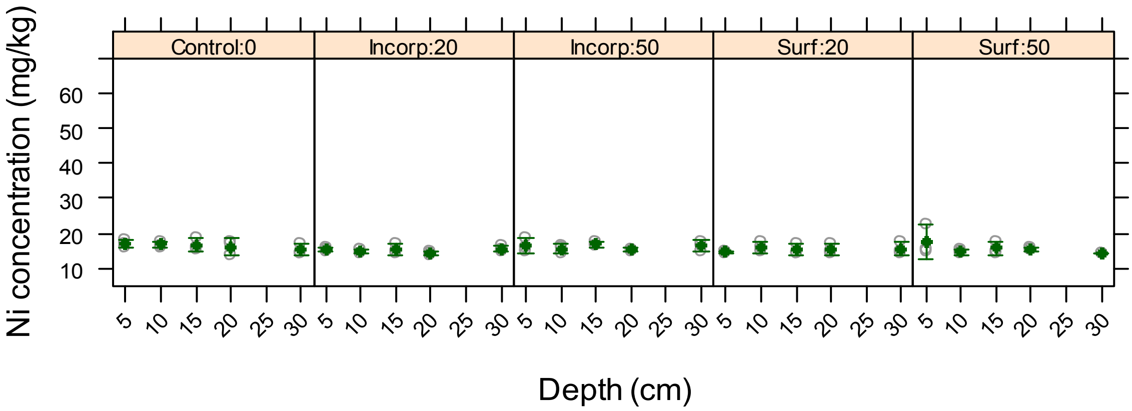

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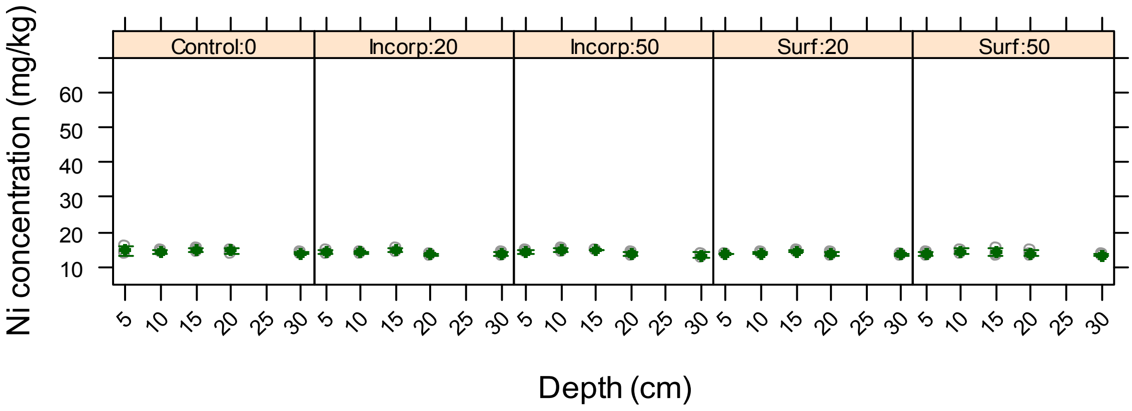
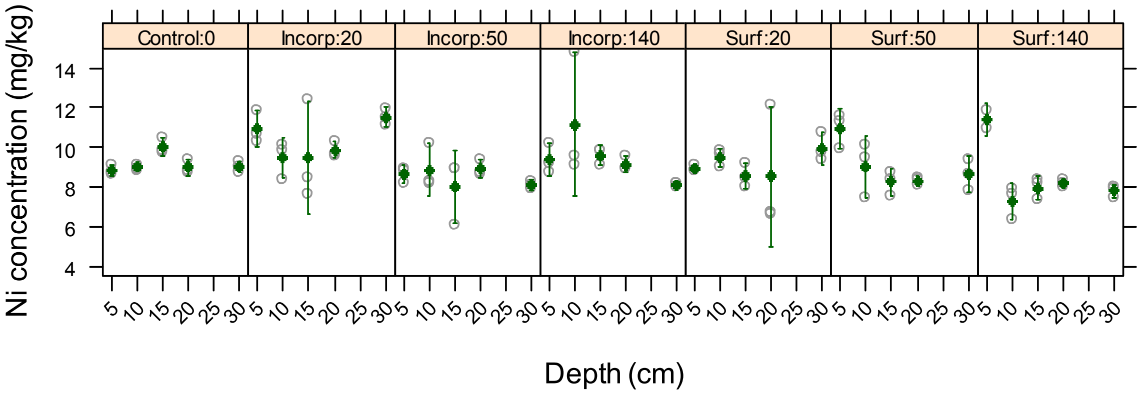


18 month

Figure 2.43. Ni concentration in SL1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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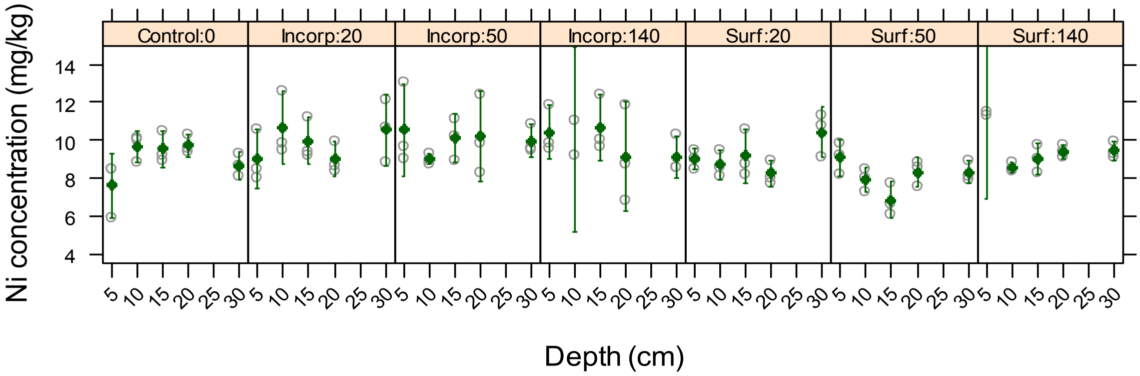

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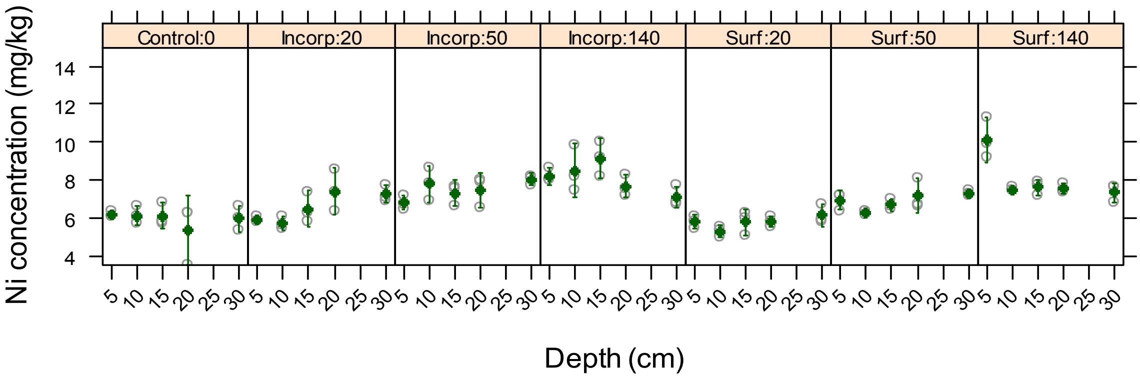
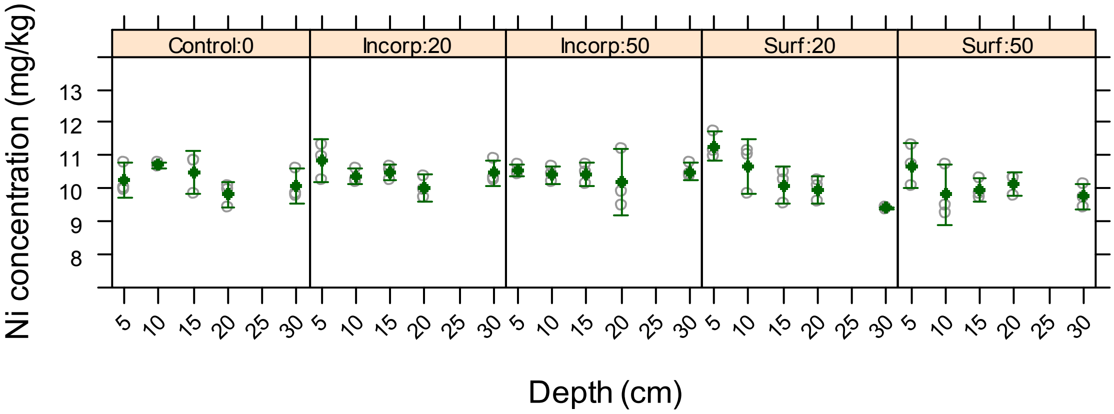


18 month

Figure 2.44. Ni concentration in SL2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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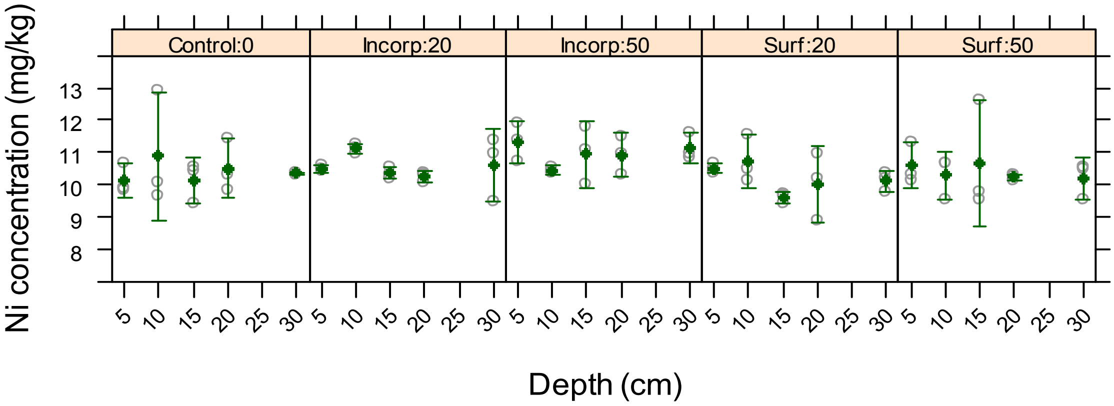
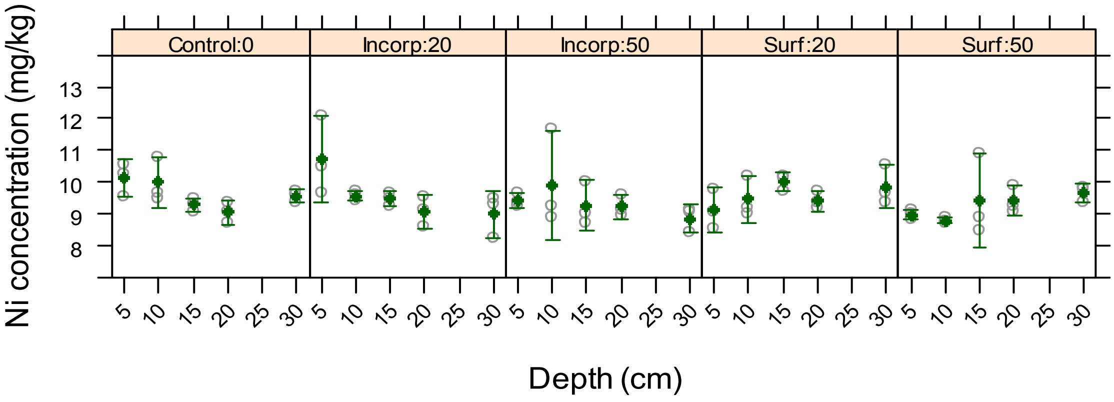
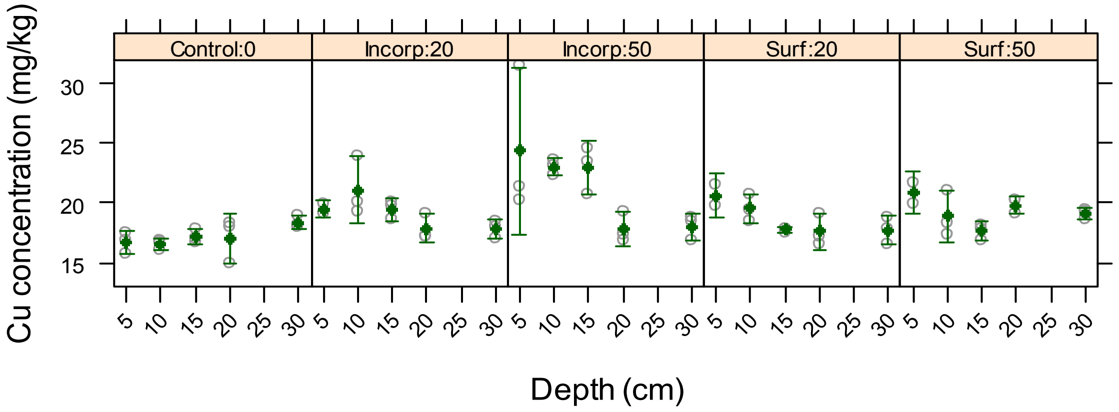


12 month

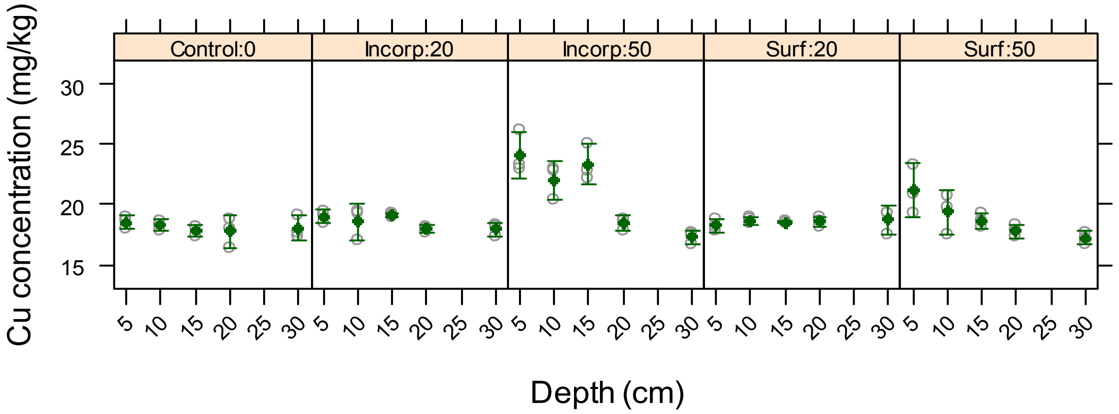


18 month

Figure 2.45. Ni concentration in SL3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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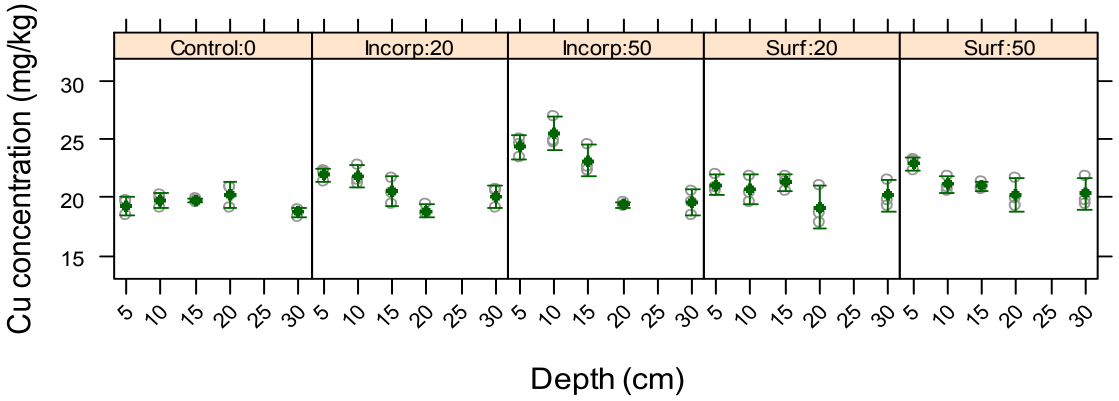
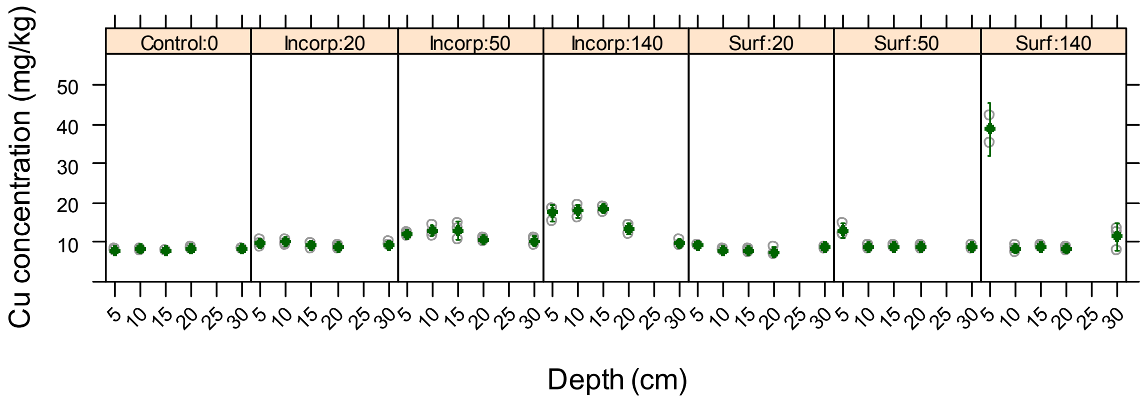


18 month

Figure 2.46. Cu concentration in SL1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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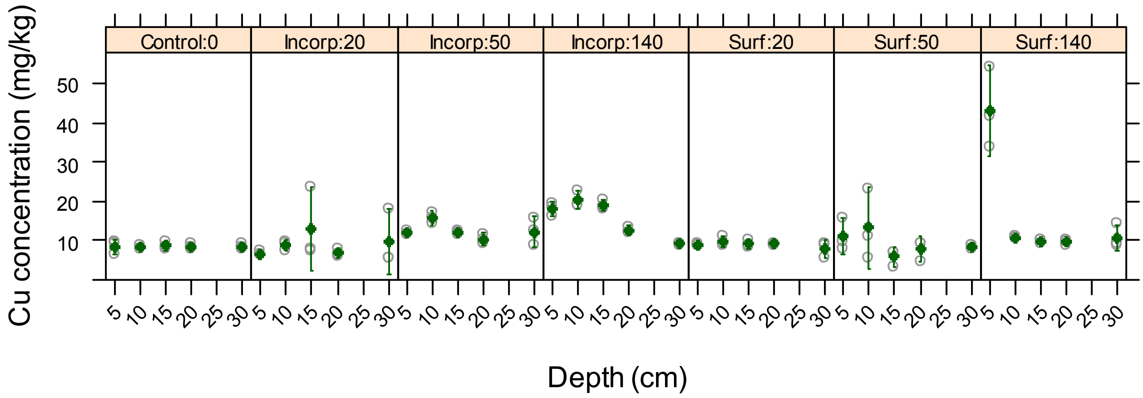

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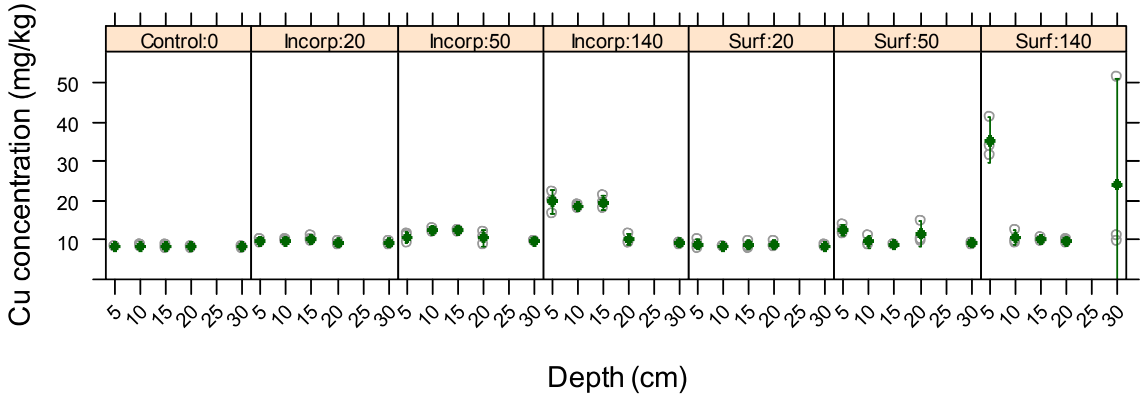
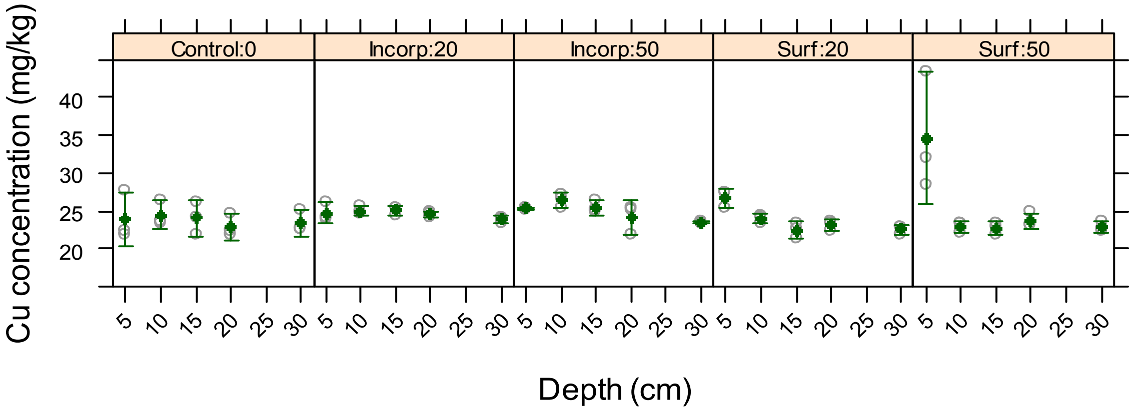


18 month

Figure 2.47. Cu concentration in SL2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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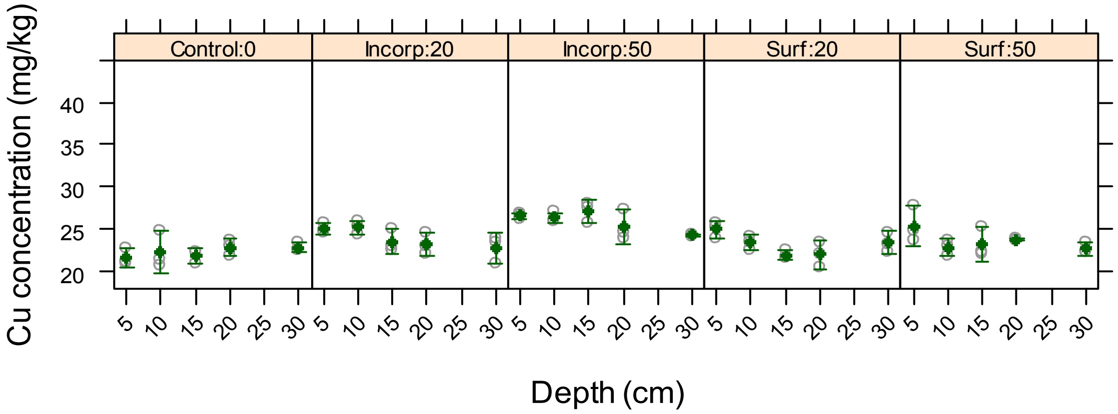
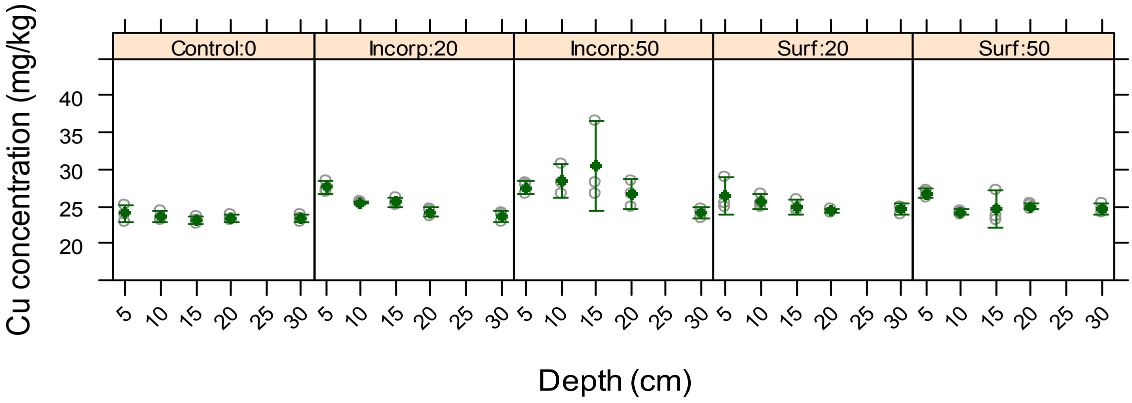
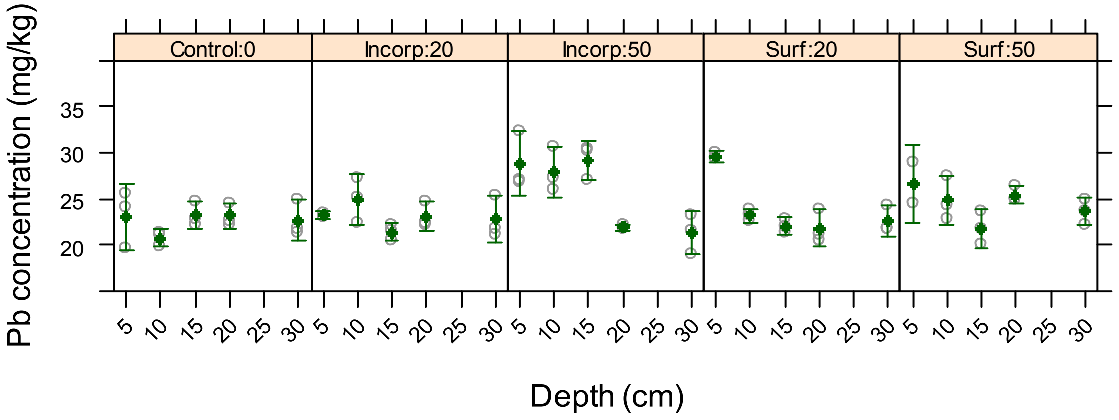


12 month

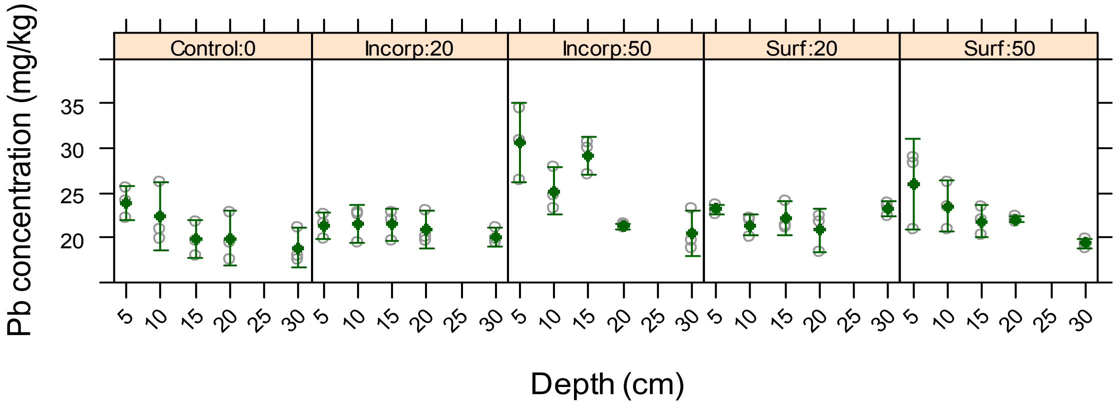


18 month

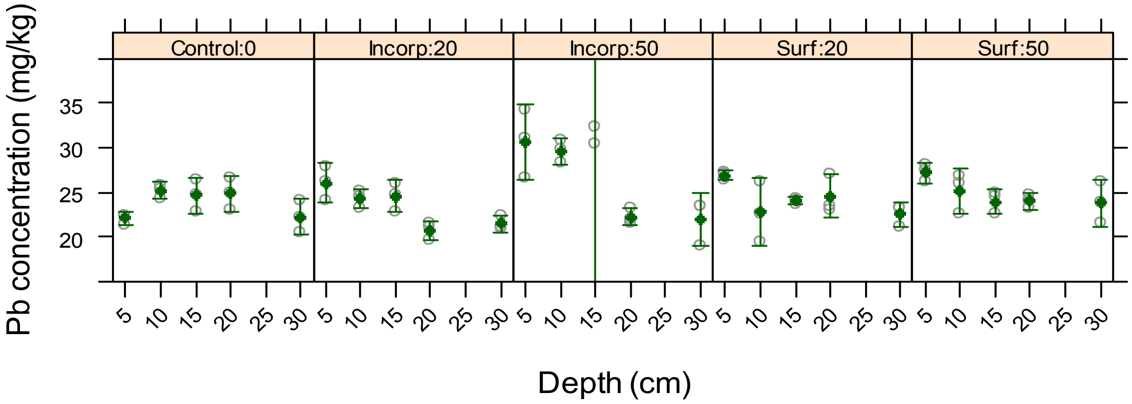
Figure 2.48. Cu concentration in SL3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

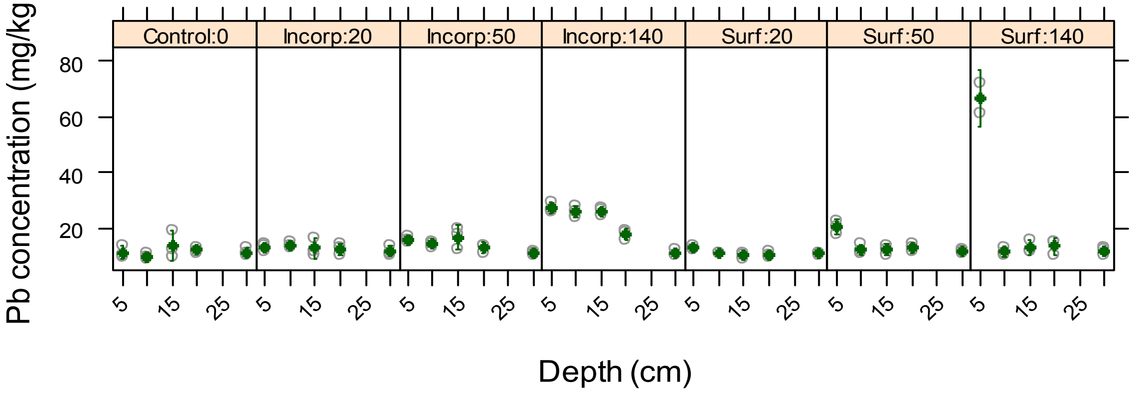


12 month



18 month

Figure 2.49. Pb concentration in SL1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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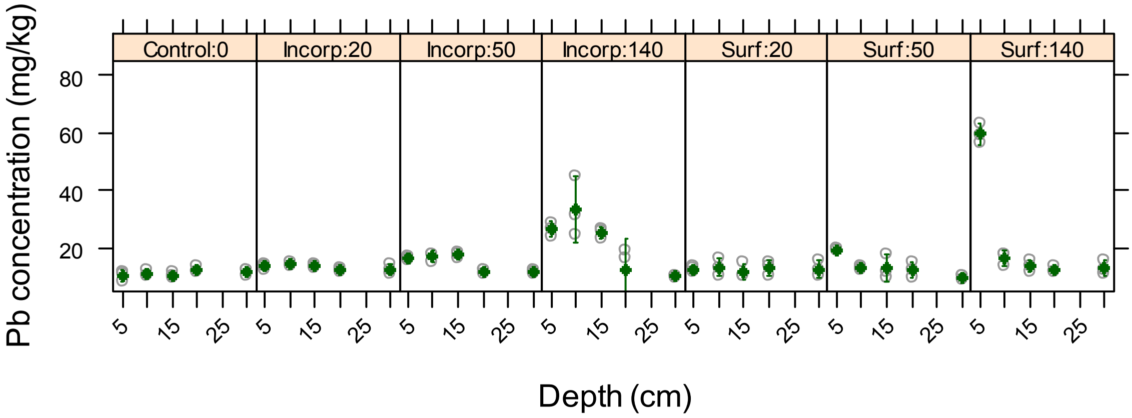

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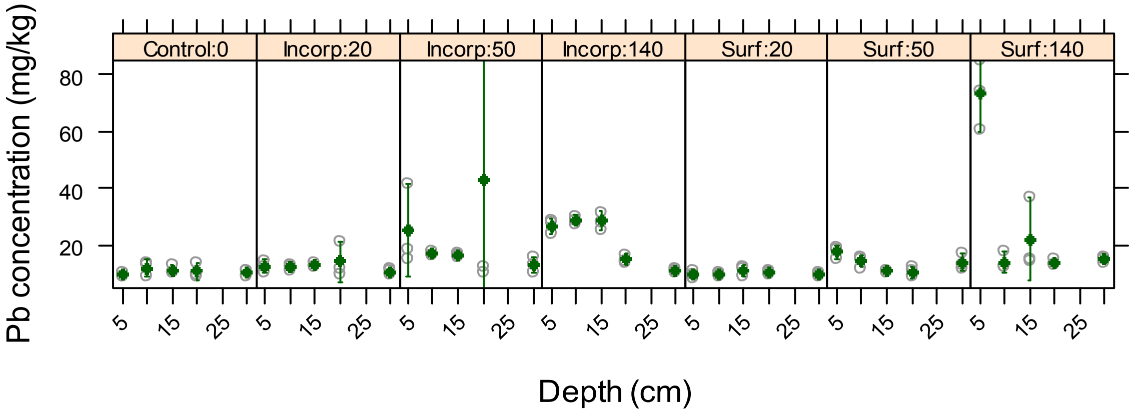
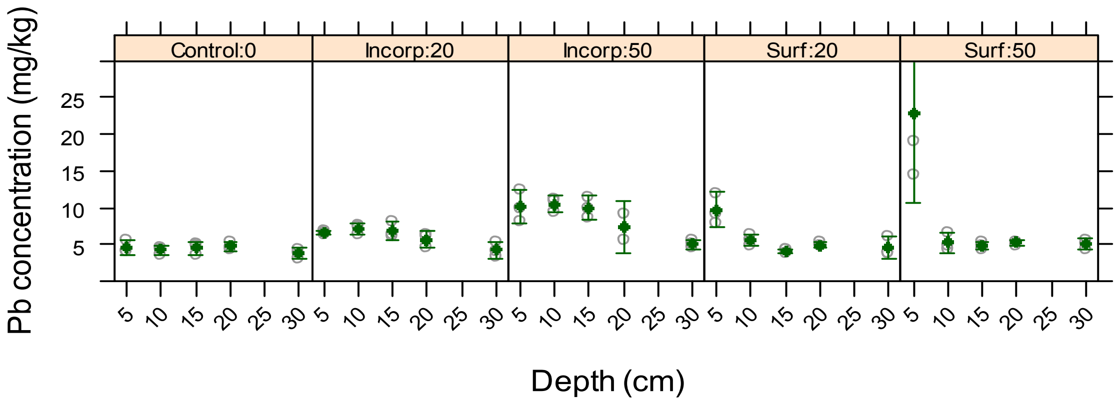


18 month

Figure 2.50. Pb concentration in SL2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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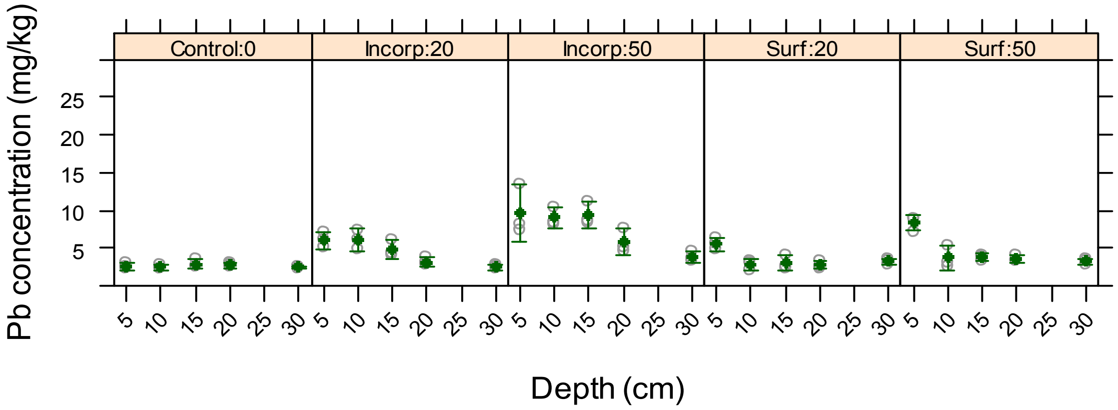
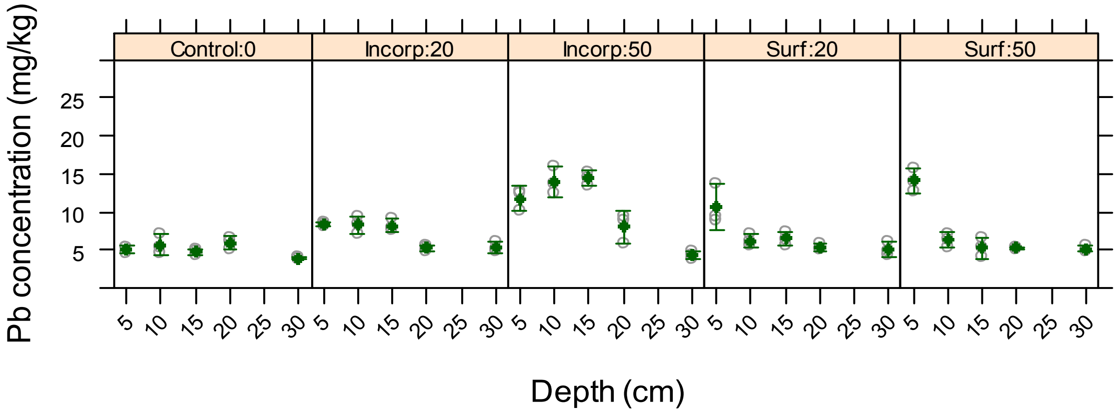
6 month

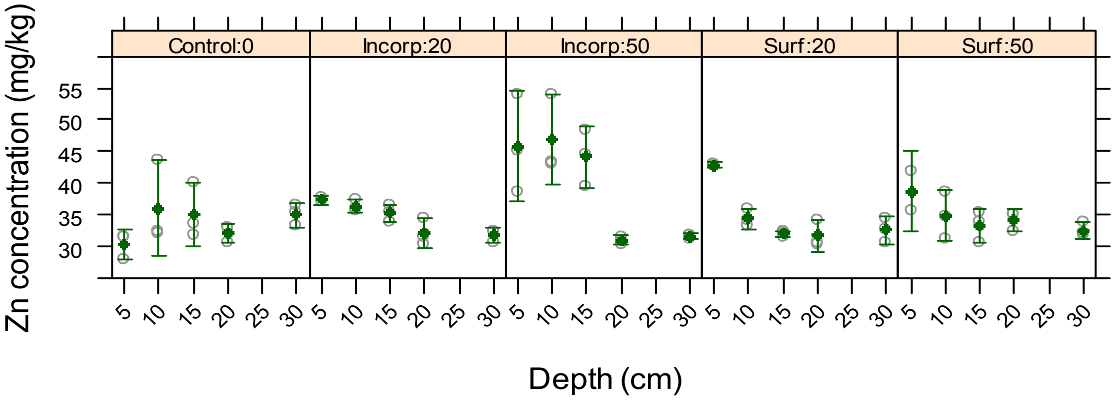


12 month



18 month

Figure 2.51. Pb concentration in SL3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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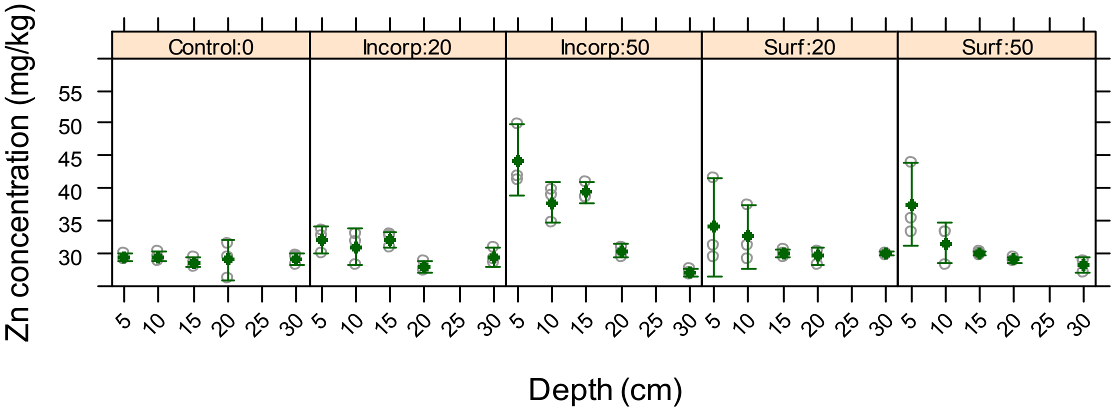

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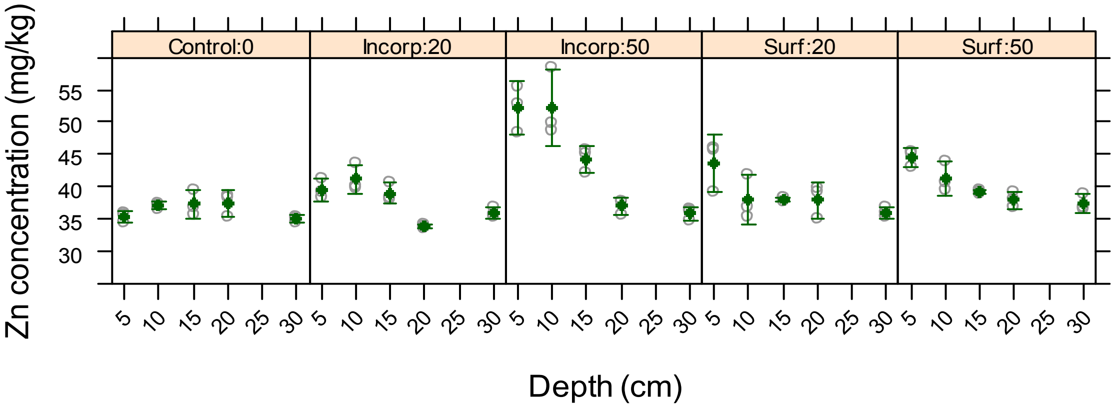
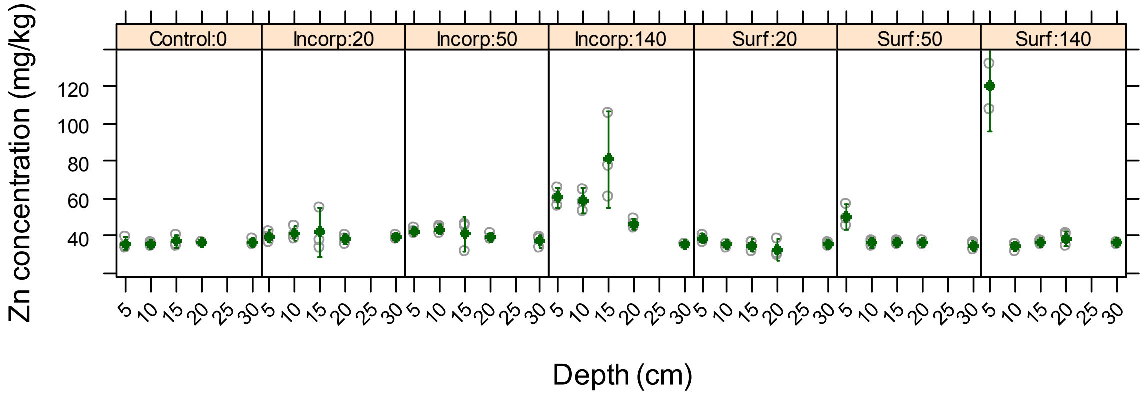


18 month

Figure 2.52. Zn concentration in SL1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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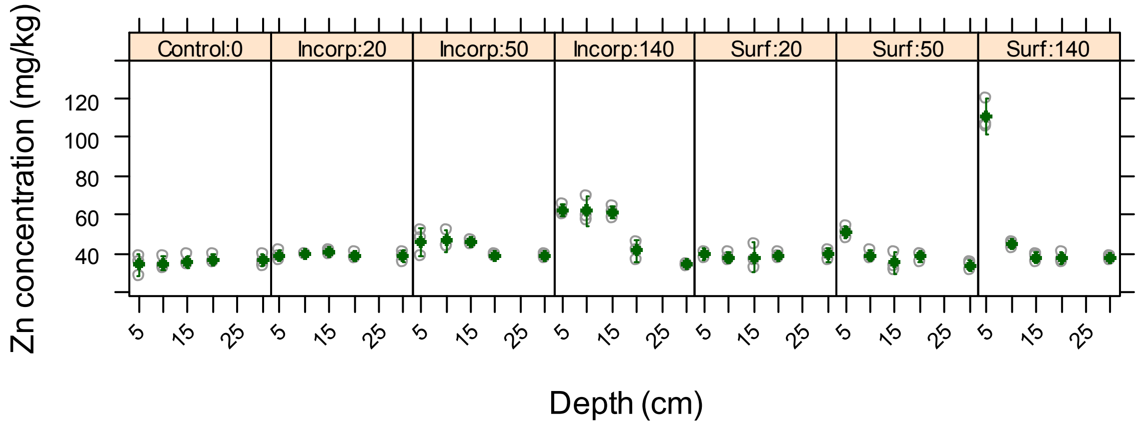

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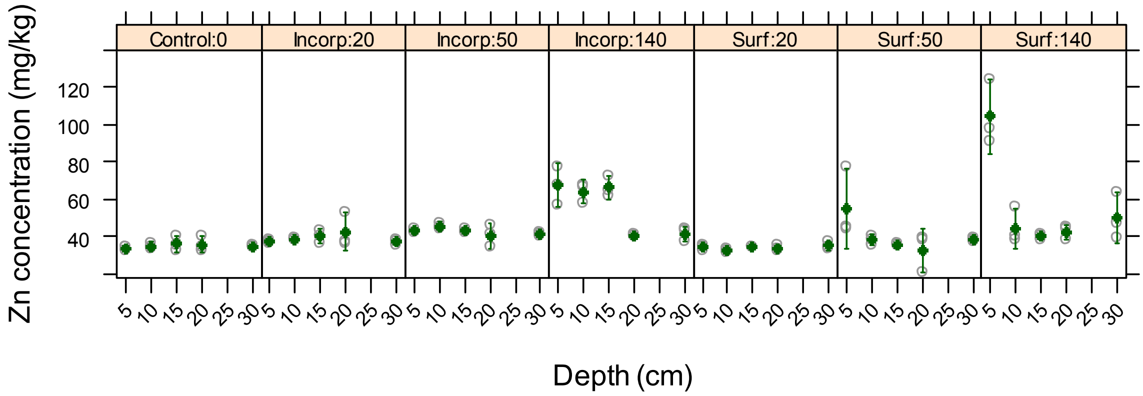
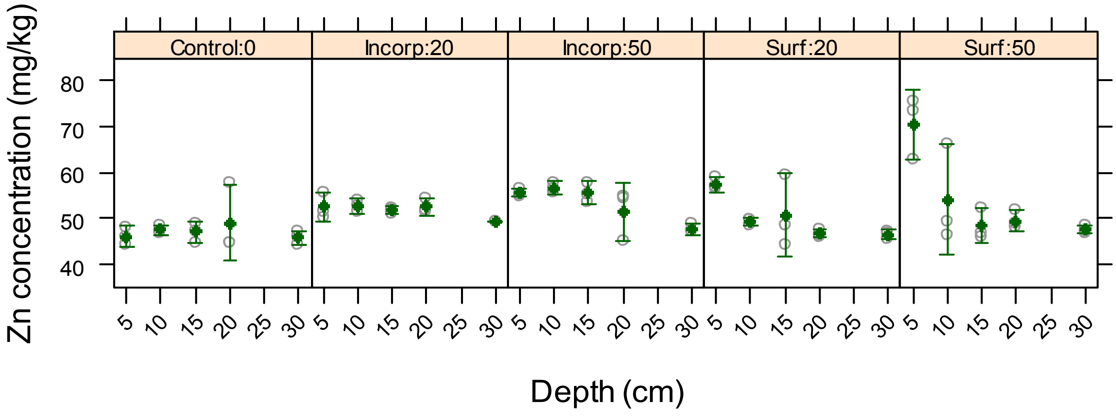
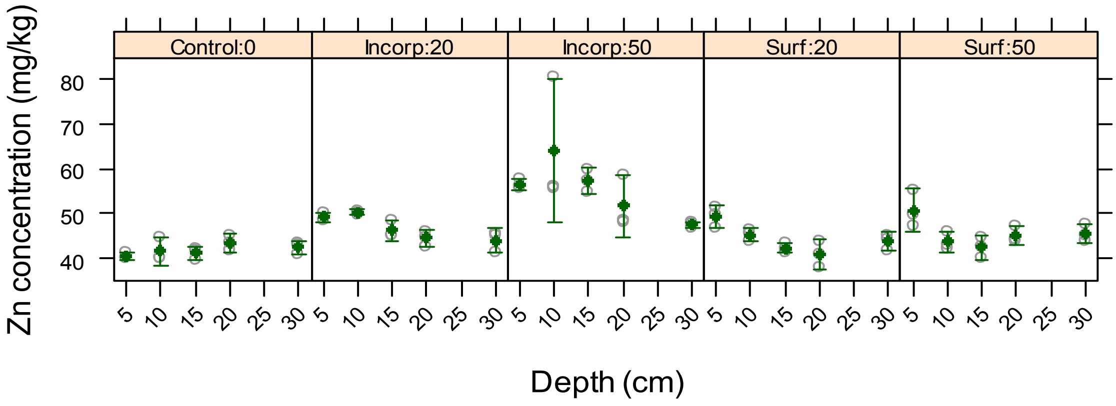


18 month

Figure 2.53. Zn concentration in SL2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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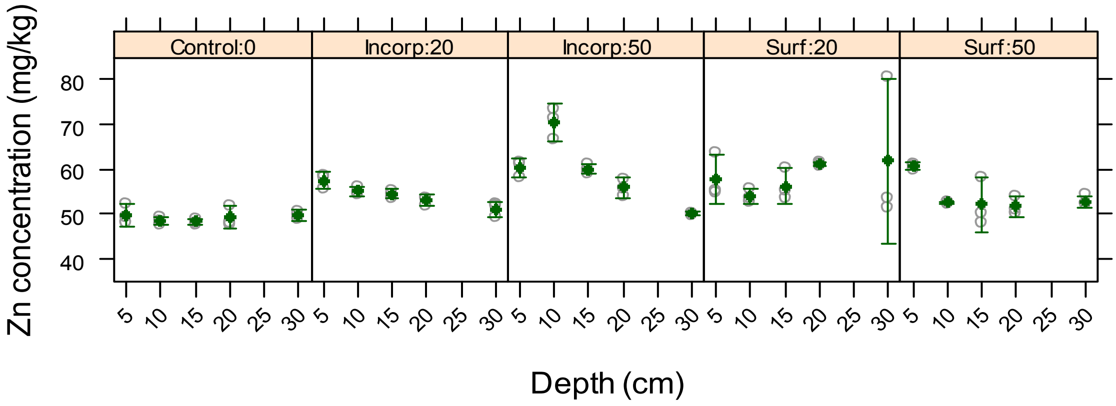
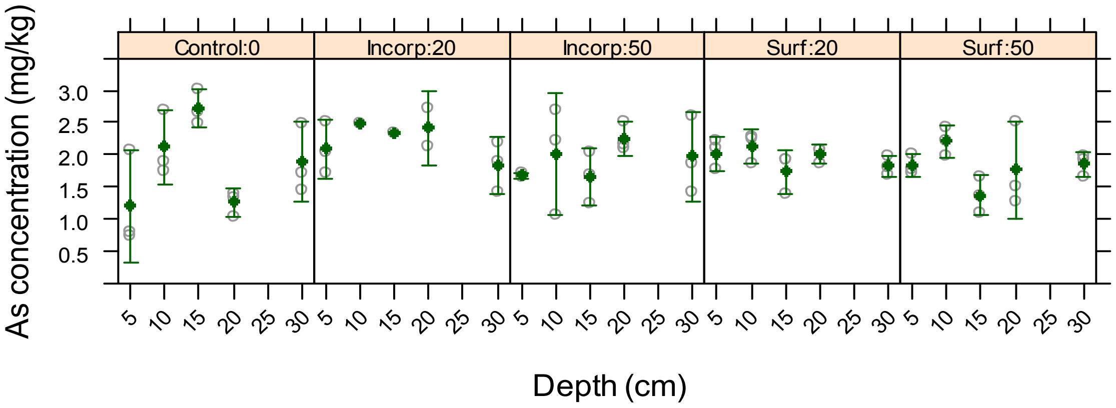


18 month

Figure 2.54. Zn concentration in SL3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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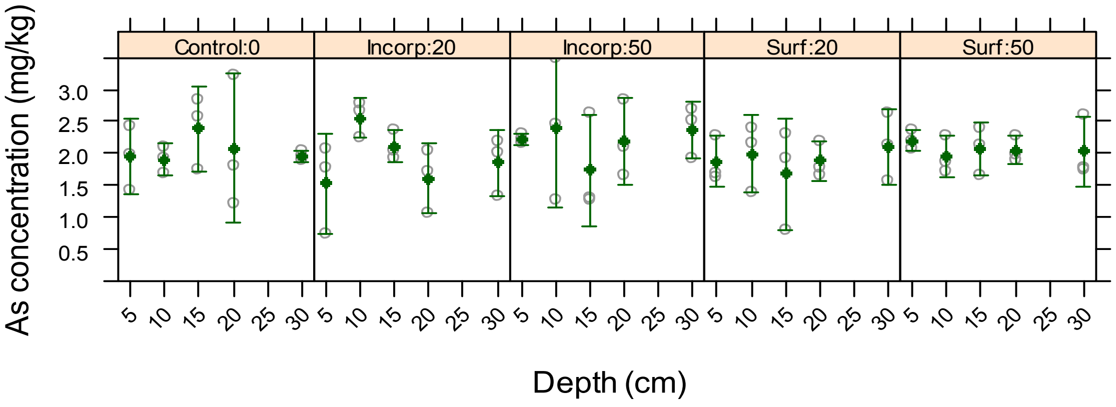

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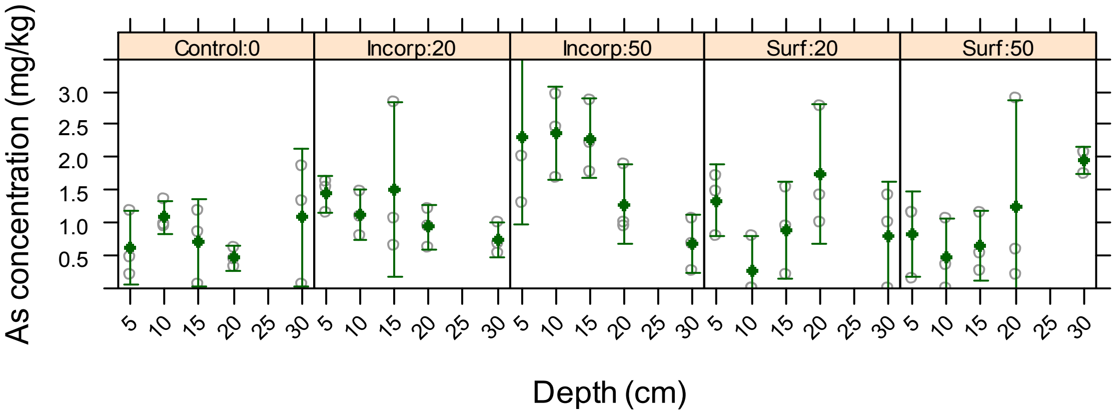
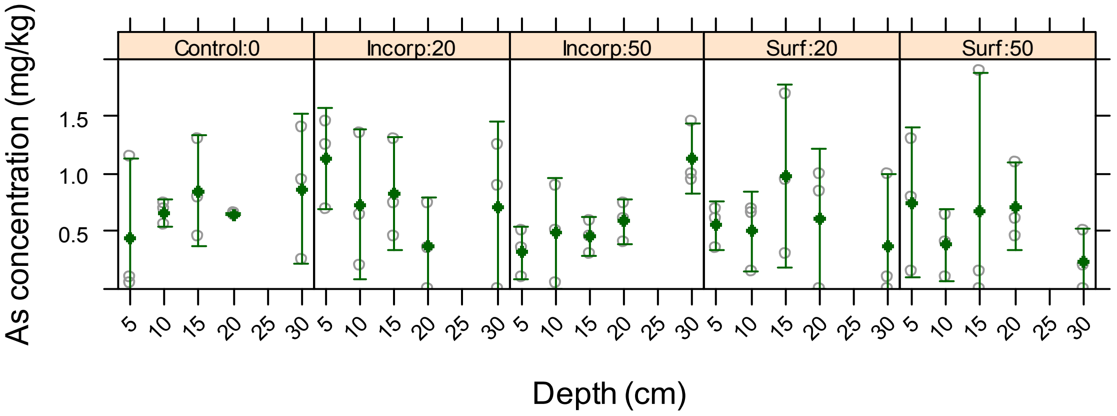
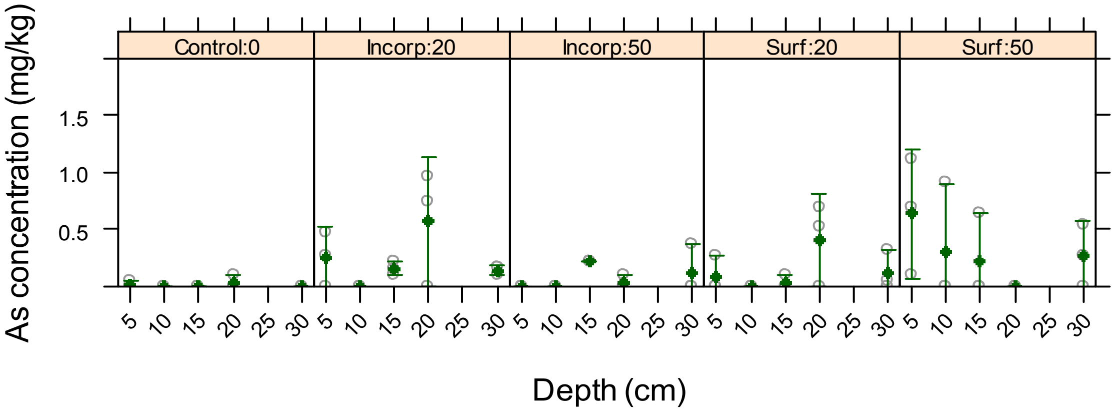


18 month

Figure 2.55. As concentration in C1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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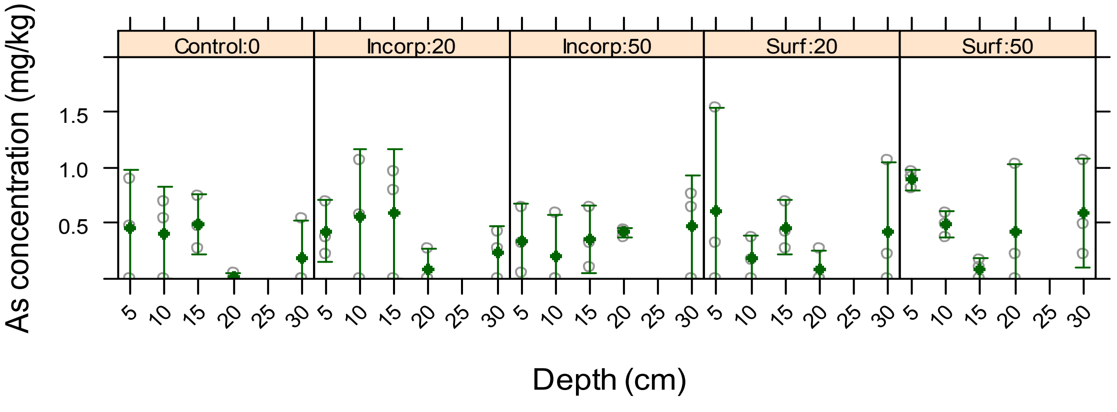
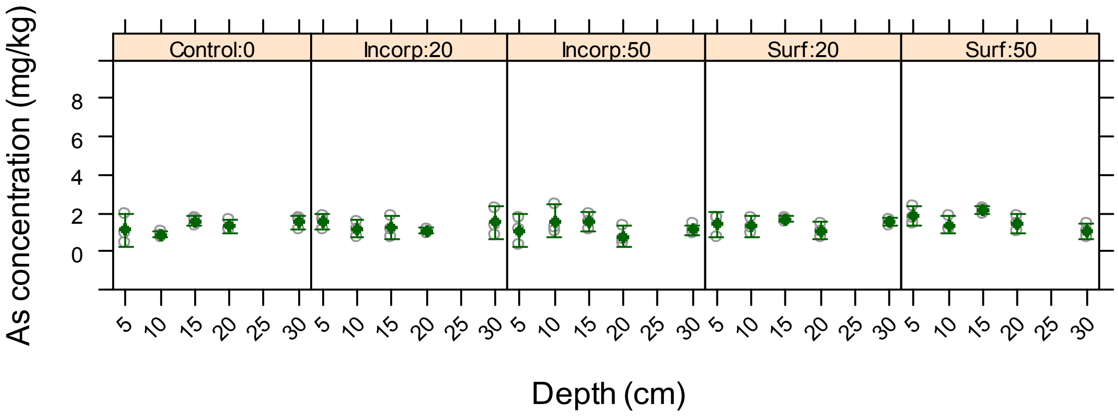
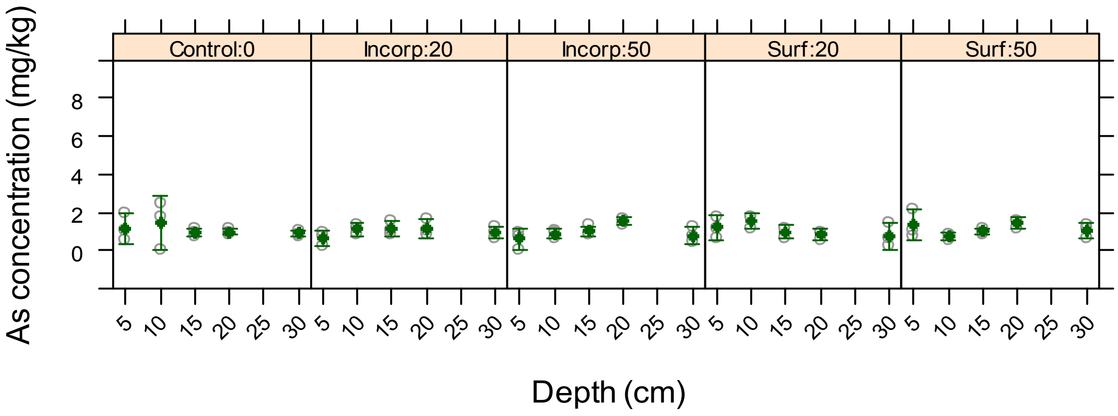


18 month

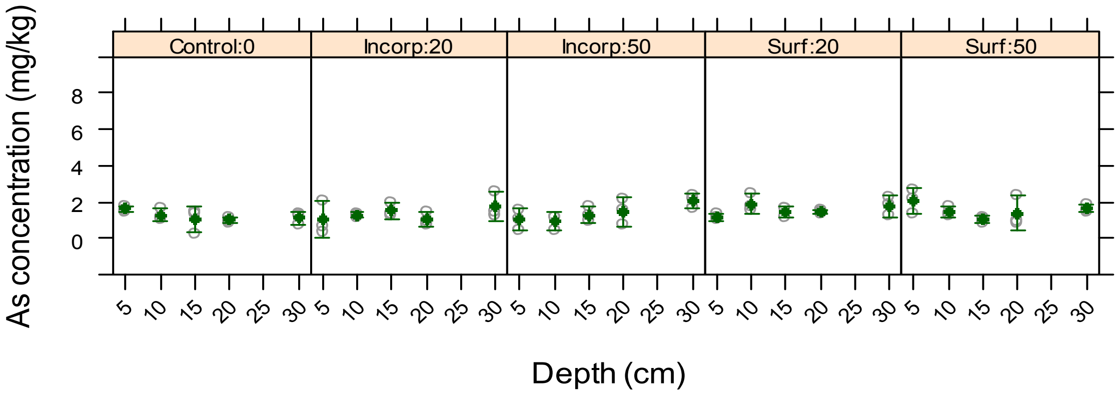
Figure 2.56. As concentration in C2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

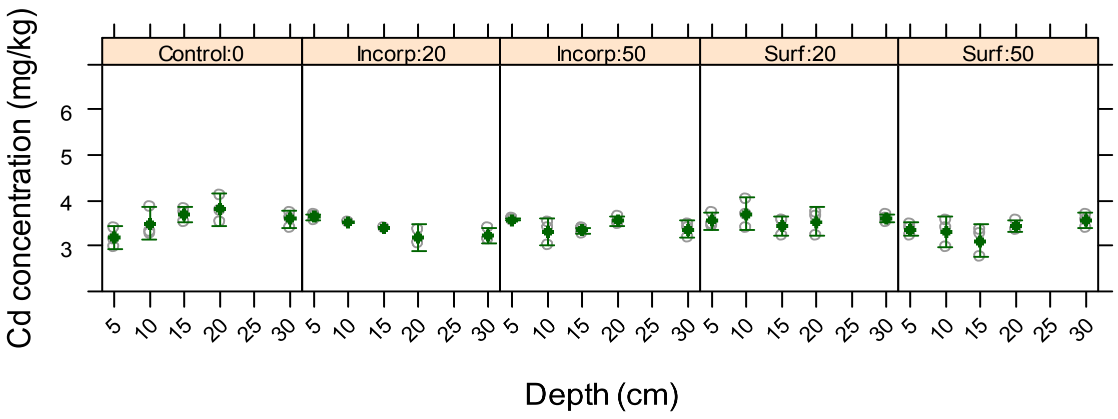


12 month

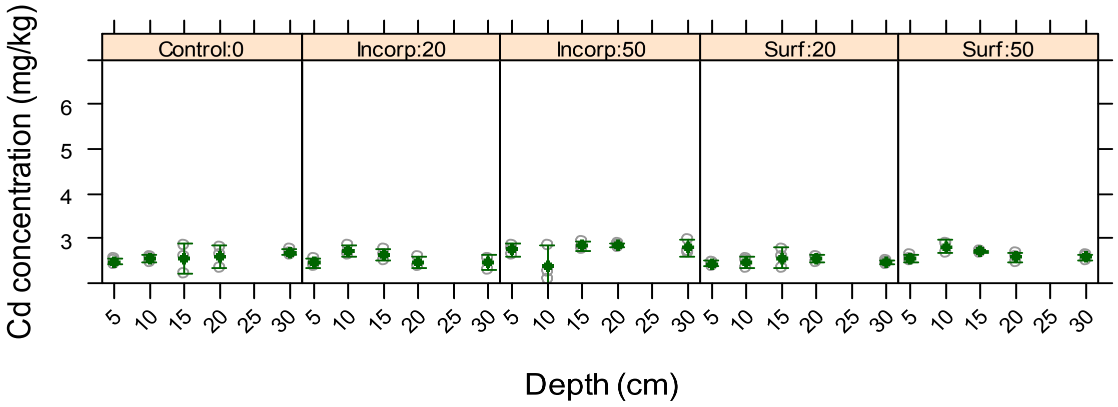


18 month

Figure 2.57. As concentration in C3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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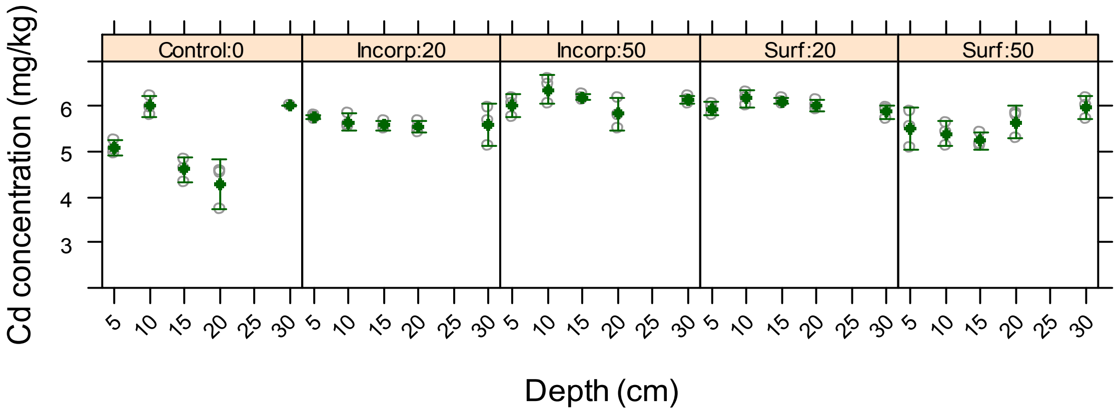
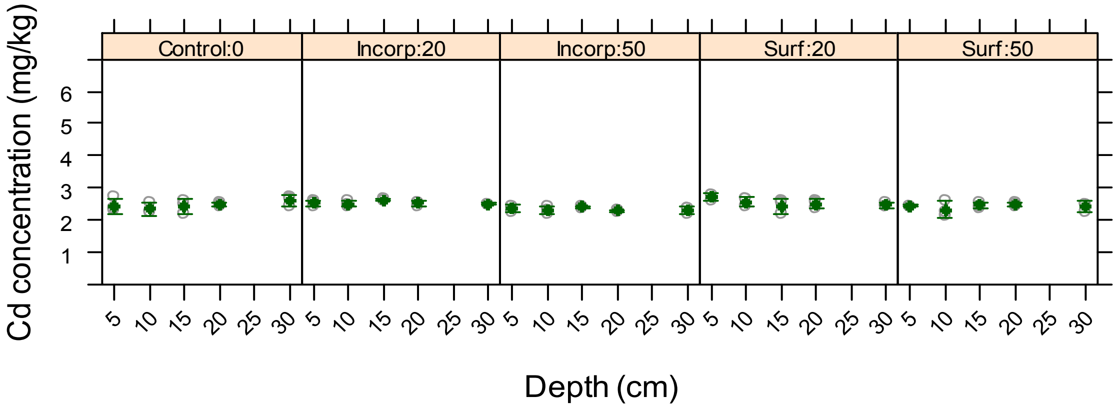
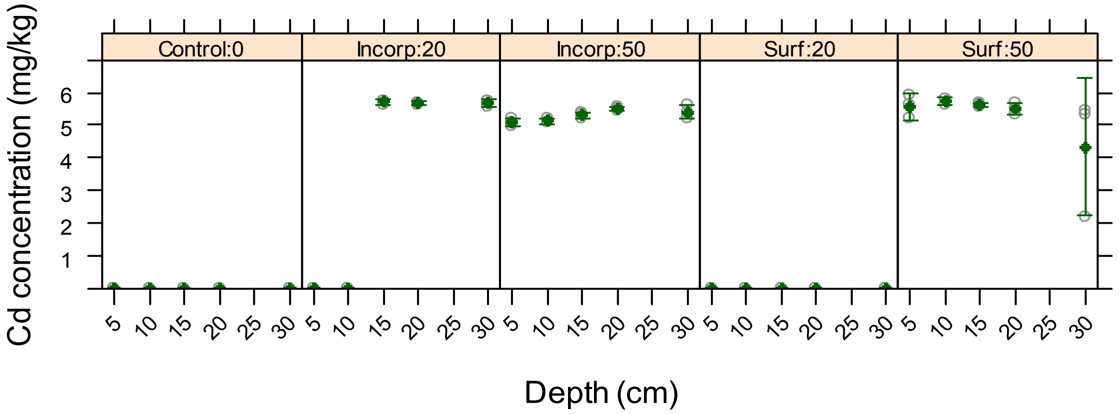


12 month

Figure 2.58. Cd concentration in C1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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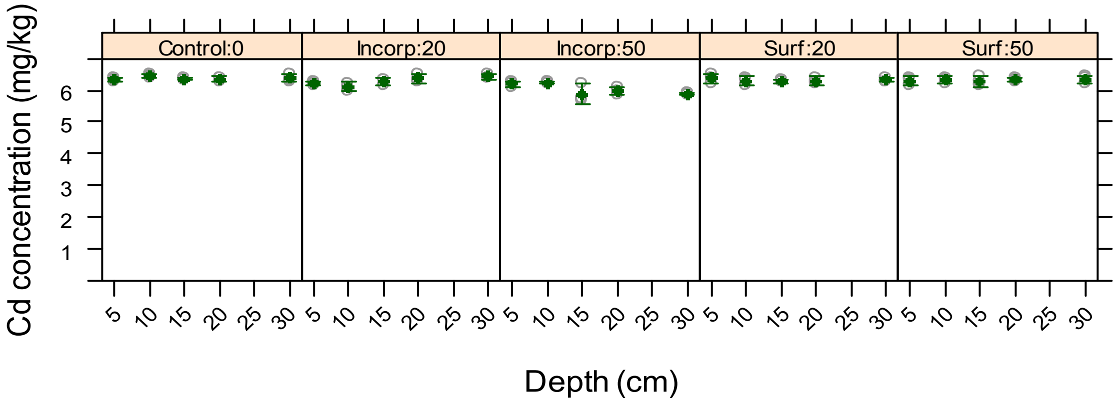
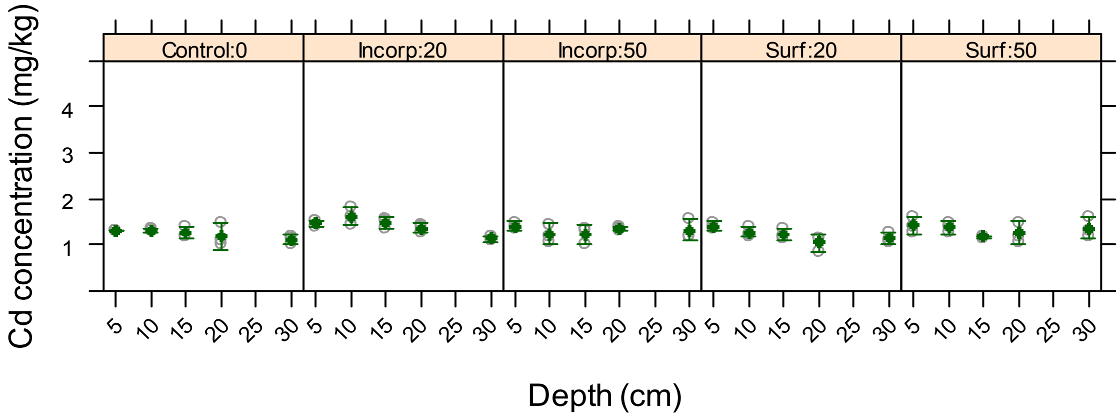
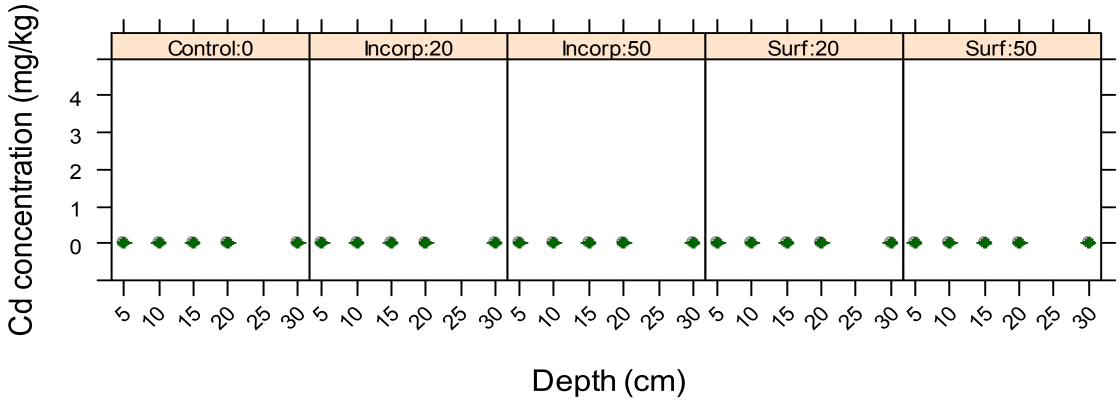


18 month

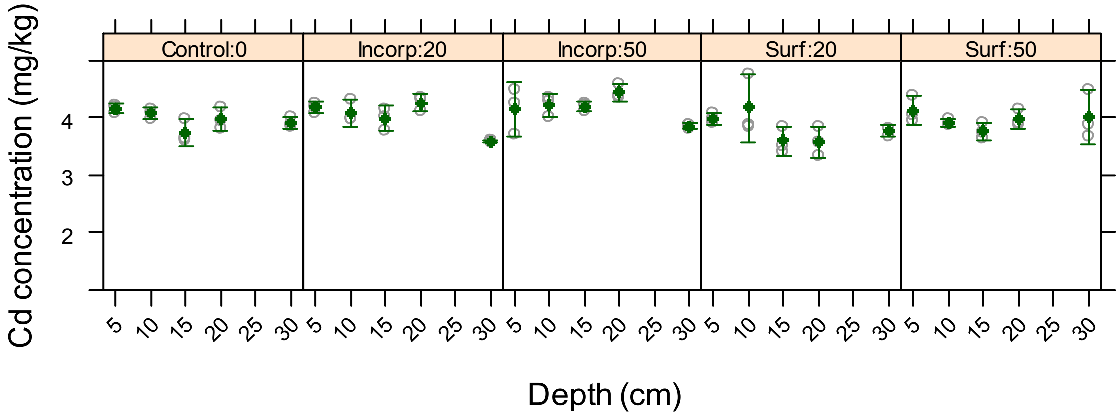
Figure 2.59. Cd concentration in C2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

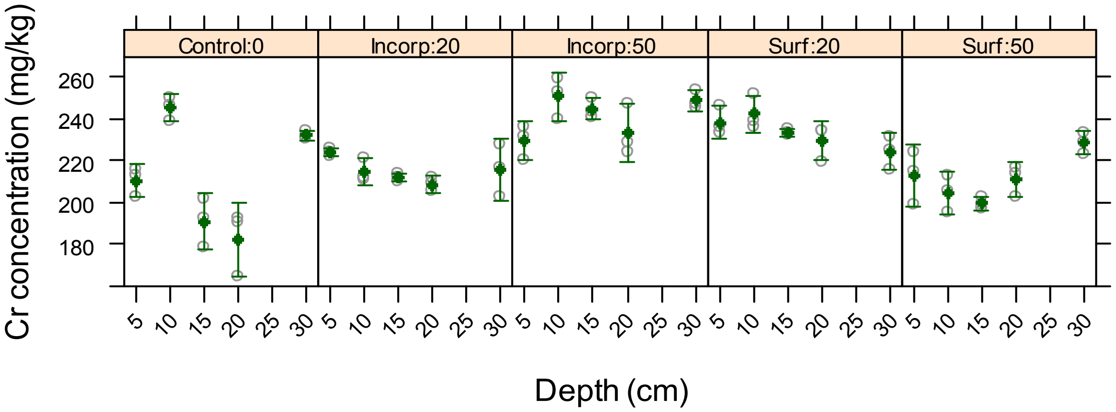


12 month

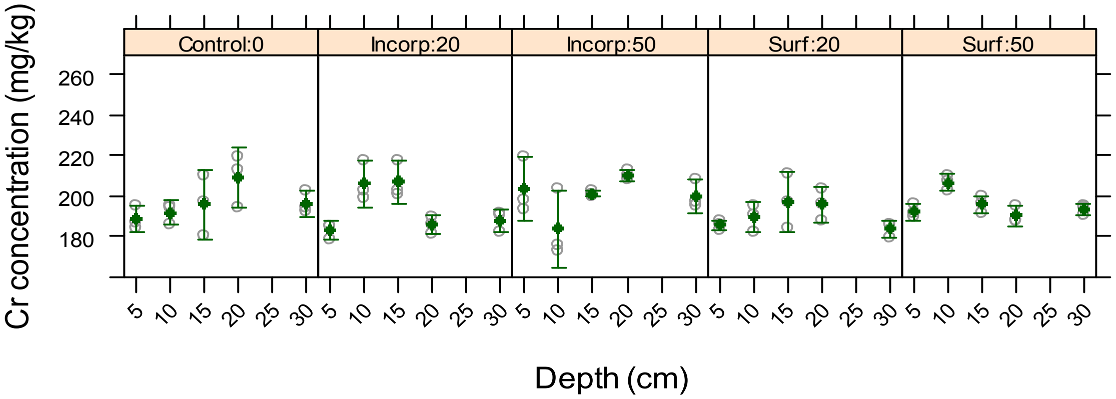


18 month

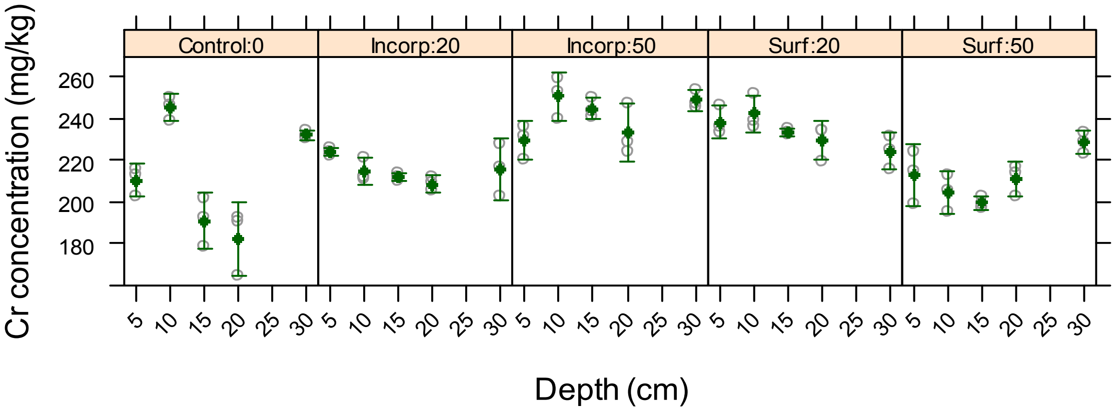
Figure 2.60. Cd concentration in C3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

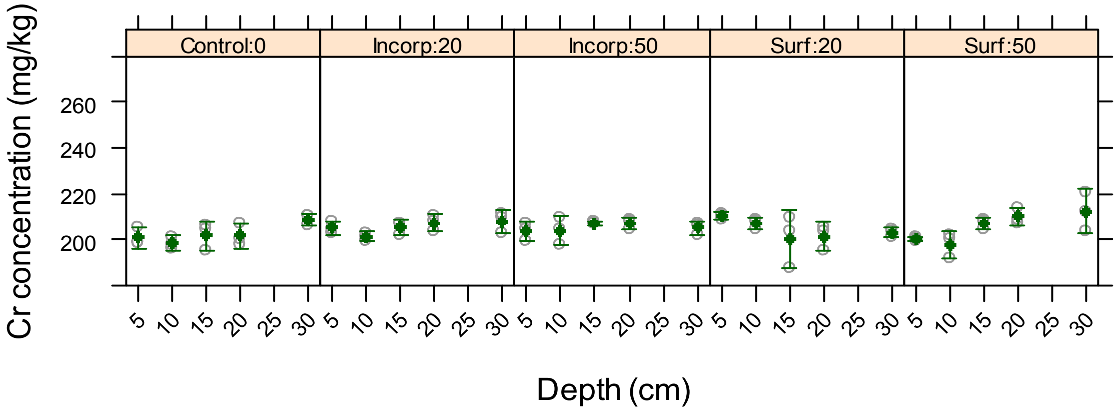


12 month

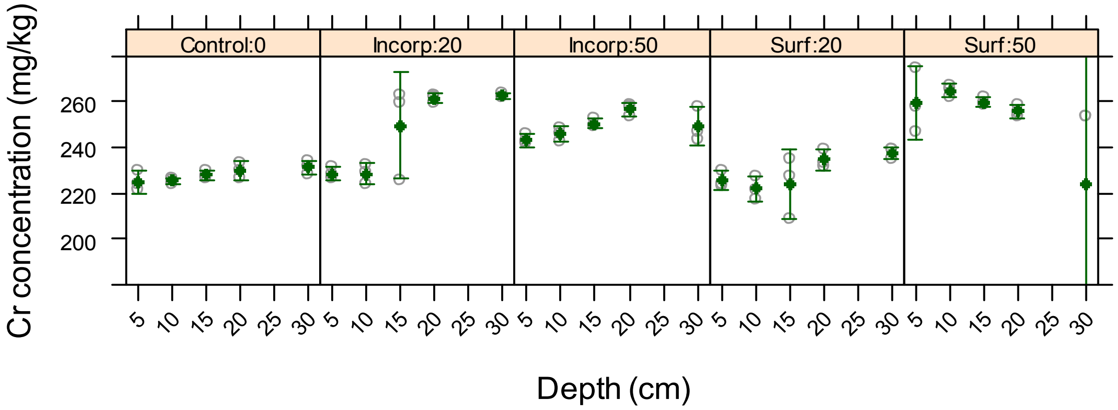


18 month

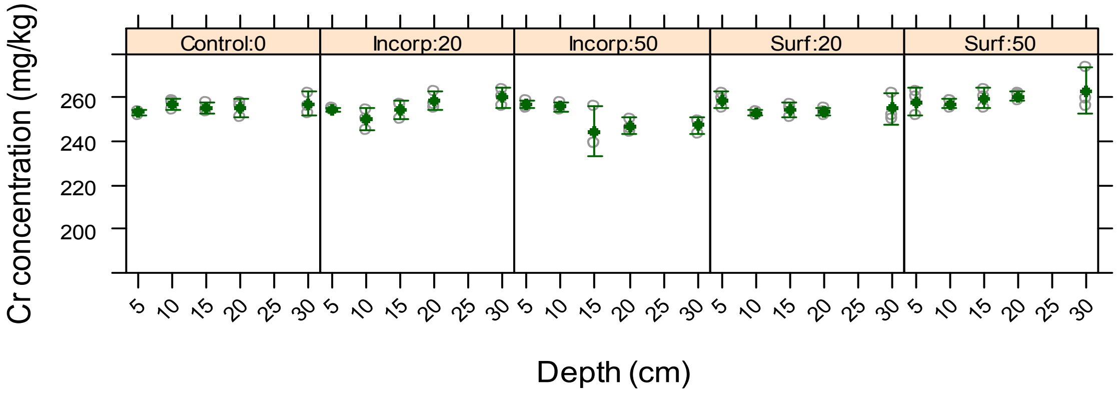
Figure 2.61. Cr concentration in C1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

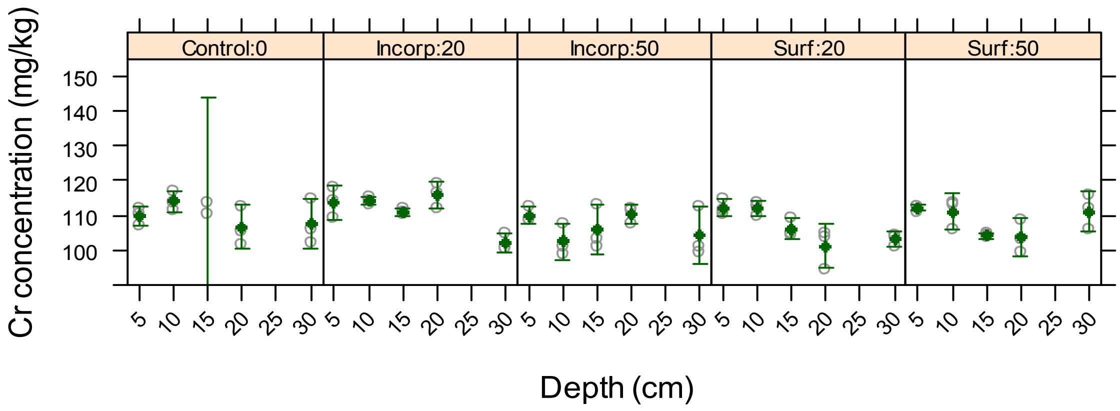


12 month

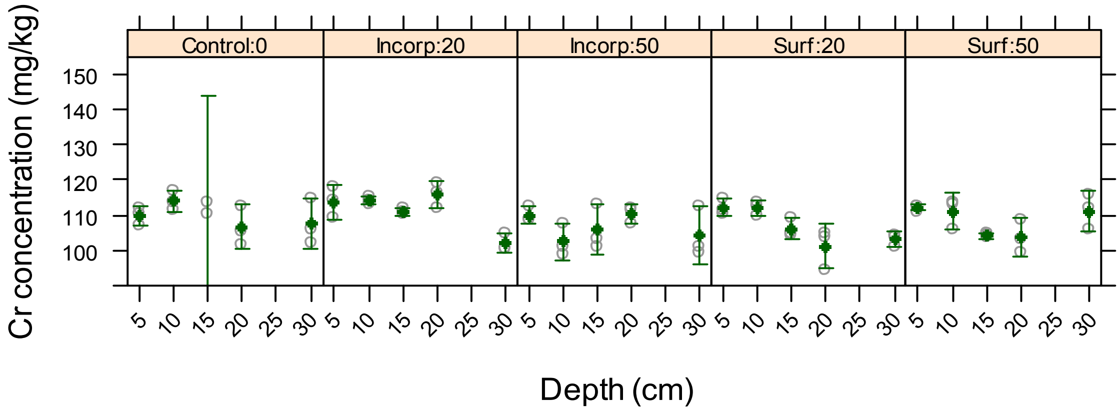


18 month

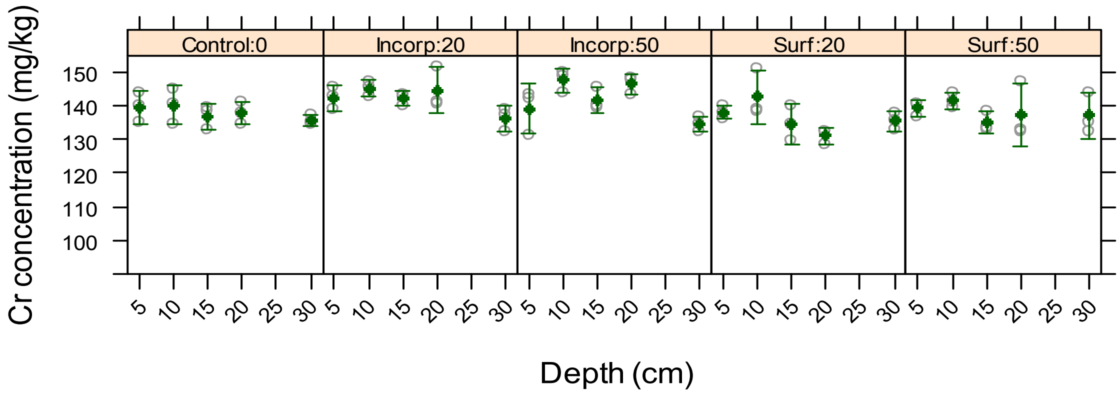
Figure 2.62. Cr concentration in C2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

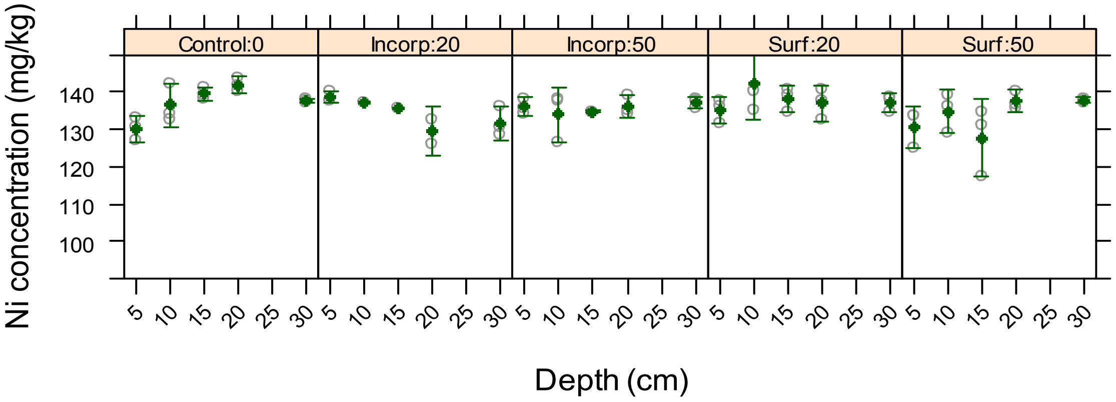


12 month



18 month

Figure 2.63. Cr concentration in C3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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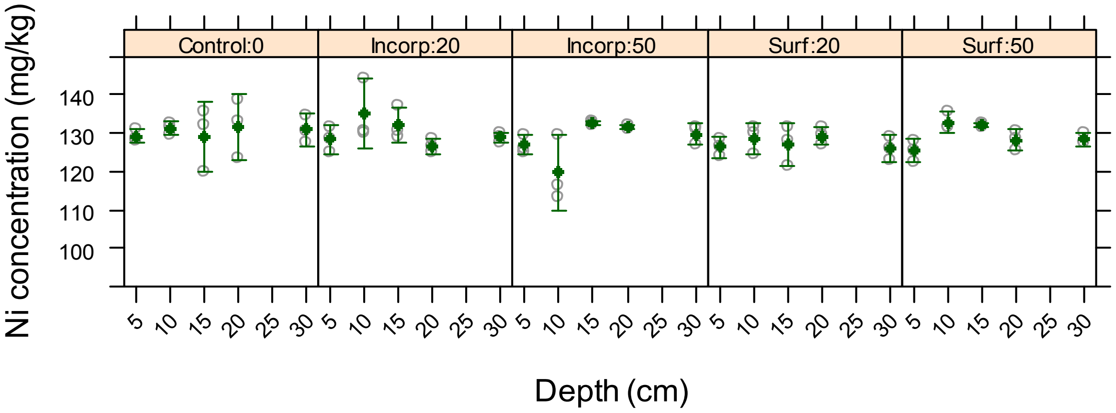

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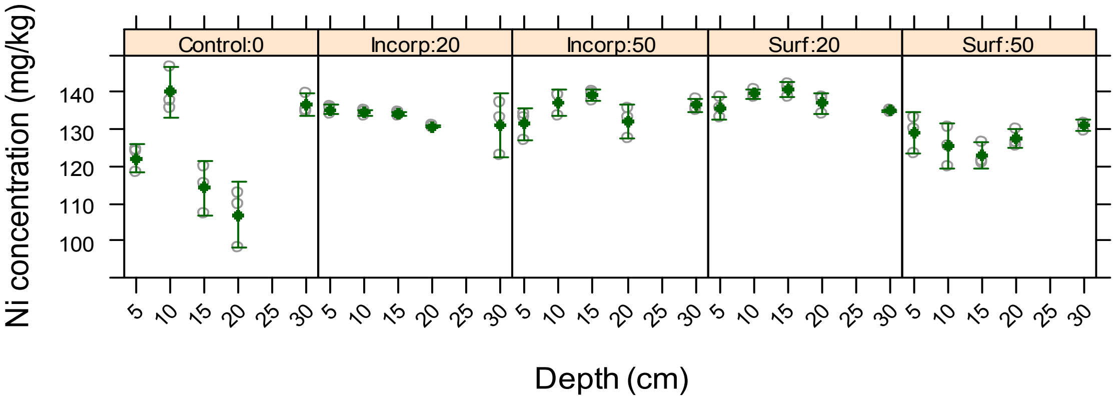
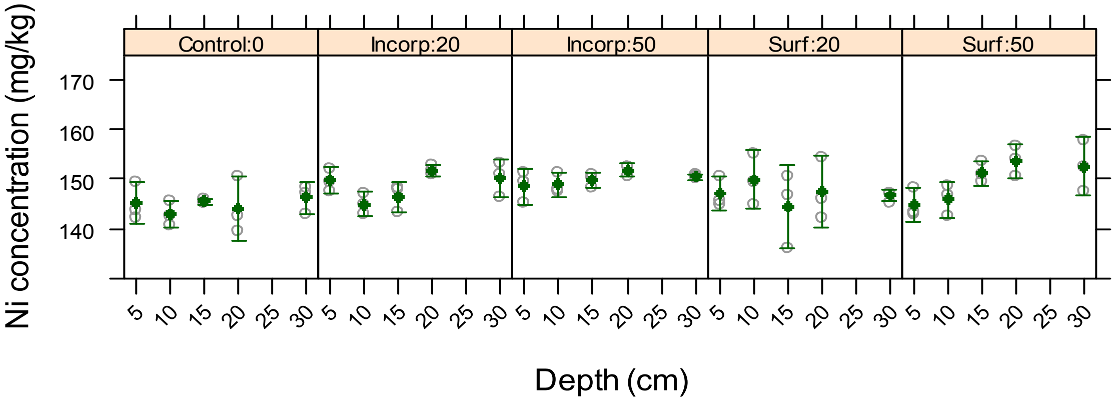
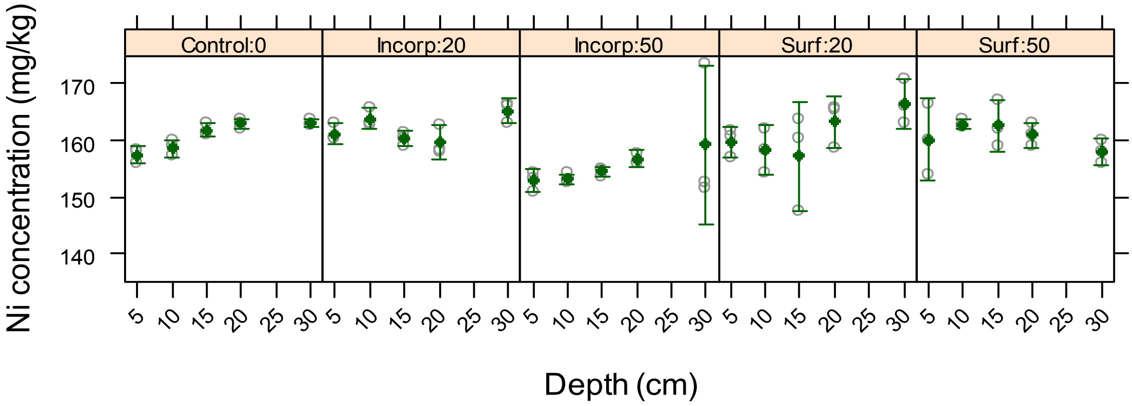


18 month

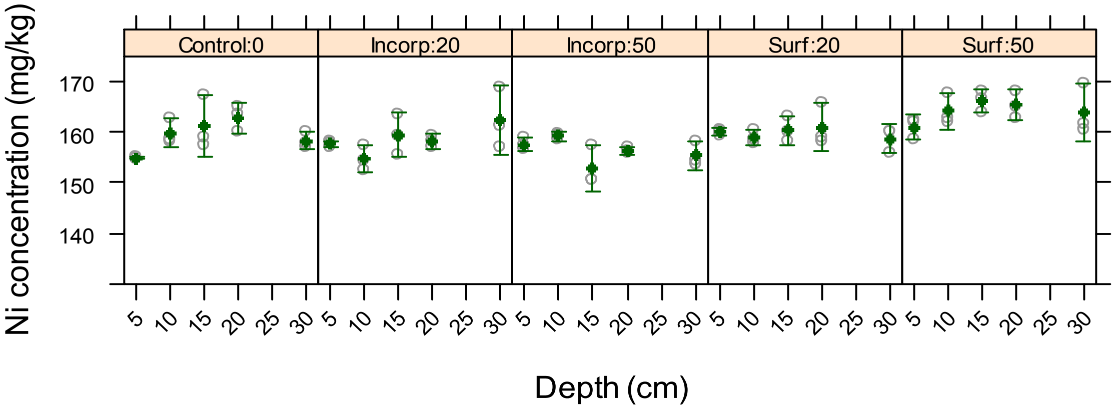
Figure 2.64. Ni concentration in C1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

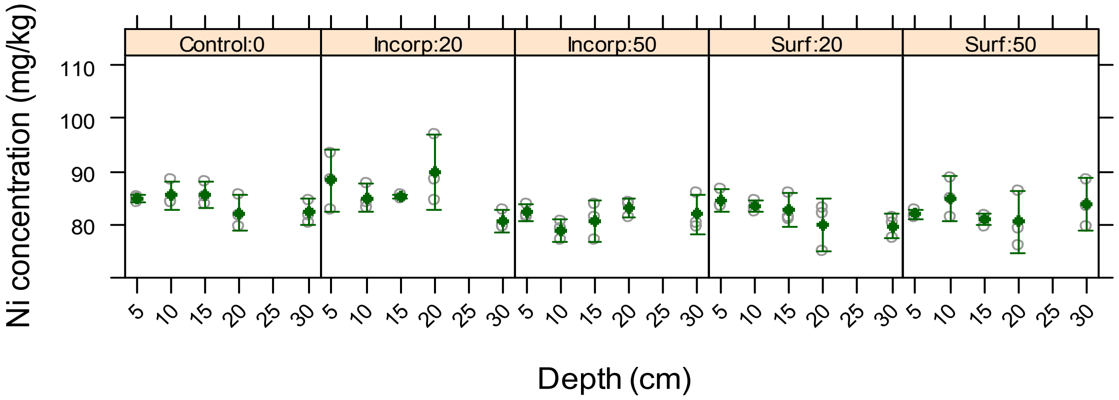


12 month



18 month

Figure 2.65. Ni concentration in C2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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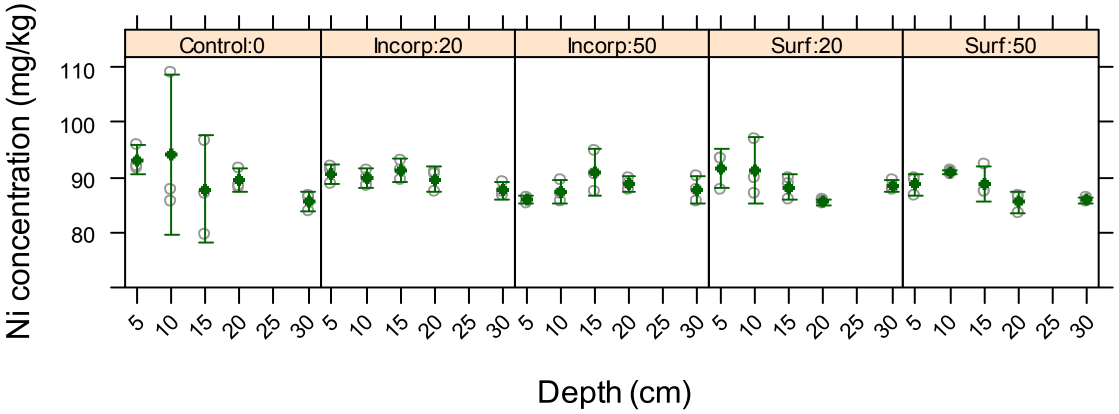

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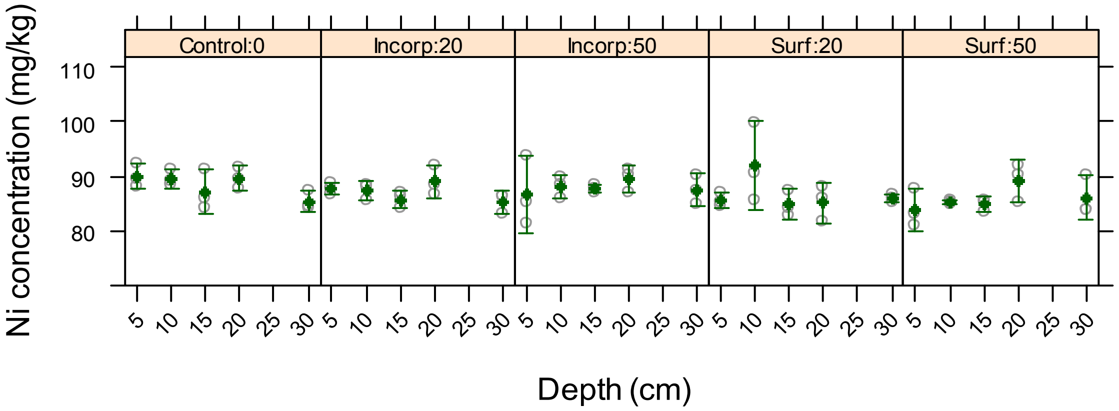


18 month

Figure 2.66. Ni concentration in C3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals

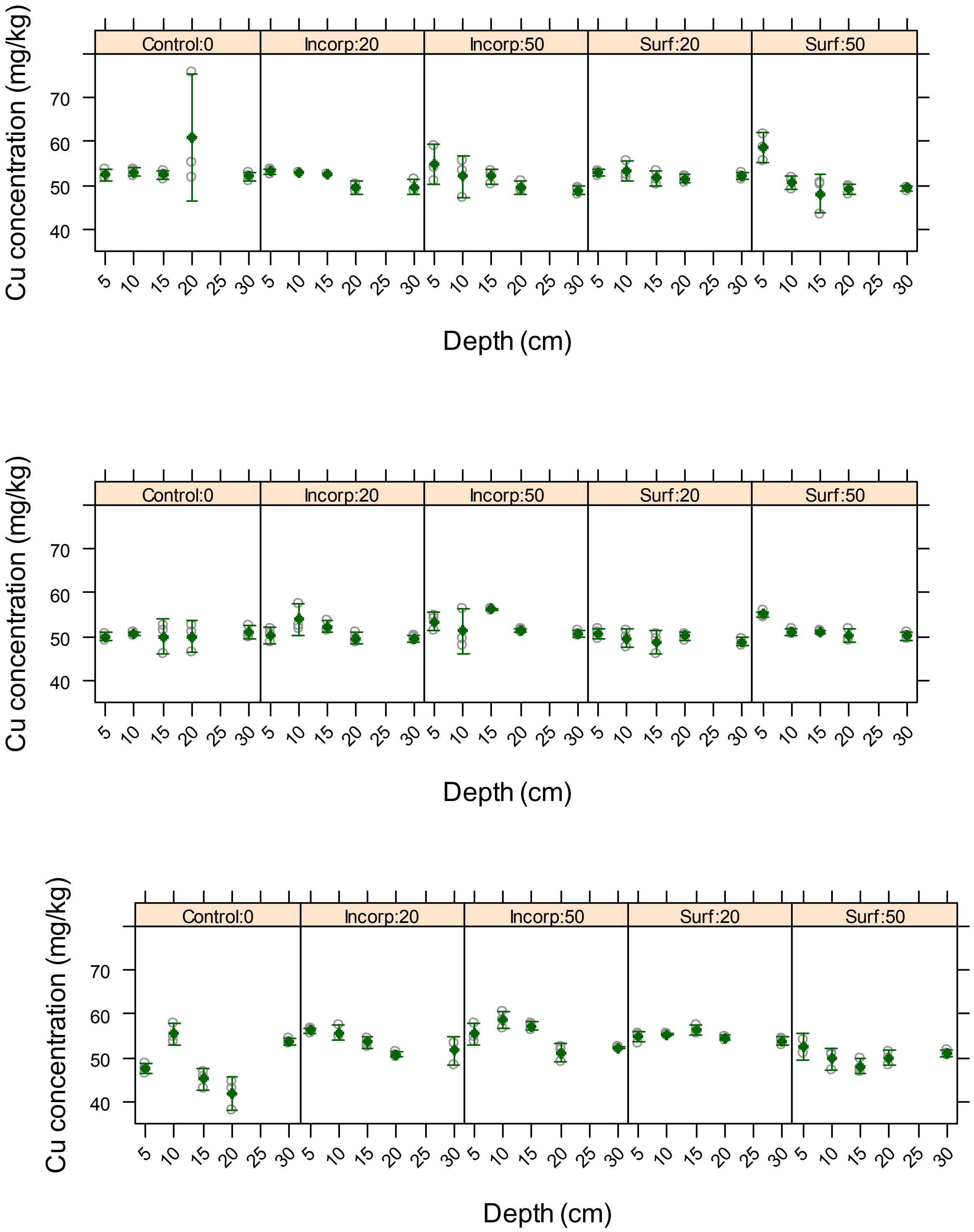
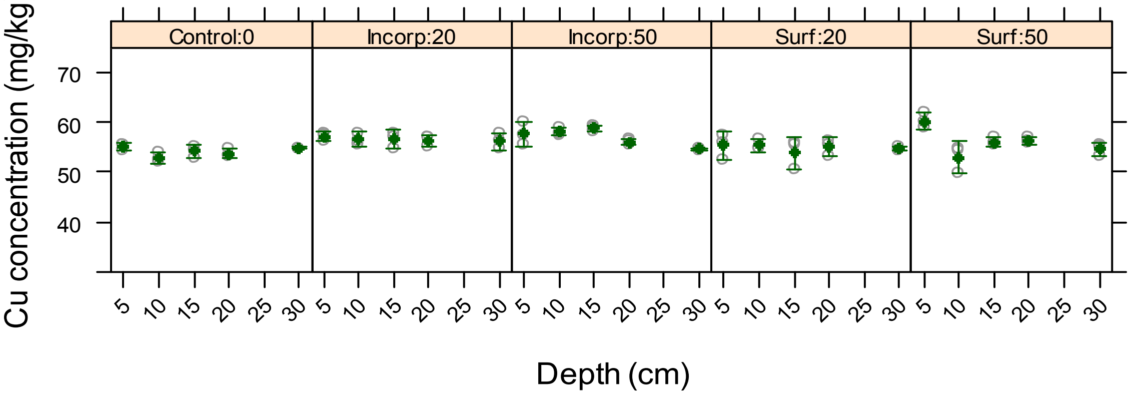


Figure 2.67. Cu concentration in C1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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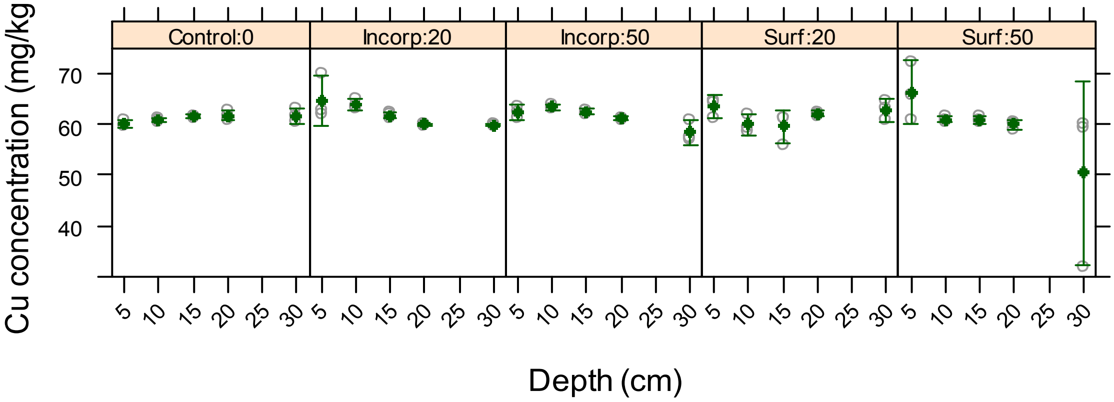
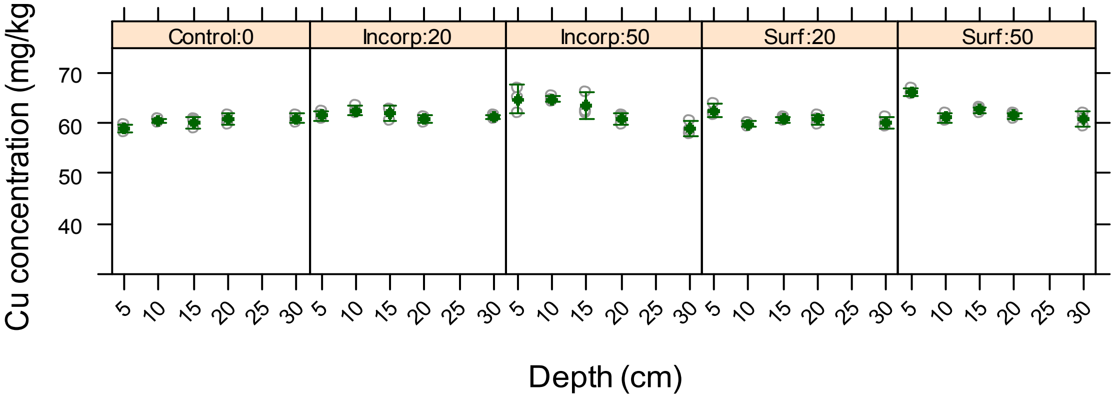
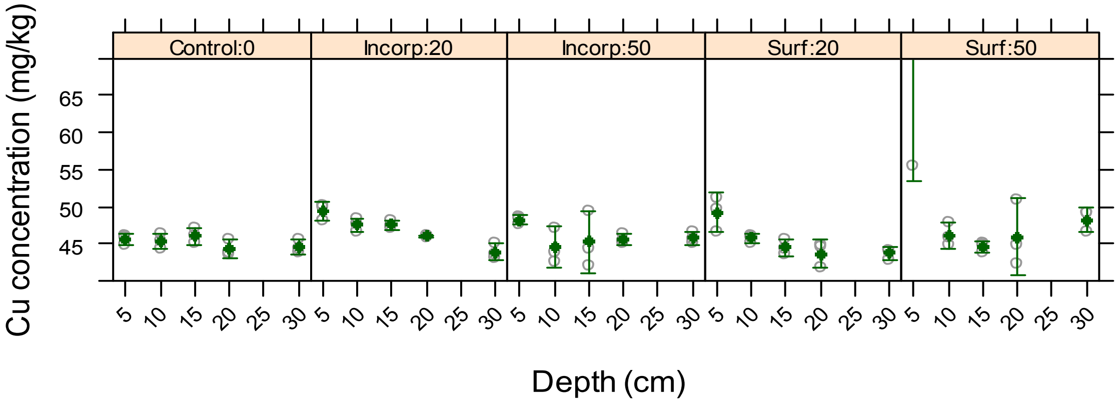


12 month

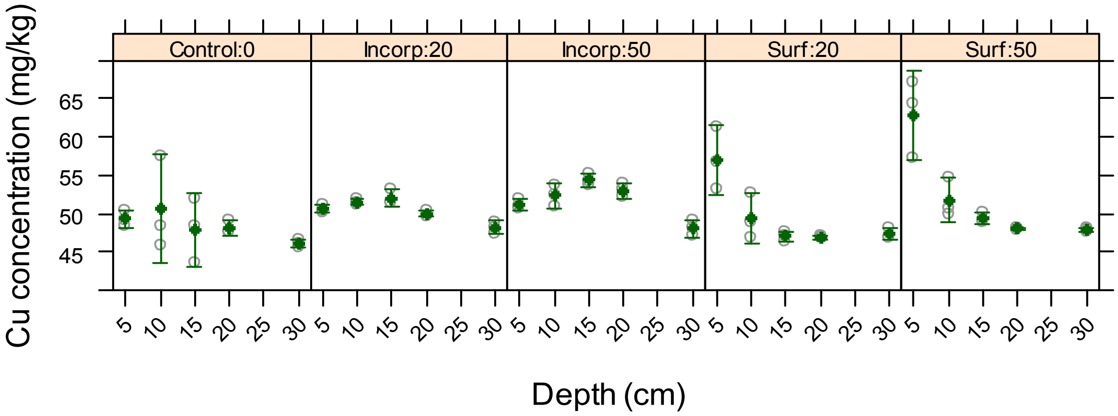


18 month

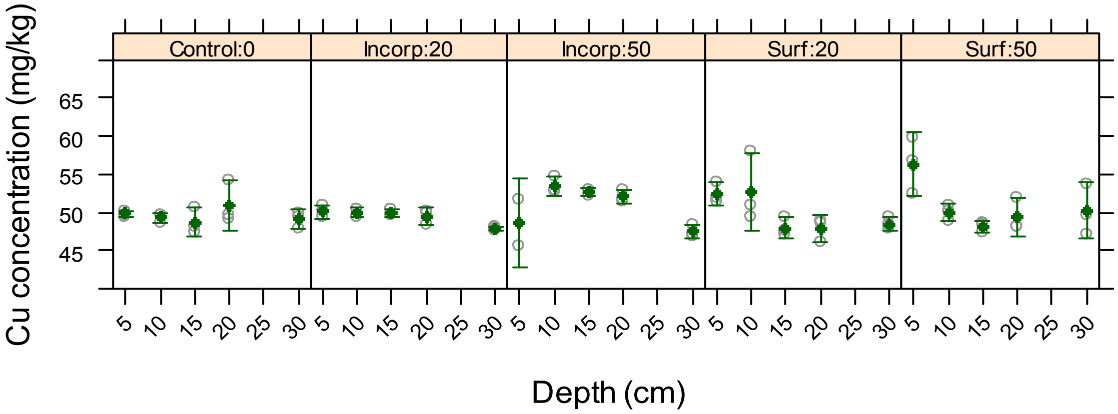
Figure 2.68. Cu concentration in C2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

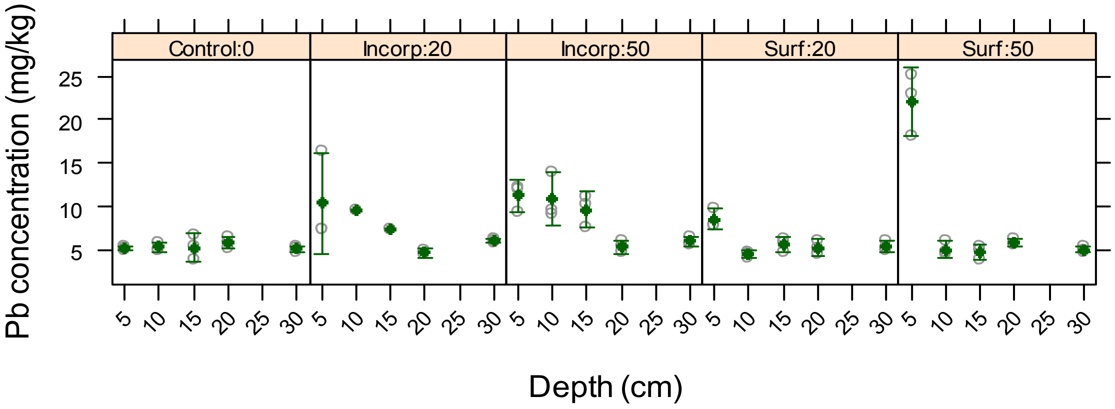


12 month

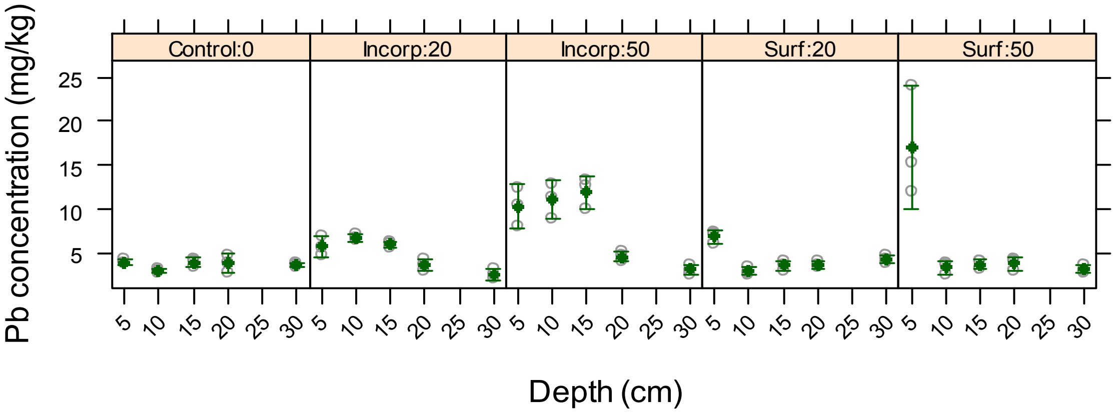


18 month

Figure 2.69. Cu concentration in C3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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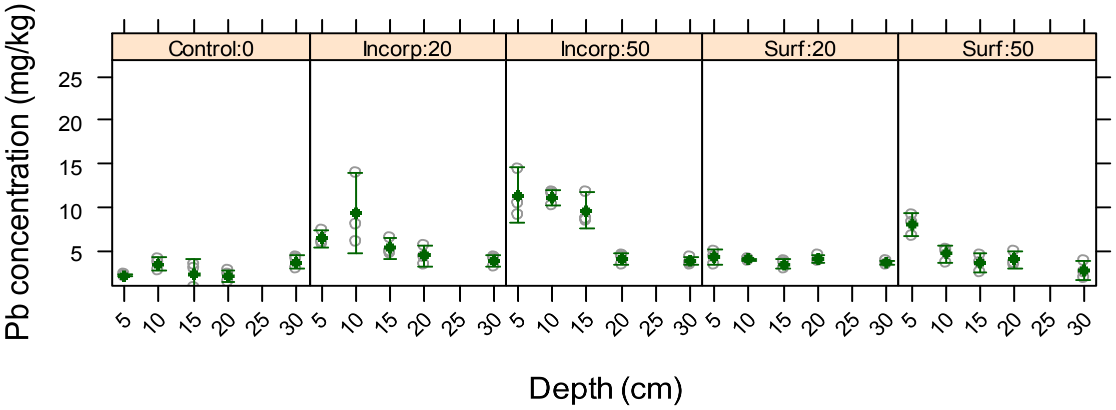
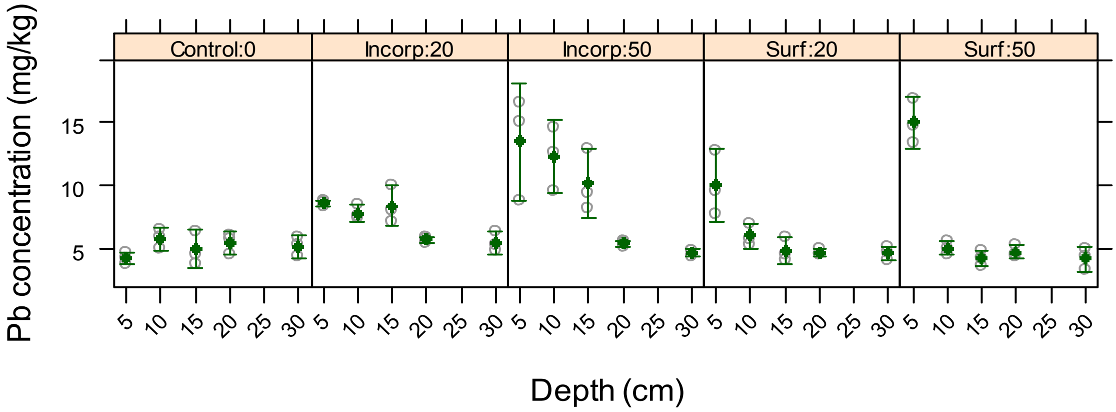
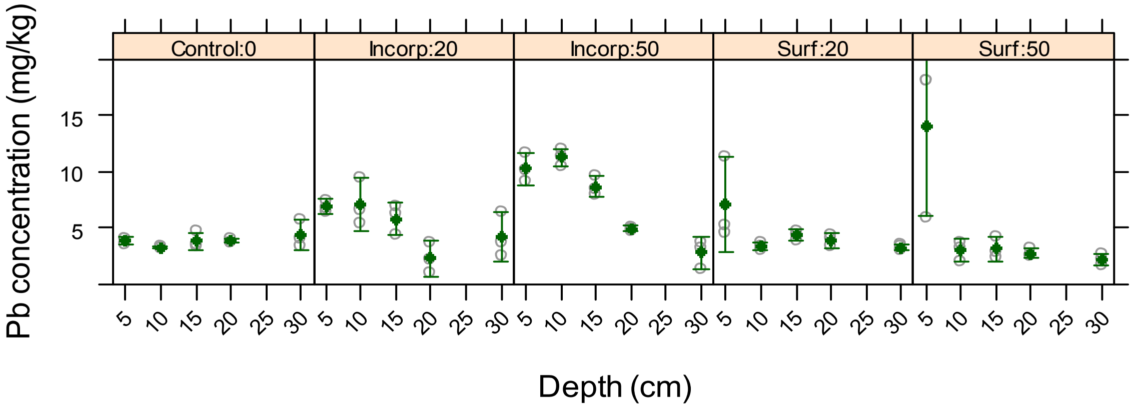


18 month

Figure 2.70. Pb concentration in C1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



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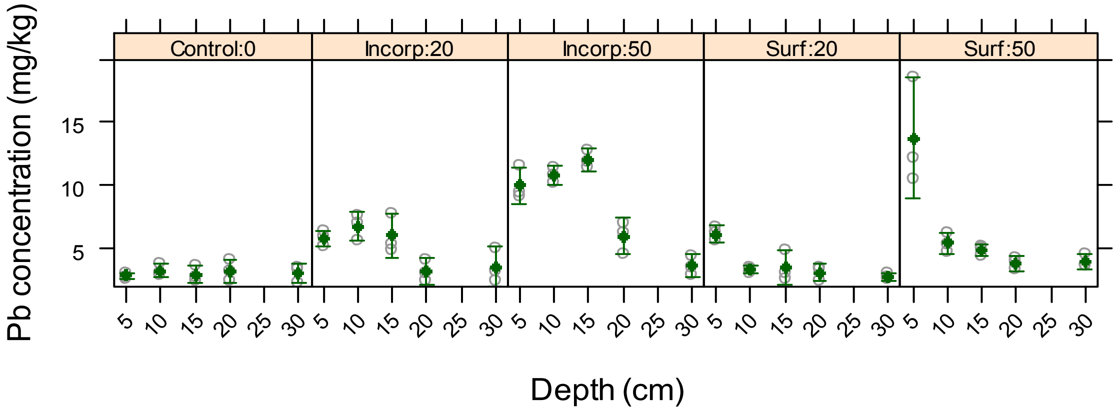
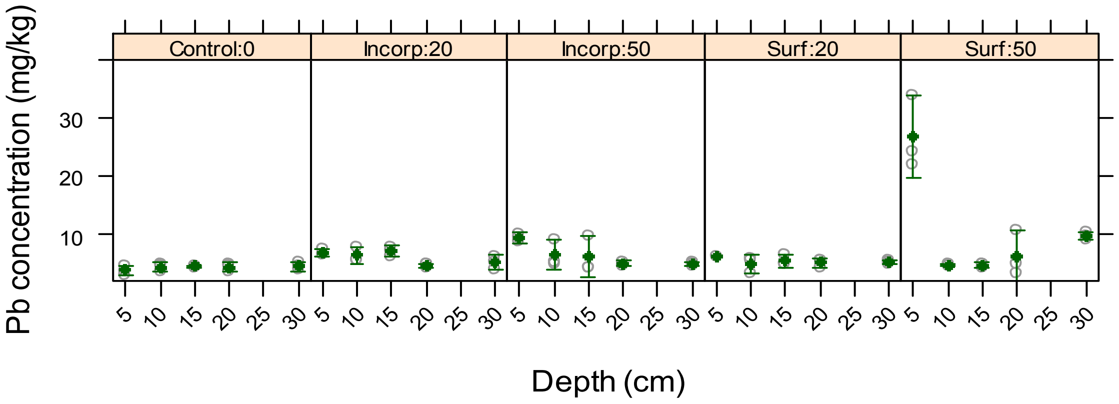


18 month

Figure 2.71. Pb concentration in C2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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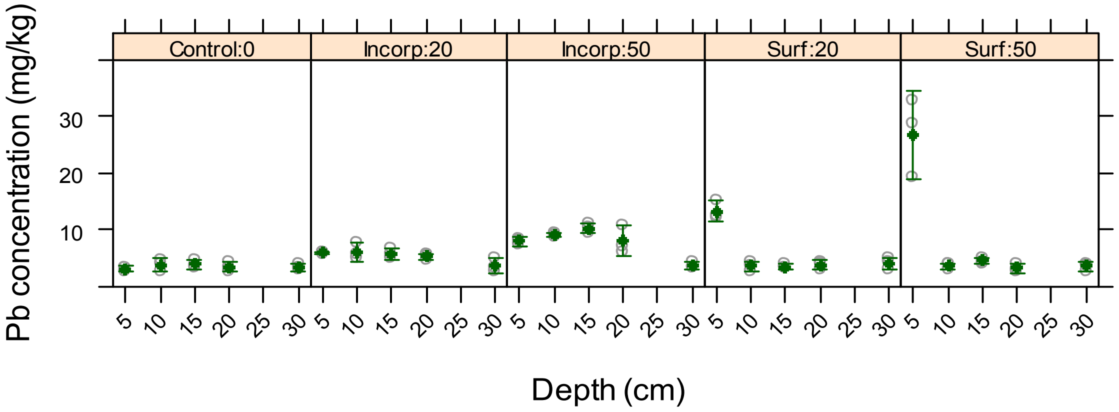

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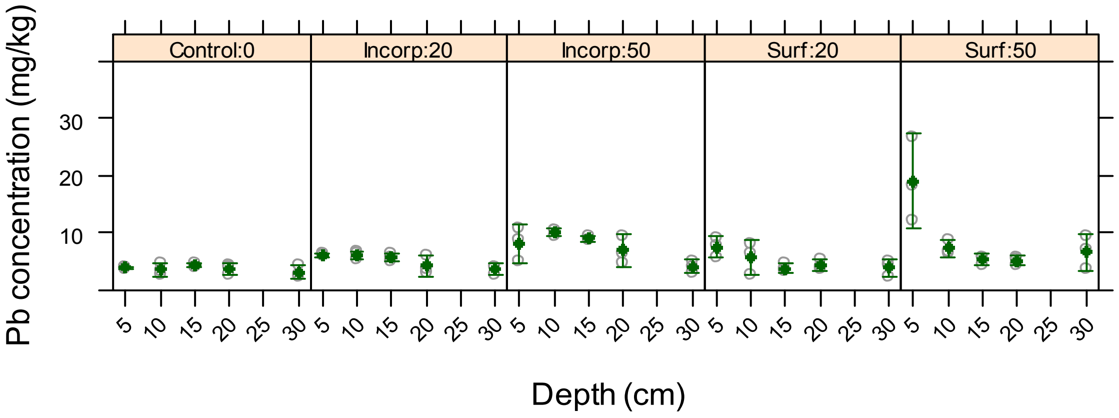
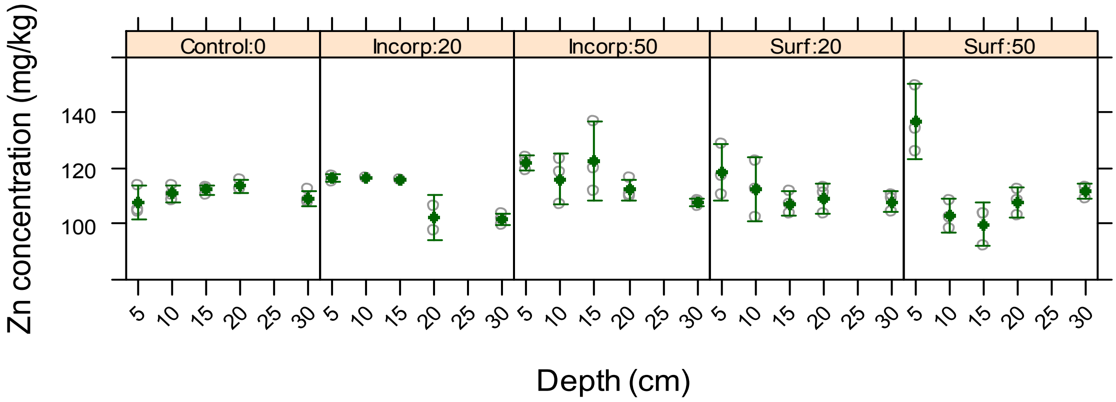
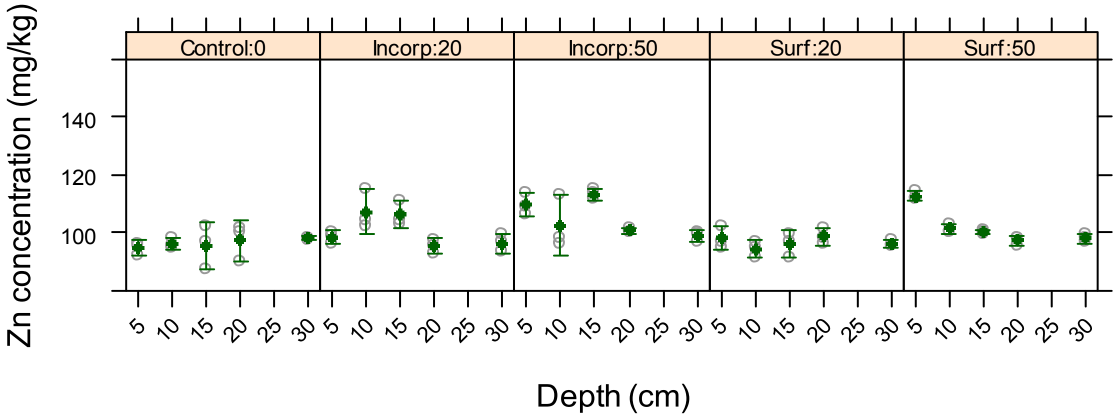


18 month

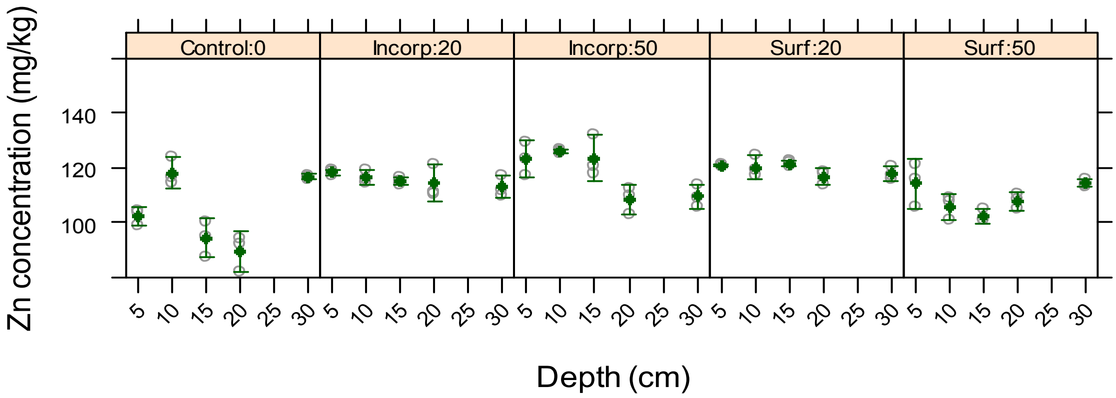
Figure 2.72. Pb concentration in C3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

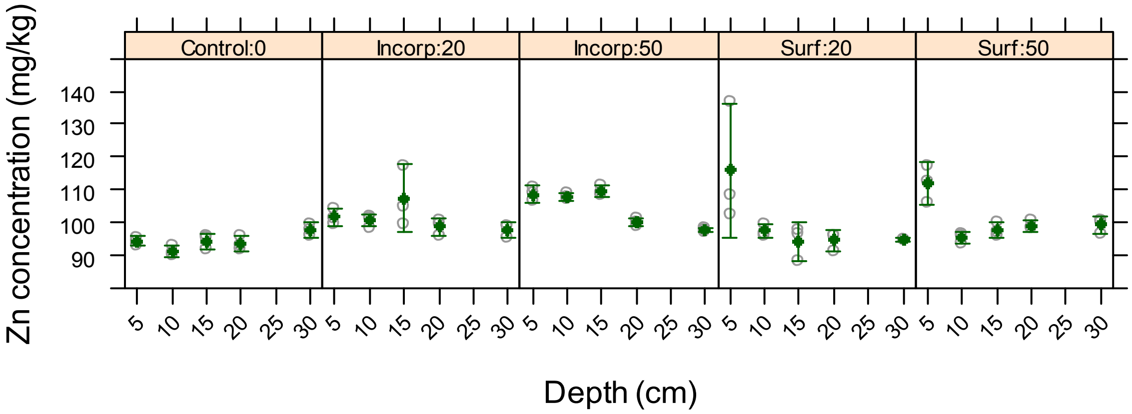


12 month



18 month

Figure 2.73. Zn concentration in C1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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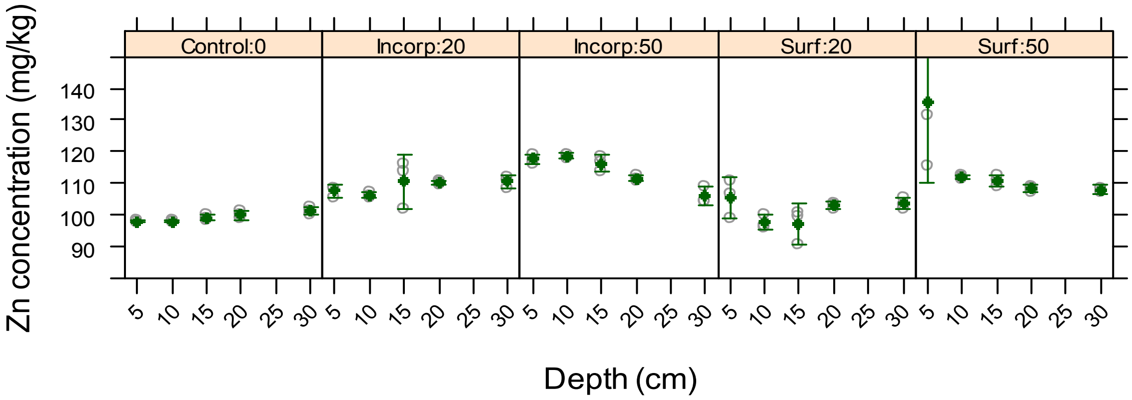

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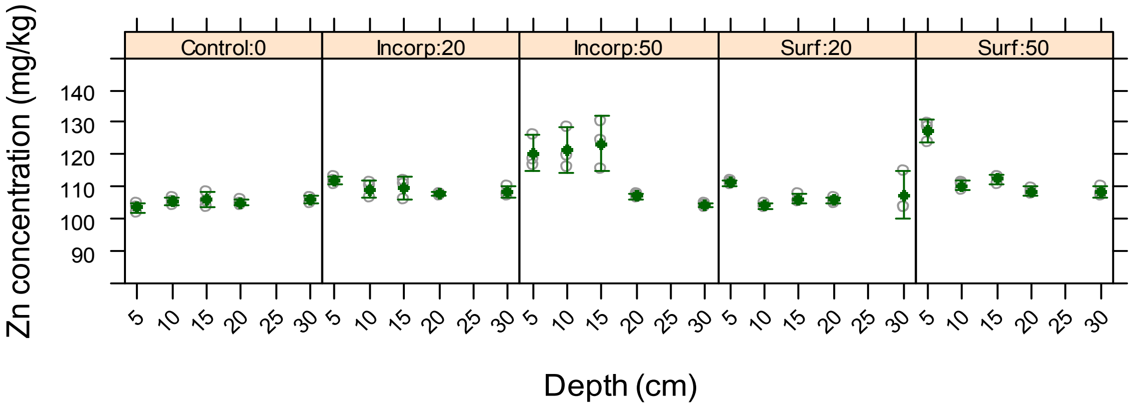
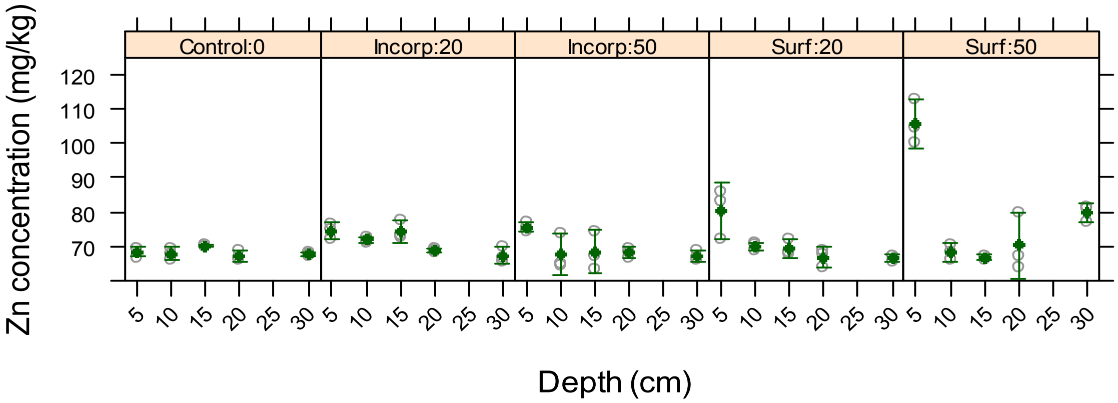


18 month

Figure 2.74. Zn concentration in C2 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



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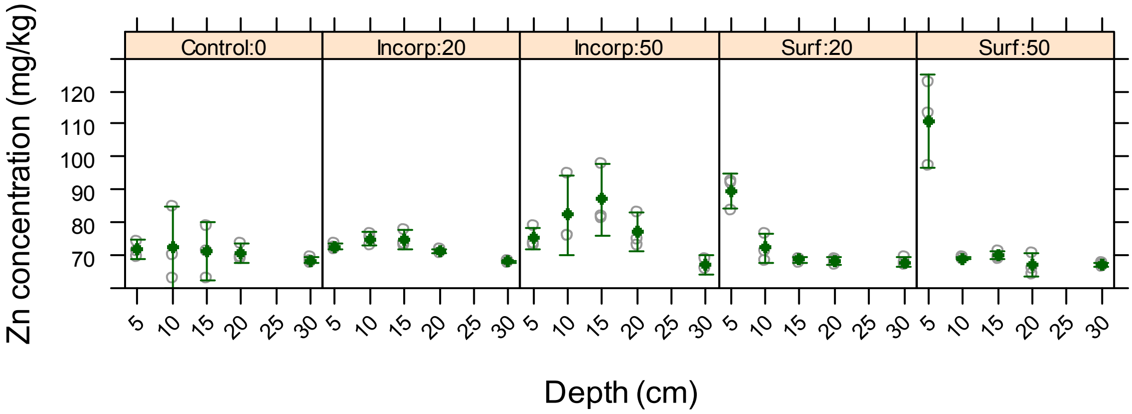

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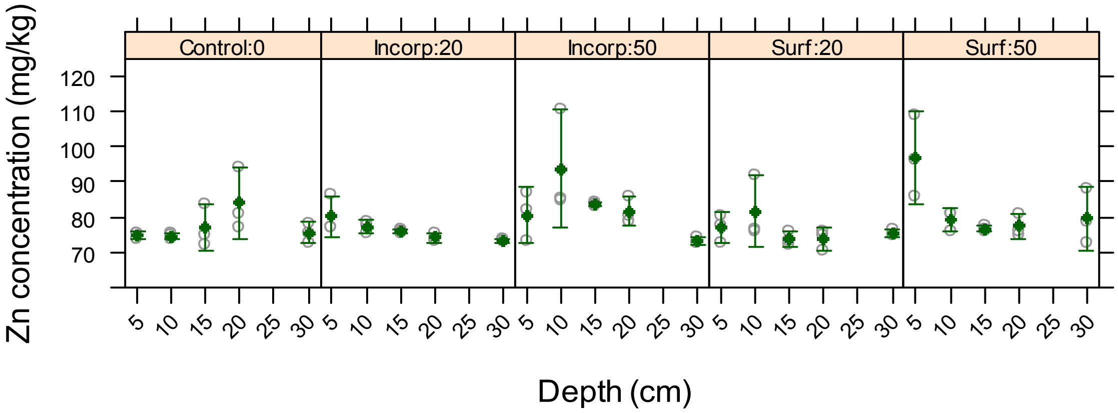
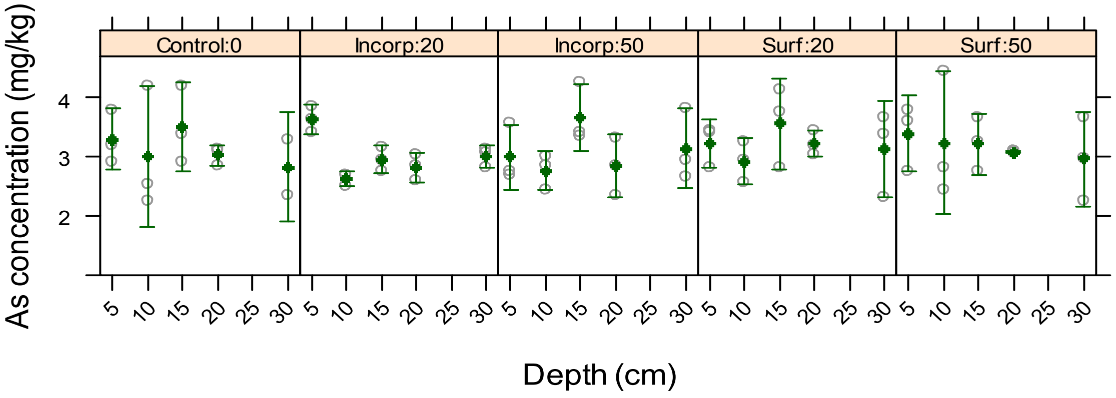
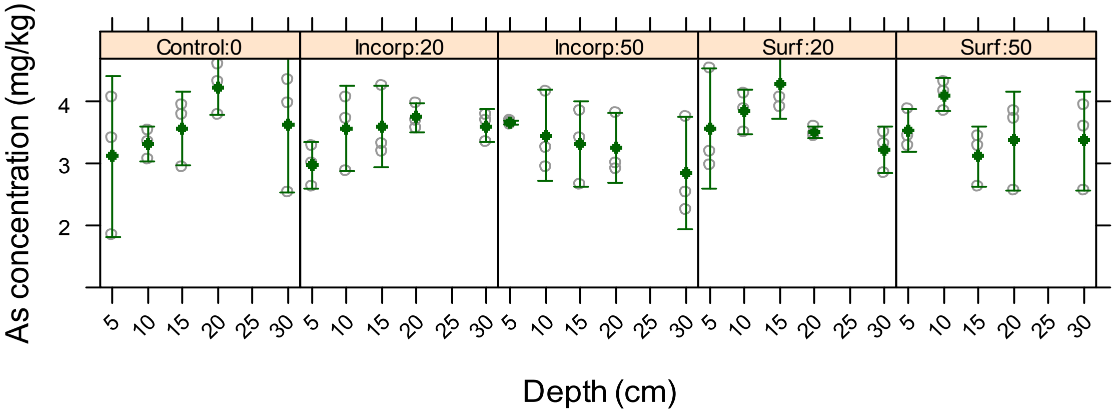


18 month

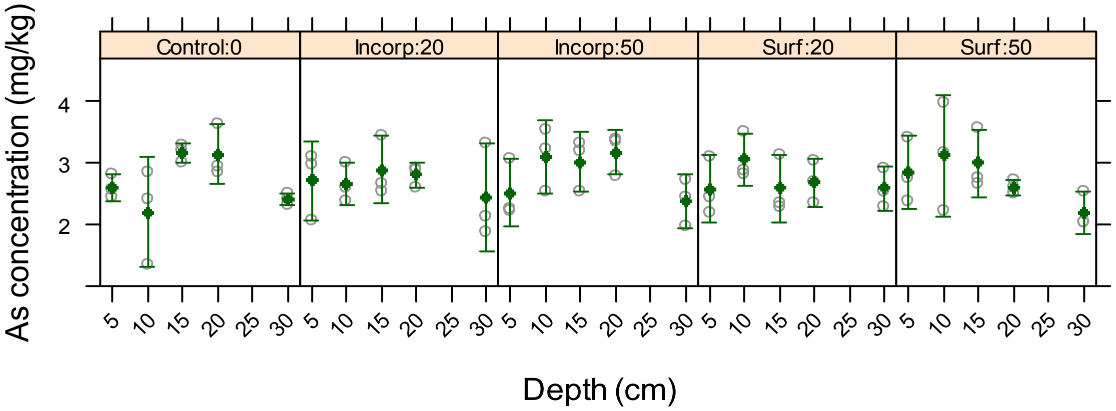
Figure 2.75. Zn concentration in C3 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

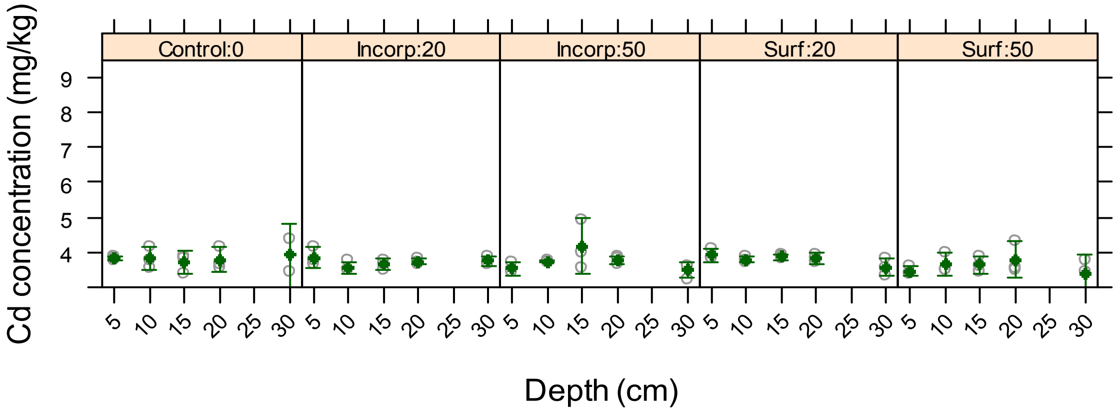


12 month

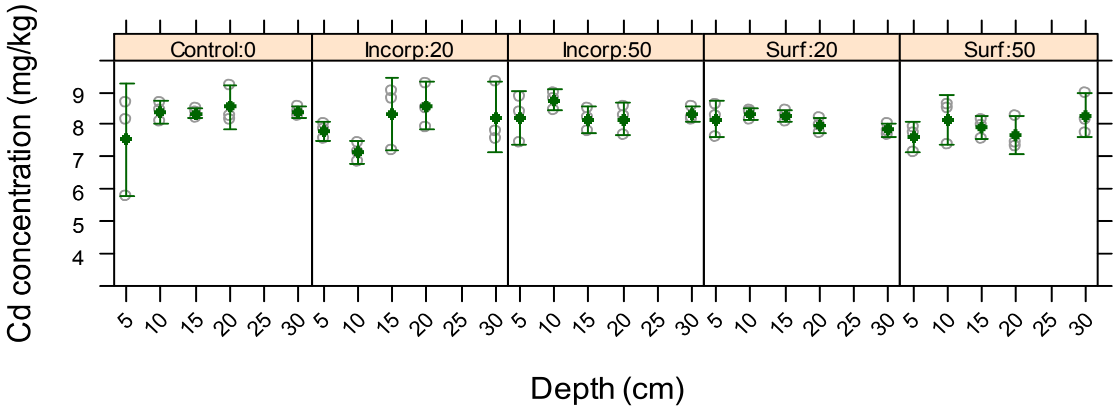


16 month

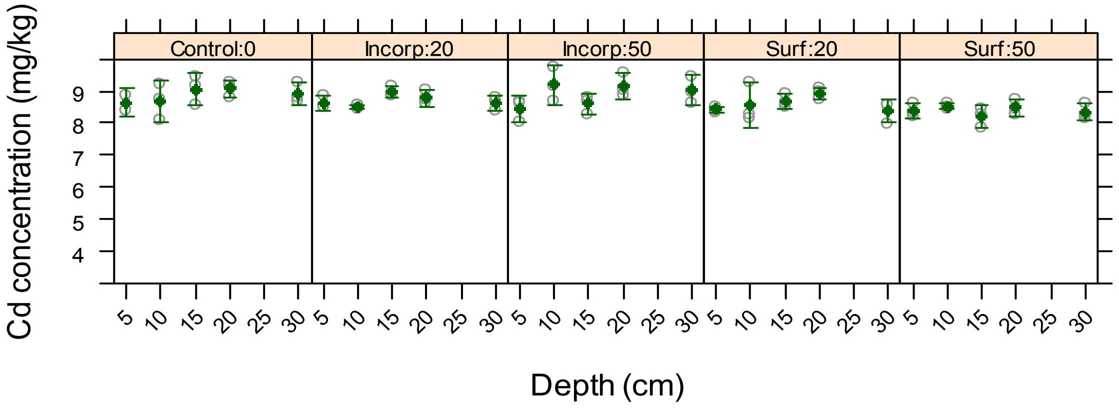
Figure 2.76. As concentration in OM1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

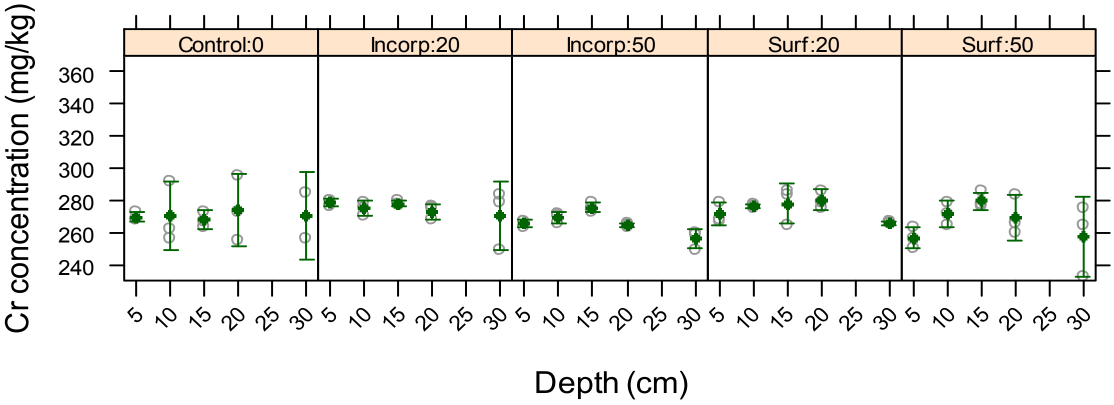


12 month

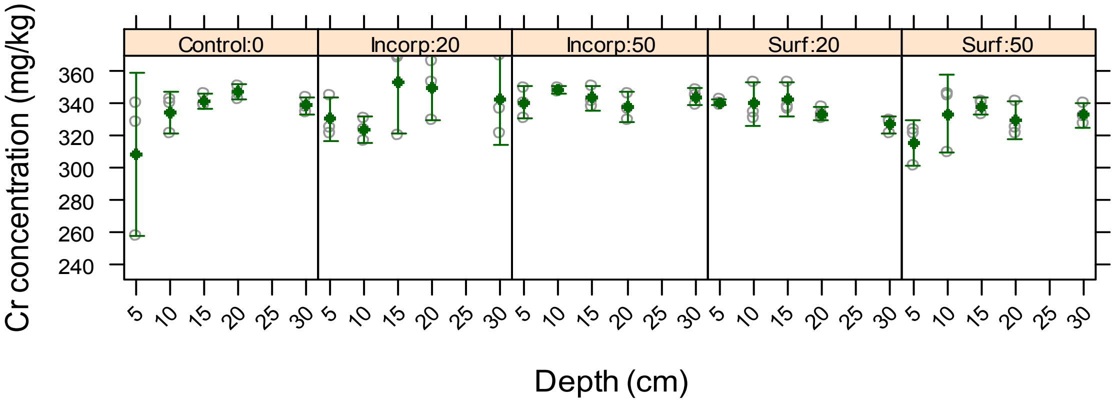


18 month

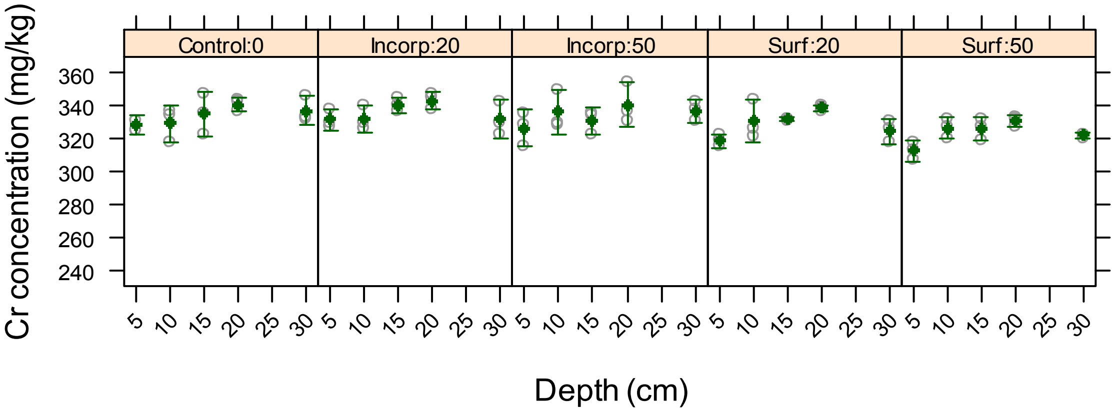
Figure 2.77. Cd concentration in OM1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

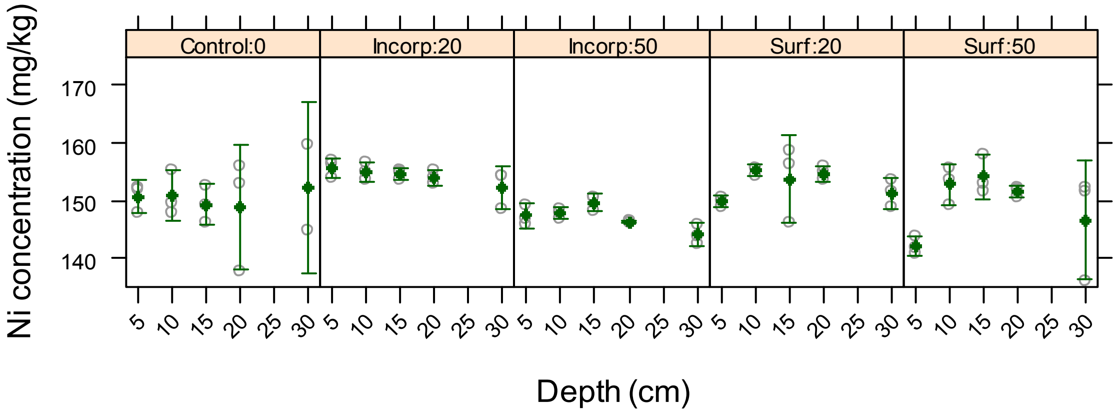


12 month

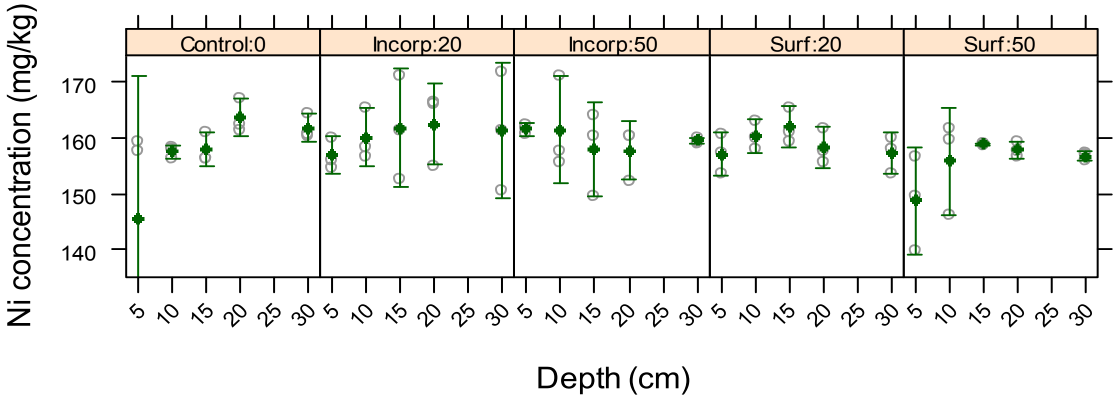


18 month

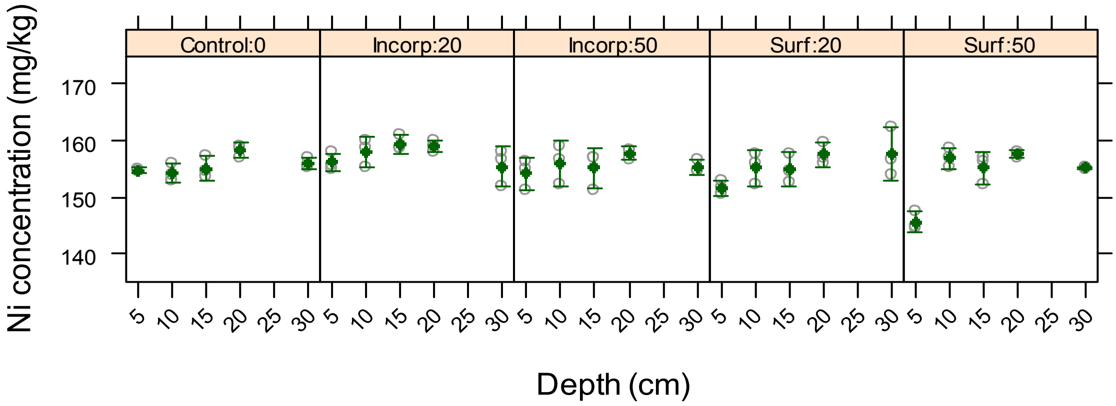
Figure 2.78. Cr concentration in OM1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

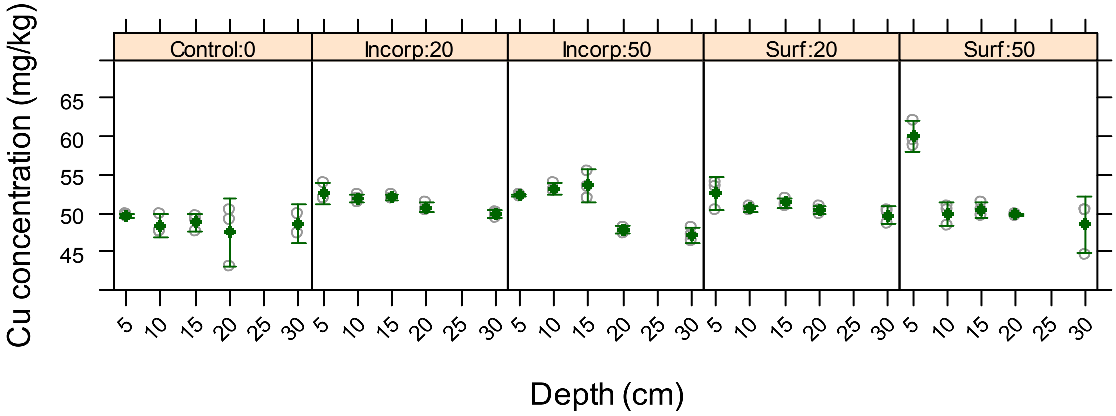


12 month

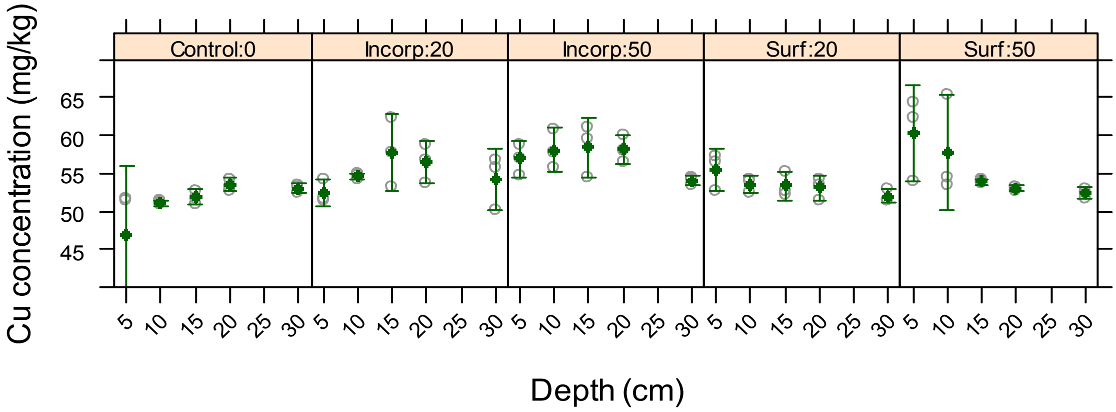


18 month

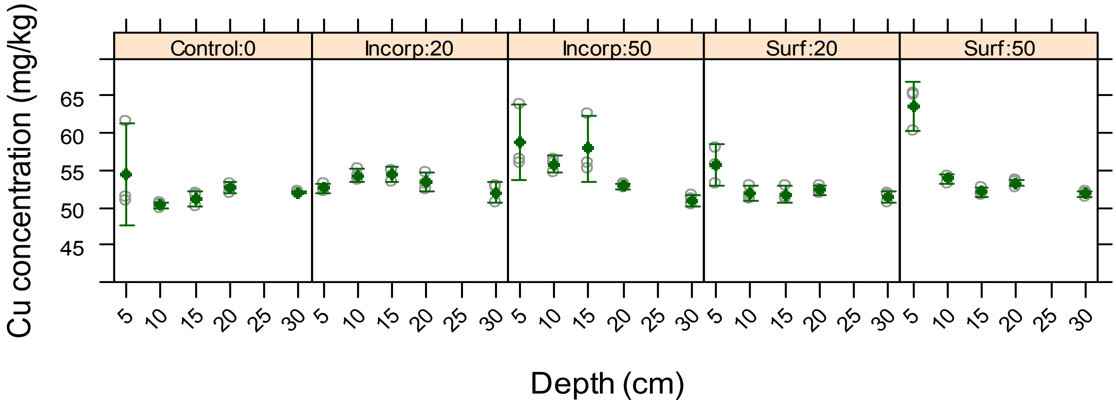
Figure 2.79. Ni concentration in OM1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

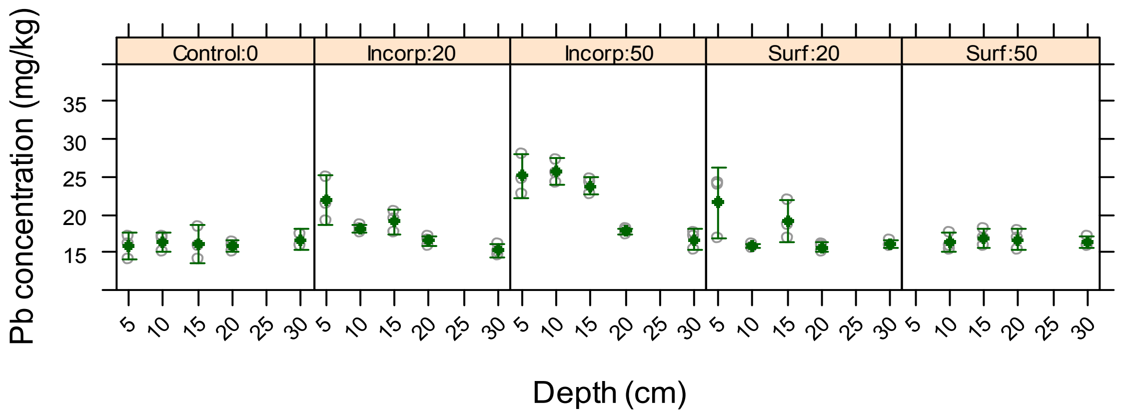


12 month

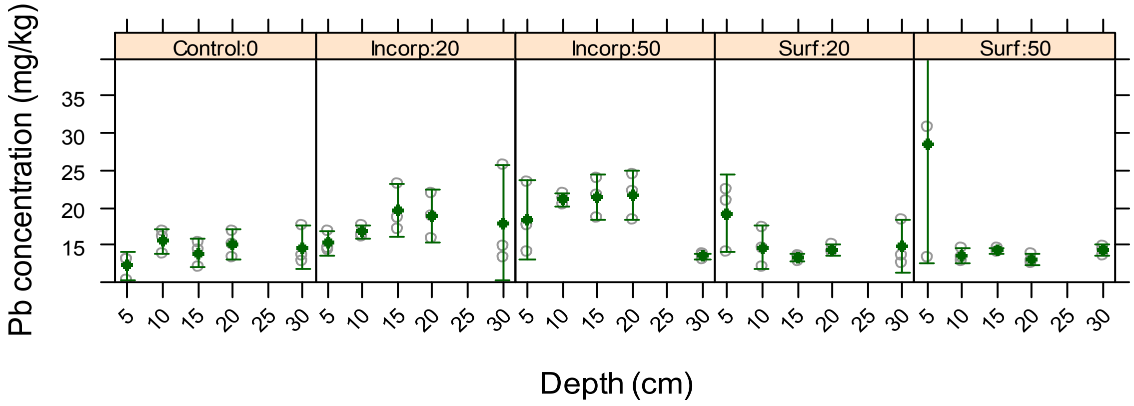


18 month

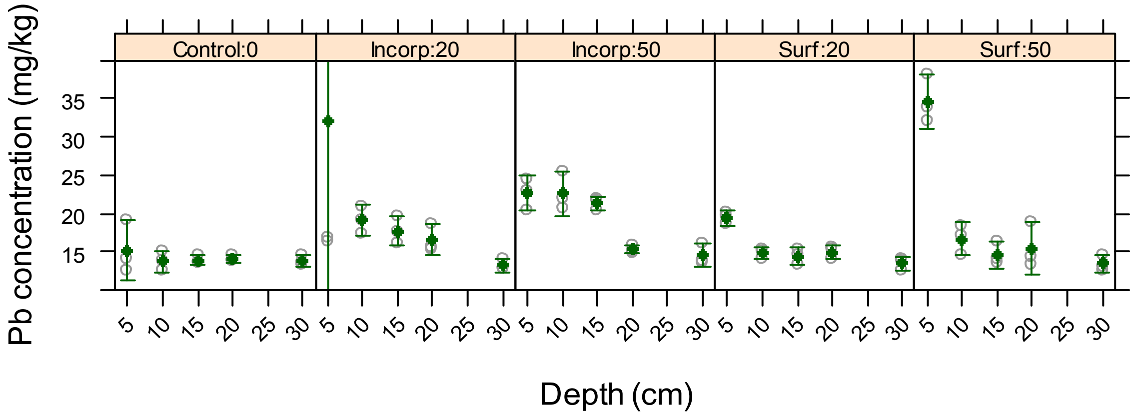
Figure 2.80. Cu concentration in OM1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month

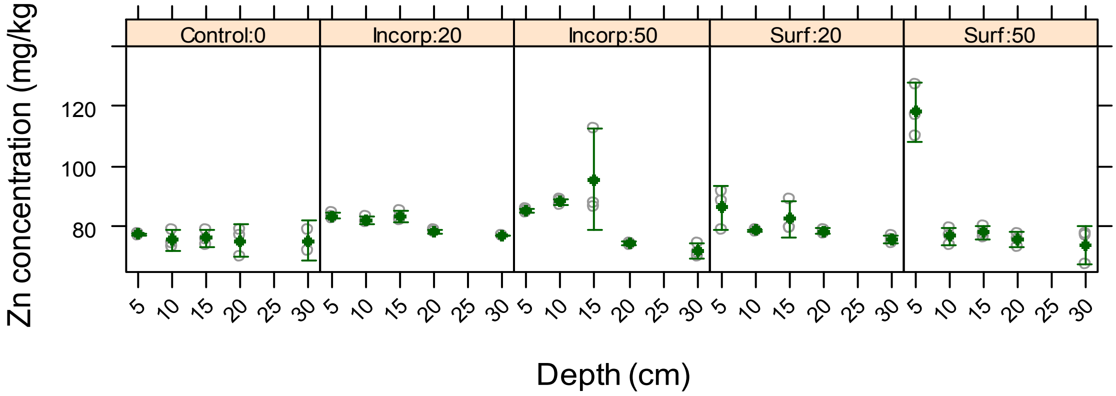


12 month

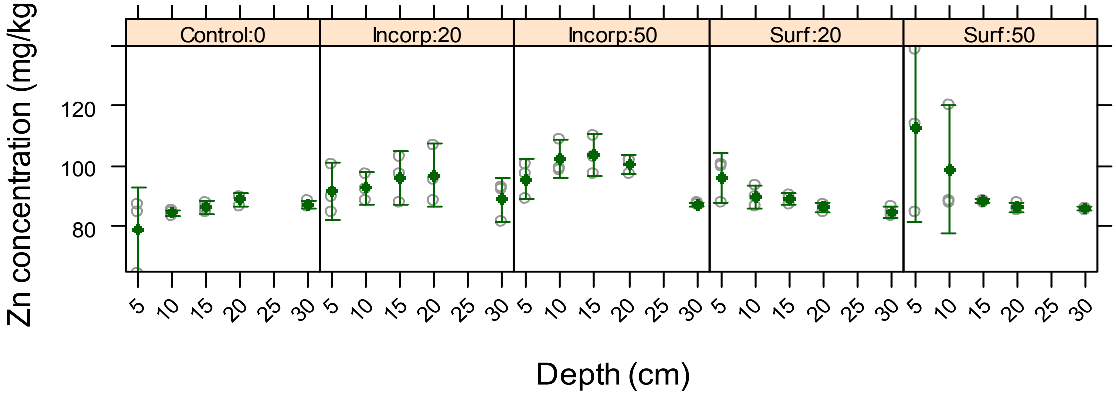


18 month

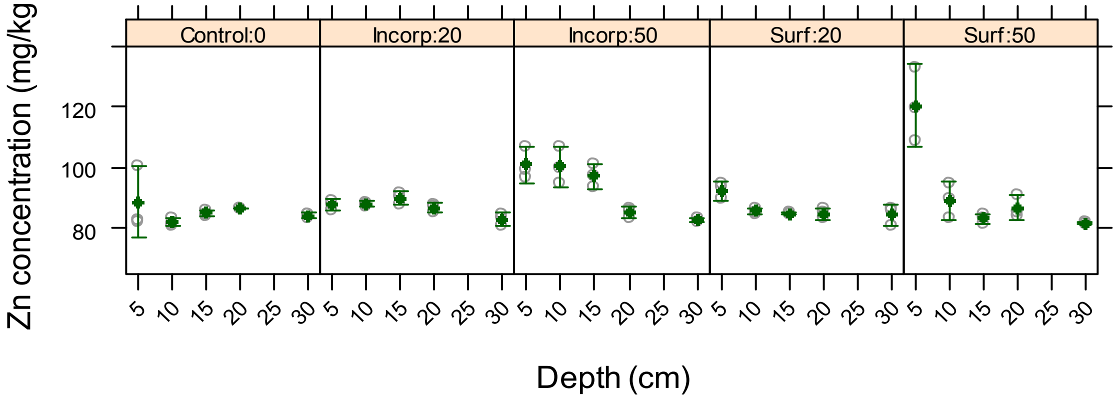
Figure 2.81. Pb concentration in OM1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals



6 month



12 month



18 month

Figure 2.82. Pb concentration in OM1 soil following MWOO application during three period of time (6, 12 and 18 months), 0, 20 and 50 t ha^{-1} = application rates, Incorp = Incorporation applied treatment, Surf = Surface applied treatment, Raw data is shown (○) with means and 95% confidence intervals

compared with controls in all three soils. Any differences observed at 6 months were not apparent at the later sampling periods.

Cadmium in MWOO was present at < 2.3 mg kg^{-1} and was again similar to the background Cd in S1 and S2 (P = 0.06) and S3 (P = 0.4). Cadmium in all S1 and S2 controls and MWOO treatments was less than detection limits at the 6 and 12 month sampling periods. At 18 months Cd was detected in both these soils in controls and some treatments at close to the method detection limit (0.02 mg kg^{-1}) but < 1 mg kg^{-1}, with no significant change observed with MWOO application at any depth in any treatment compared to the control. In S3, however, Cd was detected in all treatments at all sampling periods, albeit at low concentrations. At 6 months Cd in the 50 t ha^{-1} MWOO surface applied treatment was detected at significantly higher concentrations in the surface contact depth (0-5 cm) and also subsurface depths (5-10 and 10-15 cm) (up to 1.9 mg kg^{-1}) compared to the control samples (0.99 mg kg^{-1}). The only other significant difference to the control was an increase in Cd in the 65-70 cm depth of the 20 t ha^{-1} MWOO incorporated application (1.65 mg kg^{-1} compared to 0.7 mg kg^{-1} in the control) although increase at this depth was not observed in other MWOO treatments for this soil. At 12 and 18 months, with increased variability on the data, significant differences in MWOO treatments compared to the control were no longer apparent.

Chromium in MWOO was significantly higher than the background soil concentrations for all these soils (P < 0.001). Chromium concentration and distribution in S1, S2 and S3 soils, however, showed no significant change with MWOO application compared to the representative control samples at MWOO contact depth for any treatment at any sampling time to 18 months. The increase in Cr at 65–70 cm depth that was observed in the control was not apparent in the MWOO treatments at 6 months, with Cr at this depth being slightly lower in MWOO treatments (approximately 5 mg kg^{-1} lower) than the control concentration. At 12 months however, Cr at 65 – 70 cm in the MWOO incorporated 20 t ha^{-1} treatment was significantly elevated above the control variability but this effect was not significant in other MWOO treatments, nor at 18 months, due to greater variability on the data.

The MWOO nickel concentration was significantly greater than soil background concentrations, for S1 and S2 (P = 0.001) and for S3 (P = 0.04) but when applied to soil it was not possible to identify any significant changes in soil Ni concentration at MWOO contact depths compared with inherent control variability at any of the three sampling periods. At 6 months for S2, the sandy soil with the lowest Ni background values and highest sand content, a trend of increased concentrations was evident in some MWOO contact depths, although not significantly increased, but this was not evident at 12 and 18 months for this soil, and the trend was not observed in S1 and S3. The increase in Ni at 65-70 cm, below MWOO contact depth, observed in the controls of S1 and S2 at 6 months was not observed in the MWOO treatments for these soils and Ni at 65–70 cm depth in MWOO treatments for these soils was at times significantly lower than the control concentration (e. g in S1 at 6 months 11.7 mg kg^{-1} to 7.7 mg kg^{-1}). This effect was not observed at 12 and 18 months.

The metals Cu, Pb and Zn were all present in MWOO at very significantly greater concentrations (for all P < 0.001) than the background values for these sandy soils (Table 2.1) and as a consequence significant changes in soil concentration with MWOO application were more often apparent than the other metals examined. Considering Figures 2.25 to 2.27, in the more acidic soil S1, at 6 months an increase in Cu concentration was observed in the MWOO contact depths for the 50 t ha^{-1} incorporated treatment (but not in the 20 t ha^{-1} incorporated treatment) and the surface applied treatments compared to the inherent variability on the control samples, the increase was greatest at 0 – 5 cm depth in the 50 t ha^{-1} surface applied treatment (up to 20.5 ± 1.13 mg kg^{-1}) compared to control. At 12 and 18 months, Cu concentrations in MWOO treatments were not significantly different to the 6 month values detected in the same treatment, but because variability on control samples was lower, significant increases compared to control treatments were observed at 12 months in MWOO incorporated 50 t ha^{-1} (0 – 15 cm), and both surface applied treatments at 0 – 5 cm with the increase greatest at 50 t ha^{-1} to 19.8 ± 1.02 mg kg^{-1}, and at 18 months in the incorporated treatments and the 50 t ha^{-1} surface applied treatment (to 20.6 ± 1.09 mg kg^{-1}). In S2, the near neutral pH sand, again, increases in Cu concentration were observed in MWOO contact depths for all MWOO treatments, with a few just significantly greater than the control inherent variability (e.g. 0 -5 cm depth in 20 t ha^{-1} surface applied; 5 -10 cm depth in 20 t ha^{-1} incorporated) but the clearly significant and greatest increase was in the 0 – 5 cm depth at 50 t ha^{-1} MWOO surface applied (up to 15.1 ± 0.9 mg kg^{-1}). Similar to S1, for S2 at 12 and 18 months Cu soil concentrations did not significantly change in comparable MWOO treatments over the whole sampling period. At 18 months significant elevation was still detected in some MWOO contact depths for MWOO incorporated at 50 t ha^{-1}, but not the 20 t ha^{-1} treatments, and Cu remained at greatest concentration at 0 -5 cm in the surface applied 50 t ha^{-1} treatment at (up to 17.3 ± 2.15 mg kg^{-1}). Total Copper accumulation in MWOO treatments relative to the control for S3, the soil with higher background Cu concentration and the most alkaline soil, were less evident. In S3, the greatest increase in Cu was at 0-5 cm depth for the 50 t ha^{-1} surface application (to 27.2 ± 1.11 mg kg^{-1}), but this was not significant above inherent soil variability, nor was any other apparent increase in MWOO contact depths with MWOO application. At 12 and 18 months Cu concentrations in comparable S3 MWOO treatments were not significantly different to the concentrations at 6 months. At 18 months, however, Cu at one depth (5 – 10 cm) in MWOO incorporated 50 t ha^{-1} and also, the greatest increase in MWOO contact depth concentration, in the 50 t ha^{-1} surface applied treatment, was significant at 31.6 ± 1.7 mg kg^{-1} due in part to lower variability on control samples. There was evidence in S2, similar to Cr and Ni, where Cu increased in the control at 6 months at 65- 70 cm, that Cu at this depth in some MWOO treatments was significantly lower than the control sample e.g 20 t ha^{-1} incorporated treatment, and 20 and 50 t ha^{-1} surface application treatments. This effect was not observed at the later sampling times.

Due to high MWOO Pb concentrations relative to soil background values increases in Pb in contact depths for MWOO applied treatments were relatively clear supported also by relatively lower variability for the Pb soil concentration data. At 6 months in S1, Pb significantly increased in the MWOO contact depths for all MWOO treatments. For incorporated treatments this was only just significant for some of the 0 – 15 cm depth samples, with a greater and more significant increase at 50 t ha^{-1} (to 12.9 ± 1.5 mg kg^{-1}). In the surface applied treatments, however, increase at 0 – 5 cm was greater and significant at both application rates with the highest concentration (to 21.8 ± 3.1 mg kg^{-1}) in the 50 t ha^{-1} surface application. In S2, the more neutral sandy soil, similarly Pb increased slightly but significantly above inherent control variability in some MWOO contact depths (at 0 – 5 cm, 5 – 10 cm, 10 – 15 cm for 50 t ha^{-1}; at 10-15 cm for 20 t ha^{-1}) in both incorporated treatments (to approximately 9.9 ± 1.7 mg kg^{-1}) but increases for the two different rates were not significantly different from each other. In surface applied treatments the increase at 0 – 5 cm depth was significant at both rates but the increase at 0 – 5 cm depth at 50 t ha^{-1} was greatest (to 22.6 ± 3.5 mg kg^{-1}). In S3, apparent increases in the incorporated applied 20 t ha^{-1} treatment was not significant, but in surface applied 20 t ha^{-1} and at 50 t ha^{-1} increases in soil Pb became significant (for MWOO incorporated at 0 – 15 cm depths) with the greatest increase at 0 – 5 cm 50 t ha^{-1} surface applied (to 16.8 ± 0.5 mg kg^{-1}). Similar trends were observed at 12 and 18 months, where it was apparent that Pb concentrations in the same MWOO treatments in the three different soils did not change over the period of the trial. In S1, significant increases in Pb in MWOO contact depths were still observed at each time period with the increases in the 50 t ha^{-1} treatments greater, and the greatest increase at 18 months remaining at 0 – 5 cm depth in the 50 t ha^{-1} MWOO surface applied treatment (19.9 ± 3.9 mg kg^{-}).

In S2, in the 50 t ha^{-1} surface applied treatment soil Pb also slightly but significantly compared to control increased at 5 – 10 cm depth, below the MWOO contact depth and this increase was also observed to 18 months although was not significant compared to the control variability at this time period. Also for Pb in S2 where Pb increased in the control at 65 – 70 cm , there was evidence that Pb concentration was lower than the control at 65-70 cm depth in a number of MWOO treatments (incorporated 20 and 50 t ha^{-1} and surface applied 50 t h^{-1}). This effect was not observed at later time periods.

For Zn, which was also present in MWOO at a significantly higher value than background soil concentrations (for all P < 0.001), at 6 months in S1, the more acidic of the sandy soils, there was a clear increase in Zn soil concentration in all MWOO treatments at depths where MWOO had contact with soil. Zinc significantly increased at 0-5 cm depth for both 20 and 50 t ha^{-1} MWOO surface applied with the greater increase at 50 t ha^{-1} to a maximum of 43.5 mg kg^{-1}. In the incorporated treatments, Zn also increased significantly at 10-15 cm depth for 20 t ha^{-1} and at all incorporated depths (0-15 cm) for 50 t ha^{-1} with the extent of increase higher in the 50 t ha^{-1} treatment (to 22.1 ± 1.9 mg kg^{-1}), but not as high as observed in the 0 – 5 cm depth for the surface applied treatments. Similar results were observed at 12 months with the same treatments showing no significant change in Zn concentration or distribution over the 6 months although at 18 months due to higher variability on control samples it was not possible to identify any significant changes in MWOO treatments compared to the control although concentrations in the same MWOO treatments did not change significantly from 6 to 18 months. A significant increase in soil Zn, however, was not evident in S2 with incorporation MWOO application at 6 months due to high control sample inherent variability but this was observed at 0 – 5 cm depth in both surface applied treatments with the greatest increase at 50 t ha^{-1} (to 35.4 ± 2.28) and also at 12 and 18 months with lower control variability. At these time periods soil Zn was observed to have slightly but significantly increased in the 20 t ha^{-1} incorporated treatment MWOO contact depths (0 – 15 cm) compared to controls, significantly increased at 50 t ha^{-1} incorporated treatment for all contact depths, significantly increased in the 20 t ha^{-1} surface applied treatment at 0 -5 cm depth and significantly increased (to 37.8 ± 5.5 mg kg^{-1}) in the 50 t ha^{-1} surface applied treatment at 0 – 5 cm depth. In S3, a significant increase in soil Zn was only observed for the 0 – 5 cm depth in the 50 t ha^{-1} surface applied treatment at both 6 months to 68.4 ± 1.4 mg kg^{-1} and this increase was consistent over the 18 month sampling period. There was evidence in S1, the most acidic soil, that soil Zn increased also at depth 65

– 70 cm in all treatments, similar to the control. This was not observed in the other soils nor at later time periods in S1.

Loam soils - SL1, SL2, SL3

All MWOO treatments and controls for the loam soils were analysed at 0-5, 5-10, 10-15, 15-20, and 25-30 cm depths. An examination of the distribution of metals and metalloids in the control columns of these loam soils after 18 months of maintaining moisture at 60% field capacity shows that, there was no significant difference in concentration at any depth compared with all other depths analysed for all metals and metalloids in any soil at any sampling time period, (Ni at 10 – 15 cm in SL2 was slightly increased compared to other samples but this is considered within the inherent variability when considering other samples).

The MWOO was applied to these soils at rates of 20 and 50 t ha^{-1}, both surface applied and incorporated, but in addition to SL2, the moderately acidic loam at also 140 t ha^{-1} both surface applied and incorporated because this soil was included to represent a typical soil used for mine site rehabilitation where MWOO application at 140 t ha$_{-1}$ is acceptable in NSW.

The MWOO As concentration (5.3 mg kg^{-1}) was slightly but not significantly lower than As in SL1 (8.1 mg kg^{-1}) and was almost similar to the As concentration in SL2 and SL3 (5.6 and 4.2 mg kg^{-1}, respectively). With the low concentrations and resulting high variability on soil sample data it was not possible to detect any significant change in soil As concentrations with MWOO application for any treatment in these loam soils over the three sampling time periods to 18 months. Only for SL3, As concentration significantly increased at depth 5-10 cm in the incorporated applied 20 t ha^{-1} treatment (to 5.7 ± 0.2 mg kg^{-1}) compared to control at 6 months, although this increase was not observed at 12 and 18 months due to higher variability in the treatments. Similarly for Cd. Cadmium concentration in soil and MWOO was very low (< 0.6 mg kg^{-1}) with MWOO Cd not significantly different to soil concentrations. The MWOO application showed no significant effect on Cd concentrations or distribution in all three soils over 18 months.

Chromium in MWOO (85.4 mg kg^{-1}) was very significantly higher than the background soil concentrations for these loam soils (all $P < 0.001$), but again for all sampling periods to 18 months any apparent increases in SL1 and SL2 were smaller than the variability on control sample data, including the apparent increase at 6 months in 140 t ha^{-1} MWOO surface applied treatment in SL2 at 0-5 cm depth. In SL3, however, the loam, with the lowest background Cr concentrations, the one treatment where a significant increase in Cr concentration was evident in MWOO contact depths was at 6 months at 0-5 cm depth to 21.3 ± 0.56 mg kg^{-1}in the surface applied 50 t ha^{-1} MWOO treatment. Other apparent increases in MWOO contact depths were not significantly greater than the Cr variability in the corresponding control samples (the increase for all samples in the 50 t ha^{-1} incorporated treatment at 12 months is considered anomalous).

The MWOO nickel concentration (40.26 ± 10.8 mg kg^{-1}) was significantly ($P < 0.001$) greater that soil background concentrations. In SL1 and SL3, the loams with higher background Ni, there was, however, no significant change in Ni soil concentration in MWOO contact depths evident for any MWOO treatment at any sampling time period to 18 months, due in some treatments to variability associated with a number of the MWOO treated samples e.g 20 t ha^{-1} incorporated MWOO at 5 – 10 cm depth at 6 months. Even in SL2, the loam with the lower Ni background concentration, many apparent increases evident in MWOO contact depths were not statistically significant considering the variability associated with the data. Only in the 140 t ha^{-1} surface applied treatment, at 0-5 cm depth, was a significant increase observed at all three sampling times in this highest application rate treatment, to 11.4 ± 0.34 mg kg^{-1} at 6 months which remained consistent to 18 months. At 18 months Ni in a number of the MWOO contact depth samples for the 140 t ha^{-1} treatment was also significantly greater than the comparable control samples (which showed slightly lower concentration than controls at the previous sampling times). In soil depths below the MWOO contact zone, in some MWOO treatments at 6 months the Ni concentration was slightly but significantly lower that the Ni concentration in the control e.g. in SL1 incorporated MWOO at 50 t ha^{-1} at 25 – 30 cm depth, surface applied MWOO at 50 t ha^{-1} 15 – 20 cm; in SL2 at 25-30 cm depth in incorporated 50 and 140 t ha^{-1} treatments (down to 7.8 and 7.9 mg kg^{-1}), at 5 - 10 cm, 10 – 15, 15 – 20 cm and 25-30 cm depths in 140 t ha^{-1} surface application (to approximately 6.3 mg kg^{-1}); in SL3 at 25-30 cm in the 20 t ha^{-1} surface application treatment. Similarly in SL2 at 25 – 30 cm in MWOO incorporated 50 and 140 t ha^{-1} treatments, whilst in SL2 at 25-30 cm a significant increase in Ni was observed, in the incorporated 20 t ha^{-1} treatment.

The metals Cu, Pb and Zn were all present in MWOO at very significantly greater concentrations than the background values ($P < 0.001$) for these loam content soils (Table 2.1), and significantly greater than the other metals and metalloids analysed. Considering Figures 2.46 to 2.54, for the more acidic soil SL1, with 18.3 ± 0.5 mg kg^{-1} background Cu concentration, after 6 months a similar significant increase in Cu concentration was observed in the MWOO contact depths for surface 0-5 cm samples in both 20 and 50 t ha^{-1} MWOO surface application treatments (to 20.6 ± 0.7 and 20.8 ± 0.7 mg kg^{-1}, respectively) compared to control. For incorporated treatments increases were only significant at 50 t ha^{-1} at 5-10 and 10-15 cm depths - (maximum to 24.5 mg kg^{-1} at 10 - 15 cm). No significant difference was observed between the Cu concentration in MWOO contact depth for the 50 t ha^{-1} surface applied and incorporated treatments. The same trends were evident at 12 and 18 months with no change in soil concentrations observed through the sampling period in the same treatments. As at 6 months statistically significant increases in Cu concentration were only observed at 0 - 15 cm depth in the MWOO incorporated treatment and at 0 – 5 cm depth in the surface applied treatment for 50 t ha^{-1} MWOO application rate, with concentrations either not different or slightly greater in the incorporated treatment. In SL2, the soil with the lowest Cu background concentration (8.3 ± 0.1 mg kg^{-1} at 6 months Cu concentration significantly increased at the surface (0-5 cm depth) for both higher rate, 50 and 140 t ha^{-1}, MWOO surface application treatments. This accumulation was significantly greater at the 140 t ha^{-1} application rate (38.7 ± 2.7 mg kg^{-1}) compared to 50 t ha^{-1} (12.9 ± 0.9 mg kg^{-1}). A significant accumulation was also observed in both the higher rate (50 and 140 t ha^{-1}) MWOO incorporated treatments (to an average of 12.5 ± 0.76 and 17.9 ± 0.75 mg kg^{-1}, respectively) in the depth of MWOO incorporation (at 0-5 cm, 5-10 and 10-15 cm depths). The increase in concentration at each depth was significantly greater in the 140 t ha^{-1} treatment, whist this was still significantly lower that the increase in the 140 t ha^{-1} surface applied treatment at 0-5 cm depth. Copper concentrations and distribution in treatments were comparable at 12 and 18 months with no significant changes over the sampling period. Total Cu increase in MWOO contact depths in MWOO treatments relative to the control for SL3, the soil with highest background Cu concentration (to 23.8 ± 0.5 mg kg^{-1}) and the most alkaline pH, were little evident. After 6 months, only in the 50 t ha^{-1} surface applied MWOO treatment at 0 - 5 cm depth was an increase in soil Cu observed (to 34.6 ± 4.4 mg kg^{-1}), but with associated high variability this was not significant. At 12 and 18 months there was no significant difference observed in Cu concentration for the same treatments compared with the 6 month samples but due to in general lower variability on these control samples, the Cu concentrations in the 50 t ha^{-1} incorporated treatment 0 – 15 cm depth were significantly increased (at approximately 25.7 ± 0.4 mg kg^{-}), and at 18 months this also included the 0 - 5 cm depth at 50 t ha^{-1} surface applied and 20 t ha^{-1} incorporated treatment. Below MWOO contact depth, no significant change was observed in soil Cu concentration except in SL2, where soil Cu at 15 – 20 cm depth, just below the MWOO contact depth, was significantly increased in the 50 and 140 t ha^{-1} incorporated treatments compared to the control (to 10.6 ± 0.2 and 13.4 ± 0.2 mg kg^{-1}, respectively) which was also observed in the same 12 month sample but at only at 140 t ha^{-1}.

As with the sandy soils, due to significantly high MWOO Pb concentrations ($P < 0.001$) relative to soil background values, increases in Pb in contact depths for MWOO applied treatments were generally clear. In SL1, at 6 months soil Pb concentration significantly increased at the surface

0 - 5 cm depth in the 20 t ha^{-1} surface application (to 29.5 ± 0.25 mg kg^{-1}) but this was not observed at 50 t ha^{-1}, nor at 12 and 18 months due to high variability in the treatments. For incorporated treatments a significant increase was observed only in the 50 t ha^{-1} treatment at 10 -15 cm depth (to 28.5 ± 1.19 mg kg^{-1}). In this treatment apparent increases at 0 - 5 cm and 5 - 10 cm depths were not significant but the concentrations were not significantly different to that observed at 10 – 15 cm. Consistently at 12 and 18 months, concentrations remained similarly and usually significantly elevated in this treatment at these depths, but the increase in the 20 t ha^{-1} surface applied treatment was not observed. In SL2 at 6 months, Pb significantly increased above control variability only at the highest application rate, in the surface applied MWOO at 50 and 140 t ha^{-1} in the 0-5 cm depth (to 20.4 ± 1.35 and 66.4 ± 4.3 mg kg^{-1}, respectively) and in the top three depths (0-15 cm) for MWOO incorporated at 140 t ha^{-1} (average of three depths up to 26.4 ± 0.9 mg kg^{-1}). The increase was significantly higher in the surface applied 140 t ha^{-1} treatment. These increases were maintained at 12 and 18 months and did not significantly change. In SL3, the more alkaline of these soils and the one with the lowest background Pb, at 6 months a significant increase in Pb concentration at 0-5 cm depth was observed at 6 months in both surface applied treatments, 20 and 50 t ha^{-1} (up to 9.8 ± 0.45 and 20.1 ± 5.3 mg kg^{-1}, respectively) and this was also observed at 12 and 18 months with no significant change in concentration in treatments. Significant soil Pb increases were also observed at all MWOO contact depths (0 - 15 cm) for both incorporated 20 and 50 t ha^{-1} treatments (up to 6.1 ± 1.1 and 8.2 ± 2.01 mg kg^{-1}, respectively) at 6 months which were also maintained without change to 18 months. The increase was significantly higher in the surface applied 50 t ha^{-1} treatment compared to 20 t ha^{-1} treatment, and also in the 50 t ha^{-1} incorporated treatment than in the 20 t ha^{-1} incorporated treatment but not always significantly. Only in SL3, at 12 months, was there some evidence that Pb was slightly but significantly elevated in soil below the MWOO contact depth at 15 – 20 cm in the incorporated 50 t ha^{-1} treatment.

Zinc was also present in MWOO at a significantly higher concentration (411.15 ± 16.34 mg kg^{-1}) than background soil concentrations ($P < 0.001$). In SL1, at 6 months although soil Zn seemed to increase in some MWOO treatment in MWOO contact depths e.g. incorporated MWOO at 50 t ha^{-1}, surface applied MWOO at 20 t ha^{-1}, these were not significant because of the variability on the Zn concentrations for this soil. At 12 and 18 months however, with lower variability on control samples, the 0-15 cm depth samples in 50 t ha^{-1} MWOO incorporated were significantly elevated (to a mean of 40.5 ± 2.3 and 49.6 ± 2.1 mg kg^{-1}, respectively) and a significant increase was also observed at 18 months in the 0 – 5 cm depth sample in the 50 t ha^{-1} surface applied treatment to (44.7 ± 0.7 mg kg^{-1}). The concentrations in treatments did not significantly change, however, over the 18 month sampling period. In SL2, at 6 months, significant increases were observed in MWOO contact depths at the higher application rates, 140 t ha^{-1} for surface applied MWOO (0 – 5 cm depth to a mean of 119.7 ± 9.7 mg kg^{-1}) and incorporated MWOO (0 – 15 cm depth to a lower mean of 66.7 ± 9.2 mg $kg^{-1)}$, and also at 0 – 5 cm depth in the surface applied MWOO at 50 t ha^{-1} (slightly increased to 50.2 ± 3.5 mg kg^{-1}) which was significantly lower than the increase in the equivalent 140 t ha^{-1} sample. There was no further significant change in Zn concentrations or distribution in treatments over the 18 months of sampling. In SL3, the loam with more alkaline pH and higher background Zn, at 6 months the only significant increase in Zn in MWOO contact depth soil was at 50 t ha^{-1} surface applied 0 – 5 cm depth (to 70.5 ± 3.9 mg kg^{-1}). With slightly lower variability on control samples at 12 and 18 months significant increases were observed in also the 50 t ha^{-1} incorporated treatment contact depths (0 – 15 cm) to a mean of 59.3 ± 4.6 and 63.6 ± 3.1 mg kg^{-1}, respectively and in addition lower but significant increases in the 20 t ha^{-1} surface and incorporated treatments at MWOO contact depths to a mean of 57.8 ± 2.8 and 54.2 ± 0.7 mg kg^{-1}, respectively. There was evidence in SL2 and SL3, that soil Zn increased also at depths just lower than the MWOO contact or mixing zone with a significant increase observed at 6 months in SL2 at 15-20 cm depth in the 140 t ha^{-1} incorporated treatment (to 46.3 ± 1.5 mg kg^{-1}), and at 12 and 18 months in SL3 in the 50 t ha^{-1} incorporated MWOO treatment at 15 - 20 cm and 25 – 30 cm (to a mean of 53.0 ± 1.9 mg kg^{-1} at 18 months).

Clay soils - C1, C2, C3

There was no significant change in any of the metal or metalloid concentrations at any depth in the control samples at any sampling period. The MWOO was applied to these soils at rates of 20 and 50 t ha^{-1}, both surface applied and incorporated. Examining the sample concentrations detected shows that for some elements in these clay soils there is apparently some drift in metal and metalloid concentrations observed over the three time periods in the same soil e.g Cr and Cd although all quality control and assurance samples were within range. For these elements therefore, only concentration changes within each sampling time were considered.

The MWOO As concentration (5.3 ± 0.5 mg kg^{-1}) was not significantly higher than background As in all three clay soils C1, C2 and C3 (1.9 ± 0.5, 0.9 ± 0.6 and 1.6 ± 0.3 mg kg^{-1}, respectively) but no significant change in As concentration or distribution in any treatment was observed over the three sampling periods. Cadmium in MWOO was also present at low concentrations (2.2 ± 2.1 mg kg^{-1} in MWOO) but was not significantly different to Cd concentrations in all three clay soils. With MWOO addition to soils no significant change to Cd distribution or concentration was observed and this remained over the three sampling periods (the data for Cd in C2 at 12 months is not included here due to inconsistencies in analysis).

Chromium and Ni in MWOO (85.4 ± 12.28 mg kg^{-1} and 40.26 ± 10.84 mg kg^{-1}, respectively) were significantly lower than the background soil concentrations for all these clay soils and for these elements it was not possible to observe any significant change in concentrations and distribution with MWOO additions in the three clay soils, also compounded by variability on sample analysis at times (Cr in C2 at 12 months is considered anomalous when compared to the other two time periods for this soil).

Similar to the other soils included in this study, metals Cu, Pb and Zn were all present in MWOO at significantly greater concentrations ($P < 0.001$) than the background values for these clay soils (Table 2.1), but usually significant changes in soil concentration with MWOO additions compared to the control treatment were apparent only at the higher application rate in MWOO contact depths and when variability in samples was lower. For Cu at 6 months, this was clearly observed in all three clays at 0 – 5 cm depth for 50 t ha^{-1} surface applied MWOO treatment (to 58.5 ± 1.7 mg kg^{-1} in C1; to 57.5 ± 1.7 mg kg^{-1} in C2; 122.5 ± 11.7 mg kg^{-1} in C3). At some MWOO contact depth for the 50 t ha^{-1} incorporated MWOO samples, significant increases were also observed in some soils and in C3 (the soil with the lowest background Cu) at 20 t ha^{-1} but these were not as great as at 50 t ha^{-1}. There were no significant changes in soil Cu concentrations from 6 to 18 months with any increases observed at 6 months being maintained to 18 months (although the significance of these depended on control variability) in the three soils in MWOO contact depths. No change in concentration was observed in soil depths below the MWOO contact zone.

For Pb, 6 months following MWOO application, in all three clay soils, soil Pb concentration significantly increased at 0-5 cm depth for the surface applied 50 t ha^{-1} MWOO treatment in C1, C2 and C3 (up to 22.07 ± 2.03, 15.01 ± 1.02 and 25.1 ± 2.3 mg kg^{-1}, respectively). Lower but usually significant increases were also observed at 0 – 5 cm depth in the 20 t ha^{-1} surface application in all three soils. For incorporated treatments a slight and usually just significant increase was observed in the 0-15 cm depths at 20 t ha^{-1} in all soils, and a significant and greater increase was observed at 0-15 cm depths in 50 t ha^{-1} application (up to 10.25 ± 1.2, 12.5 ± 2.5 and 8.8 ± 0.61 mg kg^{-1}, respectively), but in C1 and C3 this was lower than the increase in the corresponding surface applied treatment. Over the longer term to 12 and 18 months, these increases were generally maintained without significant change, although in C1, the more acidic clay, there seemed to be a slight decrease in the surface depth of the 50 t ha^{-1} surface applied treatment at 18 months. In soil below MWOO contact depth, in C2, the more neutral clay, Pb slightly but significantly increased in soil immediately below MWOO contact at 15- 20 cm depth for incorporation and at 5-10 cm depth for surface applied at 18 months for 50 t ha^{-1} MWOO application which was also observed in C3.

Similarly for Zn in the clay soils, at 6 months in C1, the more acidic clay soil, despite some sample variability, in MWOO surface applied at 50 t ha^{-1} Zn significantly increased at 0-5 cm depth (to 119.4 ± 5.8 mg kg^{-1}) and slightly but significantly in some of the MWOO contact depths (0-5 cm) to 121.6 ± 1.3 in the same rate incorporated treatments, but not at 20 t ha^{-1} application. This was also the case for C2 with a similar increase at 0-15 cm depths in the incorporated 50 t ha^{-1} treatment (to 108.7 ± 0.93 mg kg^{-1}) and at 0-5 cm depth in surface applied 50 t ha^{-1} treatment (to 102.7 ± 4.4 mg kg^{-1}). In C3, the more alkaline clay, significant increases were observed in MWOO contact depths in all treatments (e.g. Zn at 0-5 cm depth for 20 t ha^{-1} surface treatment (to 80.1 ± 4.2 mg kg^{-1}), but a significantly greater than all other increases was observed at 0-5 cm depth in the 50 t ha^{-1} surface treatment (to 105.6 ± 3.6 mg kg^{-1}). Increases were maintained to 18 months, in MWOO contact depths. In C2, the more neutral clay, with in general lower sample variability, in some MWOO treatments at both 20 and 50 t ha^{-1} at 12 and 18 months, Zn in soil below MWOO contact depth was slightly but significantly increased to 20 cm compared to the control, which was not apparent in the other clay soils.

High organic content soil - OM1

An examination of the distribution of metals and metalloids in the control columns of the high organic content soil after 18 months of maintaining moisture at 60% field capacity shows that there was no significant change in any of the metal and metalloid concentrations over the depth profile over 18 months. The MWOO was applied to this soil at rates of 20 and 50 t ha^{-1}, both surface applied and incorporated. The MWOO As concentration (5.3 ± 0.5 mg kg^{-1}) was slightly but not significantly higher than background As in this soil (3.1 ± 0.4 mg kg^{-1}). Over the 18 months of sampling following MWOO application, no significant change in As distribution in any treatment compared to the controls was observed. The MWOO Cd concentration (2.25 ± 2.1 mg kg^{-1}) was slightly but not significantly lower than background Cd in this soil (4.3 ± 0.1 mg kg^{-1}) but MWOO Cr (85.4 ± 12.28 mg kg^{-1}) and Ni (40.2 ± 10.84 mg kg^{-1}) concentrations were significantly ($P < 0.001$) lower than background soil concentrations for this soil. For all these elements there was no significant change in soil concentration or distribution in any treatment over the 18 month experiment (The decreased Ni at 0 – 5 cm depth in surface applied MWOO at 50 t ha^{-1} at 18 months was considered anomalous in light of the whole Ni dataset).

The metals Cu, Pb and Zn were all present in MWOO at significantly ($P < 0.001$) greater concentrations than the background values (52.8 ± 0.0, 16.0 ± 1.3 and 82.1 ± 1.4, respectively) for this high organic content soil (Table 2.1). Considering Figures 2.70 to 2.82, after 6 months following MWOO application, a significant increase in soil Cu concentration in the surface 0-5 cm sample in 50 t ha^{-1} MWOO surface application treatment (to 53.5 ± 2.8 mg kg^{-1}) and a slight but significant increase at 0-15 cm depths in the incorporated 50 t ha^{-1} treatment (to 53.0 ± 0.63 mg kg^{-1}) was observed. The increase in the surface application was significantly greater than the increase in the same rate incorporated treatment. No increase was observed in the 20 t ha^{-1} treatments. With the high variability on control samples at later sampling times, significant increases in the MWOO contact depths compared to the controls were not evident although concentrations in treatments did not change from 6 to 18 months. Similarly, after 6 months following MWOO application, soil Pb concentration were significantly increased at the surface 0-5 cm depth in the 50 t ha^{-1} surface application (to 50.45 ± 4.1 mg kg^{-1}) and with also significant (but lower) increases in the 50 t ha^{-1} incorporated treatment MWOO contact depths (0 – 15 cm) (to 24.8 ± 1.04 mg kg^{-1}) compared to controls. Lead concentrations to 18 months remained similar to the 6 month values with no significant increases observed in the 20 t ha^{-1} treatments at any sampling period. For Zn also, there was a clear and significant increase in Zn soil concentration at depths where MWOO had contact with soil at 6 months only at 50 t ha^{-1} MWOO applications. In MWOO surface applied, Zn significantly increased at 0-5 cm depth for 50 t ha^{-1} (to 118.03 ± 4.9 mg kg^{-1}) with a lower increase at 0-10 cm depths in the incorporated 50 t ha^{-1} treatment (to 89.6 ± 5.2 kg^{-1}). Again concentrations in treatments did not change to 18 months although significance of increases relative to the control depended in control variability. For, Pb, Cu and Zn at 12 months at 15 – 20 cm depth in the 50 t ha^{-1} incorporated treatment was there indication of slight but significant concentration increases immediately below the MWOO contact depth.

2.3.4.3 Relationships between metals, metalloids and soil EC, pH and Total Organic carbon (TOC) following MWOO application

The results for pH, EC and TOC in different treatments and controls following MWOO application are presented in appendix B.1. These are only included for the 6 month sampling because for most metals little change was observed in concentration or distribution from 6 to 18 months. These parameters are not discussed in detail because the focus of this chapter are the metals and metalloids.

Increases in total organic carbon (TOC) were observed in all MWOO contact application depths (0 - 5 cm for surface applied MWOO and 0 - 15 cm depths for incorporated MWOO) in all soils. The increases were more significant at the higher rates of application and in soils with lower background TOC e.g. S2. In fact, in C1 and OM1 with higher background TOC, increases were only evident at 0 – 5 cm depth for the surface applied 50 t ha^{-1} MWOO treatment. Some increases in pH were also observed but generally were more evident in the sandy soils and those soils with lower pH. The pH did not change with MWOO application in any of the clays. Increases in EC were observed but usually in the 0 – 5 cm depth only, including the incorporated treatments, and these were not always higher at the higher rates of application. The effect of MWOO treatment on these parameters was restricted to the MWOO contact depths with little evidence of significant change in the concentrations or distribution compared to the control below the MWOO contact zone.

Table 2.2 presents the Spearman's Rho correlation coefficient and significant difference between metals, metalloids and soil EC, pH and total organic carbon (TOC) for all ten soils including all depths and all MWOO applied treatments. For the sandy soils, in S1 and S2 a highly positive correlation between Zn, Pb and Cu and EC and pH ($P \leq 0.001$), also for Zn and Pb with TOC was observed in both soils ($P < 0.001$) but this was only apparent in S1 for Cu with TOC ($P = 0.01$). Nickel showed a highly positive correlation with EC and pH ($P = 0.04$ and $P = 0.03$, respectively) and As only with pH in S1 ($P = 0.02$. Cadmium and Cr showed no correlation in either soil. In S3 only Pb was correlated with EC, pH and TOC (all $P < 0.001$), Zn and Cu with pH ($P = 0.01$) and Cu with TOC ($P = 0.03$). In all three loam soils (SL1, SL2, SL3) high positive correlations were observed between Cu, Pb and Zn and EC and TOC (all $P < 0.001$); and between As, Cr and Ni and EC ($P = 0.03$, $P < 0.001$ and $P = 0.01$, respectively) and TOC ($P = 0.05$, $P < 0.001$ and $P < 0.001$, respectively). All elements except for Cd showed a high negative correlation with pH in SL3. In OM1 a strong positive correlation between Zn, Pb and Cu and EC ($P = 0.007$, $P < 0.001$ and $P = 0.001$) and pH (all $P < 0.001$) was observed, while Ni and Cr showed a high negative correlation (both $P = 0.01$) with TOC in this soil. In all three clay soils (C1, C2 C3), a highly significant correlation between Zn, Pb and Cu and EC and TOC was observed (except for Zn with EC in C3). Zinc and Pb showed a highly significant positive correlation with pH in C1, the more acidic clay, As with pH, and Cd with EC and TOC in the more alkaline alkaline clay.

2.3.4.4 Inter factor comparisons

The repeated measures mixed model 5-way ANOVA test used to assess the interaction of the five parameters, sampling period, soil, application method, application rate and depth on the concentration of each element is presented in appendix B.3. In summary, the factors that overall had a highly significant ($P < 0.001$) influence on soil concentration were sample depth and soil. Application rate and method showed a highly significant effect on the concentration of Cu, Pb and Zn ($P < 0.001$) and also for Ni ($P < 0.002$). For As and Cd, application method was not significant ($P = 0.36$ and $P = 0.17$), while for Cr the application rate did not show a significant impact on concentration ($P = 0.14$). Based on the multiway interactions, most of the interactions were significant (table 3-8), but based on the non-significant interactions, for As, Cd, and Ni, the factor which showed the least impact on concentration was application method. For Pb and Zn the time period was the least important, for Cr application rate followed by application method the least important and for Cu time period was least important. So, for all metals the factors soil and usually application rate were the most important for influencing concentrations observed.

2.3.5. Discussion

2.3.5.1 Metals and metalloids in MWOO and soils

The metal background concentration of metals and metalloids in the soils varied widely, but was generally within background values for soils reported in Australia (except As in S1). The NSW General Exemption specifies maximum allowable soil contaminant values above which MWOO application is not allowed. Soils for this study, were selected to provide a range of texture, organic matter and pH, to better understand how MWOO amendment may affect soil properties and how this may affect metal and metalloid concentrations and distribution. To achieve the range of soil properties required inclusion of some soils with contaminant concentrations greater than these maximum allowable concentrations was necessary. It should be noted that arsenic in S1 and Cd, Cr and Ni in OM1 and the three clay soils exceeded the maximum allowable values for soils on which MWOO can be applied in NSW.

The order of metal and metalloid mean concentration for the MWOO was Zn > Pb > Cu > Cr > Ni> As > Cd, which is in agreement with other studies reviewed (Smith, 2009) and also previous Australian data (Wilson, Bayat & Wilson 2014). The concentration of Zn, Pb and Cu (411.1, 275.1 and 180.8 mean mg kg^{-1} respectively), detected in MWOO was significantly higher ($P < 0.001$) than background in all the soils and all other metals and metalloids. These elements are typically detected in MWOO at higher concentrations than other contaminant metals and were within the range of values reported for these elements in Australian MWOO (Wilson, Bayat & Wilson 2014). Total mean concentration of Pb detected in the MWOO used exceeded the maximum allowable value for MWOO in NSW. The concentrations of the other elements assessed were also within the values typically detected in Australian MWOO and within limits for MWOO use in NSW, but were not always greater than background soil concentrations. This had an important influence on detection of significant changes in concentration with MWOO application. Chromium and Ni (85.42 and 40.26 mean mg kg^{-1}respectively) in MWOO were significantly higher than background values in the sandy soils and loams but lower than background values in the clays and the organic soil. Cadmium in MWOO was only significantly greater than background soil concentrations in OM1, C1 and C2. Arsenic in MWOO was slightly (but not significantly) lower or similar in concentration to arsenic in all soils except S1.

2.3.5.2 The impact of MWOO application on metal and metalloid concentrations and distribution in soil

Metal and metalloid retention and accumulation in soil is influenced by several parameters including organic matter, mineral composition, CEC, soil redox and pH (Jones and Jarvis, 1981). The clay particles, organic matter and Fe, Mn and Al oxides and hydroxides are the main sites for metal sorption. Availability and mobility of metals and metalloids is usually lower in soil containing higher proportions of these soil fractions. A range of mechanisms such as non-specific electrostatic attraction, specific adsorption (often on Fe, Al and Mn oxides and hydroxides), formation of organic matter complexes and chemi-sorption (important in calcareous soils) control metal sorption in the soil (MacBride, 2000). An important factor which can control the importance of these mechanisms is the pH of soil. In acidic soils, the negative charge on Fe, Mn and Al oxides and hydroxides and organic matter surfaces is reduced, leading to less sorption of cationic metals such as Cu, Zn, Ni, with increasing metal mobility and availability (Smith, 2009). However, elements such as As which forms anions in acidic conditions can become less available in acidic soils.

Application of MWOO, as well as adding the MWOO derived contaminants to soil, can influence the ability of soils to retain the metals and metalloids. The MWOO can increase sorption in the soil as a result of additional sorption sites provided by organic matter through mechanisms such as chelation and ligand exchange. Metals such as Cu can be strongly bound to organic matter, although, soluble organic carbon Cu complexes can also be formed and may influence mobility (Mkhabela and Warman, 2005). Application of MWOO to soil can increase the pH and buffering capacity of the soil, although this impact can be reduced in soil with high clay content (Garcia-Gil et al. 2004, Smith 2009). Adding MWOO to soil also can provide Fe and Mn hydroxides which provide negative surfaces for sorption of cations (Illera et al. 2000). The metal and metalloid concentrations in the MWOO are also important because MWOO with low heavy metal content will tend to show a higher sorption capacity (Baldwin and Shelton, 1999; Jordao et al., 2006; Jordao et al., 2007; Smith, 2009a).

2.3.5.3 Metal and metalloid distribution in control treatments over 18 months

Considering first the control treatments a slight increase in some metals and metalloids was observed with depth at 6 months in the sandy soils. Nickel and Zn increased at 65-70 cm depth in S1, the more acidic sand and a slight but significant increase at depth was also observed for As, Cr, Cu, Ni and Pb in S2, the more neutral sandy soil with the greatest percent sand (this increase at 12 and 18 months was still apparent for some elements at times but with increased variability was not significant). Of the metals examined here Ni and Zn generally show the greatest fraction in exchangeable and more labile forms in soil with greater potential for down profile movement. In these more acidic sandy soils, where capacity for sorption was lower, it seems that water addition to maintain the water content at 60% field capacity resulted in some movement of the naturally occurring elements down the soil in these disturbed profiles, possibly by enhanced colloidal movement. However, with the MWOO amendment this was not observed.

2.3.5.4. Metal and metalloid accumulation in soil for MWOO contact depths

The influence of MWOO on soil metal and metalloid concentrations, for all elements examined, was most evident in the MWOO contact depths, which were 0 – 5 cm for MWOO surface application and 0 – 15 cm for incorporated MWOO. For all the metals and metalloids, however, the extent of the MWOO influence evident at the first sampling period, 6 months, was primarily determined by the MWOO concentration relative to the background concentration in the soil. For example, the concentration of As, Cd, Ni and Cr in MWOO was low compared to MWOO Cu, Pb and Zn, and was not always significantly different to the soil background concentrations. An increase in As, Cd, Cr and Ni in MWOO contact depths was observed in a number of MWOO treatments for the sandy soils and the loams but not for the clays and organic soil, which showed similar or greater background concentrations of these elements compared to MWOO concentrations. In the sandy soils and loams with lower background concentrations of these elements, however, an increase in soil concentration in MWOO contact depth was observed with MWOO application. This increase was usually only significant at the higher rates of application (50 t ha^{-1} and 140 t ha^{-1}) (e.g As in S3 and SL3, Cd in S3, Cr in S2, SL2 and SL3, Ni in SL2) with the greatest concentrations observed in the surface applied MWOO treatments at 0 – 5 cm depth.

The concentrations of Cu, Pb and Zn in MWOO were significantly higher than background concentrations in all the soils studied, and significant increases in soil concentrations with MWOO application were observed in MWOO contact depths for all three of these elements in all ten soils. This increase was, at times, observed at the lower rate of application (20 t ha^{-1}) in soils with lower background values (e.g. sandy soils), but more clearly and significantly at the higher application rates 50 and 140 t ha^{-1} for all the soils. The high positive correlation between these three elements and concurrent increases in soil EC, pH and TOC (table 2) in the majority of the soils with MWOO application confirms MWOO as the source. The greater increases were observed at higher application rates for the same mode of MWOO application although increases were not always proportional to application rate. The greatest increase in soil concentrations for all three elements was observed in the 0 - 5 cm depth of the surface applied treatments at the highest rates. The application rate was generally a more important influence on soil concentration than method of application.

Similarly, increases in soil concentrations with MWOO application have been reported by other authors for a range of different soils with the greater increases reported for elements with higher MWOO concentrations. For example, Weber et al. (2007) reported a significant increase in the concentration of soil Zn, Pb and Cu at 30, 60 and 120 t ha^{-1} MWOO application (up to 60, 33.2 and 13.1 mg kg^{-1}, respectively) but this was observed at only 120 t ha^{-1} for Ni, Cr and Cd (to 14.4, 13.2 and 1.3 mg kg^{-1} respectively) a year following MWOO application to a slightly acidic (6.05-6.44 pH) sandy soil. The MWOO used in this study was collected from highly industrialised area, with extremely high concentrations of Zn, Pb and Cu and high but lower Ni, Cr and Cd (1825, 972, 366, 168, 100, 11.7 mg kg^{-1} respectively). Dorahy et al. (2006) reported that application of MWOO at 100 and 200 t ha^{-1} to an Australian soil (an acidic dermosol) significantly increased Zn, Cu and Pb in soil (up to 222, 90 and 78 mg kg^{-1}) and that total soil Zn exceeded 200 mg kg^{-1}, the allowable maximum soil concentration based on NSW EPA guidelines at the time (NSW EPA 1997). Significant increases in Zn, Pb and Cu concentrations in the top 15 cm of an alkaline sandy soil one year after MWOO application in proportion to the application rates (40, 80, 120 t ha^{-1}) were reported by Walter, Martinez & Cuevas (2006) and these increased over 5 years with successive applications. Increases in Ni were only observed at the higher application rate but again increased with successive applications over 5 years although Cd and Cr did not show any change. Pinomanti et al. (1997) applied non source separated MWOO with relatively high concentrations of Zn, Pb, Cu, Ni, Cr and Cd (1206, 650, 447, 132, 61 and 3.2 mg kg^{-1}, respectively) to 0-30 cm depth of a medium sandy, calcareous soil from an orchard and reported a significant increase in total soil Zn, Cu, Ni, Pb and Cr, proportional to the application rates 80 and 160 t ha^{-1}, but with Cd remaining below the detection limit. Ayari et al. (2010) examined the long term impact of MWOO application at two rates (40 and 80 t ha^{-1}) on Tunisian clay loam soil with slightly alkaline pH (7.88). The MWOO contained Zn, Pb, Cu, Ni, Cr, Cd (1174.5, 411.5, 337, 90.8, 78.87 and 5.17 mg kg^{-1} respectively). They reported no significant increase in total concentration of Cd, Ni, Pb, Cu and Zn at 0 - 20 cm following MWOO application after one year but increases were observed in consecutive years after repeat applications even for elements with low MWOO concentrations such as Cd. Giusquani et al. (1994) reported significant increases in total soil Pb and Zn with 30, 90, 270 t ha^{-1} MWOO application to an alkaline clay soil after three years. The significant increase was also observed for Cu and Cd only at 90 and 270 t ha^{-1} MWOO application.

Importantly, the increases in metal and metalloid concentrations observed in this study at initial sampling, 6 months after MWOO addition, under the watering regime to maintain all soils at 60% field capacity remained through to 18 months. No significant increase or decrease for any element in any of the soils over the 18 month experiment was observed after the initial 6 month sampling in MWOO contact depths. This suggests that in these NSW soils, under these soil conditions, which are typical for NSW, the majority of the metal and metalloid load supplied by the MWOO remained associated strongly within the soil depth to which the MWOO was added, presumably remaining strongly associated with MWOO constituents as incorporated or surface applied to soils. This was even the case in the sandy soils with lower sorptive capacity.

The actual maximum concentration values for metal and metalloid concentrations detected in the MWOO soil contact depths for this experiment (with this one MWOO application to soils with only Pb in MWOO slightly exceeding the maximum allowable value but also a number of metalloid background concentrations exceeding maximum allowable values for soil (as defined in the NSW General Exemption)) were less than soil maximum concentrations above which MWOO cannot be applied to soil in NSW. This was even the case for surface application of MWOO which resulted in the highest soil concentrations and also for the loam used for mine site rehabilitation, SL2, to which MWOO was applied at 140 t ha^{-1}. Metal and metalloid accumulation was evident in all soils, however, especially for Pb, Cu and Zn, and in light of the fact, that the metal and metalloid concentrations did not change over the 18 month experiment in MWOO contact depths, this has important implications for capacity to apply additional MWOO to these NSW soils. On the basis of this experiment, under current guidelines, Pb accumulation with surface MWOO application would be one of the main constraints for repeat applications (e.g. Pb increased to 66.4 mg kg^{-1} in SL2 used for mine site rehabilitation and a value of 150 mg kg^{-1} Pb is used as the maximum soil concentration to which MWOO can be applied for this site use). Accumulation of other metals (e.g. Cu, Zn and Cd) to threshold concentrations acceptable for specific site uses, however, would occur with only a further one to two additional surface applications of the MWOO used in this experiment in many of the soils used. The incorporation of MWOO would extend this due to the dilution in effect of metal accumulation through soil depth.

It is well established that risk evaluation cannot be based solely on total contaminant concentration in soil. Bioavailability to organisms and phytotoxicity of the contaminant must also be integrated into any risk assessment. Clear evidence exists to show that both bioavailability and phytoavailability are influenced by soil characteristics. For example, phytotoxicity is strongly influenced by %OC, pH and CEC (Heemsbergen et al., 2009; Rooney et al., 2006). Heemsberger et al. (2009), considering soil quality guidelines derived for biosolids amended soils that incorporated phytotoxicity considerations in different soils, showed that for Cu, a soil amendment availability factor increased significantly with pH, from 1.44 at pH 4 to 2.15 at pH 8 resulting in smaller derived added contaminant limits at lower soil pH values. The data demonstrated significantly greater phytotoxic effects for Cu in amended soils with lower pH. In the study here, a number of the metal concentrations measured in the MWOO amended soil treatments (e.g. Cu) were within phytotoxic ranges as documented in the published literature and also in a recent MWOO review document (Kabata- Pendias and Pendias, 2001; Wilson et al. 2014). The NSW soils studied here showed a wide range in chemical characterisation including pH, %OC and CEC (Table 2.1). We would therefore expect a significant variation in phytotoxicity and bioavailability for the trace metals and metalloids monitored in these soils. Differences in phytoavailable contaminant for a selection of the soils has been examined, within the scope of this project, for wheat in Ch 5. It is critical that risk evaluation for MWOO amended soils in NSW includes consideration of bioavailability and phytotoxicity and the total concentrations measured here provide a baseline for realistic total contaminant loads with NSW MWOO soil amendment within this assessment. ..

2.3.5.5. Metal and metalloid movement down soil profile below MWOO contact depth.

As discussed above, when considering metal and metalloid movement down soil profile in the control treatments, there was some evidence that the addition of MWOO limited mobility of the elements significantly down soil profile in the sandy soils e.g. for Ni in the sandy soils. However, for the metals with high MWOO concentrations, Cu, Pb and Zn, at the higher application rates (usually 50 t ha^{-1} and higher) an increase in the soil concentration at depths immediately below the MWOO contact zone for both surface applied and incorporated treatments was observed in many of the soils, including the clays and OM1 with high contaminant sorptive capacity. This effect was evident for all three of these metals, albeit it to a greater extent at times for Zn, the more labile of these metals. For incorporated treatments this effect could be an artefact of some mixing with the 15 – 20 cm soil depth, but this was not considered likely for the 5 – 10 cm depth in surface applied treatments where contaminant increases were also observed. Therefore, this experiment has illustrated that contaminant increases were not limited to the MWOO contact zone, but for those metals with high MWOO concentrations applied at higher rates, over 18 months some movement to soil immediately (5 cm) below the MWOO contact zone did occur, and this was more extensive (up to 10 cm below the MWOO contact depth) for more labile metals.

Results from other authors are contradictory but some down profile movement has been observed over time, especially at higher rates of application. Breslin (1999) examined the impact of none source separated MWOO on the concentrations of Zn, Pb, Cu, As and Cd (340, 310, 120, 5.2 and 2.6 mg kg^{-1} respectively in MWOO) on the 0-5 cm depth of an acidic sandy loam. They reported increases in total soil concentration of Zn, Pb, Cu and Cd at this depth were proportional to application rate after 16 months but increases in soil below 5 cm were not observed. In a study conducted by Madrid et al. (2007) on an alkaline (pH 7.5) sandy soil sample, however, following application of MWOO three times at 21, 21 and 18 t ha^{-1} a significant increase in Zn and Pb was observed at 25-50 cm after the second application but not observed for Cu and Ni. Perez et al. (2007) examining metal concentration in an acidic clay soil samples one year after application of MWOO reported increased total Cu, Cr and Pb at 0 -5 cm and but no change for Cd and Ni. The Cu and Pb also increased at 5 - 10 cm at all four (12.5, 25, 50 and 100 t ha^{-1}) application rates but only showed an increase at 10-20 cm depth at 50 and 100 t ha^{-1}. Cambier et al. (2014) in a large scale 14 year trial also observed down profile movement and increased concentrations in leachate especially of Cu, but also Zn, in a Luvisol of the Paris Basin applied with MWOOO trace metals concentrations within regulatory limits. However, the % element leached was < 1% of respective input.

2.3.6. Conclusions

Application of MWOO significantly affected the concentration and distribution of seven priority targeted metals and metalloids in ten different NSW soils. The impact was influenced by a range of the factors including the element initial concentration in MWOO, the background soil concentration, application method and rate. Generally, the most important factor was the concentration of the metals and metalloids in MWOO compared to background soil concentrations.

Increases in soil concentrations in the MWOO contacts depths (0 – 5 cm for surface applied treatments and 0 – 15 cm for incorporated treatments) were consistently observed in all soils (regardless of soil properties) for many of the elements examined, but were most significant for the metals with higher concentrations in MWOO (Cu, Pb and Zn) and in soils with lower background concentrations. Increase in soil concentrations were, at times, observed at the lower rate of application (20 t ha^{-1}) in soils with lower background values, but were more clear and significant at the higher application rate, 50 and 140 t ha^{-1}, for all the soils. Greater increases were observed at higher application rates for the same mode of MWOO application although increases were not always proportional to application rate. The greatest increase in soil concentrations was observed in the 0 - 5 cm depth of the surface applied treatments at the highest application rates. Increases in soil concentration remained in the MWOO contact depths over the 18 months of the experiment with no significant decrease or increase in concentration in any soil during this period. This demonstrated some limitation for MWOO repeat applications on a number of the NSW soils. Accumulation of some metals with the one MWOO application e.g. Pb for surface applied MWOO, resulted in soil concentrations close to 50% of the maximum allowable value for soil to which MWOO can be applied which would limit any repeat MWOO applications. Furthermore, some of the concentrations observed after MWOO application were close to the range considered phytotoxic for plants.

Considering down profile movement, evidence in the sandy soils suggested MWOO additions limited contaminant mobility to some extent. For Cu, Pb and Zn, however, the metals present in MWOO at the highest concentrations, there was evidence especially at higher MWOO application rates (50 t ha^{-1} and higher), that over 18 months, some movement to soil immediately (5 cm) below the MWOO contact zone occurred, and this was more extensive (up to 10 cm below the MWOO contact depth) for more labile metals. It is possible that water addition to maintain 60% field capacity might have resulted in some movement of the naturally occurring elements down the soil in these disturbed profiles, possibly by enhanced colloidal movement. However, with the MWOO amendment this was not observed.

The experiment has illustrated the need for additional research to further quantify the limitations for MWOO applications in NSW soils, especially for surface MWOO application on soils with higher background metal and metalloid concentrations. This research should consider repeat MWOO applications to soils considered of greater risk and at larger scale in the field under typical NSW environmental conditions. The cumulative implications on any down profile movement and leaching with a concurrent quantification of the bioavailability and toxicity of the metals and metalloids to soil organisms and plants would provide a more accurate evaluation of risk than gained from laboratory experiments. Nevertheless, the total soil concentrations with MWOO addition measured in this experiment provide for the first time a realistic baseline for risk evaluation in NSW soils.

Table 2.2 The correlation parameters between metals and EC, pH in CaCl2 and TOC of ten soils studied following MWOO application

S1														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P value	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	0.19	0.07	-	-	0.02	0.84	0.41	<0.0001*	0.21	0.04*	0.53	<0.0001*	0.47	<0.0001*
pH	0.23	0.02*	-	-	0.16	0.12	0.71	<0.0001*	0.22	0.03*	0.88	<0.0001*	0.75	<0.0001*
TOC	0.03	0.72	-	-	-0.01	0.85	0.25	0.01*	0.11	0.26	0.33	0.001*	0.33	0.001*
S2														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	-0.03	0.75	-	-	0.16	0.12	0.61	<0.0001*	0.02	0.82	0.73	<0.0001*	0.47	<0.0001*
pH	-0.06	0.51	-	-	0.10	0.33	0.50	<0.0001*	0.01	0.85	0.67	<0.0001*	0.52	<0.0001*
TOC	0.0004	1.0	-	-	0.19	0.07	0.66	<0.0001*	0.11	0.27	0.80	<0.0001*	0.47	<0.0001*
S3														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	0.13	0.20	0.19	0.06	0.02	0.81	0.25	0.01*	0.08	0.40	0.50	<0.0001*	0.26	0.01*
pH	-0.10	0.31	-0.15	0.13	-0.02	0.79	0.09	0.36	0.02	0.80	0.34	0.0008*	0.17	0.10
TOC	-0.0005	1.0	0.01	0.91	-0.05	0.62	0.22	0.03*	0.05	0.63	0.44	<0.0001*	0.20	0.05
SL1														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	0.10	0.36	0.08	0.47	0.17	0.15	0.55	<0.0001*	0.14	0.23	0.45	<0.0001*	0.41	0.0003*
pH	0.03	0.80	0.11	0.83	0.11	0.34	0.50	<0.0001*	0.18	0.11	0.42	0.0002*	0.52	<0.0001*
TOC	0.10	0.37	0.03	0.78	0.09	0.44	0.40	0.0004*	-0.11	0.34	0.34	0.003*	0.28	0.01*
SL2														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	-0.06	0.51	-0.18	0.05*	0.19	0.04*	0.63	<0.0001*	0.12	0.20	0.58	<0.0001*	0.56	<0.0001*
pH	-0.01	0.91	-0.08	0.37	0.33	0.0004*	0.63	<0.0001*	0.25	0.01*	0.65	<0.0001*	0.65	<0.0001*
TOC	0.008	0.91	-0.05	0.27	0.22	0.04*	0.28	<0.0001*	0.14	0.01*	0.35	<0.0001*	0.36	<0.0001*
SL3														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	0.24	0.03*	0.05	0.61	0.62	<0.0001*	0.50	<0.0001*	0.30	0.01*	0.71	<0.0001*	0.70	<0.0001*
pH	-0.25	0.02*	-0.01	0.90	-0.41	0.0002*	-0.43	<0.0001*	-0.24	0.03*	-0.48	<0.0001*	-0. 51	<0.0001*
TOC	0.22	0.05*	-0.008	0.93	0.70	<0.0001*	0.70	<0.0001*	0.40	0.0003*	0.82	<0.0001*	0.75	<0.0001*

C1														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	-0.03	0.76	-0.03	0.14	0.20	0.09	0.48	<0.0001*	-0.13	0.26	0.40	0.0007*	0.52	<0.0001*
pH	-0.17	0.14	-0.17	0.52	-0.18	0.12	-0.002	0.98	-0.09	0.45	0.53	<0.0001*	0.24	0.04*
TOC	-0.10	0.36	0.04	0.71	0.11	0.35	0.38	0.001*	-0.12	0.31	0.42	0.0002*	0.43	0.0002*
C2														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	0.01	0.92	0.004	0.96	0.14	0.20	0.65	<0.0001*	0.17	0.13	0.71	<0.0001*	0.74	<0.0001*
pH	-0.08	0.44	-0.03	0.78	0.11	0.34	0.13	0.25	0.08	0.47	-0.08	0.48	0.18	0.11
TOC	-0.03	0.78	0.21	0.06	0.10	0.35	0.36	0.001*	-0.07	0.54	0.34	0.002*	0.37	0.0008*
C3														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	0.14	0.20	0.32	0.005*	0.02	0.84	0.30	0.01*	-0.07	0.50	0.30	0.008*	0.19	0.09
pH	-0.24	0.03*	-0.18	0.12	-0.14	0.23	-0.21	0.06	-0.08	0.44	-0.13	0.23	-0.15	0.18
TOC	0.13	0.24	0.37	0.0009*	0.17	0.12	0.44	<0.0001*	0.01	0.87	0.54	<0.0001*	0.46	<0.0001*
OM1														
	As		Cd		Cr		Cu		Ni		Pb		Zn	
	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P	Coe	P
EC	0.15	0.17	-0.10	0.36	-0.13	0.24	0.36	0.001*	-0.18	0.11	0.44	<0.0001*	0.30	0.007*
pH	0.05	0.61	-0.11	0.31	0.12	0.30	0.71	<0.0001*	-0.04	0.73	0.60	<0.0001*	0.67	<0.0001*
TOC	-0.15	0.20	-0.16	0.17	-0.28	0.01*	-0.07	0.52	-0.30	0.01*	0.20	0.09	-0.04	0.70

EC = Electric conductivity, pH in CaCl2, TOC = Total Organic Carbon %, TN=Total Nitrogen %, P = p values, Coe = Correlation Coefficient, significant correlation = *

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3. Effects of MWOO application on soil biological activity

3.1 Summary

A respiration experiment was conducted over a 180 day period to assess the effects of MWOO application on soil microbial activity. Four of the NSW soils were used in this experiment [Kirby Sand (S1), CROA (SL1), Minesite (SL2) and Kirby Clay (C1)] to provide a range of soil texture and pH. MWOO was applied at rates of 0 t ha^{-1}, 20 t ha^{-1}, 50 t ha^{-1} and 140 t ha^{-1} to all soils. The CO_2 evolved from soils is a measure of microbial activity in the soil and was monitored regularly over the experimental period and decomposition curves constructed.

All of the soils we examined exhibited a similar response to MWOO application with a significant initial response of increased CO2 evolution which we attribute to a supply of fresh carbon (and nitrogen) to the soil. For each soil type, this initial increase in respiration induced by the MWOO application was progressively larger at the higher application rates. This initial increase in respiration was followed by a two phase decomposition trend with respiration rate diminishing to around 34 days at which point we believe the labile, “fast pool” became limited and more resistant (slow pool) carbon began to dominate. After 34 days we could detect no difference in the respiration rate between treatments.

Addition of MWOO to soils can therefore significantly increase soil microbial activity (as measured by soil respiration). The magnitude of these changes are relative to the quantity of MWOO and presumably C and N that it provides. The benefit would however seem to be limited in its duration having diminished to control levels after 34 days regardless of soil type and application rate. This we interpret to mean the rapid decomposition of labile “fast” pool carbon diminished after this time period leaving only the more resistant carbon fractions beyond this time.

3.2 Introduction

A wide range of physical and chemical parameters have been used to examine and assess the effects of MWOO application on soils. Our previous results have indicated that soil carbon and nitrogen (soil organic matter) are significantly increased in a wide range of soils following the application of MWOO. However, soil carbon and nitrogen cannot be used alone to indicate organic matter status or the condition of the soil. Mixed waste organic outputs have also been promoted as having a beneficial effect on soil biological activity because they are potentially rich in organic compounds that have the capacity to stimulate soil microorganisms and thus activate biogeochemical nutrient cycling (Garcia et al. 1992, 1996, 1998; Pascual et al. 1997). Organic activity is believed to respond much more quickly than traditional physical and chemical indicators to soil amendments and therefore offer the potential to indicate change over much shorter timescales.

A key measure of the activity of organisms in the soil is soil respiration and this approach has been widely used to determine quantity and rate of carbon mineralisation and therefore soil microbial activity. Soil respiration refers to the production of carbon dioxide when soil organisms respire including the respiration of plant roots, the rhizosphere, microbes and fauna. Soil respiration is controlled by a range of factors including soil temperature, oxygen availability, nutrient supply and available substrate. For example, Pascual et al. (1997, 1999) reported significant increases in biological respiration in soils amended with mixed solid waste compost in Spain. Battarcharyya (2003) detected an increase in soil respiration following application of mixed solid waste compost to soils in India and that the magnitude of this effect was directly related to the application rate. However, throughout this work, respiration rates declined with increasing time following soil amendment. Ros et al. (2003) similarly demonstrated an increase in soil respiration following amendment with composted urban waste but also found that although this effect diminished with time, respiration of amended soils remained higher than that of untreated soils for up to two years.

Addition of organic substrate to the soil can potentially enhance soil respiration but the long-term effect will be dependent largely upon the nature of the material added, the inherent and added microbial population and the nature and quantity of the substrate that is added. A number of studies have reported significant, and in some cases lasting, increases in soil microbial activity following the addition of organic amendments.

The residence time and total quantity of carbon in soil depends to some degree on the carbon forms present and their rate of decomposition (Amlinger et al., 2007). Carbon in soil is typically categorised into a range of ‘fractions’. These fractions range from relatively fresh, readily degradable or ‘labile’ carbon through to more resistant products of decomposition. Each of these fractions has a discreet decomposition rate, and residence time in the soil is therefore dependent upon its chemical characteristics and point in the decomposition continuum. Amlinger et al. (2007) reported relative decomposition rates and approximate residence times for a range of different fractions (Table 3.1).

Table 3.1 Approximate turnover times for organic soil fractions (Amlinger et al., 2007)

Organic fraction	**Turnover time (years)**
Litter/crop residues	0.5-2
Microbial biomass	0.1-0.4
Macro-organisms	1-8
Particulate	5-20
Light fraction	1-15
Stable humus	20-1000

The relative proportion of the various fractions is critical to the decomposition, cycling and residence time of organic matter through the soil system. Soil respiration can be used to track the rate and magnitude of soil carbon mineralisation in response to soil amendments and therefore the extent to which amendments of mixed waste organic outputs can enhance soil microbial activity.

Few studies however, have been undertaken in Australia to determine the effects on soil respiration and therefore microbial activity of MWOO application. Soil respiration can be measured by containing soils within a sealed environment and determining the CO_2 evolution from the sample in the presence or absence of an amended material (Pascual et al., 1997, Connant et al., 2004). In order to determine the effect of MWOO application on soil biological activity, a respiration experiment was undertaken at the University of New England, carbon laboratory.

3.3 Methods

Four soil types were selected from the ten soils from across NSW. These were: Kirby Sand (S1), CROA (SL1), Minesite (SL2) and Kirby Clay (C1) soils. These soils were selected in order to provide a texture and pH range, factors that are known to affect soil biological activity.

For each soil type and treatment, 25g soil samples (sieved and air dried at ambient temperature and humidity)were weighed in triplicate and placed in sealable 250ml polypropylene vessels each with a silicon septum fitted to the lid. Soils were initially watered (using distilled and de-ionised water) to 60% field capacity prior to MWOO addition.

MWOO was incorporated into these soils at rates of: 0 t ha^{-1}, 20 t ha^{-1}, 40 t ha^{-1} and 140 t ha^{-1} (equivalent to 0, 11, 28 and 80 g kg^{-1} dry soil determined by bulk density and assuming incorporation to 15cm) corresponding to application rates used in other components of the work. Each treatment within each soil type was replicated in triplicate. Blank samples (no soil, no MWOO) vessels were also included in the experiment to determine ambient CO_2 concentration.

These were also replicated in triplicate. Once prepared, each vessel was placed in a constant temperature environment (25°C) with all light excluded for the duration of the incubation experiment.

An initial (7 day) pre-incubation was undertaken to ensure that all soils and MWOO treatments had reached equilibrium. From this point, samples of headspace gas were collected at: 3, 7, 10, 17, 34, 62, 90, 118 and 182 days. From this, a time-series CO_2 evolution could be determined.

Headspace gas within the vessel was extracted using a two-way hypodermic needle and collected in a 12ml evacuated container. Gas thus collected was analysed using a Gas Chromatograph-Mass Spectrometer to determine CO_2 concentration. From this, CO_2 evolution from each sample over the sampling period could be determined.

After each sample was collected, each vessel was opened to the ambient atmosphere for at least one hour in order to re-equilibrate with the atmosphere and watered (using distilled and de-ionised water) to target mass. Vessels were then re-sealed and returned to the 25°C constant temperature (light excluded) environment.

From the time-series measures determined through the duration of the experiment, CO_2 evolution curves could be fitted to describe the biological activity in the soil. This was undertaken using a Marquardt iterative non-linear curve fitting procedure. Resultant non-linear models represent the rate of CO_2 evolution and therefore carbon decomposition within the soil. The nature of the model also provides an indication of the relative fractions of carbon that are being decomposed – (i.e. active and slow pools) providing an estimation of the longevity of any soil respiration effect following treatment.

3.4 Results

All four soils selected demonstrated a similar pattern of CO_2 respiration through the duration of the experiment (Figure 3.1). Each soil exhibited a two-phase decline in respiration with time expressed in a rapid decrease in soil respiration in the initial decomposition phase followed by a slower rate of decomposition and CO_2 evolution in the second phase.

Despite the apparent difference between the soil types, no significant difference ($P=0.159$) in the respiration could be detected over the entire experiment. However, a significant Soil Type x Time interaction indicated that in the early part of the experiment where MWOO effects were largest, Kirby Sand (S1) and CROA (SL1) soils had similar but higher initial respiration rates (108 and 126 μgCO_2 g soil^{-1} day^{-1} respectively) compared with the Kirby Clay (C1) and Minesite Soil (SL2). Both of the latter had statistically similar with initial CO_2 respiration rates in the order of 85 μgCO_2 g soil^{-1} day^{-1}.

For each of the soils examined, there was a significant ($P<0.001$) application rate effect with the respiration being significantly higher for successively higher application rates ($0<20<50<140$ t ha^{-1}). A significant ($P<0.001$) Application Rate x Time interaction however, indicated that these differences diminished with time until day 34 ($P=0.048$), beyond which, the respiration rate for each soil type and for each application rate were statistically similar.

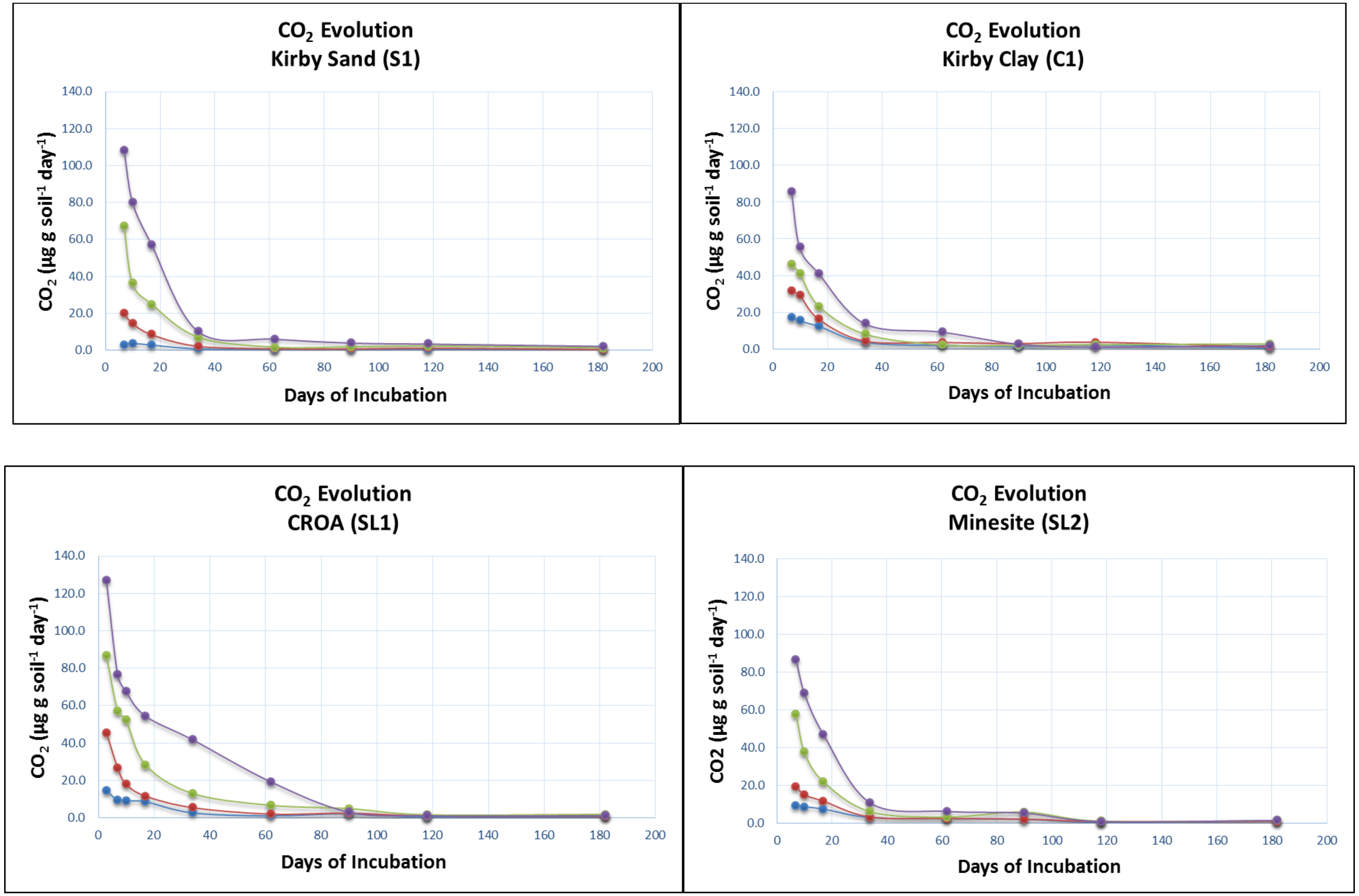

Figure 3.1 CO_2 Evolution during 180 days incubation of soil and MWOO

3.5 Discussion

All of the soils we examined exhibited the same response to MWOO application. An initial increase in respiration was followed by a two phase decomposition trend. The cause of the higher initial respiration response in the sandy (S1) and loam (SL1) soils to the MWOO application is not clear but might be attributable to a higher initial microbial population. The smaller respiration values in the clay soil, in the early (<34 days) of the experiment, might be attributed to the physical structure of clay soils which are known to protect added carbon and therefore inhibit microbial respiration (Bouma and Bryla 2000) and restrict CO_2 evolution from soil pores (Lohila et al. 2003).

For each soil type, the increase in respiration induced by the MWOO application was progressively larger at the higher application rates. This suggests that the respiration rate was related to the supply of fresh carbon (and nitrogen) provided by the MWOO.

The initial rapid phase of CO_2 evolution, we infer to be associated with the initial decomposition of the more labile fraction or “fast” pool of carbon contributed by the MWOO material. The MWOO materials are associated with a significant soluble C component which is known to initiate rapid microbial activity. The respiration rate diminished at around 34 days at which point we believe the labile, “fast pool” became limited and more resistant (slow pool) carbon began to dominate. After 34 days we could detect no difference in the respiration rate between treatments.

While Ros et al. (2006) observed that soil respiration was higher in amended soils up to two years after application of composted urban waste, we could find no detectable difference in soil respiration between treated and control soils after 34 days of incubation, regardless of soil type, and application rate. However, our results from Section 2 suggest that although soil carbon declined in the soils through an 18 month period, there was still significantly more carbon in the treated soils by comparison with the controls. These combined results suggest that what remains after a 34 day period is progressively dominated by the more resistant “slow” pool of carbon which degrades at a much slower rate, not unlike the native carbon in the control soil.

3.6 Conclusions

Addition of MWOO to soils can significantly increase soil microbial activity (as measured by soil respiration). The magnitude of these changes are relative to the quantity of MWOO and presumably C and N that it provides. The benefit would however seem to be limited in its duration having diminished to control levels after 34 days regardless of soil type and application rate. This we interpret to mean the rapid decomposition of labile “fast” pool carbon diminished after this time period leaving only the more resistant carbon fractions beyond this time.

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4. Effects of MWOO on soil properties and plant growth

4.1 Summary

In this component of the work, a ten week glasshouse study was conducted to assess the effects of MWOO on soil properties and crop growth. The study was conducted with wheat (*Triticum aestivum*) on four New South Wales (NSW) soils [Kirby Sand (S1), CROA (SL1), Minesite (SL2) and Kirby Clay (C1)] amended with three rates (0, 20, 50 and 140 t ha^{-1}) of MWOO using two methods of application (surface and incorporated). Results relating to change in soil properties conformed with those reported in Section 2. The application of MWOO significantly increased pH, EC, total C, total N, CEC and P ($P < 0.05$) of all MWOO amended soils. The increase was observed to be proportional to the application rate and these effects were restricted to the MWOO contact layers.. Wheat root growth and aboveground biomass were significantly increased ($p < 0.05$) by the application of MWOO at 20 and 50 t ha^{-1} application rates. The aboveground biomass was observed to be significantly higher ($P < 0.01$) overall in the incorporated application than in the surface application. A decline in the aboveground biomass was observed for the high application rate (140 t ha^{-1}) with average biomass below that of the control Although this effect was not significant ($P>0.05$) at the broad scale across the soils studied, individual soils – particularly Kirby Sand and CROA soils showed a significant decline in wheat growth at the high rates (140 t ha^{-1}) of MWOO application.

4.1 Introduction

The production of MWOO is an efficient alternative to landfill disposal of waste (Montemurro et al., 2005) and a method of diverting waste disposal from landfill (Warman et al., 2009). This allows the recycling of organic materials for agricultural use. Application of organic materials, like MWOO, to the soil as an inexpensive soil conditioner and source of nutrients in agricultural soil has been promoted as a management practice with the potential to improve soil condition (Sharma and Buhushan, 2001; Petersen et al., 2003). For example, Sikora and Yakovchenko (1996) demonstrated that adding MWOO to the soil had a positive influence on plant yield and that increase in yield was not only due to nutrients supplied by MWOO but also because of the improvement of physical properties of the soil.

The use of organic soil amendments in agriculture can have beneficial effects on soil properties (Araujo et al., 2008) including higher cation exchange capacity (CEC), higher plant available water holding capacity and lower bulk density. Applying organic amendments to the soil also promotes beneficial microorganisms (Bulluck et al., 2002). Moderation of soil pH and enhanced soil aggregation and the subsequent improved water infiltration rate have also been demonstrated following MWOO application (Stamatiadis et al., 1999). Further, soil chemical properties can be improved using soil amendments. Soil organic matter (SOM) is globally recognized as an important factor influencing soil fertility and crop growth (Senesi et al., 2007). Applying MWOO to the soil could potentially restore, maintain or improve SOM (Castaldi et al., 2004; Mondini et al., 2007).

In New South Wales (NSW), Australia, there is limited published work on the effects on soil properties and crop yield of MWOO application to different soils. Therefore the aims of this study were to assess and evaluate the potential for MWOO to modify soil properties and, in so doing, enhance crop growth, when applied to four different NSW soils.

4.2 Materials and Methods

4.2.1 Experiment Design

A glasshouse experiment was carried out between April and August 2014 in the Agronomy and Soil Science Department in the School of Environmental and Rural Sciences at the University of New England (UNE). The University geographically lies at latitude 30.497778 and longitude 151.642500. It lies at an altitude of 1023 metres and is located in Armidale city in the New England Tablelands of northern New South Wales in Australia (Figure 4.1).

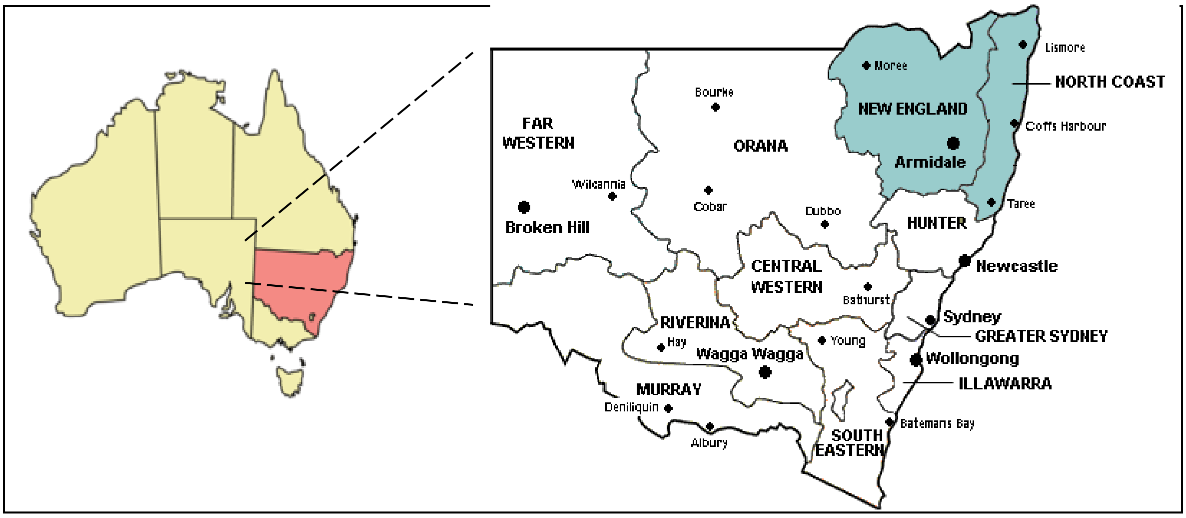

Figure 4.1: Location map of Armidale in NSW, Australia.

MWOO and Bulk Soil Sampling and Analysis

The MWOO used in this study was matured compost derived from mixed solid waste. It was sourced from the Global Renewables UR-3R facility, a Mechanical and Biological Treatment (MBT) facility in Campbelltown, Sydney, New South Wales. MWOO was collected from the production process and kept in a cool room at the university prior to use.

The soils and MWOO materials used in this experiment were sub-sampled from the bulk samples collected at the initiation of the project. As a consequence, physical and chemical properties differ slightly, though not substantially, within each experiment. This reflects the inherent variability of the soils and materials used.

The study involved two separate experiments, the soil fertility and plant biomass experiment and the root biomass experiment, and was carried out using four different soils of NSW which were collected from 0 – 10 cm depth of surface soil bulked over 5–10 square metres. These included a selection of those soils described in Table 2.1.and included: i) Kirby sand (S1), ii) Kirby clay (C1), iii) CROA (SL1) and Minesite (SL2).. The Kirby clay was derived from a Dermosol soil while the Kirby Sand, CROA and Minesite soils were derived from Chromosols (Isbell 2002). The Kirby clay was collected from UNE Kirby farm (30.435463^{O}S, 151.633636^{O}E), the Kirby sand was collected from the UNE Newholme farm (30.426087^{O}S, 151.652707^{O}E), the Minesite loam was collected from Ashton Coal, Singleton (32.460897^{O}S, 151.087095^{O}E) and the CROA loam was collected from NSW DPI Elizabeth McArthur Agricultural Institute, Camden (34.080957^{O}S, 150.704249^{O}E). The bulk soils were sieved to < 4 mm using a mechanical sieve, air dried and mixed thoroughly prior to use (Figure 4.2).

Samples (10 g) of each of the air dried soils were weighed into aluminium trays in triplicates and then oven dried at 105^{O}C. The oven dry weights were used to calculate the gravimetric moisture contents of the soils (Schmugge et al, 1980). Separate samples of the bulk soil were added in triplicates into pots that had drainage holes lined with paper towel to prevent the soil from leaking out from the bottom of the pots. Each pot was filled with 1 kg of each soil type and soaked in a water bath until the soil was saturated. The pots were then removed from the water bath and the water was allowed to drain by gravity. After 24 hours the wet soils were weighed and the weights together with the gravimetric moisture contents were used to calculate the field capacity of the soils and amount of water needed to bring moisture content to field capacity. Soil that could fill the experiment pots to 25 cm depth for each soil type was weighed and the weights were used to calculate the amount of water needed to bring each soil to 60% and 80% field capacity.

Characterization of the bulk soils and MWOO was carried before MWOO application to the soil (before setting up the experiment). For the characterization, samples of the air dried bulk (< 4 mm) soils were mechanically ground to < 2 mm and < 0.2 mm. The MWOO was mixed thoroughly and homogenised. Three samples (triplicates) of soils and the MWOO were stored in acid washed yellow-top vials. The soils and the MWOO were analysed and results obtained for pH, electrical conductivity (EC), cation exchange capacity (CEC), total carbon, total nitrogen and extractable phosphorus. For pH and EC 10 g of the < 2 mm soil was weighed and a 1:5 soil:water was shaken for 1 hour and then allowed 20-30 minutes to settle. A calibrated electrode was placed in the solution and after stabilisation the readings were taken. The pH meter was a TPS model 901-CP and the EC meter was a TPS model labCHEM cond. Exchangeable cations were analysed by weighing 2 g of the < 2 mm soil into a 50 mL centrifuge tube and adding 40mL of 1M NH_4Cl before being shaken for 1 hour. Samples were then filtered through Whatman no. 42 filter paper and stored in a cool room until Ca, Mg, Na and potassium (K) were analysed on the ICP-OES (Rayment and Higginson, 1992). Total carbon and total nitrogen were analysed by weighing 0.2 g of the < 0.2 mm soil into a tin foil cups. The tin cups are crimped closed and loaded to LECO TruSpec (LECO Corporation, 2009).

Figure 4.2: Bulk soil being air-dried. (Photo: Maseko).

4.2.2 Soil Fertility and Plant Biomass Experiment Design

The experiment involved two methods of MWOO application (surface and incorporated) using three different application rates (20, 50 and 140 t ha^{-1} equivalents) and the control treatment (0 t ha^{-1}), with three replicates for each treatment in cylindrical PVC pots (90 mm diameter; 300mm height) filled to a depth of 25 cm with the four different NSW soils. The pots were thoroughly washed using 2% extran and were rinsed with de-ionized water then allowed to dry before the soils and MWOO were added into them. There were 12 pots for the control treatment (4 soils X 3 replicates); 36 pots of incorporated compost (4 soils X 3 application rates X

3 replicates); 36 pots of surface application (4 soils X 3 application rates X 3 replicates). This generated to 84 pots altogether (Figure 4.3).

In the surface application method, air-dried soils were weighed and added to the pots to a depth 25 cm. Equivalents of 20, 50 and 140 t ha^{-1} of MWOO were applied to the surface and no compost (0 t ha^{-1}) was applied to the control treatments then water was added to 60% field capacity. In the incorporated method the bottom 10 cm of the pots was filled with soil while the soil that would fill the top 15 cm were put into plastic bags with the corresponding amounts of MWOO (20, 50, and 140 t ha^{-1}). The soil and MWOO in the bags were thoroughly mixed and added to the pots. Water was added to 60% field capacity.

Figure 4.3: Experiment pots laid in a completely randomized design in the glasshouse (Photo: Maseko).

All pots were then placed on two rectangular benches (tables) in the greenhouse (Figure 4.3). The greenhouse temperature was set to range between 15^{o}C and 25^{o}C. One day after the compost was added, six wheat (*Triticum aestivum*) seeds were sown into each pot. In some pots only five seeds had germinated therefore thinning was done in the pots where all six seeds had germinated, leaving five plants per pot. Water was added by hand on regular basis to the pots to 60% field capacity. Two weeks after emergence soil water was then maintained at 80% field capacity until harvesting. Occasionally, weeds that appeared were removed by hand. The wheat plants were allowed to grow for ten weeks and were then harvested. By this stage the wheat plants had developed heads (Figure 4.4).

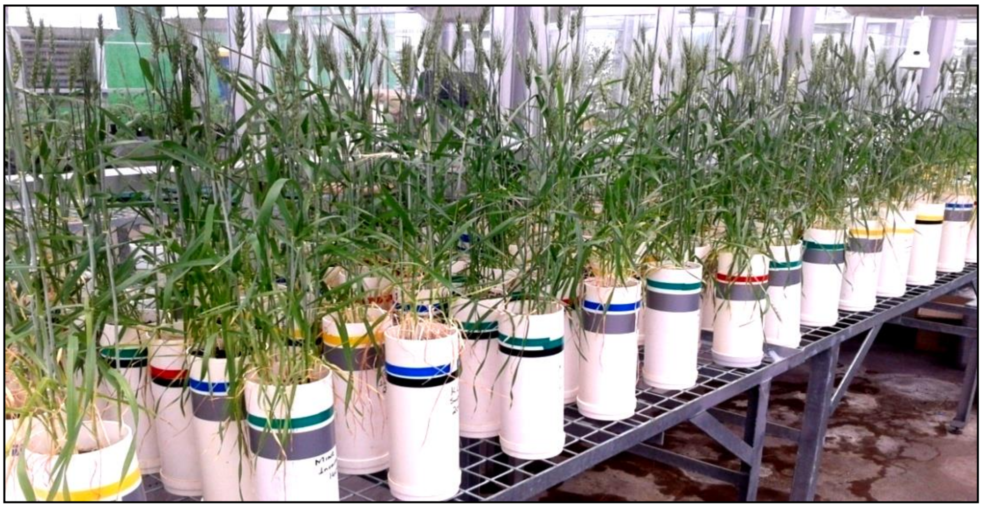

Figure 4.4: Ten weeks old wheat plants at harvesting (Photo: Maseko).

4.2.2.1 Plant and Soil Sampling

The aboveground parts of the plants were harvested using a pair of scissors which was first washed with 2% extran and rinsed off with de-ionized water. The pair of scissors was rinsed off between samples to minimise cross-contamination of plant samples. The plants were washed with de-ionized water after harvesting to remove adhering soil particles. The heads (grains) were separated from the rest of the plants parts (stems and leaves) and fresh weights were taken and recorded. The plant parts were oven-dried at 60°C to a constant weight (Figure 4.5). Roots were removed from the soil and thoroughly washed using de-ionized water, weighed, then oven dried at 60°C and re-weighed.

Figure 4.5: Wheat plant material oven-drying at 60°C (Photo: Maseko).

After harvesting aboveground plant parts, soil columns (cores) were removed from the pots and divided into three depth increments of 0-5, 5-15 and 15-25 cm using a shark knife (Figure 4.6). The soil samples were placed in separate aluminium trays and then crushed by hand to recover the roots. The soils were then weighed and oven dried at 40°C until a constant weight was reached. The samples were re-weighed to determine the dry weights (Wilson et al., 2009). The dry soils were then ground with pestle and mortar to pass through a 2 mm sieve and then stored in yellow top vials in preparation for laboratory analysis. Some of the < 2 mm soil was further ground down using a ball-mill to pass through a 0.2 mm sieve and stored in yellow top vials in preparation for laboratory analysis.

Figure 4.6: Soil column from one of the pots used in the experiment (Photo: Maseko).

4.2.2.2 Plant and Soil Analysis

The oven dry aboveground plant parts were weighed and the weights were used to calculate the aboveground biomass on dry weight basis. This was determined by dividing the total dry weight per pot by the number of plants per pot and was expressed as mg/plant.

The 2 mm and < 0.2 mm soils obtained from the experiment after harvesting were analysed in triplicates and results obtained for pH, electrical conductivity (EC), cation exchange capacity (CEC), total carbon, total nitrogen and extractable phosphorus. For pH and EC 10 g of the < 2 mm soil was weighed and a 1:5 soil:water was shaken for 1 hour and then allowed 20-30 minutes to settle. A calibrated electrode was placed in the solution and after stabilisation the readings were taken. The pH meter was a TPS model 901-CP and the EC meter was a TPS model labCHEM cond. Exchangeable cations were analysed by weighing 2 g of the < 2 mm soil into a 50 mL centrifuge tube and adding 40mL of 1 M NH_4Cl before being shaken for 1 hour. Samples were then filtered through Whatman no. 42 filter paper and stored in a cool room until Ca, Mg, Na and potassium (K) were analysed on the ICP-OES (Rayment and Higginson, 1992). Total carbon and total nitrogen were analysed by weighing 0.2 g of the < 0.2 mm soil into a tin foil cups. The tin cups are crimped closed and loaded to LECO TruSpec (LECO Corporation, 2009). Extractable phosphorus was determined using a Colwell, bicarbonate extraction procedure.

4.2.3 Root Growth Experiment Design

This experiment was designed to assess the root biomass of wheat following MWOO amendment to sandy soil (S1). The experiment was carried out in cylindrical PVC pots of 50 mm diameter and 250 mm height filled to a depth of 20 cm with the Kirby sand. The experiment involved two methods of MWOO application (surface and incorporated) using two different application rates (20 and 50 t ha^{-1} equivalents) and the control treatment (0 t ha^{-1}), with three replicates for each treatment. There were 15 pots altogether which were laid out as a completely randomized design There were3 pots for the control treatment; 6 pots of incorporated compost (2 application rates X 3 replicates); 6 pots of surface application (2 application rates X 3 replicates).

In the surface application method, air-dry soils were weighed and added to the pots to 20 cm depth then wetted to 60% field capacity. Equivalents of 20 and 50 t ha^{-1} of MWOO were applied to the surface and no compost (0 t ha^{-1}) was applied to the control treatments. In the incorporated method, the bottom 10 cm of the pots was filled with soil, while the soil that would fill the top 15 cm were put into plastic bags with the corresponding amounts of MWOO (20 and 50, t ha^{-1}). The soil and MWOO in the bags were thoroughly mixed and added to the pots. Water was added to 60% field capacity.

All pots were then placed on a rectangular bench (tables) in the same greenhouse where experiment 1 was running. One day after the compost was added, three wheat (*Triticum aestivum*) seeds were sown into each pot. Water was added on regular basis to the pots by hand to 60% field capacity. Two weeks after emergence soil water was then maintained at 80% field capacity until harvesting. Occasionally, weeds were removed by hand. The wheat plants were allowed to grow for ten weeks and were then harvested.

4.2.3.1 Root Sampling and Analysis

The rhizosphere was carefully removed from the pots and all soil adhering to the roots was thoroughly washed off using tap water. The roots were then rinsed off with de-ionised water, kept in plastic bag and stored in a cool room awaiting analysis. Root growth was assessed by determining root length and diameter. The roots were then scanned and analysed using the WinRHIZO to determine root length and root diameter.

4.2.4 Quality Control

Internal standard reference soil and blanks were included in the analysis of pH, EC, CEC and Colwell phosphorus and the analysis was carried out at 25ºC. For the carbon and nitrogen analysis using LECO, internal standard reference soil, lucerne and blanks were used. These standards have known values/concentrations of the various fertility characteristics.

4.2.5 Statistical Analysis

Statistical analyses of the whole dataset were carried out with analysis of variance (ANOVA) using the R program version 3.1.1 (R Core Team, 2014). Assumptions of heterogeneous variances and normal distributions were confirmed and any necessary transformations are noted in the results section. Data were tested for significant differences at $P = 0.05$. Significantly different means were separated using 95% confidence intervals (1.96 × standard errors). Post Hoc analysis was performed for each measured parameter using LSD analysis to determine significance of MWOO effects by factor: MWOO application rate, method of application and sampling depth. Data were further categorised by soil type and further ANOVA and post hoc analysis undertaken to determine the effects within particular soil types.

4.3 Results

4.3.1 Soil and MWOO Characterisation

The characterisation of each soil and MWOO before application of MWOO in this study is shown in Table 4.1 and Table 4.2, respectively. The data show that three of the soils [Kirby clay (C1), CROA (SL1) and Minesite (SL2)] were acidic while the Kirby sand was slightly alkaline. The Minesite soil had the highest EC compared to the other soils with the Kirby sand showing the lowest EC. The Kirby clay showed higher CEC, carbon, nitrogen and phosphorus concentrations compared to the other soils. This is consistent with fact that soils with high clay content have higher CEC, carbon, nitrogen and phosphorus concentrations compared to soils with lower clay content.

Table 4.1: Selected chemical characteristics of the soils used in the experiment before MWOO application.

Soil Parameters	Kirby Sand	Kirby Clay	CROA	Mine Site
pH	6.28 ± 0.03	6.21 ± 0.08	5.99 ± 0.82	6.19 ± 0.01
EC (µS/ cm)	126.37 ± 0.62	154.47 ± 6.29	150.50 ± 0.82	243.43 ± 3.98
Exch. Ca (cmol+/kg)	1.02 ± 0.01	25.55 ± 0.16	4.81 ± 0.12	4.66 ± 0.09
Exch. K (cmol+/kg)	0.90 ± 0.00	0.85 ±0.02	0.58 ±0.02	0.41 ± 0.01
Exch. Mg (cmol+/kg)	1.12 ± 0.01	24.09 ± 0.14	4.63 ± 0.11	5.81 ± 0.15
Exch. Na (cmol+/kg)	0.24 ± 0.01	0.35 ± 0.01	0.30 ± 0.01	0.95 ± 0.03
CEC (cmol$_+$/kg)	2.47 ± 0.03	50.83 ± 0.16	10.33 ± 0.25	11.84 ± 0.30
Total C (%)	0.488 ± 0.00	3.340 ± 0.01	2.050 ± 0.02	1.690 ± 0.05
Total N (%)	0.039 ± 0.00	0.252 ± 0.00	0.163 ± 0.01	0.106 ± 0.00
Ext. P (mg kg^{-1})	6.22 ± 0.24	77.19 ± 7.59	17.33 ± 0.58	10.87 ± 0.19

Values are the Mean ± SE (*n* = 3)

Table 4.2: Selected chemical characteristics of the MWOO used in the experiment.

Parameters	MWOO
pH	7.36 ± 0.26
EC (µS/ cm)	6220 ± 0.15
Exch. Ca (cmol+/kg)	28.01 ± 0.45
Exch. K (cmol+/kg)	12.45 ± 0.30
Exch. Mg (cmol+/kg)	7.00 ± 0.14
Exch. Na (cmol+/kg)	24.53 ± 0.67
CEC (cmol$_+$/kg)	72.00 ± 1.51
Total C (%)	24.903 ± 1.88
Total N (%)	1.460 ± 0.09
Ext. P (mg kg^{-1})	584.2 ± 16.36

Values are the Mean ± SE (*n* = 3)

4.3.2 Fertility Characteristics

4.3.2.1 pH

The pH values of the four soils after application of MWOO are shown in Table 4.3 and indicate that for all the soils examined, the addition of MWOO had a significant effect on soil pH for both the surface and incorporated application methods and at the various soil sampling depths. The soils had significantly different pH with Minesite showing significantly higher pH compare to the other soils ($P < 0.001$). Across all the soils studied, there was an increase in soil pH following MWOO application and this was proportional to the rate of MWOO application (i.e. 20<50<140t ha^{-1}). However, these effects were only statistically significant ($P < 0.01$) for the Kirby Sand and Minesite soil and only at the 50 and 140 t ha^{-1} application rates. Effects on soil pH were consistently restricted to the contact layers (i.e. surface application = 0-5cm; incorporated = 0-15cm). Within these layers, the net effect on pH in the soil was statistically larger in the surface sample depth of the surface application rate while the effect was more diffuse through the sampling layers of the incorporated treatment.

Table 4.3: The pH of the soil after harvesting following the application MWOO.

pH			Method of application					
Soil			Surface			Incorporated		
	Depth/ plant part	Control (0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 – 5	5.68 ± 0.04	6.74 ± 0.35	7.47 ± 0.03	8.04 ± 0.05	6.54 ± 0.03	7.37 ± 0.11	8.18 ± 0.07
	5 – 15	5.59 ± 0.04	6.00 ± 0.05	5.82 ± 0.04	5.47 ± 0.08	6.95 ± 0.05	7.60 ± 0.08	7.96 ± 0.08
	15 - 25	5.60 ± 0.12	5.53 ± 0.18	5.33 ± 0.08	5.56 ± 0.35	6.05 ± 0.04	5.71 ± 0.23	5.67 ± 0.15
Kirby Clay	0 – 5	6.24 ± 0.01	6.49 ± 0.03	6.61 ± 0.04	6.95 ± 0.09	6.40 ± 0.01	6.52 ± 0.04	6.88 ± 0.07
	5 – 15	6.23 ± 0.03	6.25 ± 0.06	6.04 ±0.06	5.76 ± 0.03	6.33 ± 0.07	6.46 ± 0.03	6.92 ± 0.05
	15 - 25	6.25 ± 0.07	6.26 ± 0.08	6.10 ± 0.07	5.80 ± 0.02	6.13 ± 0.09	6.04 ± 0.11	5.86 ± 0.05
CROA	0 – 5	5.91 ± 0.06	6.20 ± 0.05	6.48 ± 0.04	7.34 ± 0.03	6.38 ± 0.01	6.50 ± 0.06	7.19 ± 0.03
	5 – 15	5.86 ± 0.02	5.77 ± 0.02	5.81 ± 0.04	5.38 ± 0.02	6.34 ± 0.01	6.76 ± 0.07	7.47 ± 0.04
	15 - 25	5.84 ± 0.05	5.76 ± 0.02	5.62 ± 0.07	5.10 ± 0.02	5.90 ± 0.02	5.76 ± 0.06	5.68 ± 0.03
MINE	0 – 5	6.62 ± 0.10	6.77 ± 0.06	7.18 ± 0.02	7.83 ± 0.08	6.75 ± 0.03	7.23 ± 0.08	7.67 ± 0.02
	5 – 15	6.65 ± 0.05	6.43 ± 0.03	6.50 ± 0.02	6.25 ± 0.05	7.01 ± 0.04	7.45 ± 0.06	7.91 ± 0.03
	15 - 25	6.41 ± 0.08	6.36 ± 0.01	6.351 ± 0.01	6.09 ± 0.04	6.55 ± 0.07	6.58 ± 0.06	6.52 ± 0.06

Values are the mean ± SE (n = 3).

4.3.2.2 Electrical Conductivity

Application of MWOO also significantly modified soil Electrical Conductivity (EC) (Figure 4.7). Increases in EC were observed across all soils examined and although these changes appeared to be proportional to the application rate they were again only statistically significant ($P < 0.05$) at the 50 and 140 t ha^{-1} application rates. Effects were again restricted to the MWOO contact layers and within these layers, the surface application resulted in a more significant EC increase (i.e. 0-5cm surface application = 604 µS/ cm; incorporated - 260 µS/ cm. Overall, the EC of Minesite soil was significantly higher ($P < 0.001$) than that of other soil and that of Kirby sand was significantly lower ($P < 0.001$).

4.3.2.3 Total Carbon (% C)

Total carbon concentration increased in all soils in response to MWOO application (Figure 4.8). For Kirby Clay, CROA and Minesite soils, this increase was significant compared with the controls soil for the 50 and 140 t ha^{-1} application rates. However, for Kirby Sand, only the 140 t ha^{-1} application rate resulted in a significant increase ($P < 0.01$). For all soils, carbon concentration change was restricted to the MWOO contact layers and within these layers, surface application had a more profound effect (i.e. 0-5cm surface = 3.8%; incorporated = 1.5%).

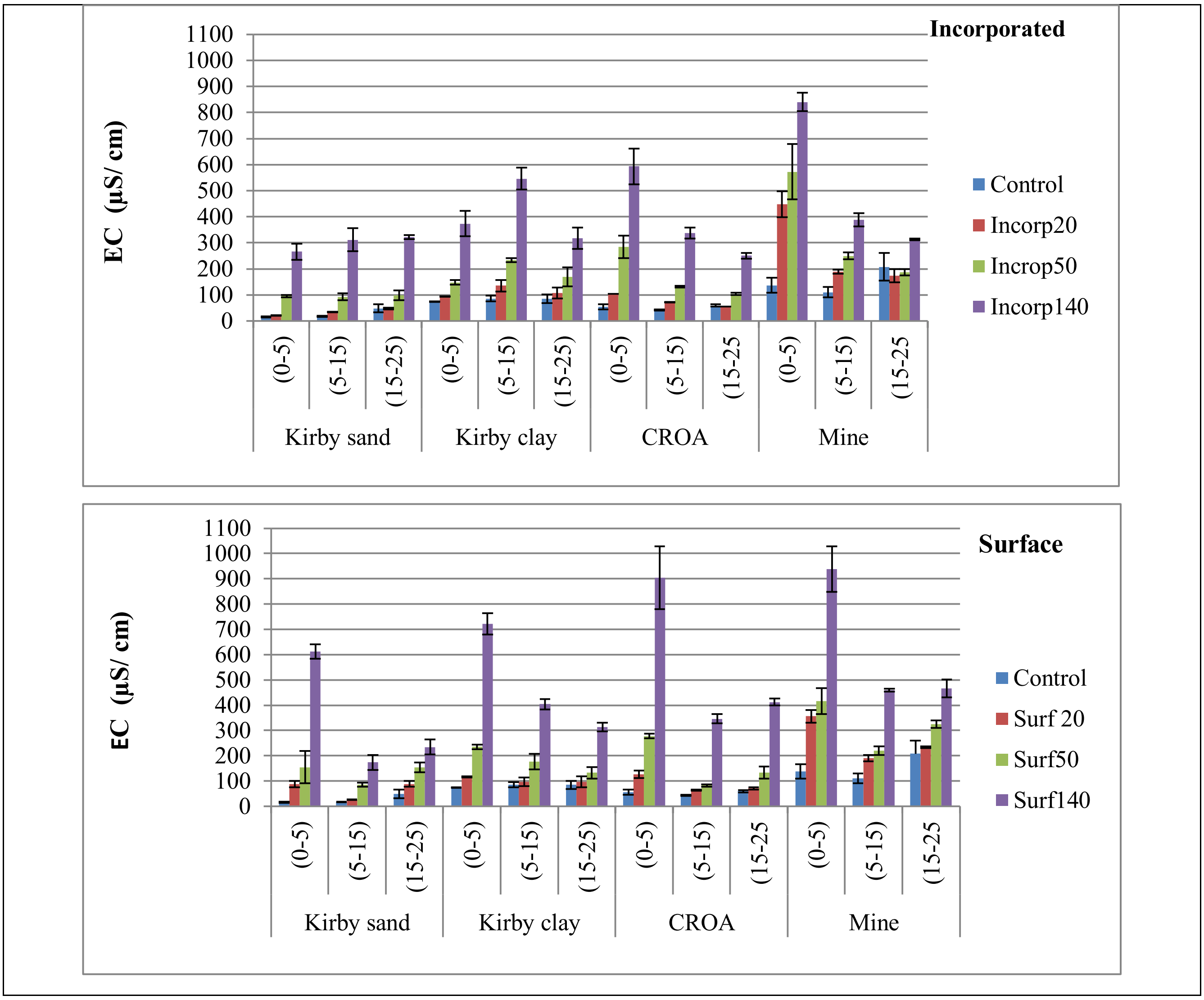

Figure 4.7: Electrical conductivity of the soil after harvesting following application of MWOO.

4.3.2.4 Total Nitrogen (% N)

The Total N concentration differed significantly (P<0.001) between the soils examined. As was the case for soil carbon, the Total N concentration was considerably larger in the Kirby Clay soil compared with the other soils. Increases in Total soil N concentration were also observed in response to MWOO application for all soils and this increase was significant (P<0.001) for all application rates (Figure 4.9). Once again, effects were restricted to the MWOO contact layers and within these layers, a more concentrated effect was measured where MWOO was surface applied resulting in larger Total N (%) concentrations overall.

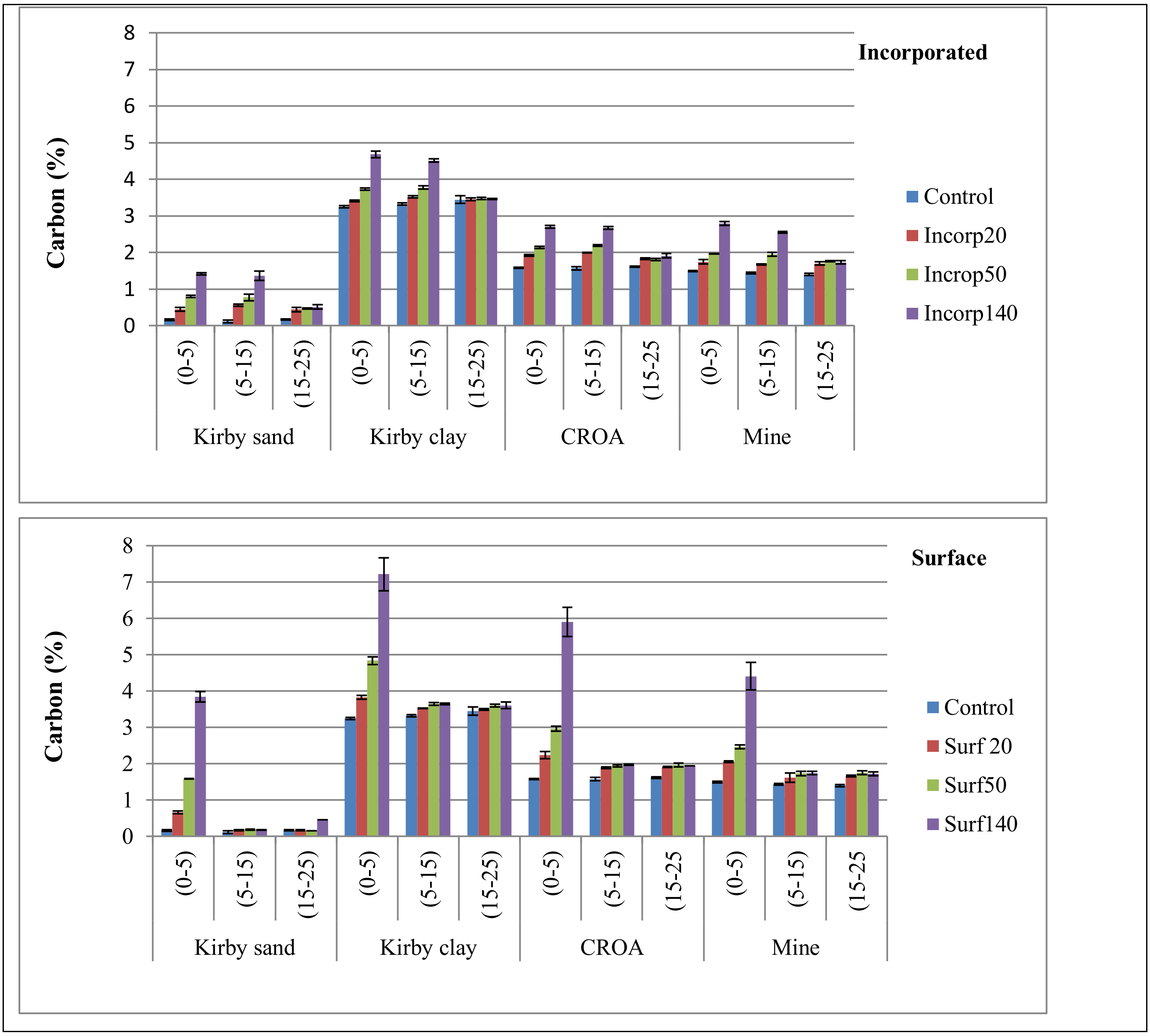

Figure 4.8: Total soil carbon, after harvesting, following application of MWOO.

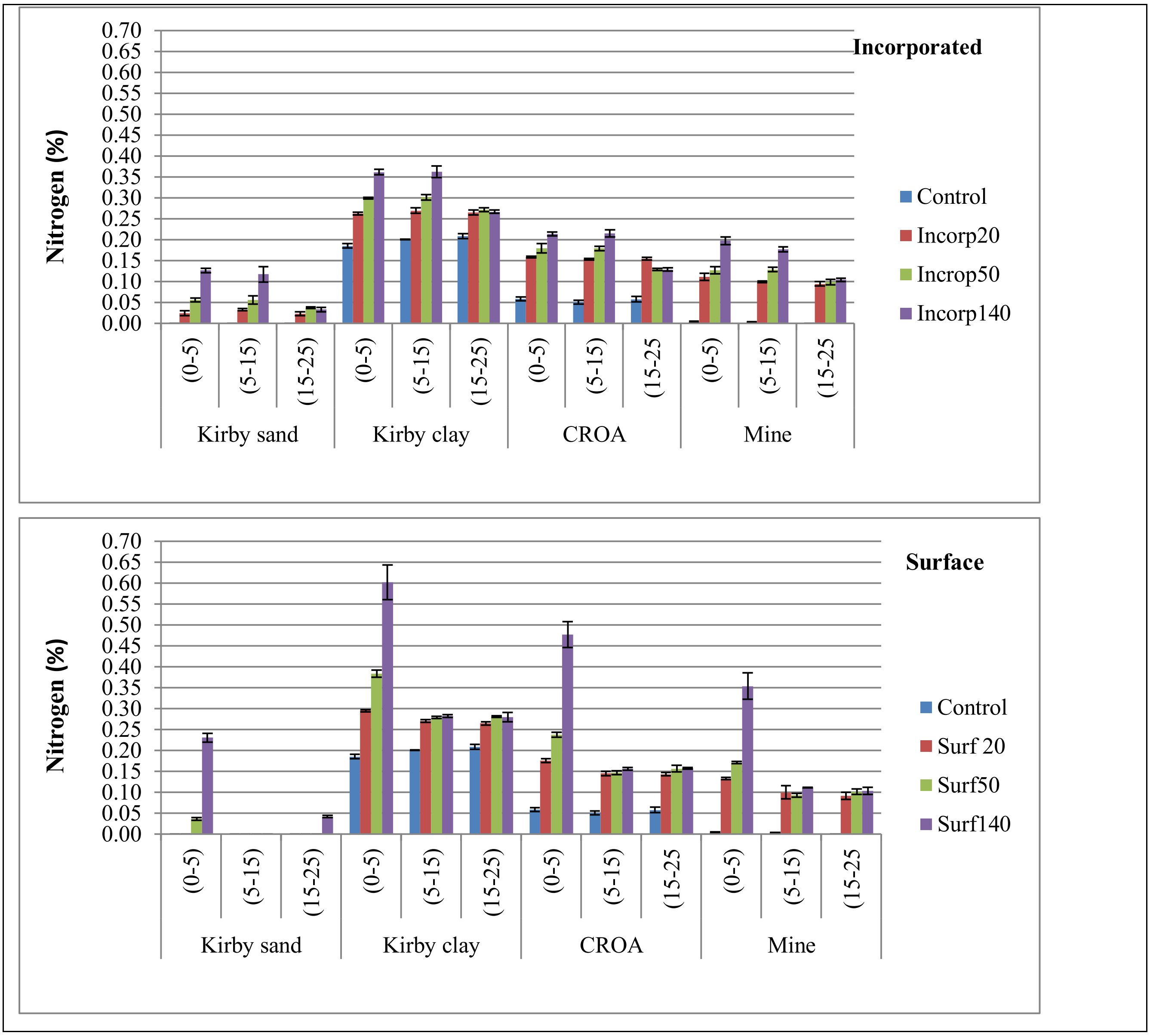

Figure 4.9: Total soil nitrogen after harvesting following application of MWOO.

4.3.2.5 Cation Exchange Capacity (CEC)

The results of the CEC of the soils following application of MWOO to the soil are shown in Figure 4.10. As might have been expected with the addition of organic material in MWOO, cation Exchange Capacity increased in all the soils studied ($P < 0.001$). The CEC of Kirby clay was observed to be consistently significantly higher ($P < 0.001$) than that of the other soils and that of Kirby sand was significantly the lowest ($P < 0.001$). The change in CEC again appeared to be proportional to the application rate but was only statistically significant at the 140 t ha^{-1} application rate. The surface application again resulted in higher CEC than the incorporated but both methods showed significantly higher CEC compared to control ($P < 0.001$)

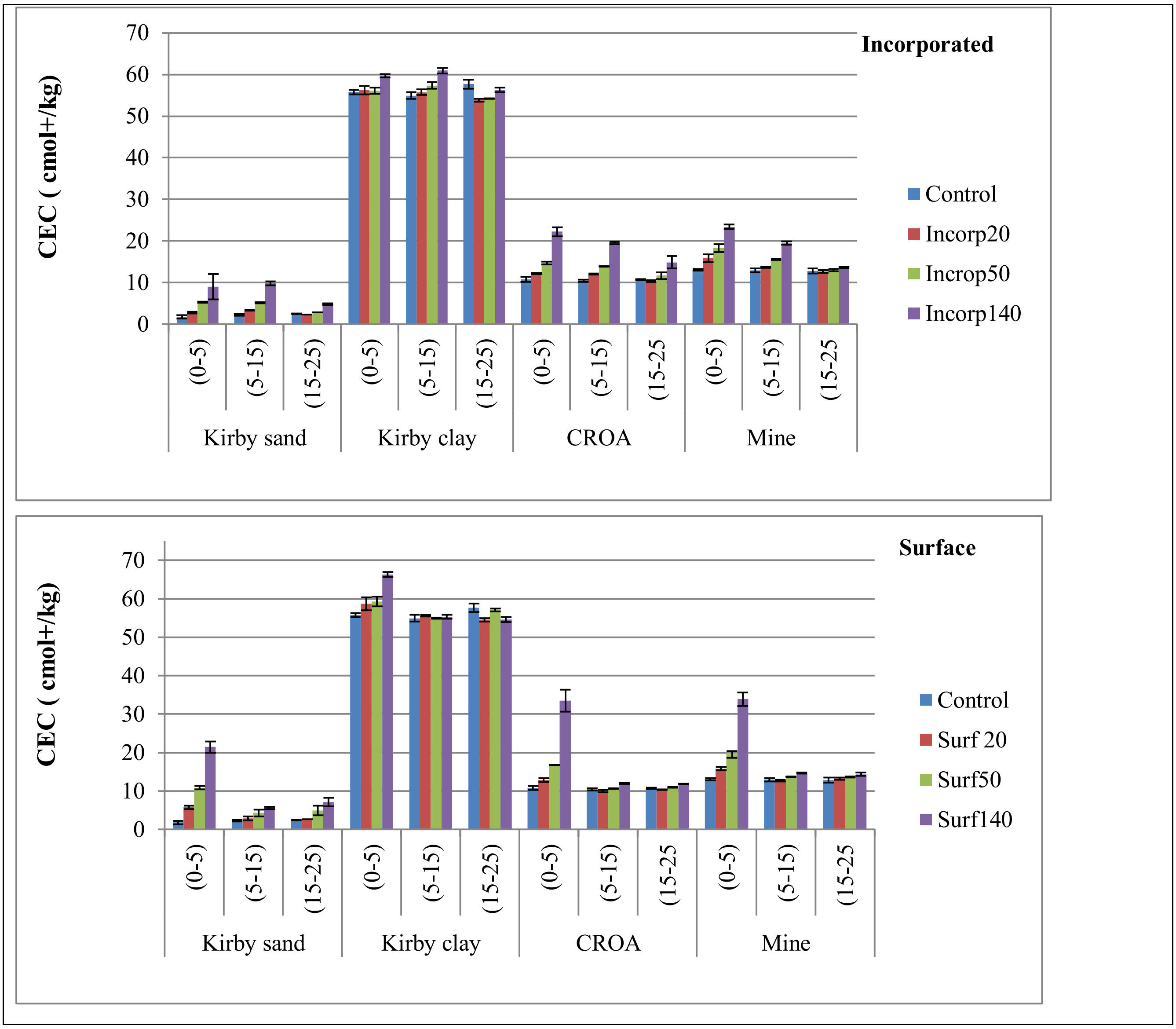

Figure 4.10: Cation exchange capacity of the soil after harvesting following application of MWOO.

4.3.2.6 Phosphorus (P)

The response of extractable P concentrations to the application of MWOO to the soil are shown in Figure 4.11. The results show that P was significantly higher in Kirby clay than in all soils ($P < 0.001$) but no significant difference between Kirby sand and Minesite soil was observed ($P > 0.05$). For all soils examined, the increase in soil extractable phosphorus was significant ($P < 0.001$) only at the 50 and 140 t ha^{-1} application rates. Both methods of MWOO application showed significantly higher P compared to the control and the surface application resulted in significantly higher P compared to incorporated ($P < 0.001$). In the surface application method, significantly high P concentration was observed in the 0-5 cm layer ($P < 0.001$) compared to the other layers but there was no significant difference in P in the 5-15 and 15-25 cm layers ($P > 0.05$). The magnitude of change in P concentrations was determined to some degree by the initial P concentrations of the soils.

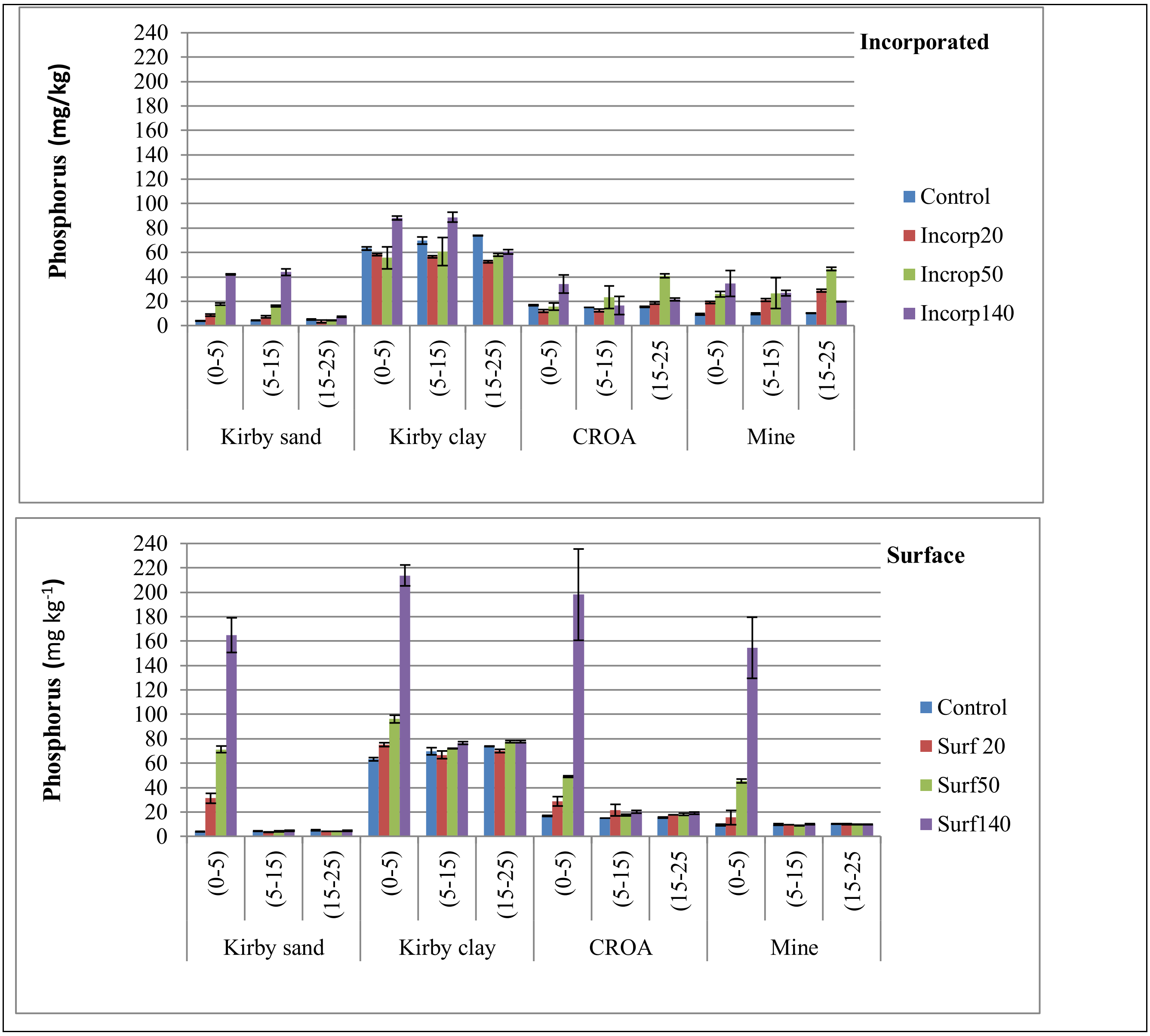

Figure 4.11: Extractable phosphorus in the soil after harvest following application of MWOO.

4.3.3 Aboveground Biomass and Root Growth

The mean values for aboveground biomass for the soil fertility and plant biomass experiment are shown in Figure 4.12 and root growth means (for the root growth experiment) are presented in Figure 4.13. In this study wheat growth was assessed by determining the aboveground biomass on dry weight basis expressed as mg/plant. The root growth was determined by assessing the root length and diameter. The application of MWOO had a significant effect on wheat biomass which was dependent on the application rate. Wheat that was grown on soils amended with 20 t ha^{-1} and 50 t ha^{-1} yielded a significantly larger biomass ($P < 0.05$) in most cases compared to the control, depending on the soil type and the application method. Growth decline was observed at the high application rate (140 t ha^{-1}) and although this result was not significant ($P > 0.05$) when all soil types were combined, where individual soils were examined, this decline was found to be significant ($P < 0.001$) for particular soils.

When MWOO was applied on the surface in Kirby Sand and CROA soils, increasing the application rate from 20 t ha^{-1} to 140 t ha^{-1} resulted in a significant ($P < 0.001$) reduction in biomass. In the Kirby Clay and Minesite soils, increasing MWOO application rate up to 50 t ha^{-1} increased the biomass compared to the control and at 140 t ha^{-1} application rate biomass again declined (although in this instance not significantly, $P>0.05$). Incorporating MWOO into the Kirby sand and Minesite, increasing the application rate up to 50 t ha^{-1} resulted in a significant increase in biomass ($P < 0.05$) whereas the 140 t ha^{-1} resulted in a significant decline in biomass ($P < 0.001$). In the CROA, only the 20 t ha^{-1} application rate significantly increased the biomass compared to the control ($P < 0.05$), and the 140 t ha^{-1} application rate again significantly reduced biomass ($P < 0.05$). Incorporating MWOO into the Kirby clay resulted in a decline in biomass as the application rate increased but no statistical difference could be identified.

The results of the study showed that soil type had a significant effect on biomass ($P < 0.01$) both before and after MWOO application. Kirby clay yielded more biomass (1083.92 mg/plant) compared to the other soils and Kirby sand showed the lowest yield (470.24 mg/plant). Plant response and biomass were larger when MWOO was incorporated into the soil than when surface applied and the control ($P < 0.01$ and $P < 0.05$ respectively) depending on the soil type. There was an exception with Kirby clay where a minimal response and a decrease was observed.

Application of MWOO also showed a significant effect on root length and diameter for both surface and incorporated applications at 20 t ha^{-1} and 50 t ha^{-1} ($P < 0.05$) compared to the control. Larger root growth response was shown when MWOO was incorporated. Literature reporting on the effects of MWOO applications on root growth is scarce. This current study showed that MWOO can indeed increase root growth.

Further research is required to study the rhizosphere following MWOO application and to quantify the extent to which MWOO can positively improve root growth.

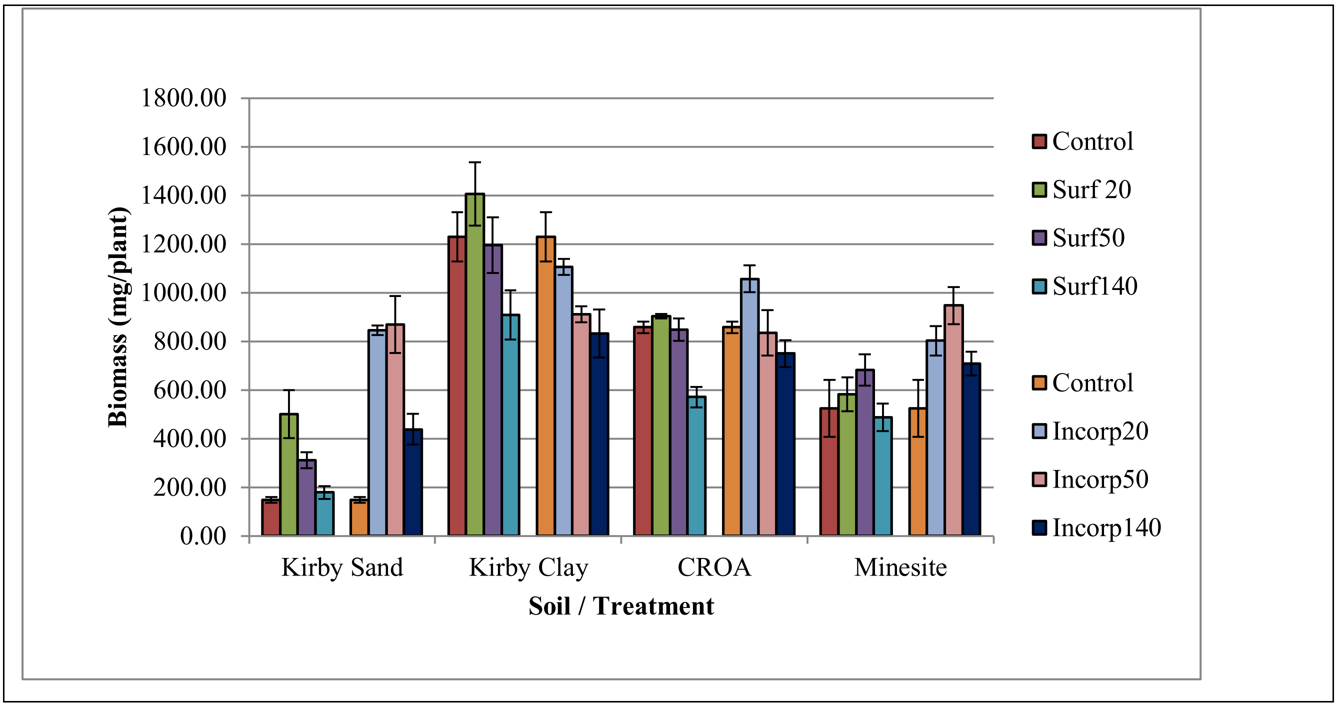

Figure 4.12: Aboveground biomass of wheat after MWOO application.

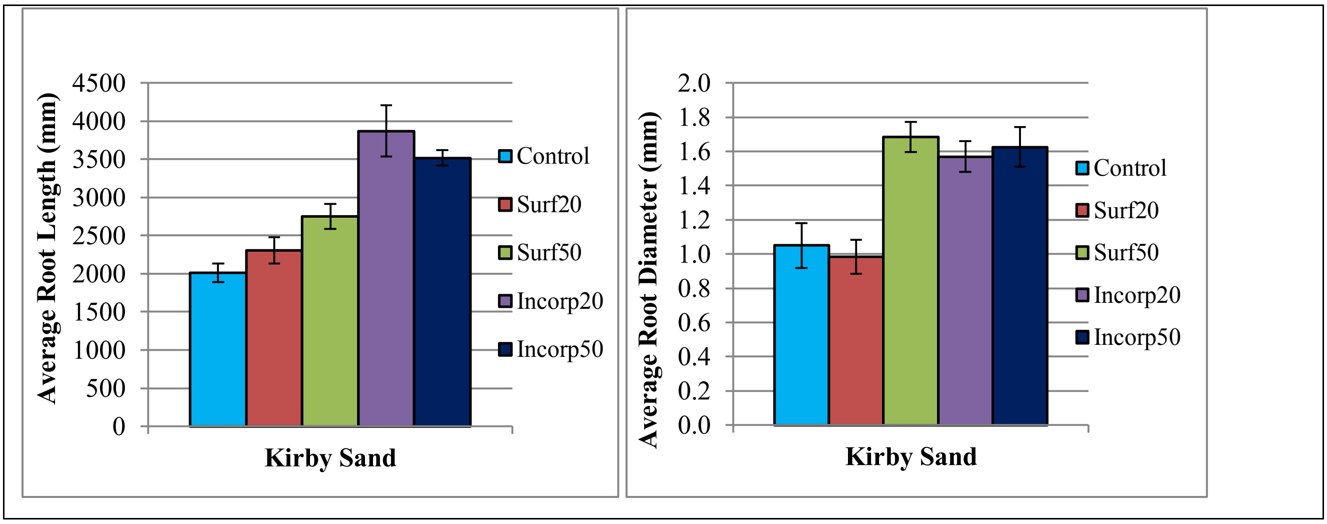

Figure 4.13: Wheat roots length and diameter following MWOO application.

4.4 Discussion

Where soils are initially acid, a common finding is that MWOO application increases soil pH (Geebelen et al. 2003; Mkhabela and Warman, 2005; Mendoza et al. 2006) and that the modification of soil pH is typically proportional to the rate of MWOO application. Mkhabela and Warman (2005) stated that the increase in pH may be due to the mineralization of carbon and the subsequent production of OH^- ions by ligand exchange as well as the introduction of basic cations such as K^+, Ca^{2+} and Mg^{2+}. Zheljazkov and Warman (2004) also reported increase in soil pH from 5.3 to 6.6 and 6.0 to 6.6. Increases from 5.8 to 6.7 and 6.1 to 6.5 have also been reported by Zhang et al. (2006). The results of our study conform with this previous work although, as was demonstrated in Section 2, this effect might be relatively short-lived and pH decline continue after initial "liming effect" with pH decline toward initial or inherent soil pH. Electrical conductivity in this study was observed to increase following application of MWOO. Similar findings were observed by Achiba et al. (2009) who reported that the supply of organic amendments to the soil increased its EC. Likewise, Walter et al. (2006) reported that applying MWOO at rates ranging from 40 t ha^{-1} to 140 t ha^{-1} increased the EC of the soil. The increase was reported to be proportional to the application rate. Zhang et al. (2006) stated that the increased EC values decline over time due to nutrient removal by the crop.

The findings of this study were consistent with the findings of other studies which also reported increased C after adding MWOO to the soil. Montemurro et al. (2006) reported that total organic carbon increased significantly after MWOO application. They suggested that the increase in organic carbon was an indication that MWOO had positive effects on organic matter, which is an indicator of soil quality (Rattan et al., 2005). Achiba et al. (2009) reported that application of MWOO significantly increased the total organic carbon, especially in the 0-20 cm soil layer compared to the 20-40 cm layer, which was similar to the current study. The increase in organic carbon was reported to be proportional to the application rate (Mohammad and Athamneh, 2004; Mendoza et al., 2006). However, results reported in Section 2 of this report would seem to suggest that such measured and reported increases in soil C in response to MWOO application will be relatively short lived.

Achiba et al. (2009) reported that application of MWOO significantly increased nitrogen concentration in the soil, especially in the 0-20 cm layer as compared to the 20-40 cm layer. This current study also showed similar findings. Increase in nitrogen concentration was also reported by Courtney and Mullen (2008) after applying 100 t ha^{-1} of MWOO. Only a fraction of total N in the MWOO is available to plant, especially in the first year of application therefore high application rates of MWOO (>50 t ha^{-1}) are often used. This can lead to the addition of excess of other nutrients and heavy metals (Hargreaves et al., 2008). Diacono and Montemurro (2010) stated that mineralization of N from MWOO is usually very limited in the short term application. The cation exchange capacity in this study was increased following application of MWOO which is consistent with the findings of other studies. In other studies, increased potassium concentration in soils treated with MWOO was reported by Rodd et al. (2002); Montemurro et al. (2006) and Weber et al. (2007). The concentrations of magnesium and calcium were reported to have increased following the application of MWOO in studies carried out by Warman et al. (2004) and Zheljazkov and Warman (2004). Increases in the concentrations of these nutrient result in increased CEC.

MWOO has also been reported in other studies to provide additional P to the soil and our results would seem to support these observations. Soil extractable P availability has been reported to increase after applying MWOO and the increase has also observed to be proportional to the application rate (Zhang et al., 2006; Hargreaves et al., 2008). Dorahy et al. (2006) also reported that addition of MWOO to the soil increased the P concentration of the soil. The amounts of P supplied by MWOO can be equivalent to the amounts provided by mineral fertilizers (Hargreaves et al., 2008). Therefore, MWOO may be effective as fertilizers in supplying available soil P (Cooperband et al., 2002; Mkhabela and Warman, 2005) although the equivalence with inorganic fertilisers has not yet been demonstrated. What also remains uncertain is the longevity of MWOO derived extractable P in the soil and its long lasting effect.

We detected an increase in wheat growth following the application of MWOO to all the soils studied but this effect was largely found at the low application rates. Our results suggest that the 20 t ha^{-1} and the 50 t ha^{-1} application rates in the Kirby Sand and Minesite Soil had the largest improvement in plant growth. The incorporation method showed the greatest improvement in plant growth compared to the surface application of MWOO in the soil with the exception of Kirby clay. Crop growth as a result of organic amendments has been reported elsewhere. For example, Lakhdar et al. (2010) reported significant plant growth of 93% and 123% following application of 40 t ha^{-1} and 80 t ha^{-1} of compost respectively. Similarly, Zheljazkov and Warman (2004) reported increased Swiss chard yield in amended soils compared to unamended soil. Carbonell et al. (2011) reported improved *Zea mays* biomass (16 – 17%) on plants grown in treated soil compared to those grown in control. Dorahy et al. (2006) reported that MWOO application had a significant effect on both root fresh and dry weights and that root dry weight was significantly higher ($P < 0.01$) for 100 t ha^{-1} treatment compared to other treatment. However, in all soils, there was an apparent decline in wheat growth at the 140 t ha^{-1} application rate and this was statistically significant in the Kirby Sand and CROA soils.

The reduction in aboveground biomass at the highest application rates has also been reported by other studies. For example, Lillywhite et al. (2009) reported that grain yield and total dry weight of barley increased in response to some MWOO products applied to the soils but decreased in response to others. The reduction in growth in other studies has been attributed to high C: N ratio (Gajdos, 1997; Lillywhite et al., 2009). The MWOO used in this study had a C:N ratio of 17.8 : 1, which is within the recommended optimal range of 20-

25:1 suggesting that N immobilization may not have been the cause of the reduction in growth. Dorahy et al. (2006) reported similar results for the low application rate of 25 t ha^{-1}. N immobilisation would seem unlikely in our experiment since the C:N ratio of the MWOO material did not exceed 20. Other potential causes of this reduction in growth at high application rates might be oxygen deficiency resulting from rapid microbial activity, ammonia toxicity (Wolkowski, 2003), phytotoxicity due to heavy metals (Paradelo et al., 2009) or a range of other potential factors associated with the high application rates. Further research to determine the actual cause (or cumulative causes) of the growth reduction is necessary.

4.5 Conclusion

This study showed that MWOO application to the four NSW soils had some significant effects on both soil condition and plant growth. Soil pH, C, N, P, EC, Extractable P and CEC were all found to increase although mainly at the higher MWOO application rates and the magnitude of change was moderated by the initial concentrations of these characteristics in the soil. Plant growth and yield were also observed to increase following addition of MWOO. Aboveground and root biomass were observed to increase at the low (20 and 50 t ha^{-1}) application rates, suggesting that MWOO application had positive effects on growth and yield of wheat. Decline in biomass was however, observed at high application rate (140 t ha^{-1}), particularly on soils with lower clay content. Further research is required to investigate the causes of this decline.

4.6 References

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5 Effects of MWOO on Metal Accumulation and Plant Uptake

5.1 Summary

In this component of the work, a ten week glasshouse experiment with wheat (*Triticum aestivum)* was conducted to assess the accumulation and distribution of heavy metals in four NWS soils and the subsequent uptake by plants after adding MWOO to the soil. The MWOO was applied at three application rates (20, 50 and 140 t ha^{-1}) using two methods of application (surface and incorporated). The soils were analysed for Cu, Zn, Ni, Cr, Pb, Cd and As at three soil depths (0-5, 5-15 and 15-25 cm). The results showed that MWOO application significantly increased ($P < 0.05$) Zn, Ni, Cr and Pb concentrations in the soil compared to the control, particularly in the superficial soil layer (0-5 cm). The Cd soil concentration change was not significant except in one soil where the MWOO concentration was significantly lower than the background soil value and resulted in a concentration decrease. There was no significant increase in the concentrations of Cu and As. The roots, stems (stems and leaves) and grains of wheat were also analysed for the seven heavy metals. The concentrations of Cu, Ni and Cr were significantly higher in the roots ($P < 0.05$) compared to the aboveground plant parts but concentration of Zn was higher in stems than roots. The grain concentrations of Pb, Cd and As were below detection limits and for the other metals the concentrations were within allowable concentration. The translocation factors showed that translocation of heavy metals to aboveground plant parts was generally low for all the metals with the exception of Zn and Ni. The concentration of Zn was generally higher in stem than in roots for the three application rates with stem: root (S/R) ratio greater than 1, with the highest S/R of 3.9 in the CROA for the 20 t ha^{-1} application rate. Compared to the control, the S/R for the treatments was lower but there was no significant difference ($P > 0.05$). Stem concentration of Ni was higher than root concentration for the 50 t ha^{-1} incorporated MWOO with a stem: root (S/R) ratio of 4.08. Compared to the control, the treatments showed higher S/R but there was no significant difference ($P > 0.05$). The translocation factors suggested that translocation of the heavy metals to the edible parts of the crop was low.

5.2 Introduction

Waste generation is a growing issue of concern in Australia and globally, especially with ever growing population and urbanization. Australia is described as one of the larger producers of waste (Hyder, 2009). According to Australian Bureau of Statistics, Australia generated nearly 44 million tonnes of waste in 2006-2007 and NSW was estimated to be the largest producer of waste, producing approximately 15.4 million tonnes (ABS, 2010). Waste generation increased from nearly 44 million tonnes in 2006-07 to approximately 48 million tonnes in 2010-11 (Randell et al., 2014). As the population in Australia grows, the country will continue to generate more waste.

Landfill disposal of solid waste is practiced across the globe to avoid the high cost associated with incineration (Lakhdar et al., 2010). Concerns associated with disposal of biodegradable waste in landfills include long term pollution of the environment from leachate, greenhouse gas emissions, noise and odour. Due to these concerns landfills are becoming a last resort choice for waste disposal (Smahi et al., 2013). Australia is encouraging diversion of waste from landfills and this has led to the total waste diversion rate increasing from 45% in 2002-3 to 63% in 2010-11. The Environment Protection Authority (EPA) has targeted to increase the percentage of waste diverted from landfill from 63% to 75% by 2020-21 (EPA, 2013).

One means that this is being achieved is processing of the waste organic fraction of mixed waste by mechanical biological treatment of the waste to pasteurised and biologically stabilised organic outputs. This process involves the microbiological degradation of organic matter from biodegradable wastes, leading to an end product containing stabilised nitrogen, carbon and other nutrients in the organic fraction (Zwart, 2003). Production of mixed waste organic output (MWOO) and its subsequent application to agricultural land has gained popularity worldwide not only because it relieves pressure on landfill space but also because of the agronomic benefits associated with it. The application of MWOO to soil can improve soil fertility by adding organic matter and improving soil pH (Zheljazkov and Warman, 2004; Achiba et al., 2009; Zhao et al., 2013) and physical soil properties (Wells et al., 2000).

However, there is concern that MWOO application to agricultural soils may pollute the soils with heavy metals (Fekri and Kaveh, 2013). The heavy metals can be introduced into the compostable organic fraction of waste from sources such as batteries, plastic, paint, motor oils, newspapers, and electronic appliances (Hamdi et al., 2003 as cited in Ayari et al., 2010). Many authors have reported that MWOO can contain high quantities of heavy metals and that its use can lead to heavy metal accumulation in the soil (Ayari et al, 2008; Mahvi, 2008, Achiba et al., 2009; Fekri and Kaveh, 2013). Long term application of MWOO may result in accumulation of heavy metals in the soil (Zhao et al., 2013). These heavy metals may be taken up by plants and therefore threaten human health by contaminating the food chain (Chukwuji et al., 2005; Jordao et al., 2006; Smith, 2009).

The availability of heavy metals to plants in MWOO amended soil depends on the metal concentration in the soil, the organic matter (OM) content, soil pH, cation exchange capacity (CEC) (Woodbury, 1992; Smith, 2009) and redox status of the soil (Illera et al., 2000) as well as the plant itself. For instance, increase in pH and OM content can decrease the availability of heavy metals such as copper (Cu), lead (Pb), nickel (Ni) and cadmium

(Cd) (Woodbury, 1992). This happens because as the organic matter and pH increase there is an increase in the negative charge density on the sorption site which in turn increases the sorption of the metallic ion. Illera et al. (2000) stated that the complex formation between soluble organic matter and metals affects mobility of metals, such as the Fe/Mn oxide fractions and the organic fractions which will determine the availability of metals to plants and potential risk.

The aims of this study were to (a) determine the accumulation and distribution of copper, zinc, nickel, chromium, lead, cadmium, and arsenic in the 0-5, 5-15 and 15-25 cm depth of four NSW soils following application of MWOO at 20, 50 and 140 t ha^{-1} rates and (b) to determine the uptake and distribution of these heavy metals in wheat (*Triticum aestivum*) plants growing in the amended soils.

5.3 Materials and Methods

5.3.1 MWOO and Bulk Soil Sampling and Analysis

The MWOO used in this study was matured output derived from mixed solid waste. It was sourced from the Global Renewables UR-3R facility, a Mechanical and Biological Treatment facility at Eastern Creek south of Sydney, New South Wales. MWOO was collected from the production process and kept in a cool room at the university prior to use.

This study was carried out using four different soils of NSW which were collected from 0-10 cm depth of surface soil bulked over 5–10 square metres. These included a selection of those soils described in Table 2.1.and included Kirby sand (S1), which is a sandy loam with 13.2% clay; 10.5% silt and 76.3% sand, Kirby clay (C1), which is a clay soil with 55.0% clay; 26.0% silt; 19.0% sand, CROA (SL1), which is a silty loam with 27.6% clay; 41.4% silt and 30.0% sand and Minesite (SL2), which is a silty loam with 14.3% clay; 29.2% silt and 56.5% sand. The Kirby clay is classified as a Dermosol and the Kirby sand, CROA and Minesite are Chromosols (Isbell 2002). The Kirby clay was collected from UNE Kirby farm (30.435463^{O}S, 151.633636^{O}E), the Kirby sand was collected from the UNE Newholme farm (30.426087^{O}S, 151.652707^{O}E), the Minesite loam was collected from Ashton Coal, Singleton (32.460897^{O}S, 151.087095^{O}E) and the CROA loam was collected from NSW DPI Elizabeth McArthur Agricultural Institute, Camden (34.080957^{O}S, 150.704249^{O}E). The bulk soils were sieved to < 4 mm using a mechanical sieve, air dried and mixed thoroughly prior to use.

The soils and MWOO materials used in this experiment were sub-sampled from the bulk samples collected at the initiation of the project. As a consequence, physical and chemical properties differ slightly, though not substantially, within each experiment. This reflects the inherent variability of the soils and materials used.

Samples (10 g) of each of the air dried soils were weighed into aluminium trays in triplicates and then oven dried at 105^{O}C. The oven dry weights were used to calculate the gravimetric moisture contents of the soils (Schmugge et al, 1980). Separate samples of the bulk soil were added in triplicates into pots that had drainage holes lined with paper towel to prevent the soil from leaking out from the bottom of the pots. Each pot was filled with 1 kg of each soil type and soaked in a water bath until the soil was saturated. The pots were then removed from the water bath and the water was allowed to drain by gravity. After 24 hours the wet soils were weighed and the weights together with the gravimetric moisture contents were used to calculate the field capacity of the soils and amount of water needed to bring moisture content to field capacity. Soil that could fill the experiment pots to 25 cm depth for each soil type was weighed and the weights were used to calculate the amount of water needed to bring each soil to 60% and 80% field capacity.

Characterization of the bulk soils and MWOO was carried before MWOO application to the soil (before setting up the experiment). For the characterization, samples of the air dried bulk (< 4 mm) soils were mechanically ground to < 2 mm and < 0.2 mm. The MWOO was mixed thoroughly and homogenised. Three samples (triplicates) of soils and the MWOO were stored in acid washed yellow-top vials. The soils and the MWOO were analysed and results obtained for pH, electrical conductivity (EC), cation exchange capacity (CEC), total carbon, total nitrogen and extractable phosphorus. For pH and EC 10 g of the < 2 mm soil was weighed and a 1:5 soil:water was shaken for 1 hour and then allowed 20-30 minutes to settle. A calibrated electrode was placed in the solution and after stabilisation the readings were taken. The pH meter was a TPS model 901-CP and the EC meter was a TPS model labCHEM cond. Exchangeable cations were analysed by weighing 2 g of the < 2 mm soil into a 50 mL centrifuge tube and adding 40mL of 1 M NH_4Cl before being shaken for 1 hour. Samples were then filtered through Whatman no. 42 filter paper and stored in a cool room until Ca, Mg, Na and potassium (K) were analysed on the ICP-OES (Rayment and Higginson, 1992). Total carbon and total nitrogen were analysed by weighing 0.2 g of the < 0.2 mm soil into a tin foil cups. The tin cups are crimped closed and loaded to LECO TruSpec (LECO Corporation, 2009). For metal analysis 4.5 mL of hydrochloric acid (36%) and 1.5 mL of nitric acid (70%) were added to 0.5 g of soil, the solution was swirled and then left to pre-digest in a fume hood for 1 hour. Soils were sonified for 2 minutes and then analysed using a Milestone Ultrawave and the EPA 3051 microwave digestion method (USEPA, 2007). Extractable phosphorus was determined using the Colwell Bicarbonate Test.

5.3.2 Experimental Design

The experiment involved two methods of MWOO application (surface and incorporated) using three different application rates (20, 50 and 140 t ha^{-1} equivalents) and the control treatment (0 t ha^{-1}), with three replicates for each treatment in cylindrical PVC pots (90 mm diameter; 300mm height) filled to a depth of 25 cm with the four different NSW soils. The pots were thoroughly washed using 2% extran and were rinsed with de-ionized water then allowed to dry before the soils and MWOO were added into them. There were 12 pots for the control treatment (4 soils X 3 replicates); 36 pots of incorporated MWOO (4 soils X 3 application rates X 3 replicates); 36 pots of surface application (4 soils X 3 application rates X 3 replicates). This amounted to 84 pots altogether.

In the surface application method, air-dried soils were weighed and added to the pots to 25 cm depth then wetted to 60% field capacity. Equivalents of 20, 50 and 140 t ha^{-1} of MWOO were applied to the surface and no MWOO (0 t ha^{-1}) was applied to the control treatments. Meanwhile, in the incorporated method the bottom 10 cm of the pots was filled with soil while the soil that would fill the top 15 cm were put into plastic bags with the corresponding amounts of MWOO (20, 50, and 140 t ha^{-1}). The soil and MWOO in the bags were thoroughly mixed and added to the pots. Water was added to 60% field capacity.

All pots were then placed on two rectangular benches (tables) in the greenhouse in a randomized design. The greenhouse temperature ranged between 15°C and 25°C diurnally. One day after the MWOO was added, six wheat (*Triticum aestivum*) seeds were sown into each pot. In some pots only five seeds had germinated therefore thinning was done in the pots where all six seeds had germinated, leaving five plants per pot. Water was added by hand on regular basis to the pots to maintain 60% field capacity. Two weeks after emergence, water was then maintained at 80% field capacity until harvesting. Occasionally, weeds were removed by hand. The wheat plants were allowed to grow for ten weeks and were then harvested. By this stage the wheat plants had developed heads.

5.3.3 Plant and Soil Sampling

The aboveground parts of the plants were harvested using a pair of scissors which had been washed with 2% extran and rinsed off with de-ionized water. The pair of scissors was rinsed off between samples to minimise cross-contamination of plant samples. The plants were washed with de-ionized water after harvesting to remove adhering soil particles. The heads (grains) were separated from the rest of the plants parts (stems and leaves) and fresh weights were taken and recorded. The plant parts were oven-dried at 60°C to a constant weight. Roots were removed from the soil and thoroughly washed using de-ionized water, weighed, then oven dried at 60°C and re-weighed. The oven-dry plant materials (roots; stems and leaves; heads) were mechanically ground, separately, to less than 1 mm and stored in plastic bags before being sent to a commercial laboratory for heavy metals analysis.

After harvesting aboveground plant parts, soil columns (cores) were removed from the pots and divided into three depth increments of 0-5, 5-15 and 15-25 cm using a sharp knife. The soil samples were placed in separate aluminium trays and then crushed by hand to recover the roots. The soils were then weighed and oven dried at 40^{o}C until a constant weight was reached. The samples were re-weighed to determine the dry weights (Wilson et al., 2009). The dry soils were then ground with pestle and mortar to pass through a 2 mm sieve and stored in acid washed yellow-top vials in preparation for laboratory analysis.

5.3.4 Plant and Soil Analysis

All plant parts were analysed for tissue concentrations of zinc (Zn); copper (Cu); lead (Pb); nickel (Ni); chromium (Cr); Cadmium (Cd); and Arsenic (As) at Australian Laboratory Services (ALS) in Sydney using inductively coupled plasma mass spectrometry(ICP-MS method) (aqua regia digestion using USEPA Method 200.2). Limit of recovery was 0.1 mg kg $^{-1}$ for all elements except Zn which was 0.5 mg kg^{-1}.

The 2 mm soil samples obtained from the experiment after harvesting were analysed in triplicates and results obtained the heavy metals zinc (Zn); copper (Cu); lead (Pb); nickel (Ni); chromium (Cr); Cadmium (Cd); and Arsenic (As). In the heavy metal analysis, 4.5 mL of hydrochloric acid (36%) and 1.5 mL of nitric acid (70%) were added to 0.5 g of soil, the solution was swirled and then left to pre-digest in a fume hood for 1 hour. Soils were sonified for 2 minutes and then analysed using a Milestone Ultrawave and the EPA 3051 microwave digestion method (USEPA, 2007). Extractable phosphorus was determined using the Colwell Bicarbonate Test.

5.3.5 Translocation of Heavy Metals

Metal uptake and translocation from the roots of wheat plants to the aboveground plant parts was determined by calculating the translocation factors (TFs). The translocation factor from roots to the stems is given by the ratio of metal concentration in the aboveground plant parts and the metal concentration in the roots (Bose and Bhattacharyya, 2008). The translocation factor for the stems (stems and leaves) was calculated as the ratio of metal concentration in the stems and the metal concentration in the root and was expressed as S/R. The translocation factor for the grains was calculated as the ratio of metal concentration in the grain and the metal concentration in the stems and was expressed as G/S (Carbonell et al., 2011).

5.3.6 Quality Control

The analysis of soil samples for heavy metals was done in the Agronomy and Soil Science laboratory at the University of New England. A standard reference soil (NIST SRM 2711 Montana Soil) and blank samples were included in the analysis of heavy metals in the soil for quality control purposes. The plant heavy metal analysis was done in a NATA accredited commercial laboratory in Sydney (ALS Environmental).

5.3.7 Statistical Analysis

Statistical analyses were carried out with analysis of variance (ANOVA) using the R program version 3.1.1 (R Core Team, 2014). Assumptions of heterogeneous variances and normal distributions were confirmed and any necessary transformations are noted in the results section. Data were tested for significant differences at P = 0.05. Significantly different means were separated using 95% confidence intervals (1.96 × standard errors). Post Hoc tests using Tukey were performed (Goldstein and Healy, 1995).

5.4 Results

5.4.1 Soil and MWOO Characterisation

The characterisation of each soil and MWOO used in this study is shown in Table 5.1 and Table 5.2, respectively. The recovery of all the heavy metals from all the soils and MWOO using the aqua regia digestion technique was > 80%(95.8, 96.0, 92.0, 82.1, 92.4, 94.7and 94.9% for Cu, Zn, Ni, Cr, Pb, Cd and As respectively).The concentration of Cr was higher compared to the other heavy metals reported in this study in all the other soils except in Kirby sand where As concentration was higher. The concentrations of the heavy metals Cu, Zn, Ni, Cr and Cd were higher in the Kirby clay compared to the other soils. The concentration of Pb was higher in the CROA whereas the concentration of As was higher in the Kirby sand. All heavy metals MWOO were observed to be within the NSW General Exemption limit values (OEH, 2011b) except for Zn (831.97 mg kg^{-1}) and Pb (303.02 mg kg^{-1}) for plantation forestry/non-contact agricultural and broad acre agricultural.

Table 5.1: Selected chemical characteristics of the soils used in the experiment.

Soil Parameters	Kirby Sand	Kirby Clay	CROA	Minesite
pH	6.28 ± 0.03	6.21 ± 0.08	5.99 ± 0.82	6.19 ± 0.01
Total Cu (mg kg^{-1})	15.50 ± 0.45	45.60 ± 0.68	17.05 ± 0.25	8.66 ± 0.04
Total Zn (mg kg^{-1})	21.28 ± 0.55	86.57 ± 1.56	32.19 ± 0.31	37.01 ± 0.89
Total Ni (mg kg^{-1})	14.87 ± 0.27	124.52 ± 1.60	14.67 ± 0.20	15.79 ± 0.73
Total Cr (mg kg^{-1})	37.61 ± 1.29	195.11 ± 4.22	45.14 ± 3.43	41.96 ± 2.40
Total Pb (mg kg^{-1})	9.38 ± 0.68	4.08 ± 0.22	21.11 ± 1.10	12.07 ± 0.31
Total Cd (mg kg^{-1})	BDL	3.73 ± 0.10	0.90 ± 0.23	0.98 ± 0.16
Total As (mg kg^{-1})	66.46 ± 4.89	1.27 ± 0.55	7.68 ± 0.82	6.37 ± 0.60

Values are the Mean ± SE (*n* = 3). BDL= Below Detection limit.

Table 5.2: Selected chemical characteristics of the MWOO used in the experiment.

Parameters	MWOO	NSW General Exemption limit value (OEH, 2011b)
pH	7.36 ± 0.26	-
Total Cu (mg kg^{-1})	202.69 ± 52.82	375
Total Zn (mg kg^{-1})	831.97 ± 287.15	700
Total Ni (mg kg^{-1})	31.76 ± 6.68	60
Total Cr (mg kg^{-1})	88.78 ± 18.10	100
Total Pb (mg kg^{-1})	303.02 ± 29.80	420, 300, 250 †
Total Cd (mg kg^{-1})	1.10 ± 0.09	3
Total As (mg kg^{-1})	5.62 ± 0.45	20

† Limit values for - mines sites: plantation forestry/non contact agricultural and broad acre agricultural until 31 Dec 2011: plantation forestry/non contact agricultural and broad acre agricultural from 1 Jan 2012.Values are the Mean ± SE (*n* = 3)

5.4.2 Heavy Metals in the Soil

5.4.2.1 Copper (Cu)

The total concentration of Cu in the soil after application of MWOO is shown in Table 5.3. The recovery of Cu from the soil was 94.5%. The initial concentration of Cu in the soil determined the magnitude change in the concentration. The results showed that MWOO application significantly increase ($P < 0.05$) Cu concentration in the 0-5 cm layer compared to the other layers in the surface application. In the incorporated application method, MWOO addition resulted in a significant increase ($P < 0.05$) in Cu concentration in both the 0-5 and the 5-15 cm layers. The 50 t ha^{-1} and 140 t ha^{-1} application rate showed significant increases in Cu concentration ($P < 0.05$) compared to the control. Generally, the method of application had no significant difference in the concentration of Cu except at the highest doses in the 0-5 cm layer where the surfaces application method was significantly higher than in the incorporated method.

Table 5.3: The concentration of Cu (mg kg^{-1}) in the soil after harvesting following MWOO application.

Cu		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 – 5	13.38 ± 0.54	16.78 ± 0.32	32.50 ± 7.71	51.11 ± 1.98	13.61 ± 0.38	16.14 ± 0.54	20.59 ± 0.96
	5 – 15	13.12 ± 0.51	13.26 ± 0.34	13.30 ± 0.22	13.13 ± 0.34	13.83 ± 0.60	16.38 ± 0.59	24.40 ± 0.96
	15 - 25	13.33 ± 0.31	13.76 ± 0.18	12.16 ± 0.76	12.94 ± 0.61	11.51 ± 0.20	12.96 ± 0.38	13.49 ± 0.33
Kirby Clay	0 – 5	44.11 ± 0.91	50.99 ± 0.57	58.15 ± 0.60	76.14 ± 4.62	51.81 ± 0.93	54.16 ± 1.01	64.16 ± 1.69
	5 – 15	46.61 ± 1.46	49.03 ± 0.71	50.57 ± 0.51	49.92 ± 0.32	52.00 ± 0.77	55.33 ± 1.17	61.00 ± 0.49
	15 - 25	48.69 ± 0.93	49.15 ± 0.47	49.00 ± 0.71	48.86 ± 0.34	49.16 ± 0.43	49.27 ± 0.44	49.40 ± 0.40
CROA	0 - 5	18.48 ± 0.49	18.45 ± 0.72	24.93 ± 0.44	513.6 ± 465.7	52.27 ± 34.4	20.24 ± 0.61	26.54 ± 0.40
	5 - 15	17.46 ± 0.31	16.45 ± 0.28	17.68 ± 0.06	17.96 ± 0.46	18.38 ± 0.63	18.64 ± 0.51	40.39 ± 11.82
	15 - 25	16.97 ± 0.10	16.75 ± 0.33	18.00 ± 0.38	17.55 ± 0.19	16.97 ± 0.27	17.35 ± 0.51	18.00 ± 0.53
MINE	0 - 5	8.23 ± 0.22	13.12 ± 1.95	17.19 ± 0.94	110.99 ± 70.81	9.40 ± 0.32	11.01 ± 0.16	17.14 ± 0.54
	5 - 15	7.85 ± 0.11	8.53 ± 0.21	8.61 ± 0.06	8.30 ± 0.41	9.05 ± 0.16	19.75 ± 6.41	17.96 ± 0.58
	15 - 25	7.93 ± 0.09	8.03 ± 0.24	8.01 ± 0.09	8.13 ± 0.43	7.78 ± 0.31	8.32 ± 0.29	7.85 ± 0.15

Values are the mean ± SE (n = 3). Stems: stems and the leaves together.

5.4.2.2 Zinc (Zn)

The concentrations of Zn in the soils following application of MWOO are presented in Table 5.4. The recovery of Zn from the soil was 88.5%. The results show that the application of MWOO significantly increased Zn concentration in all the soils ($P < 0.001$). A strong increase was observed in the sandy so and no significant increase was observed in the clay ($P > 0.05$). The surface application resulted in a slightly higher but not significantly different ($P > 0.05$) Zn concentration than the incorporation, especially in the 0-5 cm layer. Both methods were significantly higher than the control ($P < 0.001$). The 140 t ha^{-1} treatment significantly increased Zn concentration ($P < 0.05$) compared to the other treatments and the control. The increase in Zn was observed to be proportional to the application rate. The Zn concentration was significantly higher in the 0-5 cm soil layer in the surface application method whereas in the incorporated method, increase was observed to have occurred in the 0-5 and the 5-15 cm layers. The magnitude of change in the concentration was determined by the initial concentration of Zn in the soil.

Table 5.4: The concentration of Zn (mg kg^{-1}) in the soil after harvesting following MWOO application.

Zn		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 - 5	9.62 ± 2.15	13.39 ± 0.99	38.78 ± 5.21	89.89 ± 4.43	12.65 ± 3.89	17.00 ± 1.56	29.50 ± 2.51
	5 - 15	3.64 ± 1.12	3.37 ± 1.00	5.19 ± 0.40	4.39 ± 0.31	22.62 ± 2.39	16.64 ± 1.55	37.08 ± 1.88
	15 - 25	4.84 ± 1.07	3.89 ± 0.62	2.79 ± 0.40	4.00 ± 1.07	13.32 ± 1.40	7.52 ± 1.25	12.29 ± 1.50
Kirby Clay	0 - 5	66.93 ± 1.87	87.07 ± 1.51	109.05 ± 1.27	157.19 ± 12.79	86.88 ± 1.58	92.58 ± 1.04	112.92 ± 1.18
	5 - 15	72.05 ± 2.46	80.08 ± 0.74	85.73 ± 0.16	80.74 ± 0.94	88.76 ± 2.17	103.08 ± 9.07	119.09 ± 2.29
	15 - 25	89.94 ± 6.79	83.06 ± 1.03	79.92 ± 1.69	81.75 ± 0.60	84.08 ± 2.02	83.76 ± 0.84	82.43 ± 0.44
CROA	0 - 5	34.25 ± 2.44	23.68 ± 1.62	44.42 ± 3.05	153.10 ± 23.90	21.71 ± 1.15	29.73 ± 1.51	71.90 ± 18.12
	5 - 15	17.45 ± 0.42	17.34 ± 0.22	21.00 ± 0.67	21.69 ± 1.03	24.25 ± 0.78	31.29 ± 1.16	52.93 ± 0.732
	15 - 25	17.41 ± 0.32	18.03 ± 0.46	20.42 ± 0.52	21.30 ± 0.84	20.88 ± 1.08	24.76 ± 2.30	22.87 ± 1.45
MINE	0 - 5	23.94 ± 0.95	35.42 ± 0.72	53.93 ± 3.65	142.41 ± 35.49	29.04 ± 1.37	34.08 ± 1.51	50.10 ± 0.76
	5 - 15	22.29 ± 2.43	26.95 ± 1.40	29.55 ± 3.33	26.90 ± 2.69	30.86 ± 2.95	99.57 ± 65.03	50.24 ± 0.92
	15 - 25	22.90 ± 1.51	26.84 ± 0.43	27.401 ± 1.66	28.82 ± 1.70	29.23 ± 3.48	28.47 ± 1.20	24.28 ± 0.53

Values are the mean ± SE (n = 3). Stems: stems and the leaves together.

5.4.2.3 Nickel (Ni)

The concentrations of Ni in the soils following application of MWOO are presented in Table 5.5. The recovery of Ni from the soil was 86.0%. The magnitude of change in the Ni concentration was determined by the initial concentration in the soil. These results show that the surface application resulted in a slightly higher Ni concentration compared to the incorporation but there was no significant difference ($P > 0.05$) except in the Kirby sand where it was significantly higher ($P < 0.05$). However, both methods were significantly higher than the control ($P < 0.001$). All application rates were significantly higher than the control ($P < 0.001$) in the other soils except in CROA but there was no significant difference between the application rates (20, 50 and 140 t ha^{-1}). It was observed that Ni was significantly higher in the 5-15 and 15-25 cm layers compared to the 0-5 cm layer.

Table 5.5: The concentration of Ni (mg kg^{-1}) in the soil after harvesting following MWO application.

Ni		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 - 5	6.90 ± 0.18	11.90 ± 0.07	13.44 ± 0.62	17.55 ± 1.20	5.92 ± 0.28	6.77 ± 0.43	6.90 ± 0.29
	5 - 15	6.92 ± 0.23	13.78 ± 1.08	21.40 ± 7.66	13.65 ± 0.75	23.07 ± 7.25	16.64 ± 5.99	7.62 ± 0.03
	15 - 25	7.20 ± 0.36	22.86 ± 9.10	10.65 ± 0.54	17.60 ± 3.54	20.38 ± 5.50	18.60 ± 6.40	6.92 ± 0.60
Kirby Clay	0 - 5	114.06 ± 2.19	132.85 ± 2.61	130.16 ± 0.61	117.16 ± 1.74	130.14 ± 0.43	130.02 ± 0.45	127.53 ± 1.46
	5 - 15	121.31 ± 3.32	134.35 ± 1.96	138.16 ± 1.46	136.51 ± 1.61	133.87 ± 0.77	131.20 ± 1.19	126.21 ± 1.03
	15 - 25	128.23 ± 2.36	131.99 ± 0.16	134.03 ± 2.33	133.86 ± 1.26	134.19 ± 1.74	133.21 ± 1.17	133.71 ± 1.26
CROA	0 - 5	14.67 ± 0.40	13.25 ± 0.46	14.51 ± 0.29	17.27 ± 0.68	13.21 ± 0.36	13.86 ± 0.22	14.82 ± 0.20
	5 - 15	13.00 ± 0.32	13.66 ± 0.12	14.53 ± 0.66	14.62 ± 0.13	13.32 ± 0.20	13.03 ± 0.15	21.79 ± 6.47
	15 - 25	13.06 ± 0.37	14.57 ± 0.53	15.06 ± 0.15	14.85 ± 0.24	14.59 ± 0.06	15.14 ± 0.64	14.31 ± 0.33
MINE	0 - 5	7.05 ± 0.14	8.59 ± 0.31	14.62 ± 1.26	22.73 ± 4.34	14.35 ± 0.65	13.89 ± 1.50	17.19 ± 1.49
	5 - 15	7.24 ± 0.13	14.32 ± 1.07	13.74 ± 1.55	14.67 ± 0.41	13.07 ± 0.79	19.72 ± 4.12	14.20 ± 1.17
	15 - 25	7.57 ± 0.09	13.68 ± 0.45	14.07 ± 1.35	13.52 ± 1.13	14.87 ± 1.01	16.54 ± 1.34	6.89 ± 0.22

Values are the mean ± SE (n = 3). BDL: Below detection limit. Stems: stems and the leaves together.

5.4.2.4 Chromium (Cr)

The concentrations of Cr in the soils following application of MWOO are presented in Table 5.6. The recovery of Cr from the soil was 94.8%. The magnitude of change in the Cr concentration was determined by the initial concentration in the soil. The results show that application of MWOO significantly increased Cr concentrations in the all soils ($P < 0.001$). The Cr concentration of the soils was significantly different ($P < 0.001$) following the order: Kirby clay > CROA > Minesite > Kirby sand. The surface application resulted in a slightly higher Ni concentration compared to the incorporation but there was no significant difference ($P > 0.05$). However, both methods were significantly higher than the control ($P < 0.001$). With the exception of CROA, in all soils all the application rates (20, 50 and 140 t ha^{-1}) resulted in significantly higher Cr concentration compared to the control ($P < 0.001$) but they did not differ significantly ($P > 0.05$) from each other. The 50 t ha^{-1} resulted in slightly higher Cr compared to the other treatments. It was observed that Cr in the 15-25 cm layer was significantly higher than in the 0-5 cm layer ($P < 0.01$) but not significantly higher than in the 5-15 cm layer ($P > 0.05$). No significant difference was observed in between the 0-5 and the 5-15 cm layers ($P > 0.05$).

Table 5.6: The concentration of Cr (mg kg^{-1}) in the soil after harvesting following MWOO application.

Cr		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 - 5	25.21 ± 0.90	35.60 ± 0.23	38.81 ± 2.47	48.58 ± 2.72	25.26 ± 1.03	27.92 ± 0.92	28.42 ± 0.48
	5 - 15	28.15 ± 1.36	40.06 ± 1.92	51.91 ± 14.05	38.16 ± 1.66	56.59 ± 13.19	45.41 ± 11.15	30.54 ± 0.23
	15 - 25	26.14 ± 0.37	55.83 ± 16.57	32.19 ± 1.52	44.76 ± 5.80	51.04 ± 9.73	49.17 ± 11.83	26.74 ± 0.68
Kirby Clay	0 - 5	197.36 ± 3.89	242.62 ±2.22	246.25 ± 2.35	216.58 ± 0.41	250.22 ± 2.79	235.97 ± 3.02	236.32 ± 2.77
	5 - 15	211.39 ± 5.91	239.80 ± 1.03	257.25 ± 0.71	242.45 ±4.21	249.50 ± 5.10	240.48 ± 1.74	229.67 ± 1.33
	15 - 25	225.55 ± 1.80	244.74 ± 1.57	242.32 ± 4.47	252.15 ± 0.93	241.80 ± 2.25	258.68 ± 2.89	244.51 ± 2.39
CROA	0 - 5	69.66 ± 3.17	58.84 ± 1.86	63.33 ± 1.66	70.67 ± 2.39	57.50 ± 1.54	62.02 ± 3.21	68.47 ± 4.82
	5 - 15	55.59 ± 3.64	62.01 ± 2.10	70.97 ± 4.33	64.32 ± 3.87	60.82 ± 2.45	53.48 ± 4.32	69.01 ± 4.02
	15 - 25	59.84 ± 0.44	60.15 ± 2.31	67.83 ± 4.91	62.98 ± 3.74	63.19 ± 4.10	73.33 ± 7.61	69.00 ± 7.28
MINE	0 - 5	42.27 ± 4.20	39.87 ± 1.32	61.67 ± 4.57	76.49 ± 9.85	59.83 ± 0.68	58.72 ± 5.13	64.51 ± 3.06
	5 - 15	35.93 ± 2.02	54.60 ± 4.46	55.02 ± 4.06	59.83 ± 9.85	55.84 ± 2.20	62.32 ± 4.17	57.15 ± 5.17
	15 - 25	39.12 ± 1.49	58.31 ± 1.43	60.63 ± 6.40	62.12 ± 7.03	62.83 ± 2.86	67.17 ± 5.16	42.71 ± 2.86

Values are the mean ± SE (n = 3). Stems: stems and the leaves together.

5.4.2.5 Lead (Pb)

The concentrations of Pb in the soils following application of MWOO are presented in Table 5.7. The recovery of Pb from the soil was 92.1%. The magnitude of change in the Pb concentration was determined by the initial concentration in the soil. The results show that application of MWOO at 140 t ha^{-1} significantly increased Pb concentrations of all soils ($P < 0.05$) in both methods compared to the other application rates and the control, especially in the upper layers in all soils. The surface application of MWOO resulted in slightly higher Pb concentration compared to the incorporation. The concentration of Pb in surface application was significantly higher in the 0-5 cm layer ($P < 0.001$) compared to the other layers (5-15 and 15-25) whereas in the incorporated method there was no significant difference in the Pb concentration in the 0–5 and 5-15 cm layers.

Table 5.7: The concentration of Pb (mg kg^{-1}) in the soil after harvesting following MWOO application.

Pb		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 - 5	6.63 ± 0.35	13.75 ± 0.46	46.57 ± 16.71	61.64 ± 6.27	8.73 ± 0.90	13.00 ± 0.33	23.09 ± 1.09
	5 - 15	8.57 ± 1.50	7.28 ± 0.68	7.20 ± 0.40	7.05 ± 0.18	8.78 ± 0.51	22.17 ± 8.34	25.01 ± 1.34
	15 - 25	6.90 ± 0.22	6.93 ± 0.16	6.53 ± 0.30	6.96 ± 0.22	6.82 ± 0.23	6.95 ± 0.06	7.27 ± 0.42
Kirby Clay	0 - 5	2.18 ± 0.30	5.91 ± 1.16	17.74 ± 2.12	58.08 ± 7.66	2.63 ± 0.28	8.68 ± 0.62	25.25 ± 1.15
	5 - 15	1.72 ± 0.38	0.63 ± 0.23	1.13 ± 0.12	1.23 ± 0.22	4.62 ± 0.94	7.85 ± 0.78	21.92 ± 1.32
	15 - 25	1.52 ± 0.19	0.10 ± 0.0.67	0.88 ± 0.22	0.47 ± 0.24	0.78 ± 0.12	1.07 ± 0.15	1.60 ± 0.14
CROA	0 - 5	122.13 ± 2.57	23.90 ± 0.98	37.00 ± 3.31	80.51 ± 5.29	19.63 ± 0.27	26.23 ± 2.32	36.99 ± 0.56
	5 - 15	18.43 ± 0.95	18.60 ± 0.78	21.06 ± 0.67	20.43 ± 0.71	20.59 ± 0.67	22.67 ± 0.97	40.24 ± 1.80
	15 - 25	18.44 ± 0.35	19.51 ± 0.78	20.35 ± 1.08	19.66 ± 0.95	19.74 ± 0.93	21.00 ± 1.08	21.18 ± 0.65
MINE	0 - 5	11.68 ± 1.57	16.05 ± 0.96	28.74 ± 1.14	290.53 ± 227.34	12.45 ± 0.88	16.30 ± 0.53	26.92 ± 0.24
	5 - 15	8.98 ± 0.40	11.86 ± 1.18	11.13 ± 0.56	10.33 ± 0.43	12.54 ± 1.00	16.61 ± 1.29	27.23 ± 0.70
	15 - 25	10.05 ± 0.74	10.96 ± 0.16	11.11 ± 0.69	12.10 ± 0.94	11.78 ± 1.41	11.58 ± 0.95	10.06 ± 0.66

Values are the mean ± SE (n = 3). BDL: Below detection limit. Stems: stems and the leaves together.

5.4.2.6 Cadmium (Cd)

The concentrations of Cd in the soils following application of MWOO are presented in Table 5.8. The recovery of Cd from the soil was 93.6%. The results show that background Cd soil concentration was greatest in Kirby Clay (which was at a value greater than the maximum allowable value for soil onto which MWOO can be applied) but lowest in Kirby sand ($P < 0.001$). The background Cd concentration in Kirby clay was significantly greater than the MWOO Cd concentration. The MWOO Cd concentration was comparable to Cd background concentrations in the other soils. Consequently, similar to observations in Section 2.3, this resulted in a moderation of the MWOO amendment influence on soil Cd. For example, in Kirby clay we see small but significant decreases in soil Cd concentration compared to the control in some MWOO treatments where MWOO concentration and the MWOO dilution effect was greatest ($P < 0.05$). For the other three soils any change in soil Cd concentration with MWOO application was either not detected or not significant ($P > 0.05$).

Table 5.8: The concentration of Cd (mg kg^{-1}) in the soil after harvesting following MWOO application.

Cd		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 - 5	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	5 - 15	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	15 - 25	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Kirby Clay	0 - 5	4.78 ± 0.16	4.92 ± 0.03	4.95 ± 0.05	4.23 ± 13	5.06 ± 0.04	4.87 ± 0.03	4.90 ± 0.09
	5 - 15	5.23 ± 0.23	4.97 ± 0.03	5.15 ± 0.05	5.07 ± 0.09	4.98 ± 0.09	4.88 ± 0.05	4.77 ± 0.06
	15 - 25	5.70 ± 0.15	4.93 ± 0.06	4.97 ± 0.14	5.07 ± 0.04	4.97 ± 0.03	5.18 ± 0.07	5.10 ± 0.06
CROA	0 - 5	1.98 ± 0.35	0.63 ± 0.07	1.17 ± 0.42	1.08 ± 0.13	0.55 ± 0.03	0.87 ± 0.20	1.12 ± 0.38
	5 - 15	1.25 ± 0.18	0.78 ± 0.19	1.23 ± 0.18	0.90 ± 0.18	0.60 ± 0.15	0.28 ± 0.21	1.02 ± 0.20
	15 - 25	1.55 ± 0.10	0.62 ± 0.14	1.03 ± 0.34	0.77 ± 0.21	0.82 ± 0.22	1.48 ± 0.37	1.25 ± 0.44
MINE	0 - 5	1.60 ± 0.58	0.23 ± 0.17	1.22 ± 0.29	1.42 ± 0.62	0.95 ± 0.13	0.92 ± 0.31	0.92 ± 0.17
	5 - 15	0.70 ± 0.18	0.47 ± 0.25	0.63 ± 0.20	0.82 ± 0.37	0.80 ± 0.40	1.18 ± 0.54	0.67 ± 0.35
	15 - 25	0.98 ± 0.17	1.08 ± 0.03	1.20 ± 0.66	1.47 ± 0.56	1.17 ± 0.39	1.35 ± 0.33	0.75 ± 0.33

Values are the mean ± SE (n = 3). BDL: Below detection limit. Stems: stems and the leaves together.

5.4.2.7 Arsenic (As)

The concentrations of As in the soils following application of MWOO are presented in Table 5.9. The recovery of As from the soil was 91.2%. The magnitude of change in the As concentration was determined by the initial concentration in the soil. The soil that had higher initial As had higher As levels even after MWOO application. The results show that As concentration in Kirby sand was significantly higher and that of Kirby clay was significantly lower ($P < 0.001$) than concentration in the other soils. Concentration in CROA was higher than in Minesite soil but there was no significant different ($P > 0.05$). There was no significant difference between the two methods of application and the control ($P > 0.05$) but the concentration in the control was slightly higher than in the two methods. Concentration of As was slightly higher in the 50 t ha^{-1} treatment and the other application rates were slightly lower than the control but there was no significant difference ($P > 0.05$). There was no significant difference in As concentration in different depths ($P > 0.05$).

Table 5.9: The concentration of As (mg kg^{-1}) in the soil following MWOO application.

As		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	0 - 5	59.65 ± 4.67	60.32 ± 3.23	60.65 ± 2.90	57.81 ± 1.95	67.48 ± 0.77	68.98 ±3.10	58.81 ± 0.60
	5 - 15	83.83 ± 25.04	65.47 ± 5.01	69.49 ± 6.89	69.32 ± 8.09	67.66 ± 2.04	73.33 ± 5.23	56.66 ± 1.89
	15 - 25	56.80 ± 6.65	61.15 ± 3.18	70.47 ± 3.76	69.13 ± 8.46	69.15 ± 1.20	67.32 ± 2.80	57.67 ± 1.74
Kirby Clay	0 - 5	1.38 ± 0.13	0.33 ± 0.20	0.50 ± 0.23	1.62 ± 0.49	0.23 ± 0.14	0.02 ± 0.02	0.42 ± 0.21
	5 - 15	0.52 ± 0.32	0.70 ± 0.38	0.78 ± 0.32	0.73 ± 0.32	0.37 ± 0.07	0.35 ± 0.20	0.60 ± 0.25
	15 - 25	1.13 ± 0.44	0.58 ± 0.31	0.33 ± 0.161	0.57 ± 0.28	0.35 ± 0.26	0.13 ± 0.11	0.00 ± 0.00
CROA	0 - 5	9.13 ± 1.03	6.87 ± 0.31	7.70 ± 0.30	7.85 ± 0.78	6.32 ± 0.69	7.47 ± 0.72	8.03 ± 0.81
	5 - 15	7.51 ± 0.61	7.42 ± 0.22	8.63 ± 0.67	7.78 ± 0.38	6.80 ± 0.16	5.55 ± 0.53	7.22 ± 0.59
	15 - 25	8.32 ± 0.09	7.20 ± 0.74	8.00 ± 0.91	7.93 ± 0.71	7.60 ± 0.33	9.07 ± 0.82	8.25 ± 1.07
MINE	0 - 5	6.10 ± 0.81	4.74 ± 0.20	6.37 ± 0.30	6.48 ± 0.82	5.73 ± 0.56	5.45 ± 0.25	6.55 ± 0.86
	5 - 15	4.63 ± 0.22	5.87 ± 1.02	6.55 ± 0.23	6.15 ± 0.09	4.95 ± 0.20	5.22 ± 0.58	5.25 ± 0.20
	15 - 25	5.26 ± 0.20	5.83 ± 0.75	5.88 ± 0.63	6.58 ± 0.51	6.330 ± 0.86	5.53 ± 0.38	4.75 ± 0.25

Values are the mean ± SE (n = 3). Stems: stems and the leaves together.

5.4.3 Heavy Metals in the Plants

5.4.3.1 Copper (Cu)

The concentrations of Cu in the plants parts (roots, stems and grains) following the application of MWOO to the soil are presented in Figure 5.1. The results show that there was a significant difference in the Cu concentration of the different plant parts ($P < 0.01$) but no significant differences were observed between treatments (20 t ha^{-1}, 50 t ha^{-1}, 140 t ha^{-1} and the control). Plants growing in surface application had significantly higher Cu concentration compared to the incorporated and the control ($P < 0.001$). Highest concentrations of Cu were found in the roots followed by grains > stems. The highest observed Cu concentration was 25.10 mg kg^{-1} for the 140 t ha^{-1} application rate in the surface application method, which is within the critical phytotoxicity concentration range of 10 – 30 mg kg^{-1} (Kabata-Pendias, 2001).

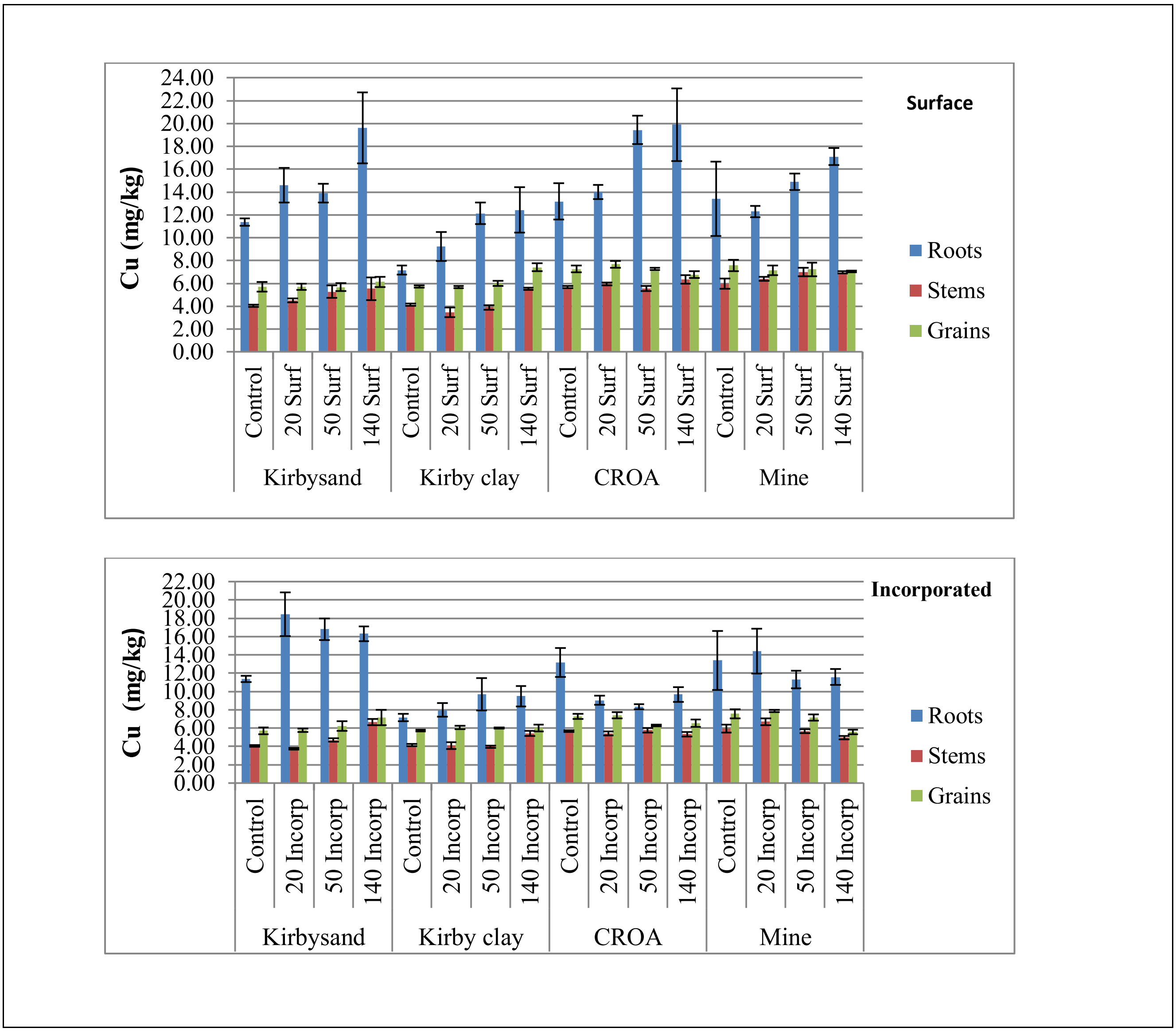

Figure 5.1: Concentration of Cu in wheat plant parts following application of MWOO.

5.4.3.2 Zinc (Zn)

The concentrations of Zn in the plants parts (roots, stems and grains) following the application of MWOO to the soil are presented in Figure 5.2. The results show that the stem concentrations of Zn were significantly higher than that of other parts ($P < 0.05$). The concentration distribution followed the order: stem > grain > roots. The 20 and 140 t ha^{-1} application rates showed higher Zn concentration compared to the 50 t ha^{-1} and the control but there was no significant differences ($P > 0.05$). Surface application resulted in slightly higher Zn concentration in plants there was no significant difference ($P > 0.05$). The Zn concentration detected in this study did not exceed 200 mg/ka which mean that the Zn concentration was within the critical concentration range of 100 – 500 mg kg^{-1} for phytotoxicity stated by Kabata-Pendias (2001).

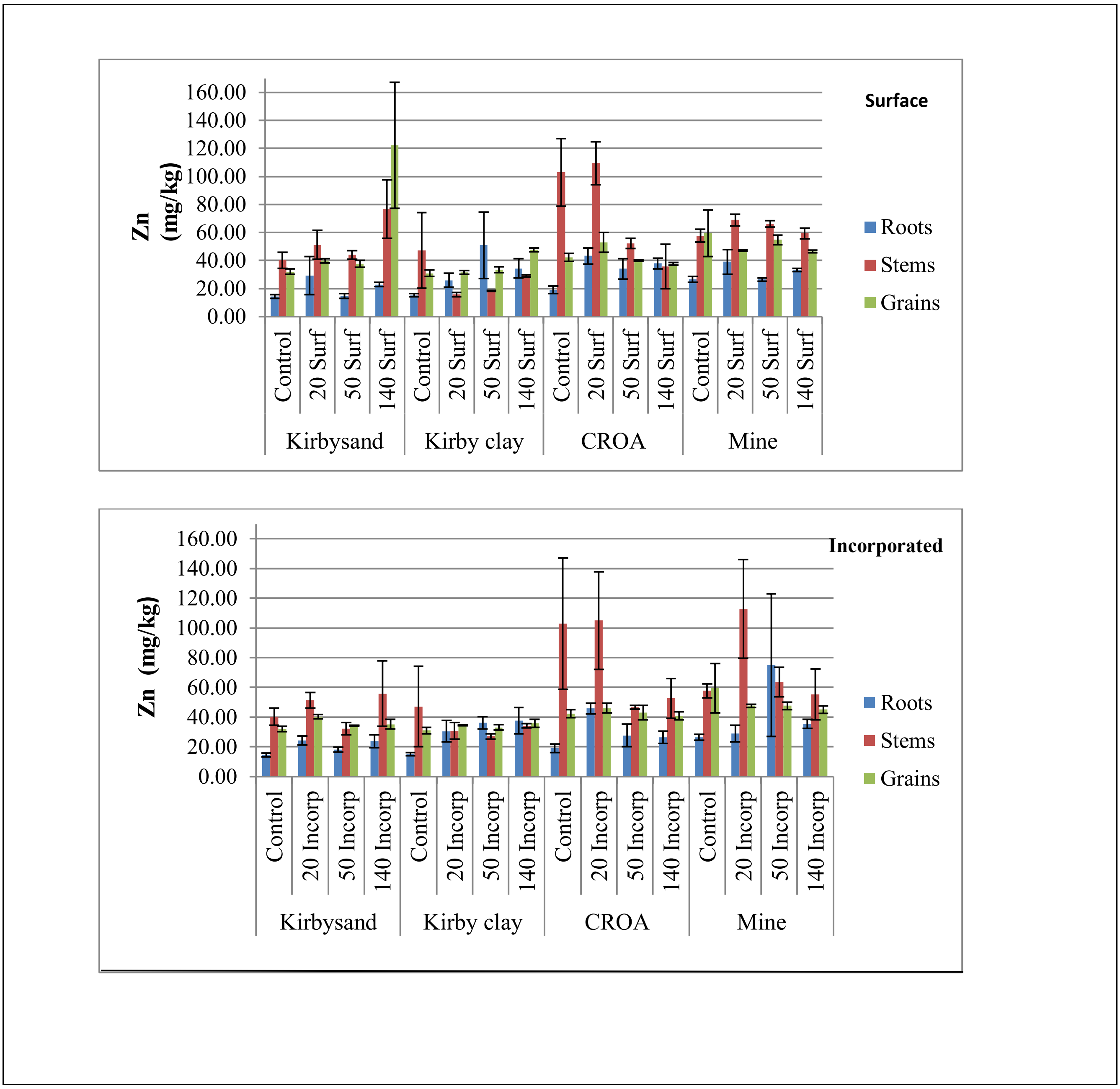

Figure 5.2: Concentration of Zn in wheat plant parts following application of MWOO.

5.4.3.3 Nickel (Ni)

The concentrations of Ni in the plants parts (roots, stems and grains) following the application of MWOO to the soil are presented in Figure 5.3. The results show that there was a difference in the Ni concentration of the different plant parts. Plants growing in Kirby clay showed significantly higher ($P < 0.001$) Ni concentrations compared to those growing in other soils. Application rates and methods had no significant effect on Ni concentration in plants (P > 0.05). The roots had significantly higher (P < 0.001) Ni concentrations followed by the grains > stems. In some cases the concentration of Ni was observed to be below detection limits. Even though Ni was detected in the plant, it is not likely to cause phytotoxicity because the highest concentration (13.3 mg kg^{-1}) was within critical range (10 – 30 mg kg^{-1}) for phytotoxicity (Kabata-Pendias, 2001).

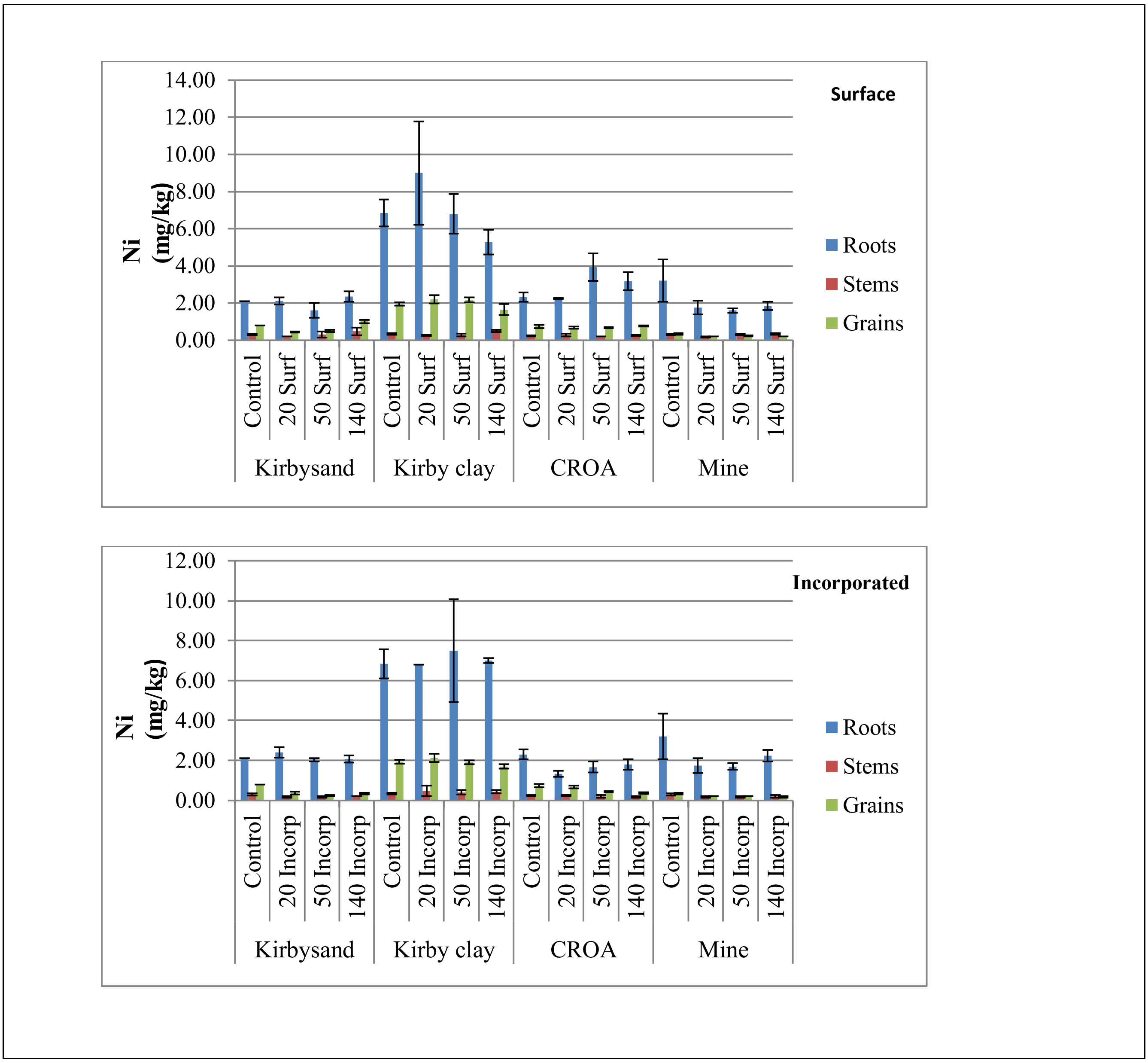

Figure 5.3: Concentration of Ni in wheat plant parts following application of MWOO.

5.4.3.4 Chromium (Cr)

The concentrations of Cr in the plants parts (roots, stems and grains) following the application of MWOO to the soil are presented in Figure 5.4. The root Cr concentration was significantly higher compared to the other plant parts ($P < 0.001$). It was observed that in most cases the stem concentration of Cr was higher than that of grain. A different trend was observed in the Kirby sand where Cr concentration in grains was higher than concentration in stems although there was no significant difference in the concentration. The critical range for Cr plant toxicity is 1–10 mg kg^{-1} (Kabata-Pendias, 2001). The Cr concentration of plants growing in the different soils was not significantly different ($P > 0.05$). Application rate had no significant effect on Cr plant concentration ($P > 0.05$). The different trends observed in translocation of Cr from stems to grain requires further investigation.

5.4.3.5 Lead (Pb)

The concentrations of Pb in the plants parts (roots, stems and grains) following the application of MWOO to the soil are presented in Table 5.10. The critical concentration range for plant toxicity is 30 – 300 mg kg^{-1} (Kabata-Pendias, 2001).The values in this table show that the concentration of Pb in the grains was below detection limits. There was no significant difference in Pb concentrations in plants growing in the different soils ($p > 0.05$). The Pb concentration of plants growing in soils where MWOO was surface applied was significantly higher than those in the incorporated treatment and control ($P < 0.01$). The 140 t ha^{-1} treatment resulted in significantly higher Pb concentrations compared to the 20 t ha^{-1} and the control ($P > 0.01$) but not significantly higher than the 50 t ha^{-1} treatment ($P > 0.05$).Accumulation of Pb was observed to occur mostly in roots but a few cases were below detection limits (Table 18). The root concentration was significantly higher compared to the aboveground plant parts ($P < 0.001$). The stems had higher Pb concentration than the grains but there was no significant difference ($P > 0.05$).

5.4.3.6 Cadmium (Cd)

The critical plant toxicity concentration range for Cd is 10 – 20 mg kg^{-1} The maximum residue limit established for Cd in leafy vegetables and root and tuber vegetables and wheat in Australia is 0.1 mg kg_{-1} (FSANZ, 2016). The concentrations of Cd in the plants parts (roots, stems and grains) following the application of MWOO to the soil are presented in Table 5.11 and show that for grain all concentrations were less than the maximum residue limit (which was also the method limit of recovery). Cadmium was detected in some plant parts, often roots, usually at concentrations close to the method limit of recovery or not significantly different to the control.The MWOO addition seemed to show some influence on root Cd concentration for Kirby sand and

CROA in some treatments but this was not evident in increased grain concentrations at the limits of recovery available. .

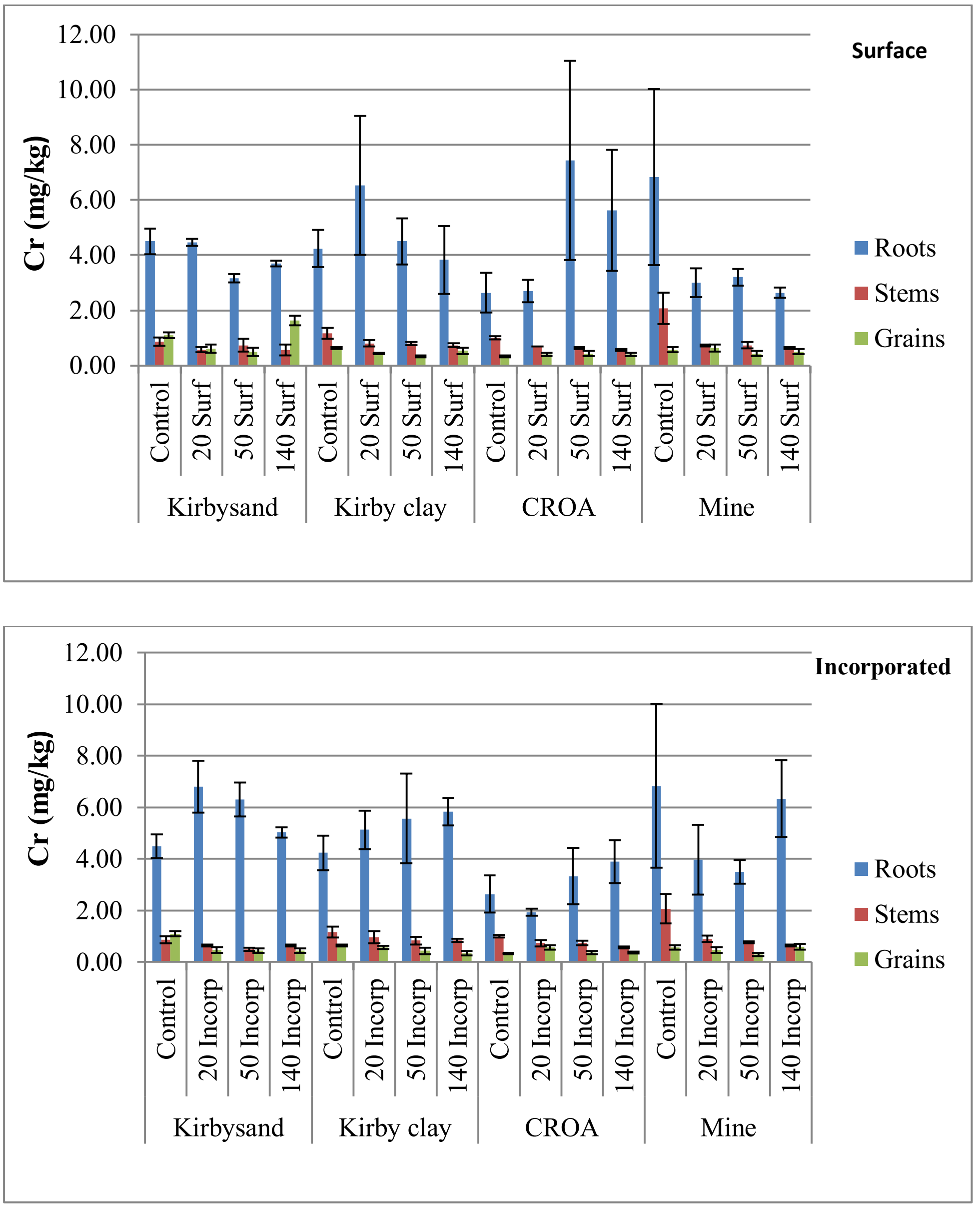

Figure 5.4: Concentration of Cr in wheat plant parts following application of MWOO.

Table 5.10: The concentration of Pb (mg kg^{-1}) in wheat plant parts following MWOO application.

Pb		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	Roots	1.90 ± 0.3	2.70 ± 0.3	2.03 ± 0.15	4.70 ± 1.04	3.37 ± 0.47	4.13 ± 0.33	4.07± 0.52
	Stems	0.3 ± 0.00	0.20 ± 0.0	0.33 ± 0.13	0.33 ± 0.13	0.13 ± 0.03	0.20 ± 0.00	0.17 ± 0.03
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Kirby Clay	Roots	0.7 ± 0.25	2.13 ± 0.47	5.90 ± 1.44	4.77 ± 2.12	0.97 ± 0.24	0.9 ± 0.08	1.77 ± 0.55
	Stems	BDL	BDL	0.10± 0.0	0.2 ± 0.0	BDL	BDL	0.3 ± 0.08
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL
CROA	Roots	1.23 ± 0.27	2.77 ± 0.54	4.30 ± 2.15	5.1 ± 3.9	1.03 ± 0.09	1.23 ± 0.49	1.83 ± 0.23
	Stems	0.1. ± 0.00	BDL	0.10 ± 0.00	0.10 ± 0.00	0.15 ± 0.04	BDL	BDL
	Grains	BDL	BDL	BDL	BDL	0.10 ± 0.0	BDL	BDL
MINE	Roots	1.25 ± 0.00	2.30 ± 0.90	3.90 ± 0.75	11.1 ± 6.55	1.37 ± 0.33	3.73 ± 1.43	3.53 ± 0.81
	Stems	BDL	0.10 ± 0.00	0.20 ± 0.1	0.10 ± 0.00	BDL	BDL	BDL
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Values are the mean ± SE (n = 3). BDL: Below detection limit. Stems: stems and the leaves together.

Table 5.11: The concentration of Cd (mg kg^{-1}) in wheat plant parts following MWOO application.

Cd		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	Roots	BDL	BDL	BDL	BDL	0.20 ± 0.00	0.50 ± 0.00	0.30 ± 0.00
	Stems	BDL	BDL	BDL	BDL	BDL	0.1 ± 0.00	0.17 ± 0.03
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Kirby Clay	Roots	BDL	BDL	BDL	BDL	BDL	BDL	0.20 ± 0.0
	Stems	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL
CROA	Roots	BDL	0.20 ± 0.00	0.33 ± 0.03	0.50 ± 0.00	0.20 ± 0.00	BDL	0.20 ± 0.00
	Stems	0.13 ± 0.03	0.13 ± 0.03	0.20 ± 0.00	0.37 ± 0.03	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL
MINE	Roots	BDL	BDL	BDL	BDL	BDL	BDL	1.10 ± 0.73
	Stems	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	BDL	BDL
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Values are the mean ± SE (n = 3). BDL: Below detection limit. Stems: stems and the leaves together.

5.4.3.7 Arsenic (As)

The concentrations of AS in the plants parts (roots, stems and grains) following the application of MWOO to the soil are presented in Table 5.12. The values in this table show that the concentration of As in the stems and grains was below detection limits in the Kirby clay, CROA and Minesite soils and only in a few cases was it observed to have accumulated in the roots. In the Kirby sand As was found to be significantly higher ($p > 0.001$) than in the other soil and had accumulated in all plant parts. Although the application rates slightly increased As, there was no significant difference compared to the control ($P > 0.05$).The concentration of As was observed to be significantly higher in roots ($P < 0.001$) compared to stems and grains. Although concentration was slightly higher in stems compared to grains, the difference was not significant. The concentration of As in plants growing in the incorporated MWOO was significantly higher than in those growing in the surface application and control ($P < 0.05$)

Table 5.12: The concentration of As (mg kg^{-1}) in wheat plant parts following MWOO application.

As		Method of application						
Soil	Depth/ plant part	Control	Surface			Incorporated		
		(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Kirby Sand	Roots	2.97 ± 0.64	2.93 ± 0.64	2.63 ± 0.52	2.15 ± 0.04	6.07 ± 0.30	6.00 ± 2.08	3.70 ± 0.75
	Stems	0.53 ± 0.12	0.57 ± 0.09	0.83 ± 0.15	0.77 ± 0.13	1.27 ± 0.09	1.97 ± 0.07	1.57 ± 0.15
	Grains	BDL	BDL	0.15 ± 0.04	0.35 ± 0.20	0.17 ± 0.03	0.33 ± 0.09	0.33 ± 0.09
Kirby Clay	Roots	0.20 ± 0.00	0.40 ± 0.08	0.30 ± 0.00	0.40 ± 0.00	0.25 ± 0.04	0.50 ± 0.00	BDL
	Stems	BDL	BDL	BDL	0.10 ± 0.00	BDL	BDL	BDL
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL
CROA	Roots	0.40 ± 0.00	0.57 ± 0.18	1.47 ± 0.97	2.40 ± 1.31	0.27 ± 0.03	0.50 ± 0.00	0.30 ± 0.001
	Stems	BDL	BDL	BDL	0.10 ± 0.00	BDL	BDL	BDL
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL
MINE	Roots	BDL	BDL	BDL	BDL	0.60± 0.08	0.90 ± 0.29	0.93 ± 0.32
	Stems	BDL	BDL	BDL	0.0	BDL	BDL	0.10 ± 0.00
	Grains	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Values are the mean ± SE (n = 3). BDL: Below detection limit. Stems: stems and the leaves together.

5.4.4 Translocation Factors

The translocation factors for the heavy metals in this study are shown in Table 5.13. This table presents the TFs of metals from the roots to the stems (S/R) and from the stems to the grains (G/S). The table shows that for Zn S/R was often > 1 compared to Ni, Cr and Cu. Values of S/R which are > 1 show that the concentration of the metals is higher in the stem than in the roots and so for Ni, Cr and Cu most element was restricted to the roots. In this experiment Zn translocation to stems was greater than observed for the other elements. The G/S was typically > S/R in treatments except for Zn in some treatments and suggest once the element is within the stem movement to the grain is less restricted (Carbonell et al., 2011). The influence of MWOO on metal translocation was often not significant, nor was there any obvious relationship with application rate. In some MWOO treatments, however, especially for Zn, the S/R ratio was slightly reduced compared to control, possibly due to the high concentrations of this element in the MWOO with relatively greater root accumulation compared to that translated.

Table 5.13: Translocation factors of heavy metals from roots to stems (S/R) and from stems to grains (G/S).

			Method of application						
Metal	Soil	Translocation Factor	Control	Surface			Incorporated		
			(0 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})	(20 t ha^{-1})	(50 t ha^{-1})	(140 t ha^{-1})
Zn	Kirby Sand	S/R	3.68	3.29	3.11	2.08	1.37	1.90	1.53
		G/S	0.59	0.66	0.78	5.50	1.05	0.84	0.94
	Kirby Clay	S/R	1.50	0.63	0.68	0.58	1.07	0.79	1.41
		G/S	1.60	2.08	2.00	1.81	1.33	1.23	0.83
	CROA	S/R	8.97	3.90	1.82	1.34	3.14	2.51	1.97
		G/S	0.19	0.31	0.74	0.64	0.27	0.76	1.08
	MINE	S/R	2.11	1.36	2.91	1.65	0.13	1.91	1.20
		G/S	0.66	0.62	0.89	0.79	0.27	0.91	15.56
Ni	Kirby Sand	S/R	N/A	0.09	0.25	0.11	0.08	0.10	0.10
		G/S	N/A	1.86	1.00	3.00	1.43	1.43	1.43
	Kirby Clay	S/R	0.05	0.06	0.03	0.08	N/A	4.08	N/A
		G/S	5.38	8.57	11.43	2.94	2.12	0.06	3.85
	CROA	S/R	0.08	0.10	0.08	0.09	0.13	0.16	0.14
		G/S	2.86	2.86	3.29	2.70	2.86	1.86	1.86
	MINE	S/R	N/A	N/A	0.20	0.28	0.10	0.11	0.10
		G/S	1.30	2.33	1.00	0.54	1.00	1.00	1.00
Cr	Kirby Sand	S/R	0.17	0.08	0.37	0.16	0.08	0.05	0.13
		G/S	1.33	1.77	0.33	3.00	1.00	1.00	0.50
	Kirby Clay	S/R	0.27	0.39	0.14	0.10	0.23	0.11	0.17
		G/S	0.40	0.48	0.37	1.00	0.36	0.85	0.57
	CROA	S/R	0.23	0.29	0.23	0.00	0.43	0.48	0.16
		G/S	0.43	0.74	0.50	0.50	0.85	0.33	0.65
	MINE	S/R	0.45	0.30	0.27	0.24	0.26	0.28	0.13
		G/S	0.38	1.11	0.37	1.00	0.27	0.48	1.35
Cu	Kirby Sand	S/R	0.35	0.17	0.29	0.38	0.28	0.43	0.22
		G/S	1.16	1.56	1.18	0.90	1.20	0.80	1.09
	Kirby Clay	S/R	0.53	0.46	0.28	0.35	0.57	0.40	0.75

	G/S	1.46	1.78	1.60	1.30	1.40	1.48	0.98
CROA	S/R	0.39	0.43	0.17	0.34	0.53	0.78	0.62
	G/S	1.17	1.30	2.43	0.96	1.41	1.02	1.21
MINE	S/R	0.95	0.57	0.45	0.45	0.40	0.53	0.35
	G/S	1.01	1.12	1.29	0.97	1.18	1.25	1.29

Translocation Factors of Pb, As and Cd were not calculated because their concentrations in some plant parts were below detection limits. The values are ratios of the means (n = 3). BDL is below detection limits.

5.5 Discussion

Giusquiani et al., (1988) observed that low rates of MWOO did not affect available soil Cu concentration. Larger increases of Cu were reported to occur in the organic fraction in the soil following application of MWOO (Zheljazkov and Warman 2004). On the other hand, Achiba et al. (2009) reported that MWOO application significantly increased Cu concentration in soil, especially in the 0-15 cm layer of clay loam soil. This increase was observed to be proportional to the application rate and highest application rate caused the highest increase compared to the control, which is similar to the findings of this study. Other authors have also reported increase in Cu concentration in different soils after adding MWOO to the soils (Perez et al., 2007; Weber et al., 2007; Carbonell et al., 2011). Significant increase in total Cu concentration was only observed in acidic sandy soil after 15 t ha^{-1} of MWOO was added to neutral loam soil, acidic sandy soil and alkaline sandy soil (Sebastiao et al., 2000). Carbonell et al. (2011) also reported that highest concentrations of Cu were observed in the root, although the order of distribution was different from the current study. In their study they found that grain concentration of Cu was the lowest. Woodbury (1992) reported that Cu concentration in crops did not increase significantly even with an increase of nearly 350% in the soil. This suggests that very little increase in crop Cu concentration will occur even with considerable amounts of MWOO addition.

The findings of this study are similar to the findings of other studies. For example, increased Zn concentration following the application of MWOO was observed in the 0-15 and 15-30 cm layers of Gray Luvisols (Zhang et al., 2006). The increase was observed to be proportional to application rate. Similarly, Carbonell reported that Zn concentration increased in amended loam soil. Other authors also reported similar results (Weber et al., 2007; Achiba et al., 2009). The findings of this study shows that following application of MWOO to soil, wheat plants growing in the amended soil will accumulate more Zn in the stem and less is translocated to grain. Other authors have reported the uptake of Zn by plant as Zn concentrations were detected in pepper fruits (Ozores-Hampton et al., 2005), maize grains (Lakhdar et al., 2010) and barley grains (Lillywhite et al., 2009).

Similarly to this study, increased Ni concentration in the soil was reported, particularly in soil treated with 80 t ha^{-1}yr^{-1} of MWOO (Ayari et al., 2010). In contrast, Illlera et al. (2000) observed that application of MWOO had little effect on Ni concentration in amended soil. They attributed this to the low availability of Ni in the MWOO they used in their study.

The findings of this study are consistent with the findings of other studies. Ayari et al. (2010) also observed that Ni accumulated mostly in the roots and transfer to the above ground plant parts was low. Similarly, Carbonell et al. (2011) reported that Ni concentration was higher in the roots compared to other plant parts. Many authors reported that even though Ni was detected in plant parts the concentrations were within acceptable concentrations (Woodbury, 1992; Ozores-Hampton et al., 2005; Jordão et al., 2007), suggesting that MWOO application is unlikely to cause due to Ni. The findings of the current study are broadly consistent with earlier studies. For example, accumulation of Cr was reported to be higher in roots of wheat and sorghum than in the shoots (López-Luna et al., 2009). Many other authors reported that more Cr was found in the root than in the aboveground plant parts (Scoccianti et al., 2006; Paiva et al., 2009; Ayari et al., 2010; Sundarmoorthy et al., 2010). Similarly, more than 70% Cr in wheat plants accumulated in roots and little translocated to the shoots (Ayari et al., 2010). They reported that the average Cr concentration ranged from 1.7 – 12.9 mg kg^{-1}. The higher value can be toxic. Generally the current study and the other studies suggest that MWOO addition is unlikely to cause toxicity to plant because the foliar and grain concentrations were low.

Similarly to this study, other studies reported that MWOO increased Pb concentration in the soil Perez et al., 2007 observed that Pb significantly concentrated in the 0-20 cm soil layer. Carbonell et al. (2011) reported that application of MWOO increased Pb concentration in amended soil. Similar findings were reported by Ozores-Hampton et al. (2005) and Ayari et al. (2010). Other Authors also reported increased Pb concentration following the application of MWOO (Garcia-Gil et al., 2000; Walter et al., 2006). The results of this study on accumulation of Pb in plants were consistent the findings of other studies. Carbonell et al. (2011) reported that the accumulation of was high Pb in the roots compared to other plant parts in maize plants. They further reported that Pb was not easily translocated from roots to shoots or grains of maize plants. Ozores-Hampton et al. (2005) reported that no accumulation of Pb was observed in pepper fruits growing in amended soil. This suggests that there is little increase in the Pb concentration of crops even with considerable MWOO addition therefore the uptake of Pb is unlikely to be a serious problem.

Low MWOO Cd values in this study resulted in little change to soil Cd concentrations with MWOO application even at 140 t ha^{-1} application rate. In fact a decrease was observed in some treatments where background soil Cd was greater that the MWOO concentration. In contrast, Ayari et al. (2010), observed an increase in soil Cd particularly when MWOO was applied at a rate of 80 t ha^{-1}yr^{-1}. Similarly, Illera et al. (2000) reported increased Cd concentration in MWOO amended soils. With regard influence of MWOO application on plant Cd, Ayari et al. (2010) reported that no Cd was detected in wheat plants following application of 80 t ha^{-1} of MWOO. Likewise, Cd was not detected in barley grains growing in MWOO amended soil (Lillywhite et al., 2009). Bhattacharyya et al. (2003) also reported that Cd in straw and grains of rice was not detected after MWOO application to soil and Woodbury (1992) reported that Cd concentrations in wheat, and barley were observed to decrease with an increase in MWOO application rate. In contrast, Carbonell et al. (2011) reported significant translocation of Cd to the shoots in maize. These studies and also the work reported here suggest that phytotoxicity due to Cd in MWOO with soil amendment is unlikely. Our results show that Cd concentrations in wheat grain were below limit of recovery (0.1 mg kg^{-1}) for all treatments and all soils tested.

Little research has reported the accumulation of As in the soil following application of MWOO. Zhang et al. (2006) found that As concentration in acidic loam soil was not affected by addition of MWOO. There is little literature that has reported As accumulation in plants following application of MWOO. Woodbury (1992) stated that As is not readily taken up by plants. Similarly to this study, a study by Zheljazkov and Warman (2004) reported no significant differences between treatments in basil and Swiss chard plant tissue growing in MWOO amended soil. Decreased Ac concentration in crops was observed even though soil concentration increased (de Haan, 1981). Accordingly, considerable uptake of As by plants in amended soils is unlikely.

5.6 Conclusions

This study used MWOO application to four NSW soils to understand the distribution of Cu, Zn, Ni, Cr, Pb, Cd and As in amended soils and the subsequent accumulation of these heavy metals by wheat plants. The translocation of the heavy metals from roots to stems and grain was assessed. The results show that MWOO application to agricultural soils has a potential to increase certain heavy metal concentrations in the soil and in plant tissue. Significant increases in Zn, Ni, Cr and Pb concentrations were observed mostly in the superficial layers of the soils (0 – 5cm). No significant increases were observed for Cu and As in the soil and MWOO application did not increase the concentration of Cd, even at higher application rate (140 t ha^{-1}). The methods of application were found to have a significant effect on the accumulation of some heavy metals in the soil compared to the control but no significant difference between the surface and the incorporated applications in this experiment. Roots of wheat in this study showed the greater concentrations of heavy metals with increases in some elements related to MWOO application. Translocation of the heavy metals to the grains was observed for Zn, Cr, Ni and Cu, but not Pb, As and Cd. Grain concentrations of Pb, As and Cd were below limits of recovery and maximum residue limits for foodstuffs in Australia. Should maximum residue limits be reduced or General Exemption criteria altered, then further assessment for Cd would be required because in this study the LOR for Cd was the same as the current maximum residue limit.

5.7 References

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6. Runoff and leaching of metals and metalloids from MWOO amended soils using simulated rainfall

6.1 Summary

Very little research has been undertaken on the effects of MWOO application to metal and metalloid concentrations in leachate and runoff. In this component of the work, an experiment was undertaken to assess how MWOO application, using different MWOO application rates (control, 20 t ha^{-1}, 50 t ha^{-1} and 100 t ha^{-1}), different MWOO application methods (surface application and incorporation to 15 cm (adjusted to 3 cm soil depth)), and two different soil types (an acidic sandy loam and a neutral clay), influences metal and metalloid concentrations and chemical constituents in runoff and leachate. A small scale leaching and runoff experiment using a rainfall simulator was conducted. The variables that were measured included: time to runoff, total runoff/leachate, runoff/leachate rate, sediment yield, pH, electrical conductivity, dissolved organic carbon, nitrate, ammonia and seven metals and metalloids; arsenic, cadmium, chromium, copper, nickel, lead and zinc.

The findings demonstrated that MWOO application increased trace metal and metalloid concentrations in surface water runoff and leachate from the two soils studied and that generally, sandy loam soils had higher concentrations than the clay. Metal and metalloid concentrations in runoff and leachate were generally higher when MWOO was surface applied and at higher application rates. Our results suggest some concern for water resources associated with application of this MWOO to soil, since metal concentrations in runoff and leachate were increased. Risk is apparently lowest with incorporation of this MWOO at a low application rate of 20 t ha^{-1} in a clay soil and higher at greater application rates especially for surface MWOO application which varies with soil type. Although dilution and attenuation processes would modify concentrations in field applications, this will depend on environmental and soil characteristics and water monitoring would be recommended to ensure water quality guidelines are not exceeded.

6.2 Introduction

This experimental component of the work was designed to investigate the effects of MWOO amendment on runoff and leachate from two soil types; a sandy loam and clay, using both surface applied and incorporation methods of MWOO soil application. The aims of this experiment were to assess:

- the influence of MWOO addition to two different soil types on metals, metalloids and the chemical constituents in runoff and leachate,
- the influence of MWOO addition with different application rates on metals, metalloids and the chemical constituents in runoff and leachate, and
- the influence of MWOO addition using different application methods (surface applied and incorporated) on metals, metalloids and the chemical constituents in runoff and leachate.

6.3 Methods

6.3.1 Soil and MWOO Characterisation

The soils used included a selection of those soils described in Table 2.1. comprising the acidic sandy loam (S1) and the more acidic clay (C1), as well as MWOO sourced from the UR3R MWOO plant in Sydney after the composting process was complete.

The soils and MWOO materials used in this experiment were sub-sampled from the bulk samples collected at the initiation of the project. As a consequence, physical and chemical properties differ slightly, though not substantially, within each experiment. This reflects the inherent variability of the soils and materials used.

6.3.2 Experimental Set Up

Alfoil trays (16 x 21 x 6 cm) perforated with evenly distributed holes in the bottom were lined with Whatman No. 1 filter paper, filled to 3 cm depth with the soil/MWOO treatments and elevated in a second tray to collect leachate. For each of the two soil types, three rates of MWOO were applied (20, 50 and 100 t ha^{-1}) using two application methods: surface applied and incorporated (by mixing well with the 3 cm depth of soil (1000 g for the sandy loam; 800 g for the clay). Because MWOO incorporation usually occurs to a depth of 15 cm (equivalent to the plough layer) and since the soil in this experiment was only 3 cm deep the volume equivalent surface applied MWOO (38 g for 20 t ha^{-1}; 95 g for 50 t ha$^{-1;}$ 190 g for 100 t ha^{-1}) was incorporated to ensure the equivalent application rate was applied. A control was also prepared for each soil and there were three replicates of each treatment, giving a total of 42 experimental units. In this experiment the soil treatments were wetted to 100% FC just prior to a simulated rainfall event. This was carried out to ensure that cracks did not form in the clay and runoff could be collected. Rainfall was applied at 75 mm hr^{-1} with trays placed on a 20% slope. During rainfall application, leachate and runoff were collected until approximately 50 mL was collected. Time to runoff, total runoff and total leachate were recorded.

6.3.3 Laboratory Methods

In this experiment pH was measured using a TPS 901-CP pH meter and EC was measured using a TPS Model WP-81 conductivity meter. Nitrate, ammonia and DOC analysis was performed on 5 mL of the leachate sample diluted to 30 mL, using a Sievers InnovOx Laboratory TOC Analyzer. Sediment yield was analysed by drying weighed leachate samples at 105° C overnight and recording the weight of dry residue. Elemental analysis of a 20mL filtered (0.45 µm) and nitric acid preserved sample was undertaken at ALS in Brisbane using ICP-MS. Detection limits for the metals and metalloids, as identified by ALS, were 1 µg/L for As, Cr, Cu, Ni and Pb, 0.1 µg/L for Cd and 5 µg/L for Zn.

6.3.4 Statistical Analysis

Statistical analysis was performed using Rstudio and version 3.0.3 of R (R Core Team, 2014). Application rate and application method were combined to create one factor (called treatment), for example, the surface applied 20 t ha^{-1} was assigned as one treatment and labelled 20S. Analysis of variance was undertaken for each variable to determine differences between treatments within each soil, differences in application rate treatments between soil types and differences between leachate and runoff. Pearson's correlations were performed for variables that showed similar trends and post-hoc analysis was undertaken using a Tukey's test. Total metal lost as a mass and as a percentage of the total metal initially present in the soil after MWOO application was also calculated and statistical analysis on differences between runoff and leachate were undertaken. Assumptions of the model were checked and if necessary data was transformed to meet normal distribution requirements. Values identified as outliers were removed from the data if they had standardised residuals less than -3 or greater than 3 in the normal Q-Q diagnostics plot (Simkiss et al., 2014). If metal or metalloid concentrations in treatments were at or below detection limits for all three replicates, they were removed from the data prior to statistical analysis, with the exception of the control treatments, which were given the value of the detection limit.

6.4 Results

6.4.1 Soil and MWOO Characterisation

Results show that pH was neutral in the sandy loam and slightly acidic in the clay (Table 6.1). The clay had a higher N and C content than the sandy loam, as well as cation exchange capacity and percentages of Fe, Al and Mn (Table 6.1). Metal concentrations were generally more elevated in the clay but As concentrations were much higher in the sandy loam (Table 6.1).

The pH of MWOO was neutral and nitrogen and carbon percentages were relatively high compared to the two soils (Table 6.1). The MWOO had a high *p* concentration and for the metals and metalloids, Cu, Pb and Zn concentrations were highest (Table 6.1). Because MWOO can only be applied to soil in NSW if it complies with the NSW General Exemption Guidelines (EPA, 2014), a comparison of the metal content in the MWOO with the guideline values have been provided in Table 6.1. The results show that MWOO exceeded the guidelines for Cd, Cu, Pb, Zn for agricultural soils and for Pb and Zn for Minesite soils.

Table 6.8. Physical and chemical characteristics of the sandy loam, clay soil and MWOO. The maximum allowable concentrations (mg kg^{-1}) outlined in the NSW General Exemption Guidelines (EPA, 2014) are provided for comparison.

				Maximum allowable concentration (mg kg^{-1})	
	Sandy Loam [Kirby Sand (S1)]	Clay [Kirby Clay (C1)]	MWOO	Minesite	Plantation forestry; non-contact; broad-acre agriculture
pH	6.28 ± 0.03	6.21 ± 0.04	7.36 ± 0.16	-	-
EC (µS/ cm)	126.37 ± 0.62	154.47 ± 6.30	6220 ± 0.15	-	-
Clay (%)	13.2	55.0	-	-	-
Silt (%)	10.5	26.0	-	-	-
Sand (%)	76.3	19.0	-	-	-
Soil Texture	Sandy loam	Clay	-	-	-
Nitrogen (%)	0.04 ± 0.00	0.25 ± 0.00	1.46 ± 0.09	-	-
Carbon (%)	0.49 ± 0.00	3.34 ± 0.01	24.90 ± 1.88	-	-
Ca (cmol_+/kg)	1.017 ± 0.01	25.55 ± 0.16	28.014 ± 0.45	-	-
K (cmol_+/kg)	0.09 ± 0.00	0.85 ± 0.02	12.45 ± 0.30	-	-
Mg (cmol_+/kg)	1.12 ± 0.01	24.09 ± 0.14	7.00 ± 0.14	-	-
Na (cmol_+/kg)	0.24 ± 0.01	0.35 ± 0.01	24.53 ± 0.67	-	-
P (µg/g)	6.22 ± 0.24	77.19 ± 7.59	584.20 ± 16.36	-	-
Al (%)	1.49 ± 0.06	5.19 ± 0.09	0.95 ± 0.01	-	-
Fe (%)	1.32 ± 0.06	6.41 ± 0.10	0.93 ± 0.04	-	-
As (mg kg^{-1})	66.49 ± 4.89	1.29 ± 0.55	5.64 ± 0.45	20	20
Cd (mg kg^{-1})	0.00 ± 0.00	3.73 ± 0.10	1.10 ± 0.09	5	1
Co (mg kg^{-1})	3.01 ± 0.11	64.93 ± 1.34	3.61 ± 0.20	-	-
Cr (mg kg^{-1})	37.61 ± 1.29	195.11 ± 4.22	88.78 ± 18.10	250	100
Cu (mg kg^{-1})	15.50 ± 0.45	45.60 ± 0.68	202.69 ± 52.82	375	100
Mn (mg kg^{-1})	166.11 ± 3.38	1709.67 ± 22.92	232.51 ± 4.78	-	-
Ni (mg kg^{-1})	15.11 ± 0.27	124.77 ± 1.61	32.01 ± 6.68	125	60
Pb (mg kg^{-1})	9.38 ± 0.68	4.08 ± 0.22	303.02 ± 29.80	150	150
Sb (mg kg^{-1})	2.71 ± 1.75	3.37 ± 0.76	3.57 ± 0.43	-	-
Zn (mg kg^{-1})	21.28 ± 0.55	86.57 ± 1.56	831.97 ± 287.15	700	200

6.4.2 Runoff

6.4.2.1 Runoff Descriptors

Tables 6.2 and 6.3 provide runoff results for the sandy loam and clay soils, respectively, and include time to runoff, runoff volume, runoff rate, sediment yield, and chemical properties such as pH, EC, DOC, nitrate and ammonia.

Table 6.9. Runoff results for non-metal variables in the sandy loam soil (mean ± standard error).

		MWOO Surface Applied			MWOO Incorporated		
	Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**100 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**100 t ha^{-1}**
Time to runoff (min)	1.44 ± 0.51^{a}	3.78 ± 1.93^{a}	4.21 ± 2.45^{a}	0.68 ± 0.09^{a}	6.97 ± 2.17^{ab}	3.59 ± 0.72^{a}	13.97 ± 0.95^{b}
Total runoff (mL)	61.65 ± 3.54^{a}	61.90 ± 1.56^{a}	54.53 ± 3.19^{a}	58.05 ± 8.08^{a}	52.84 ± 1.91^{a}	58.19 ± 3.65^{a}	59.59 ± 2.87^{a}
Runoff rate (mL/min)	5.74 ± 0.76^{b}	5.13 ± 0.99^{ab}	1.31 ± 0.18^{a}	5.45 ± 1.55^{ab}	2.84 ± 1.14^{ab}	3.05 ± 0.63^{ab}	1.96 ± 0.46^{ab}
Sediment yield (g/L)	0.75 ± 0.21^{a}	2.86 ± 0.25^{a}	2.74 ± 0.80^{a}	5.39 ± 0.46^{b}	1.87 ± 0.90^{a}	0.79 ± 0.23^{a}	0.44 ± 0.11^{a}
pH	6.77 ± 0.41^{a}	6.04 ± 0.29^{a}	6.56 ± 0.08^{a}	6.30 ± 0.03^{a}	6.39 ± 0.15^{a}	6.48 ± 0.13^{a}	7.09 ± 0.24^{a}
EC (µS/ cm)	24.10 ± 5.66^{a}	1676.67 ± 119.75^{b}	1533.33 ± 467.58^{b}	3019.33 ± 575.56^{b}	25.39 ± 4.30^{a}	47.94 ± 18.61^{a}	44.90 ± 8.36^{a}
DOC (µg/mL)	12.24 ± 1.11^{a}	487.20 ± 35.04^{b}	455.00 ± 150.25^{b}	616.40 ± 51.50^{b}	15.42 ± 0.39^{a}	22.42 ± 5.26^{a}	17.32 ± 2.87^{a}
Nitrate (µg/mL)	1.66 ± 0.38^{b}	1.74 ± 0.12^{b}	0.60 ± 0.03^{a}	0.96 ± 0.07^{ab}	0.64 ± 0.07^{a}	0.70 ± 0.02^{a}	0.56 ± 0.07^{a}
Ammonia (µg/mL)	0.18 ± 0.07^{a}	2.87 ± 1.32^{b}	5.64 ± 0.54^{bc}	8.74 ± 1.46^{c}	0.36 ± 0.03^{a}	0.24 ± 0.12^{a}	0.02 ± 0.02^{a}

In each row, the same letter indicates that the difference among treatments was not significant ($p > 0.05$).

Table 6.10. Runoff results for the non-metal variables in the clay (mean ± standard error).

		MWOO Surface Applied			MWOO Incorporated		
	Control	**20 t ha^{-1}**	**50t/h t ha^{-1}a**	**100 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**100 t ha^{-1}**
Time to runoff (min)	2.31 ± 0.09^{ac}	4.20 ± 0.43^{bc}	1.72 ± 0.51^{ab}	0.76 ± 0.12^{a}	5.41 ± 1.31^{bc}	4.66 ± 0.64^{bc}	7.17 ± 3.81^{c}
Total runoff (mL)	102.70 ± 29.98^{a}	58.95 ± 9.59^{a}	70.31 ± 15.43^{a}	57.90 ± 1.71^{a}	62.68 ± 4.60^{a}	92.60 ± 33.75^{a}	87.04 ± 28.71^{a}
Runoff rate (mL/min)	11.03 ± 2.41^{a}	5.90 ± 0.96^{a}	4.94 ± 2.58^{a}	2.52 ± 1.45^{a}	3.74 ± 0.27^{a}	5.94 ± 2.39^{a}	8.11 ± 3.36^{a}
Sediment yield (g/L)	1.59 ± 0.16^{a}	3.53 ± 0.40^{b}	3.91 ± 0.73^{b}	3.93 ± 0.78^{b}	1.15 ± 0.03^{a}	1.23 ± 0.12^{a}	1.50 ± 0.09^{a}
pH	6.86 ± 0.69^{ab}	6.22 ± 0.09^{ab}	6.24 ± 0.07^{ab}	7.71 ± 0.26^{b}	6.21 ± 0.61^{ab}	7.02 ± 0.30^{ab}	5.77 ± 0.17^{a}
EC (µS/ cm)	50.87 ± 15.47^{a}	1779.33 ± 256.47^{b}	2388.00 ± 541.42^{b}	2322.67 ± 439.73^{b}	102.2 ± 12.32^{a}	100.73 ± 21.00^{a}	81.03 ± 25.69^{a}
DOC (µg/mL)	24.99 ± 3.01^{a}	542.40 ± 67.07^{b}	479.10 ± 56.10^{b}	648.2 ± 107.90^{b}	31.04 ± 2.85^{a}	36.16 ± 6.00^{a}	40.98 ± 4.83^{a}
Nitrate (µg/mL)	1.10 ± 0.17^{a}	2.12 ± 0.61^{a}	1.53 ± 0.27^{a}	1.18 ± 0.02^{a}	1.66 ± 0.43^{a}	0.68 ± 0.10^{a}	0.62 ± 0.02^{a}
Ammonia (µg/mL)	0.06 ± 0.03^{a}	4.02 ± 0.81^{b}	8.40 ± 1.98^{c}	35.58 ± 8.28^{d}	0.00 ± 0^{a}	0.04 ± 0.04^{a}	0.22 ± 0.12^{a}

In each row, the same letter indicates that the difference among treatments was not significant ($p > 0.05$).

6.4.2.2 Total Runoff, Runoff Volume, Runoff Rate and Sediment Yield

For the sandy loam, the only significant effect of MWOO application on time to runoff was for MWOO incorporated at 100 t ha^{-1} , which had a mean time to runoff of 13.97 minutes; significantly higher than the control (1.44 mins) and the 50 t ha^{-1} (3.59 mins) treatments ($F_{6,14}$ = 8.68, *p* = 4.60x10^{-4}) (Table 6.2). In contrast, when MWOO was applied to the surface only, the 100 t ha^{-1} treatment recorded the lowest mean time to runoff (0.68 mins) of all treatments, which was more than 13 minutes less than the incorporated treatment.

Total runoff volume in the sandy loam ranged between 52.84 mL (20 t ha^{-1} incorporated) to 61.90 mL (20 t ha^{-1} surface applied) for all treatments (Table 6.2), none of which were significantly different. For the runoff rate (mL/min), the treatments with MWOO applied were all lower than the control (5.74 mL/min), but this was only significant for the 50 t ha^{-1} surface applied treatment which had the lowest mean runoff rate of all treatments at 1.31 mL/min ($F_{6,14}$ = 4.25, *p* = 0.01) *p* (Table 6.2). Similarly to runoff volume and runoff rate, MWOO application had little effect on sediment yield from the sandy loam. The only treatment that was significantly different from the control (0.75 g/L) and other treatments, was the 100 t ha^{-1} surface applied treatment, which yielded 5.39 gL/ ($F_{6,14}$ = 9.70x10^{-5}) (Table 6.2).

For the clay soil, the time to runoff following MWOO application largely depended on whether the MWOO was incorporated or applied only to the surface. The time to runoff tended to increase with increasing amounts of MWOO surface applied, but in complete contrast, decrease with increasing amounts of MWOO incorporated. However, despite these trends, time to runoff within each method of application was not significantly different to the control. Significant differences in time to runoff were only apparent between the application methods, with the 100 t ha^{-1} surface applied treatment recording the lowest mean time to runoff (0.76 minutes) and the 100 t ha^{-1} incorporated treatment the highest mean time to runoff (7.17 minutes) ($F_{6,14}$ = 8.56, *p* = 4.9x10^{-4}) (Table 6.3).

As for the sandy loam, MWOO application to the clay soil had little or no effect on total runoff and the runoff rate. These descriptors were, however, consistently highest in the control (102.70 mL and 11.03 mL/min, respectively) and lowest in the 100 t ha^{-1} surface applied treatment (57.90 mL and 2.52 mL/min, respectively) (Table 6.3). Again, as for the sandy loam, sediment yield from the clay soil with MWOO incorporated was not significantly higher than the control, but there were differences for MWOO applied only at the surface. For the sandy loam, this was the case for the 100 t ha^{-1} treatment only, however, for the clay, all treatments with MWOO surface applied yielded significantly higher amounts of sediment ($F_{6,14}$ = 20.20, *p* = 3.8x10^{-6}).

Overall, there was generally little or no significant difference between the application rates, nor the two soil types. For example, time to runoff from the sandy loam and the clay were statistically the same for each individual application rate and method. For both soils the 100 t ha^{-1} surface applied treatment recorded the fastest time to runoff and the 100 t ha^{-1} incorporated treatment the slowest. Furthermore, total runoff from soils with MWOO applied was mostly the same regardless of MWOO application rate, and even though the clay tended to have a higher runoff rate than the sandy loam, this was not significant. Equally, sediment yield mean values for both soils were not significantly different, despite the clay appearing to have higher mean values.

6.4.2.3 pH and EC

The pH of runoff from the sandy loam soil did not change significantly with MWOO application, although the 100 t ha^{-1} incorporated treatment showed the highest mean pH at 7.09, compared to the control at 6.77 (Table 6.2). Electrical conductivity in runoff from all surface applied treatments was significantly higher than the runoff from the control ($F_{6,14} = 85.70$, $p = 3.2\text{x}10^{-10}$). There was no significant difference in EC of runoff from the sandy loam between the three application rates, although the 100 t ha^{-1} surface applied application rate had the highest mean at 3019.33 µS/ cm (Table 6.2).

There was no significant difference in pH in runoff from the clay soil between the MWOO applied treatments and the control, although the greatest mean pH in the clay of 7.71 occurred in the 100 t ha^{-1} surface applied treatment (Table 6.3). Within the MWOO treatments, the 100 t ha^{-1} surface applied treatment (pH 7.71) was significantly higher than the lowest mean pH (5.77) recorded in the 100 t ha^{-1} incorporated treatment ($F_{6,14} = 3.55$, $p = 0.024$). This low pH in runoff from the 100 t ha^{-1} incorporated treatment was in contrast to the results for the sandy loam, where this treatment recorded the highest pH in runoff (7.09). Surface application of MWOO increased runoff EC in the clay significantly but there was no significant effect on EC when MWOO was incorporated. For both application methods, there was no significant difference between the three application rates ($F_{6,14} = 73.8$, $p = 8.6\text{x}10^{-10}$).

There were also no significant differences between the runoff pH values of the controls and treatments according to soil type, nor between the two soil types. Similar mean EC values were also evident in runoff for both soils for the controls and surface applied treatments, and although the clay appeared to have higher mean values with the incorporated treatments, there were no significant differences between the two soils.

6.4.2.4 DOC, Nitrate and Ammonia

Surface application of MWOO significantly increased DOC ($F_{6,14} = 101$, $p = 1.0\text{x}10^{-10}$) and ammonia ($F_{6,13} = 29.7$, $p = 7.3\text{x}10^{-7}$) in runoff from the sandy loam soil, but there was no significant difference between the control and the incorporated MWOO treatments. For the three surface applied MWOO application rates, all DOC values were statistically the same, even though the 100 t ha^{-1} surface applied application rate recorded the highest mean value of 616.40 µg/mL (Table 6.2). Ammonia concentrations, however, did increase with increasing application rate, with the 100 t ha^{-1} surface applied treatment (8.74 µg/mL) significantly higher than the 20 t ha^{-1} surface applied treatment (2.87 µg/mL), and the 50 t ha^{-1} surface applied treatment in between the two (5.64 µg/mL) (Table 6.2).

For nitrate in runoff from the sandy loam, MWOO addition significantly decreased nitrate concentrations from all three incorporated treatments compared to the control (1.66 µg/mL), and nitrate runoff results were similar for all three application rates (Table 6.2). For the surface applied treatments, only the 50 t ha^{-1} treatment was significantly lower than the control ($F_{6,13}$ = 12.7, p = 8.9x10^{-5}), with the 20 t ha^{-1} (1.74 µg/mL) and 100 t ha^{-1} (0.96 µg/mL) treatments having no effect.

The DOC and ammonia responses were nearly exactly the same for runoff from the clay as they were for the sandy loam. Surface application of MWOO to the clay soil significantly increased DOC ($F_{6,13}$ = 131, p = 7.2x10^{-11}) and ammonia ($F_{6,12}$ = 138, p = 2.3x10^{-10}) concentrations in runoff, and again, incorporation of MWOO had no significant effect. There were no significant differences in DOC concentrations between application rates in both surface applied and incorporated MWOO treatments, however, as MWOO surface application rate increased mean ammonia concentrations in runoff also increased significantly, from 0.06 µg/mL in the control to 35.58 µg/mL for the 100 t ha^{-1} treatment (Table 6.3).

In contrast to the sandy loam, nitrate concentrations in runoff from the clay soil were not significantly influenced by MWOO application. Despite the results not being significant, however, a trend was evident that was in line with that of the sandy loam, whereby the higher incorporated application rates had the lowest mean nitrate concentrations (0.68 µg/mL at 50 t ha^{-1} and 0.62 µg/mL at 100 t ha^{-1}) and the 20 t ha^{-1} surface applied treatment had the highest nitrate concentration (2.12 µg/mL) (Table 6.3).

The analysis of soil type effects suggested DOC and nitrate mean values for the sandy loam and clay were not significantly different, however, the clay did tend to have higher mean values than the sand. The only significant different between the two soil types was the concentration of ammonia in runoff in response to 100 t ha^{-1} MWOO applied at the surface, with the clay being significantly higher *p* value than the sandy loam ($F_{13,26}$ = 58.52, p = 7.3x10^{-16}).

6.4.2.5 Metals and Metalloids

In runoff from the sandy loam and the clay, of the seven metals tested (As, Cd, Cr, Cu, Ni, Pb and Zn), Zn and Cu were present at the highest concentrations in both soil types; 1520 and 1233 µg/L in the sandy loam and 985 and 922 µg/L in the clay, respectively (Figure 6.1). These were followed by Ni, Pb, Cr, As, and finally Cd, which had the lowest overall concentration at 4.07 µg/L in the sandy loam and 2.83 µg/L in the clay (Figure 6.1).

The main trends for metal and metalloid concentrations in runoff were that the incorporated MWOO treatments for all application rates were not significantly different from the control or each other. In contrast, all surface applied MWOO application rates, in both the sandy loam and the clay, showed significantly higher metal and metalloid concentrations (in most cases for all seven tested) than both the control and incorporated treatments. This pattern was also evident for DOC (see section 6.4.2.4) and further analysis revealed significant correlations between DOC in the sandy loam and Zn ($r^2 = 0.65$, $p = 2.88\text{x}10^{-5}$), Cu ($r^2 = 0.93$, $p = 9.97\text{x}10^{-12}$), Ni ($r^2 = 0.84$, $p = 1.21\text{x}10^{-8}$) and Cd ($r^2 = 0.89$, $p = 3.94\text{x}10^{-10}$), and in the clay, these same correlations were evident; DOC and Zn ($r^2 = 0.89$, $p = 4.80\text{x}10^{-10}$), DOC and Cu ($r^2 = 0.95$, $p = 2.28\text{x}10^{-13}$), DOC and Ni ($r^2 = 0.94$, $p = 2.74\text{x}10^{-12}$) and DOC and Cd ($r^2 = 0.98$, $p = <2\text{x}10^{-16}$).

In the sandy loam, differences between application rates were significant for the 100 t ha^{-1} surface applied treatment compared the two lower application rates, whereby concentrations of Zn, Cu, Pb and Cd in the runoff were higher with the 100 t ha^{-1} treatment (Figure 6.1) While no significant differences between application rates were evident for the remaining metals and metalloids (Ni, Cr, As), the 100 t ha^{-1} surface applied treatment still recorded the highest mean metal and metalloid concentrations in runoff. Although not a statistically significant difference, the 20 t ha^{-1} surface applied application rates showed a higher mean than the 50 t ha^{-1} application rate in the sandy loam for all metals and metalloids except As (Figure 6.1).

For the clay, metals and metalloid concentrations in runoff from the surface applied treatments were similar for all three application rates, although, like the sandy loam, the 100 t ha^{-1} surface applied treatment consistently had the highest mean concentration for all (except Ni) (Figure 6.1).

There were no significant differences between the two soils for As, Cd, Cr and Cu, despite the sandy loam tending to show higher means than the clay. The clay appeared to exhibit higher Ni concentrations than the sandy loam across all treatments, however only the 20 and 50 t ha^{-1} surface applied treatments had significantly higher mean concentrations in the clay than the sandy loam ($F_{13,28} = 22.63$, $p = 1.89\text{x}10^{-11}$). The control and incorporated treatments for Pb were at or below detection limits for both soils. For the surface applied treatments, the sandy loam appeared to show higher Pb concentrations, however, only the 100 t ha^{-1} application rate had significantly higher Pb concentrations in the sandy loam than the clay ($F_{13,28} = 19.26$, $p = 1.36\text{x}10^{-10}$). Similarly with Zn, the sandy loam appeared to exhibit higher concentrations in the surface applied treatments, however, only for the 100 t ha^{-1} surface applied treatment.

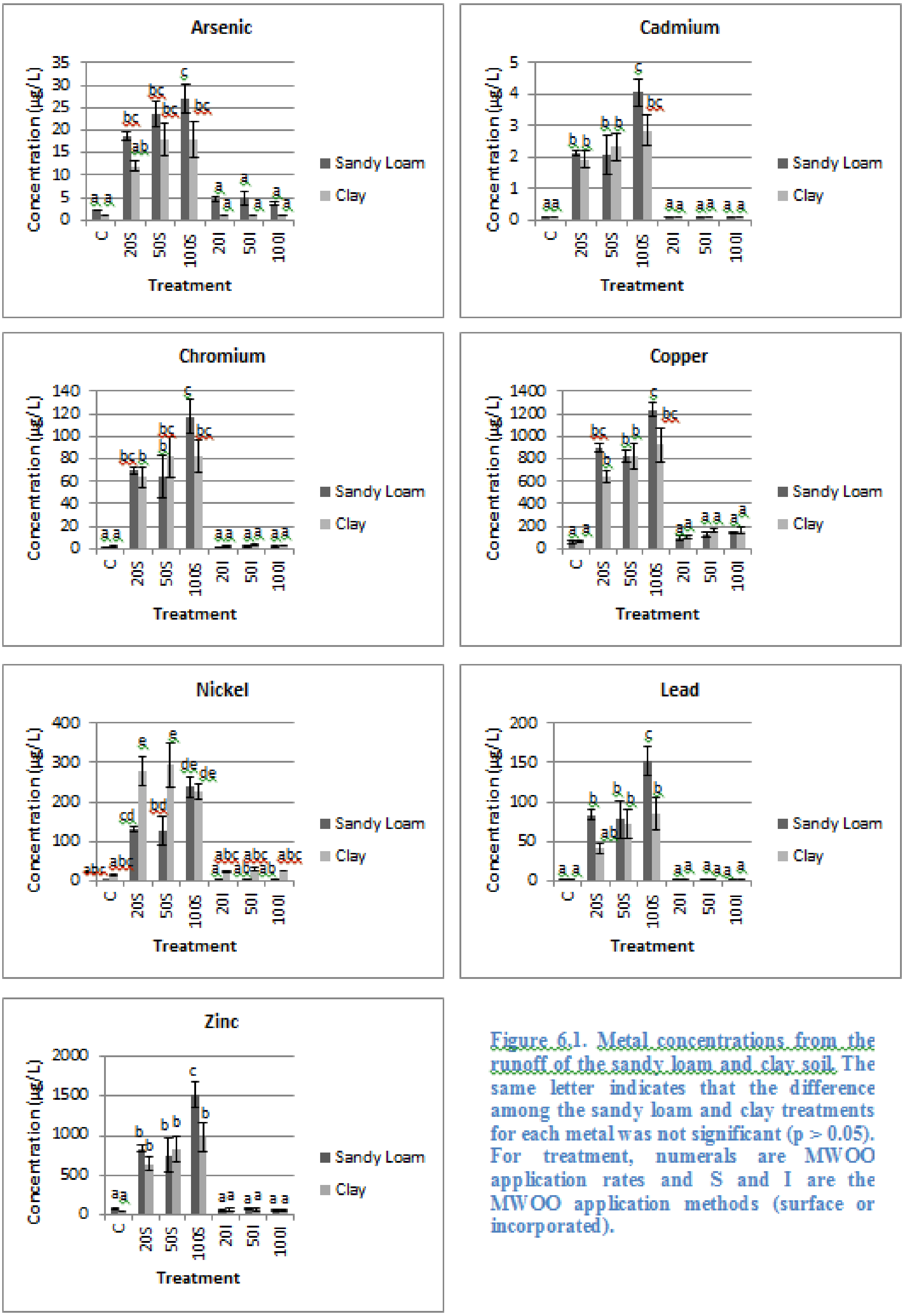


Figure 6.1. Metal concentrations from the runoff of the sandy loam and clay soil. The same letter indicates that the difference among the sandy loam and clay treatments for each metal was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

6.4.3 Leachate

6.4.3.1 Leachate Descriptors

Tables 6.4 and 6.5 provide leachate results for the sandy loam and clay soils, respectively, and include total leachate, leachate rate, sediment yield and chemical properties including pH, EC, DOC, nitrate and ammonia.

Table 6.11. Leachate results for non-metal variables in the sandy soil (mean ± standard error).

		MWOO Surface Applied			MWOO Incorporated		
	Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**100 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**100 t ha^{-1}**
Total leachate (mL)	237.03 ± 26.73^{a}	321.35 ± 95.99^{a}	914.10 ± 70.59^{b}	279.08 ± 101.66^{a}	633.51 ± 111.35^{ab}	514.14 ± 94.91^{a}	958.33 ± 35.03^{b}
Leachate rate (mL/min)	21.78 ± 2.87^{a}	23.58 ± 1.06^{a}	21.59 ± 2.24^{a}	21.30 ± 1.66^{a}	22.64 ± 4.94^{a}	25.15 ± 1.54^{a}	33.71 ± 4.04^{a}
Sediment yield (g/L)	0.88 ± 0.01^{a}	1.85 ± 0.03^{c}	2.18 ± 0.02^{c}	3.14 ± 0.09^{d}	1.25 ± 0.13^{ab}	1.66 ± 0.24^{bc}	2.04 ± 0.10^{c}
pH	5.80 ± 0.23^{a}	5.65 ± 0.17^{a}	5.93 ± 0.16^{a}	6.00 ± 0.04^{a}	5.48 ± 0.14^{a}	5.64 ± 0.21^{a}	6.14 ± 0.06^{a}
EC (µS/ cm)	379.67 ± 32.84^{a}	1362.33 ± 46.37^{bc}	1634.67 ± 16.59^{c}	2320.67 ± 30.47^{d}	640.00 ± 97.96^{a}	1120.33 ± 198.14^{b}	1358.33 ± 109.37^{bc}
DOC (µg/mL)	40.94 ± 3.67^{a}	336.8 ± 13.36^{cd}	413.00 ± 1.49^{d}	550.80 ± 13.65^{e}	149.60 ± 16.19^{b}	265.40 ± 36.92^{c}	358.30 ± 26.81^{cd}
Nitrate (µg/mL)	37.04 ± 3.31^{c}	30.14 ± 5.50^{bc}	11.48 ± 0.92^{a}	18.46 ± 1.30^{ab}	15.82 ± 3.67^{a}	21.50 ± 1.79^{ab}	11.26 ± 1.07^{a}
Ammonia (µg/mL)	BDL	3.76 ± 0.54^{b}	6.76 ± 0.38^{c}	11.20 ± 0.78^{d}	1.56 ± 0.09^{ab}	3.64 ± 0.62^{b}	7.47 ± 0.51^{c}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

Table 6.12. Leachate results for non-metal variables in the clay soil (mean ± standard error).

		MWOO Surface Applied			MWOO Incorporated		
	Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**100 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**100 t ha^{-1}**
Total leachate (mL)	114.88 ± 13.18^{a}	155.19 ± 28.18^{a}	421.91 ± 132.41^{a}	274.50 ± 72.20^{a}	416.90 ± 85.23^{a}	253.51 ± 57.84^{a}	279.34 ± 93.93^{a}
Leachate rate (mL/min)	13.42 ± 3.08^{a}	15.52 ± 2.82^{ab}	21.21 ± 2.21^{ab}	23.12 ± 1.89^{ab}	28.41 ± 2.96^{b}	15.36 ± 2.66^{ab}	21.24 ± 4.99^{ab}
pH	5.98 ± 0.26^{a}	6.02 ± 0.05^{a}	6.13 ± 0.06^{a}	7.11 ± 0.20^{b}	5.44 ± 0.34^{a}	5.95 ± 0.09^{a}	5.22 ± 0.18^{a}
EC (µS/ cm)	314.2 ± 39.97^{a}	1397.33 ± 205.88^{bcd}	1533.33 ± 35.41^{cd}	2035.33 ± 316.11^{cd}	431.67 ± 39.10^{ab}	981.00 ± 190.69^{ac}	2332.33 ± 396.68^{d}
Sediment yield (g/L)	1.20 ± 0.13^{a}	2.22 ± 0.30^{ac}	2.19 ± 0.05^{ac}	2.85 ± 0.56^{bc}	1.07 ± 0.02^{a}	1.67 ± 0.23^{ab}	3.57 ± 0.57^{c}
DOC (µg/mL)	71.1 ± 5.42^{a}	294.00 ± 60.31^{ab}	296.10 ± 6.90^{ab}	451.00 ± 91.43^{b}	78.00 ± 9.49^{a}	174.80 ± 34.40^{a}	457.05 ± 74.67^{b}
Nitrate (µg/mL)	13.56 ± 2.19^{ab}	16.24 ± 0.17^{b}	9.39 ± 2.85^{ab}	6.34 ± 0.64^{a}	7.46 ± 0.21^{a}	10.40 ± 1.77^{ab}	14.00 ± 2.25^{ab}
Ammonia (µg/mL)	BDL	0.30 ± 0.12^{ac}	0.36 ± 0^{bc}	8.84 ± 3.79^{c}	BDL	0.04 ± 0.02^{ab}	0.32 ± 0.16^{ac}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$). BDL = Below Detection Limits.

6.4.3.2 Total Leachate, Leachate Rate and Sediment Yield

For the sandy loam, total leachate tended to increase after MWOO application. Significant increases in total leachate volume were recorded for the 50 t ha^{-1} surface applied treatment and the 100 t ha^{-1} incorporated treatment. ($F_{6,14} = 12.96$, $p = 5.2\text{x}10^{-5}$), with this 100 t ha^{-1} incorporated treatment yielding the greatest mean total leachate at 958.33 mL (Table 6.4).The leachate rate, however, did not change significantly with MWOO application, although the 100 t ha^{-1} incorporated treatment did exhibit the highest mean leachate rate at 33.71 mL/min (Table 6.4). Sediment yield increased significantly with MWOO application rate for both application methods ($F_{6,14} = 39.3$, $p = 5.6\text{x}10^{-8}$) (with the exception only of the incorporated 20 t ha^{-1} treatment), and of the two methods, the incorporated treatments yielded significantly less sediment than the same application rate in the surface applied treatments. The surface applied 100 t ha^{-1} treatment demonstrated the greatest mean result at 3.14 g/L (Table 6.4).

In contrast to the sandy loam, total leachate in the clay was not significantly influenced by MWOO application; this was despite relatively large variation in the means across treatments from 114.88 mL in the control to 421.91 mL in the 50 t ha^{-1} surface applied treatment (Table 6.5). However, similar to the sandy loam, the leachate rate in the clay was again not significantly affected by MWOO application. This was for nearly all treatments with the exception of the 20 t ha^{-1} incorporated treatment only, which had a significantly higher leachate rate at 28.41 mL/min ($F_{6,14} = 2.94$, $p = 0.045$) (Table 6.5). For sediment yield, the results were different from the sandy loam in that only the 100 t ha^{-1} treatments, for both application methods (3.57 g/L incorporated and 2.85 g/L surface applied), were significantly higher than the control (1.2 g/L) ($F_{6,14} = 6.97$, $p = 1.4\text{x}10^{-3}$), and in that there was no significant difference between application methods (Table 6.5).

Overall, both soils behaved similarly, and even though the sandy loam tended to show higher mean total leachate and leachate rate results compared to the clay, this was not significant. Sediment yield was also similar for both soils across all treatments, with the only exception being the 100 t ha^{-1} incorporated application rate, where the clay recorded significantly higher sediment yield than the sandy loam ($F_{13,28} = 9.81$, $p = 2.94\text{x}10^{-7}$).

6.4.3.3 pH and EC

Even though the highest mean leachate pH was recorded in both the 100 t ha^{-1} treatments (pH 6.00 for MWOO surface applied and pH 6.14 for MWOO incorporated), this was not a significant change from pH 5.8 in the control (Table 6.4). Leachate EC in the sandy loam, however, did increase significantly with MWOO application (except for the 20 t ha^{-1} incorporated treatment only), and continued to increase in response to increasing rates of MWOO application, for both application methods. Of the two methods, the incorporated treatments had significantly lower EC results than the same application rate in the surface applied treatments ($F_{6,14}$ = 44.10, p = 2.7x10^{-8}). And as for total leachate, leachate rate and sediment yield, the surface applied 100 t ha^{-1} treatment again recorded the highest result, with a mean EC of 2321 µS/ cm (Table 6.4).

The pH of leachate from the clay was generally not significantly influenced by MWOO application, which was a similar result to the sandy loam, however in this case one of the 100 t ha^{-1} treatments (surface applied, pH 7.11) was significantly higher than the control (pH 5.98) ($F_{6,14}$ = 9.46, p = 2.9x10^{-4}) (Table 6.4). Again, similar to the sandy loam, leachate EC tended to increase with increasing rates of MWOO applied to the clay, with all surface applied treatments and the 100 t ha^{-1} incorporated treatment significantly different from the control($F_{6,14}$ = 12.00, p = 8.1x10^{-5}) (Table 6.5). However, whereas all treatments in the sandy loam were different to the control, in the clay, the two lower incorporated rates were not, even though the results followed a trend of increasing EC with increasing application rate (Table 6.5)

Overall, the difference in pH between the two soils was not significant except for the 100 t ha^{-1} surface applied treatment, whereby the clay recorded a significantly higher mean pH than the sandy loam ($F_{13,28}$ = 6.29, p = 2.41x10^{-5}). Similarly, but in this case for the incorporated treatment rather than the surface applied, the only significant difference in EC concentrations between the two soils was for the 100 t ha^{-1} incorporated treatment ($F_{13,28}$ = 15.84, p = 1.40x10^{-9}), with the clay recording a significantly higher mean EC than the sandy loam.

6.4.3.4 DOC, Nitrate and Ammonia

In the sandy loam, application of MWOO increased leachate DOC ($F_{6,14}$ = 73.4, p = 9.0x10^{-10}) and ammonia ($F_{6,14}$ = 63.1, p = 7.3x10^{-9}) concentrations significantly in all treatments, with the only exception being the incorporated 20 t ha^{-1} treatment for ammonia. The concentrations for both DOC and ammonia generally increased significantly as application rate increased, with the highest recorded in the surface applied 100 t ha^{-1} treatment (550.80 µg/mL for DOC and 11.20 µg/mL for ammonia (Table 6.4). As was the case for EC, concentrations of DOC and ammonia in the incorporated treatments were significantly lower than concentrations in leachate at the same application rate in the surface applied treatments. For nitrate, concentrations in leachate from the sandy loam were significantly higher in the control (37.04 ± 3.31 µg/mL) compared to the MWOO treatments for both application methods ($F_{6,14}$ = 10.7, p = 1.5x10^{-4}); the only exception was the 20 t ha^{-1} surface applied treatment (30.14 ± 5.50 µg/mL).

Application of MWOO to the clay soil also tended to increase leachate DOC concentrations in leachate for both application methods. This was significantly so for the surface applied and incorporated 100 t ha^{-1} treatments (451 and 457 µg/mL, respectively), which were more than six times higher than the control (71 µg/mL) ($F_{6,13}$ = 8.8, p = 5.8x10^{-4}) (Table 6.5). In contrast to the sandy loam, ammonia concentrations in leachate were not significantly affected when MWOO was incorporated into the clay soil. There was a significant change, however, for MWOO surface applied at 50 and 100 t ha^{-1} ($F_{6,14} = 7.86$, $p = 7.6x10^{-4}$), with the ammonia concentration recorded for the surface applied 100 t ha^{-1} treatment (8.84 µg/mL) considerably higher than the control (0.00 µg/mL) and other treatments (see Table 6.5). As with the sandy loam, nitrate concentrations were high in the control (13.56 g/mL), but there was no significant difference between this and the MWOO applied treatments. The highest nitrate concentration was actually recorded in the 20 t ha^{-1} surface applied treatment (16.24 g/mL), and this was significantly different to the lowest concentration recorded for the 100 t ha^{-1} surface applied treatment (6.34 µg/mL) ($F_{6,13} = 5.3$, $p = 5.8x10^{-3}$).

Generally, the sandy loam appeared to have higher DOC, nitrate and ammonia concentrations across all treatments compared to the clay, however, significant differences between the two soils were only apparent for nitrate ($F_{13,27} = 12.86$, $p = 2.32x10^{-8}$) and ammonia ($F_{13,27} = 15.82$, $p = 2.31x10^{-9}$). This was a reflection of nitrate concentrations in leachate from the sandy loam being significantly higher than the clay for the control and 20 t ha^{-1} surface applied treatment, and ammonia concentrations being significantly higher in the sandy loam than the clay in the 50 t ha^{-1} surface applied treatment and the 100 t ha^{-1} incorporated treatment.

6.4.3.5 Metals and Metalloids

Copper and Zn were present at the greatest concentrations in the leachate of both the sandy loam and the clay, followed by Ni. The next highest concentrations were for Cr and Pb, followed by As. Cadmium was present at the lowest concentrations in the sandy loam and clay leachate. The maximum mean values for all seven metals were recorded in leachate from the 100 t ha^{-1} treatments, with nearly being recorded in the surface applied treatment (Figure 7). The only exceptions were As in the sandy loam and Ni in the clay, which were both highest in leachate from the incorporated treatment.

The main trend for metal concentrations in leachate for both soils were that metal concentrations increased with MWOO application rates in both surface applied and incorporated MWOO application methods (see Figure 7). Surface applied treatments generally had significantly higher metal concentrations than the incorporated treatments, and the sandy loam generally, and in most cases significantly, had higher metal concentrations than the clay (except for Ni, which had higher concentrations in the clay) (Figure 7). As with runoff, these trends were similar to results for DOC and significant correlations were observed between DOC and Cd ($r^2 = 0.89$, $p = 3.94x10^{-10}$), Cu ($r^2 = 0.93$, $p = 9.97x10^{-12}$), Ni ($r^2 = 0.84$, $p = 1.21x10^{-8}$) and Zn ($r^2 = 0.88$, $p = 3.24x10^{-10}$) in the sandy loam. In the clay significant correlations were also found between DOC and Cd ($r^2 = 0.98$, $p =<2x10^{-16}$), Cu ($r^2 = 0.95$, $p = 2.28x10^{-13}$), Ni ($r^2 = 0.94$, $p = 2.74x10^{-12}$) and Zn ($r^2 = 0.89$, $p = 4.80x10^{-10}$).

In the sandy loam, as MWOO application rate increased metal and metalloid concentrations in leachate also increased, however, although the difference with the control was usually significant, the difference between application rates was not always so. For example, in the surface applied treatments the 50 t ha^{-1} treatment was often not significantly different from either the 20 t ha^{-1} or 100 t ha^{-1} treatment, but the 20 and 100 t ha^{-1} treatments were; this trend was observed for Cd, Cr and Cu. For As, the 50 t ha^{-1} treatment was significantly higher than the 20 t ha^{-1}, for Pb and Zn the 100 t ha^{-1} treatment was significantly higher than the 20 and 50 t ha^{-1} treatments and for Ni there was no significant difference between the 20, 50 and 100 t ha^{-1} surface applied treatments.

Although trends were similar in leachate from the sandy loam with incorporated treatments, fewer significant differences were observed, with As, Cd and Cr the only metals and metalloids with significant differences between the incorporated application rates: As concentrations in leachate were significantly higher in the 100 t ha^{-1} treatment compared to the 20 and 50 t ha^{-1} treatments; and Cd and Cr followed the same trend as the surface applied treatments whereby the 100 t ha^{-1} treatment had significantly higher concentrations than the 20 t ha^{-1} treatment, but neither were significantly different from the 50 t ha^{-1} treatment.

In the clay, metal and metalloid concentrations in leachate also increased as MWOO application rate increased. However, this was not always significantly different to the control, nor were application rates significantly different. Within the surface applied treatments, As, Cd, Cr, Cu, Ni and Zn concentrations in leachate were only significantly different between the 20 t ha^{-1} and 100 t ha^{-1} treatments, and for Pb, the 100 t ha^{-1} treatment was significantly higher than the 20 and 50 t ha^{-1} treatments. As with the sandy loam, fewer significant differences were observed in the incorporated treatments, with only Cr and Ni recording significant differences between treatments. For both, leachate from the 100 t ha^{-1} incorporated treatment contained significantly higher concentrations than both the 20 and 50 t ha^{-1} treatments, which were statistically the same.

Concentrations of metals and metalloids in leachate were generally higher from the sandy loam compared to the clay, however, this was only significant for As ($F_{13,28} = 55.24$, $p = <2.2\text{x}10^{-16}$), Pb ($F_{13,28} = 51.15$, $p = 5.4\text{x}10^{-16}$) and Zn ($F_{13,28} = 15.04$, $p = 2.56\text{x}10^{-9}$). This was also despite As in leachate from the control sandy loam being slightly higher, although not significantly higher, than the clay control. For Ni, significantly higher concentrations were present in the clay in all surface applied MWOO treatments and also in the 100 t ha^{-1} incorporated treatment ($F_{13,28} = 25.21$, $p = 4.96\text{x}10^{-12}$). Throughout all MWOO treatments the sandy loam showed significantly higher arsenic concentrations than the clay. The sandy loam exhibited significantly higher Pb concentrations than the clay for all MWOO applied treatments, except the 20 t ha^{-1} incorporated treatment, and significantly higher Zn concentrations were also evident in the sandy loam with the 100 t ha^{-1} surface applied treatment.

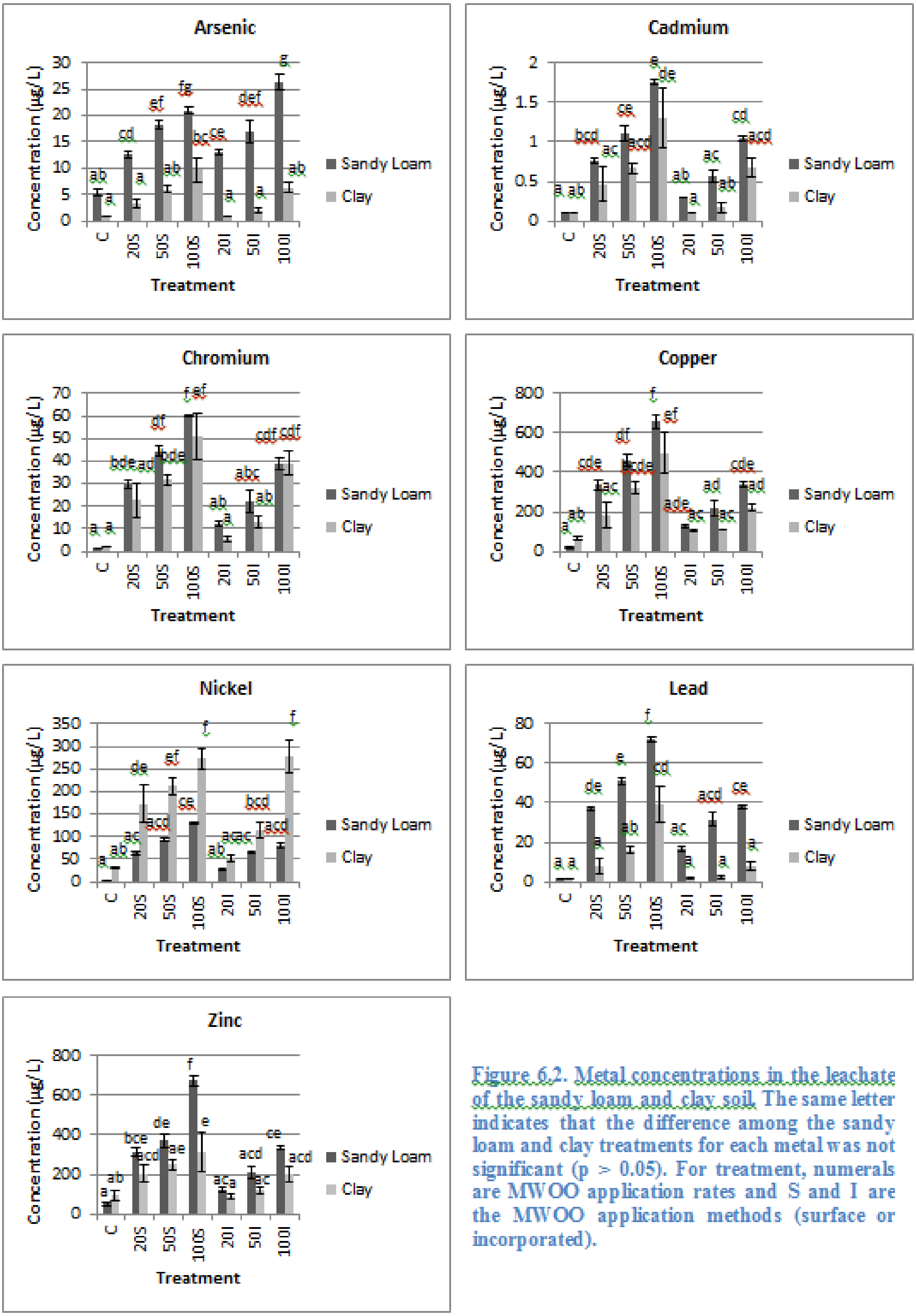


Figure 6.2. Metal concentrations in the leachate of the sandy loam and clay soil. The same letter indicates that the difference among the sandy loam and clay treatments for each metal was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

6.4.4 Comparing Runoff and Leachate

6.4.4.1 Runoff and Leachate Descriptors

Total leachate was significantly higher than total runoff in all treatments in the sandy loam soil ($F_{13,28}$ = 59.57, p = <2.2x10^{-16}), and while this trend was evident with the clay, significant results were only obtained for the surface applied 50 and 100 t ha^{-1} application rates and the incorporated 20 t ha^{-1} treatment ($F_{13,28}$ = 7.00, p = 8.86x10^{-6}). Leachate rate was also higher than runoff rate but this was only significant for the surface applied 50 and 100 t ha^{-1} application rates and the incorporated 20 and 100 t ha^{-1} application rate from both the sandy loam ($F_{13,28}$ = 26.76, p = 2.35x10^{-12}) and the clay soil ($F_{13,28}$ = 9.13, p = 6.29x10^{-7}). There were generally no significant differences in sediment yield between runoff and leachate, except in the 100 t ha^{-1} incorporated application rate where leachate was significantly higher than runoff in both the sandy loam ($F_{13,28}$ = 9.15, p = 6.10x10^{-7}) and the clay soil ($F_{13,28}$ = 15.00, p = 2.64x10^{-9}).

6.4.4.2 pH and EC

In the sandy loam, pH in leachate tended to be higher than in runoff, however this was only significant for the sandy loam control and 100 t ha^{-1} incorporated treatment ($F_{13,28}$ = 6.39, p = 2.06x10^{-5}). In the clay soil, pH tended to follow an opposite pattern whereby pH was higher in runoff, but the results were not statistically significant for any of the treatments. For EC, patterns in the pH results were essentially reversed. Runoff from the sandy loam generally recorded higher mean EC values than leachate across all treatments; however, this was not significant. And in the clay, the leachate generally recorded higher EC values compared to the runoff, significantly so in the control and all incorporated application rates ($F_{13,28}$ = 69.24, p = <2.2x10^{-16}); there was no significant difference in EC between runoff and leachate with the surface applied treatments.

6.4.4.3 DOC, Nitrate and Ammonia

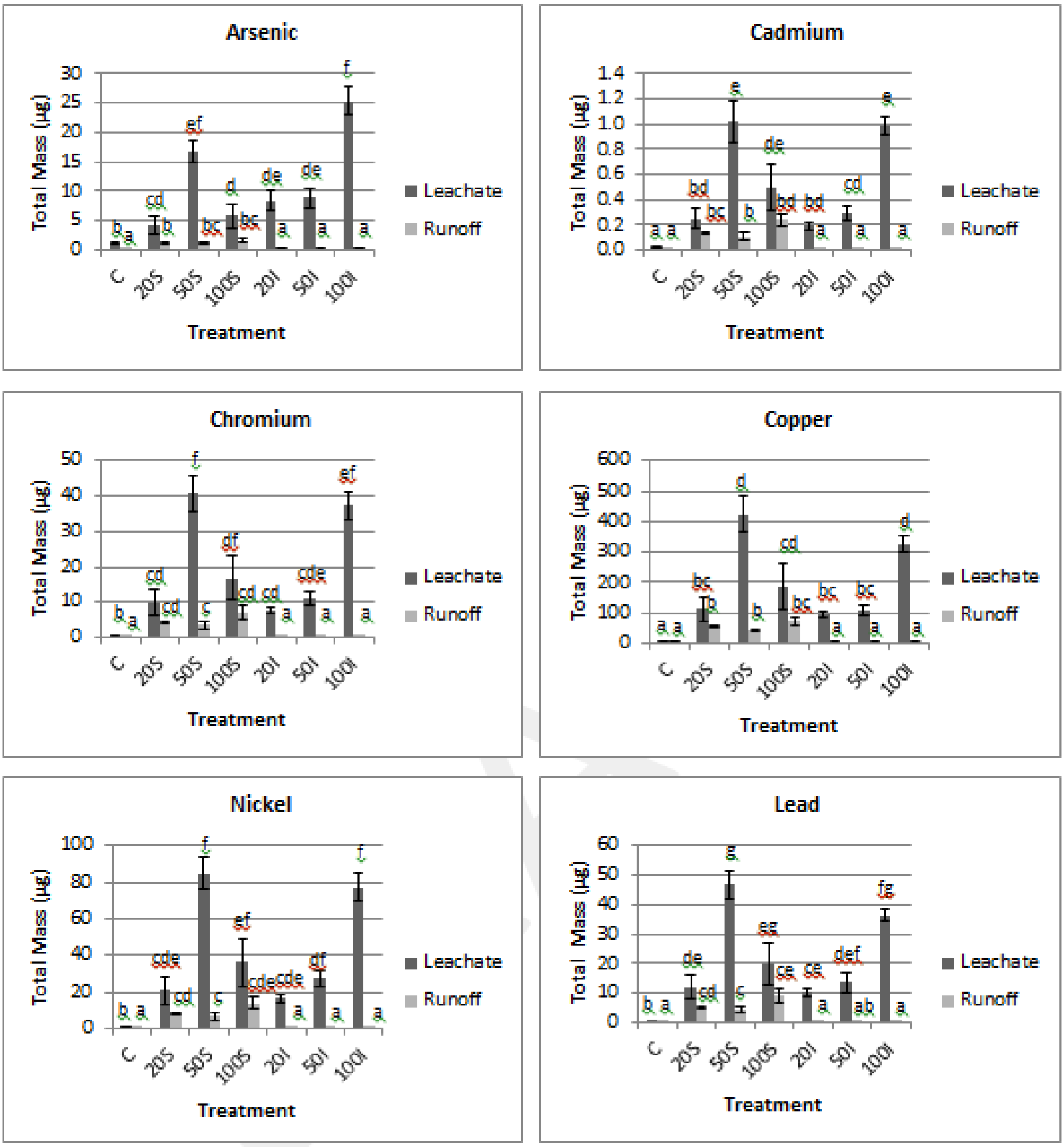
Dissolved organic carbon concentrations were significantly higher in leachate compared to runoff from both the sandy loam ($F_{13,28}$ = 115.84, p = <2.2x10^{-16}) and the clay ($F_{13,26}$ = 69.31, p = <2.2x10^{-16}) for all controls and incorporated MWOO treatments. Nitrate concentrations in leachate were also significantly higher than in runoff across all treatments in both the sandy loam ($F_{13,27}$ = 161.28, p = <2.2x10^{-16}) and the clay ($F_{13,26}$ = 49.50, p = 5.77x10^{-15}). Ammonia concentrations in leachate from the sandy loam also tended to be higher than in runoff, but this was only significant for the incorporated 50 and 100 t ha^{-1} application rates ($F_{13,27}$ = 37.42, p = 7.48x10^{-14}). The only response to differ in this pattern whereby leachate generally contained higher concentrations of DOC, nitrate and ammonia than the runoff, was for ammonia concentrations from the clay with MWOO surface applied. In this case, the concentration of ammonia in the runoff was significantly higher than in the leachate ($F_{13,26} = 68.05$, $p = <2.2\text{x}10^{-16}$).

6.4.4.4 Metals and Metalloids

The general trends for leaching and runoff of metals and metalloids from both the sandy loam and the clay were the opposite depending on how MWOO was applied. Metal concentrations from the surface applied treatments were higher in runoff than leachate, and concentrations from the incorporated treatments were higher in leachate than runoff. However, since the total leachate volumes were significantly larger than total runoff volumes, the total mass of metal lost from the soil was consistently larger in the leachate (Figures 6.3 and 6.4). By accounting for the total mass of metals lost, metals lost from the surface applied treatments now changed from being highest in runoff, to highest in leachate, and metals lost from the incorporated treatments remained highest in the leachate.

To clarify, a significantly larger mass of total metal was generally lost in leachate than runoff for every metal and metalloid in all the incorporated MWOO application rates for both soils (Sandy Loam, Figure 6.3; Clay, Figure 6.5), and the 50 t ha^{-1} surface applied treatment in the sandy loam (Figure 6.3). For the surface applied clay treatments, leachate again contained higher metal mass than runoff, however, results were quite variable and this resulted in fewer significant differences (Figure 6.4).

The results for total metals and metalloids lost from the soil/MWOO treatment as a percentage of the total amount initially present in the soil/MWOO treatment for both soils are provided in Table 6.6. The total amount of metal lost in both leachate and runoff from the sandy loam was less than 1% for As, Cr, Ni, Pb and Zn, and less than 2.5% for Cd and Cu. For the clay soil, metals lost in leachate and runoff was less than 1% for all metals and metalloids in all treatments.



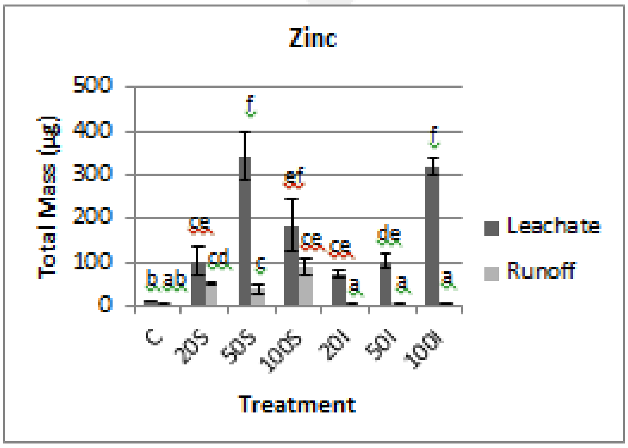


Figure 6.3. Total metal lost (µg) from leachate and runoff in the surface applied and incorporated treatments of the sandy loam soil. The same letter indicates that the difference among runoff and leachate treatments for each metal was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

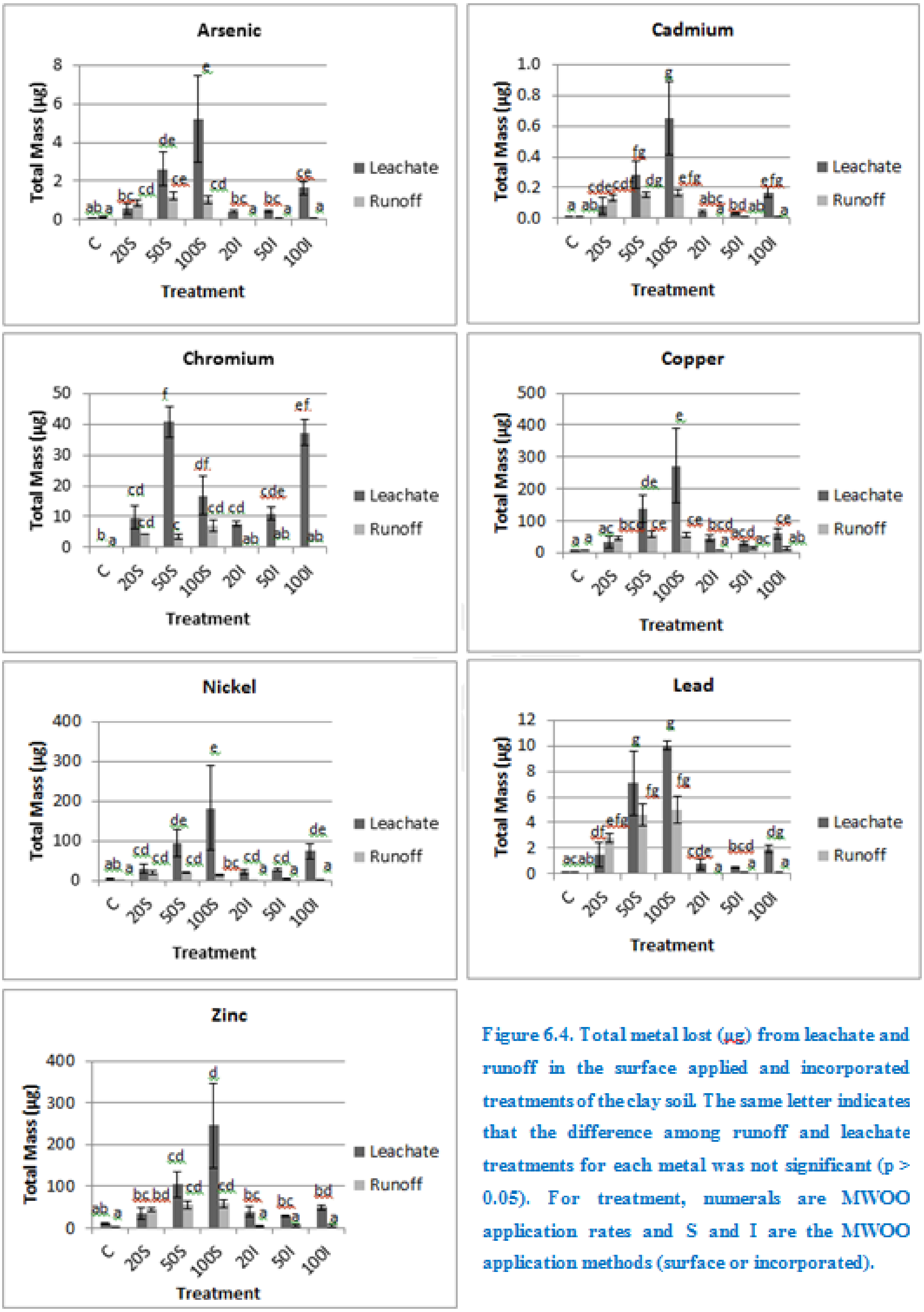


Figure 6.4. Total metal lost (µg) from leachate and runoff in the surface applied and incorporated treatments of the clay soil. The same letter indicates that the difference among runoff and leachate treatments for each metal was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

Table 6.13. Results for total metal and metalloid lost from the sandy loam soil/MWOO treatment as a percentage of the total amount initially present.

			MWOO Surface Applied						MWOO Incorporated					
	Control		20 t ha^{-1}		50 t ha^{-1}		100 t ha^{-1}		20 t ha^{-1}		50 t/h t ha^{-1}a		100 t ha^{-1}	
	Sandy Loam	Clay	Sandy Loam	Clay	Sandy Loam	Clay	Sandy Loam	Clay	Sandy Loam	Clay	Sandy Loam	Clay	Sandy Loam	Clay
As (%)	BDL	0.02	0.01	0.10	0.03	0.25	0.01	0.18	0.01	0.05	0.01	0.05	0.04	0.15
Cd (%)	BDL	BDL	0.91	0.01	1.07	0.01	0.35	0.02	2.35	BDL	1.43	BDL	2.39	0.01
Cr (%)	BDL	BDL	0.03	BDL	0.10	0.01	0.04	0.01	0.02	BDL	0.03	BDL	0.09	0.01
Cu (%)	0.05	0.04	0.70	0.15	1.34	0.35	0.47	0.25	0.50	0.13	0.61	0.11	1.44	0.17
Ni (%)	0.02	BDL	0.18	0.04	0.51	0.11	0.24	0.08	0.11	0.02	0.21	0.03	0.48	0.08
Pb (%)	BDL	0.01	0.08	0.02	0.13	0.04	0.04	0.03	0.09	0.01	0.11	0.01	0.17	0.02
Zn (%)	0.08	0.02	0.29	0.07	0.38	0.11	0.15	0.06	0.29	0.05	0.30	0.04	0.61	0.06

BDL = Below Detection Limits.

6.5 Discussion

6.5.1 MWOO

Much of the literature reports that high metal concentrations are often present in final MWOO products (Amlinger et al., 2004; Smith, 2009; Tisdell & Breslin, 1995), and by comparison the metal concentrations in the MWOO used in this research are low. Despite this, the MWOO used in this research could not be applied to agricultural soil in NSW as it exceeded guideline concentrations for Cu, Pb, Zn and also Cd. Additionally, it could not be applied to Minesites in NSW, as guideline concentrations were exceeded for Pb and Zn. These metals in particular may be high because they are widely used in disposable materials. For example, Cu, Cd, Pb and Zn are all used in electronics and due to the increased disposal of electronic waste these metals are present in high concentrations in municipal waste and MWOO (Leung et al., 2006; Wong et al., 2007). Disposal of Pb based paint and batteries, which also contain Zn, Cu and Cd, could lead to high Pb, Zn, Cu and Cd concentrations in MWOO. The harmful effects of these metals to humans include kidney, bone and skin damage, and neurotoxic and carcinogenic effects (Jarup, 2003). Environmental and ecological hazards, including fatal effects to organisms, are also associated with metal contamination to soil and water (Vinodhini et al., 2008). Despite these limitations, this MWOO was still used in this research so that an understanding of MWOO amendment to soil and effects on runoff and leachate in Australia and NSW could be gained.

6.5.2 Runoff and Leachate

Leachate and runoff results generally followed the same pattern. Addition of MWOO generally increased the magnitude and significance of changes in measured variables, and surface applied treatments generally recorded more significant effects than incorporated treatments. This could simply be because a larger amount of MWOO was added to the surface applied treatments than the incorporated treatments. The reason for this was because MWOO incorporation usually occurs to a depth of 15 cm (equivalent to the plough layer) and since the soil in this experiment was only 3 cm deep a smaller amount of MWOO was required to ensure the equivalent application rate was applied. Despite these limitations this experiment still provided a comparison of having a concentrated mass of MWOO on the soil surface versus a more dispersed incorporated mass of MWOO and the effect this had on contamination of runoff and leachate to 3 cm. A better comparison of application method would require soil with a depth of at least 15 cm so that the same amount of MWOO could be used, and this was addressed in Chapter 7 (a column leaching experiment).

6.5.2.1. Runoff and Leachate Descriptors

The effect of MWOO application on runoff is generally little researched, however studies by Martinez et al. (2003) and Glanville et al. (2004) report the effect of MWOO application on runoff water volume and quality. Glanville et al. (2004) undertook research on highway embankments where MWOO was spread in 5 and 10 cm blankets and rainfall was applied at approximately 100 mm/hr. They reported that MWOO application significantly increased the infiltration capacity of the soil. Martinez et al. (2003) applied rainfall to soil 3 and 4 years after MWOO was applied at 80 t ha^{-1} and found that total runoff after MWOO application (2021 mL) was generally lower than the control (3320 mL). Sediment yield similarly decreased after MWOO application from 56.36 g/m^2 in the control to 34.40 g/m^2 in their 80 t ha^{-1} treatment. However, in their experiment, time to runoff was increased in the MWOO treated soils compared to the control. Generally, these findings seem to contradict the results from our experiment, where total runoff volume was unaffected by MWOO application, sediment yield increased and total runoff decreased when MWOO was surface applied to soil. The differences could simply be due to changes occurring through time and in field conditions compared to the controlled short term experiment undertaken in our research.

Experiments on the effect of MWOO addition to soil in leachate were undertaken by Kaschl et al. (2002) and Giusquiani et al. (1992). Kaschl et al. (2002) undertook a column leaching experiment using a sandy soil with MWOO applied to the top 2 cm of soil at 65 t ha^{-1}, and Giusquiani et al. (1992) undertook a column leaching experiment on a sandy loam and a clay loam with MWOO incorporated to 15 cm at a rate of 90 t ha^{-1}. The main focus of both of these experiments was on metal and metalloid concentrations in leachate and no results were reported for total leachate or sediment yield in leachate. However, they did report time to runoff results, whereby surface application of MWOO decreased time to runoff and incorporation of MWOO increased time to runoff in both soils, as occurred in our experiment.

These and our results conflict with results reported by Agassi et al. (1998), who examined the effects of MWOO both surface applied and incorporated at the higher rate of 200 m^3/ha (120 t ha^{-1}), and found that surface application of MWOO increased time to runoff and incorporation of MWOO decreased time to runoff. This study also reported that 85% of the rainfall event percolated into the surface applied MWOO treatment but only 52% percolated into the incorporated MWOO treatment. They also reported that surface crusting of the soil (a clay loam) occurred in the first rainfall event.

A recent study by Bekier et al. (2014) reported that there are hydrophobic substances in MWOO, and this could potentially result in runoff occurring faster in surface applied treatments, as occurred for Kaschl et al. (2002) and Giusquiani et al. (1992), because water infiltration into the soil can be initially inhibited by the hydrophobicity of the MWOO. Despite this hydrophobicity, many studies report that MWOO addition increases the water holding capacity of the soil (Hargreaves et al., 2008; McConnell et al., 1993), which may be why in our study, the incorporated treatments had a slower time to runoff than the control and surface applied treatments.

Both total runoff and total leachate volumes were generally not significantly influenced by MWOO application in either soil, but rather by soil type. The clay soil recorded a higher total runoff than the sandy loam, whereas the sandy loam had the larger total leachate-probably due to the higher infiltration capacity of the sandy loam. Generally, leachate rate in both soils was also not significantly influenced by MWOO application, however, runoff rate was reduced with application of MWOO in the sandy loam compared to the control, although not always significantly. Runoff rate was reduced when runoff volume and time to runoff decreased, and while neither runoff volume or time to runoff were significant, their interaction caused a significant result in runoff rate (UDFCD, 2007).

The reduction in runoff rate with MWOO application to the sandy loam was a positive effect, since a reduced runoff rate results in reduced velocity and volume of runoff, which both decrease erosion. Hence, runoff rate can be used to estimate soil loss, although sediment yield is a better indication of soil loss since runoff can be clear yet still yield sediment. It was expected that sediment yield would decrease as runoff rate decreased (Yu et al., 1997). However, sediment yield in both runoff and leachate was generally higher in the MWOO surface applied treatments for both soils and no significant correlations with runoff rate or leachate rate were found. In runoff, sediment yield in the clay was significantly higher in all three application rates than the control and in the sandy loam, the 100 t ha^{-1} application rate showed a significantly higher sediment yield in runoff than the control. Sediment yield in leachate in the sandy loam increased significantly in all MWOO application rates, however, in the clay, sediment yield only increased significantly at the higher application rates. Incorporated treatments did not show a significantly different sediment yield than the control. This could be because MWOO was more concentrated in the surface applied treatments and since MWOO is very light and hydrophobic small particles were washed into runoff regardless of runoff rate. Incorporating MWOO into the soil may, therefore, not have negative effects on erosion or sedimentation of water ways, as could happen with surface applied MWOO.

Sediment yield is important because it is a measure of the suspended solids that enter the surface or groundwater. Suspended solids can be used as a water quality indicator and in large amounts can have negative effects on aquatic ecosystems (Bilotta & Brazier, 2008; Newcombe & Macdonald, 1991). Negative effects include reducing light penetration, which results in changed temperatures, reduced photosynthesis and a reduction in oxygen production. If sedimentation becomes severe enough it can also result in the clogging of fish gills and increased likelihood of disease in aquatic organisms (Newcombe & Macdonald, 1991).

6.5.2.2. pH and EC

Effects of MWOO application on pH and EC in runoff have also been reported by Martinez et al. (2003), who found that MWOO application resulted in a significant decrease in pH in runoff water from 7.33 in the control to 6.93 in an 80 t ha^{-1} MWOO amended treatment. Kaschl et al. (2002) reported that pH in leachate was generally unaffected by MWOO addition to a sandy or loamy soil but at depth pH in leachate tended to decrease slightly. In this experiment, pH in runoff and leachate was not significantly influenced by MWOO application to the sandy loam, however, for the clay, the 100 t ha^{-1} surface applied treatment recorded the highest pH in both runoff and leachate. This contradicts the findings of other authors such as Helyar et al. (1990) and Hargreaves et al. (2008), who stated that soils with higher clay content have a higher buffering capacity and hence should be less susceptible to pH change.

Our initial soil tests revealed that the sandy loam had a lower initial pH (4.44) than the clay (6.21), and that the pH of the MWOO was 7.36. Generally, MWOO contains more than 50% organic matter and has a neutral to alkaline pH, which usually causes a rise in pH when applied to acidic soils (McConnell et al., 1993). This would suggest that the pH of sandy loam should have been more likely to show a significant change rather than the clay. However, when surface applied at 100 t ha^{-1} the MWOO significantly increased pH in the runoff and leachate from the clay soil, not the sandy loam. This may have been due to the increased sediment in runoff and leachate in the 100 t ha^{-1} surface applied treatment, although no significant correlation was found between these variables and pH in runoff and leachate.

Martinez et al. (2003) reported that EC in runoff tended to increase significantly with MWOO application-from 46.7 µS/ cm in the control to 78.7 µS/ cm in the MWOO applied treatment. This literature generally corroborates the results obtained from this experiment, with runoff from both soils containing significantly higher EC for the surface applied MWOO treatments compared to the control; however, the incorporated treatments were not influenced by MWOO application. In our study, there was no significant difference in runoff EC between the three application rates, probably due to large variability among the replicates. Generally, the EC results obtained from this research were much higher than those obtained from Martinez et al. (2003), except in the incorporated sandy loam runoff results.

Kaschl et al. (2002) showed that EC in leachate also tends to spike after MWOO application and then decrease with time, and in our experiment, surface application of MWOO caused a similar significant increase in leachate EC compared to the control in both soils. However, for the clay, EC in leachate from the incorporated treatments was not significantly influenced by MWOO application, except at 100 t ha^{-1}. These findings are also somewhat supported by McConnell et al. (1993), Shiralipour et al. (1992) and Hargreaves et al. (2008), although they only reported that EC in soil increased after MWOO application and did not report leachate or runoff results.

Electrical conductivity is a measure of the total inorganic solids that passes through a 0.45 µm filter. Since EC and salt-forming cations such as Ca and Na were both present in higher amounts in the MWOO compared with the sandy loam, addition of MWOO to the soil would have added these cations to the sandy loam and hence increased EC (McConnell et al., 1993; Shiralipour et al., 1992). This could explain the result of significantly higher EC in both runoff and leachate in the surface applied treatments with progressively higher application rates. Conversely, the clay and MWOO have more similar concentrations of salt-forming cations, particularly for calcium, which may explain the lack of significant change in the incorporated treatments in runoff and leachate until the highest application rate in the incorporated treatment for leachate, where the high EC of the MWOO appeared to have an effect.

High EC has the potential to create salinity problems in both soil and groundwater, which can have negative effects on crop and livestock growth and productivity (ANZECC & ARMCANZ, 2000; Araujo et al., 2013). For this reason, there are guideline trigger values for EC in freshwater systems. The trigger value for upland rivers is 350 µS/ cm, for lowland rivers the trigger value is 2200 µS/ cm and for freshwater lakes it is 30 µS/ cm (ANZECC & ARMCANZ, 2000). For runoff in our experiment, the trigger value for freshwater lakes was generally exceeded by all MWOO applied treatments in both soils. The trigger value for upland rivers was exceeded in both soils by the surface applied treatments but not the incorporated MWOO treatments. For lowland rivers, only three treatments exceeded this higher EC trigger value and all were with MWOO surface applied: the sandy loam with the 100 t ha^{-1} surface applied MWOO treatment (3019 µS/ cm) and the clay with MWOO surface applied at 50 t ha^{-1} (2388 µS/ cm) and 100 t ha^{-1} (2323 µS/ cm).

In leachate for both soils, all MWOO application rates for both application methods exceeded trigger values for upland rivers and freshwater lakes, and the trigger value for lowland rivers was exceeded in the 100 t ha^{-1} surface applied treatment in the sandy loam, with a mean concentration of 2321 µS/ cm, and in the 100 t ha^{-1} incorporated treatment in the clay, with a mean concentration of 2332 µS/ cm. Overall, these results indicate that MWOO addition has potential to cause salinity problems in both leachate and runoff, particularly when surface applied at high application rates.

6.5.2.3. DOC, Nitrate and Ammonia

Very little is known about DOC in runoff water after MWOO application, and although Martinez et al. (2003) undertook an experiment examining the effect of MWOO application on runoff, no results for DOC in the runoff were reported. For Leachate, Kaschl et al. (2002) reported that DOC concentrations in leachate increased in response to MWOO application. It could be inferred, therefore, that DOC in runoff may also increase with MWOO application, particularly since McConnell et al. (1993), Shiralipour et al. (1992) and Hargreaves et al. (2008) all report that organic carbon can become soluble after MWOO application.

In runoff from this experiment, DOC concentrations from both soils were significantly higher in the surface applied treatments than the control, but not for the incorporated treatments, and for both application methods, there was no significant difference in DOC concentrations in runoff between application rates. These results generally support the inference outlined above, except for when MWOO was incorporated, which could be due to less interaction between MWOO and runoff in the incorporated treatments. In leachate from the sandy loam, all application rates across both application methods showed significantly higher mean values for DOC than the control, which supports the results reported by Kaschl et al. (2002), but in the clay, only the surface applied application rates and the 100 t ha^{-1} incorporated application rate had significantly higher DOC in leachate compared to the control. For the two soils, the sandy loam consistently recorded higher DOC concentrations in leachate across all MWOO treatments compared to the clay. This result is unsurprising since DOC is generally more mobile in soils with a sandy texture (Wang & Alva, 1999).

Similar to EC, DOC is a measure of the organic fraction that passes through a 0.45 µm filter and a significant increase in this fraction in runoff and leachate following MWOO application can be explained by a number of factors. Because the organic matter and organic carbon content of MWOO is high, when MWOO is applied to soil these parameters will also increase, potentially resulting in higher DOC concentrations in runoff and leachate. In addition, since DOC and organic matter are both negatively charged, the increase in organic matter from MWOO application would compete with DOC for positive sorption surfaces and DOC may therefore become more mobile (Jardine et al., 1989). Any excess DOC can have negative effects on groundwater because it affects colour, taste and odour as well as promoting bacterial growth by becoming a food source for micro-organisms (Orica, 2012). Dissolved organic carbon can also form metal complexes when positively charged cations, particularly, Cu, Cd, Ni and Zn, bind to the negatively charged DOC (Christensen et al., 1996; Temminghoff et al., 1997). Since MWOO amendment significantly increases DOC mobility, metal mobility could therefore be subsequently increased (Hargreaves et al., 2008; Kaschl et al., 2002).

Martinez et al. (2003) reported that MWOO application can also result in a significant increase in ammonia concentrations in runoff (from 3.37 mg/L to 15.0 mg/L), although they found no significant increases in nitrate concentrations in runoff (from 0.12 mg/L to 0.18 mg/L). For leachate, Kaschl et al. (2002) reported that nitrate concentrations increased after MWOO application (from 45 mg/L to 135 mg/L), however, as with most other studies, they did not report ammonia concentrations in leachate after MWOO addition. In our study, ammonia concentrations in runoff from both soils increased significantly with surface application of MWOO, but not when MWOO was incorporated. As the surface application rate increased, ammonia concentrations in runoff also increased significantly, for both soils. Nitrate concentrations in runoff were not impacted significantly with MWOO application in the clay, however, in the sandy loam, incorporation of MWOO resulted in significantly lower nitrate in runoff compared to the control, whereas the surface applied treatments tended to be same as the control. For the two soils, ammonia and nitrate concentrations in runoff were lower in the sandy loam compared to the clay. For leachate, ammonia concentrations again increased significantly with MWOO application, but there no effect on nitrate, although the lowest concentrations were generally recorded in leachate from the 100 t ha^{-1} application rates. For the two soils, in contrast to the runoff, ammonia and nitrate concentrations in leachate were lower in the clay than the sandy loam. The quantity of ammonia and nitrate concentrations in runoff from this experiment were generally lower and higher, respectively, than those reported by Martinez et al. (2003), and nitrate concentrations in leachate were all below the highest nitrate concentration reported by Kaschl et al. (2002).

Hargreaves et al. (2008), Mamo et al. (1999) and Iglesias-Jimenez and Alvarez (1993) suggested that application of MWOO could result in N immobilisation in soils, which is the conversion of inorganic and mobile forms of N, such as nitrate and ammonia, to organic forms by soil microorganisms, and our results suggest this may have occurred for nitrate, particularly when MWOO was incorporated into the sandy loam. Nitrogen immobilisation predominantly occurs in immature material with C:N ratios greater than 25, however in our study, the C:N ratio of the MWOO was 17. Iglesias-Jimenez and Alvarez (1993) proposed that even mature composts with a C:N ratio less than 12, which ours was above, can undergo immobilisation. Although their experiment was run over 7 months, this could explain the nitrate results seen in our study.

Nitrate and ammonia are important sources of N for plant growth, however excess amounts in surface and groundwater can harm human health and lead to eutrophication of water ways. This has led to the development of guidelines for concentrations of nitrate acceptable in drinking water (drinking water guidelines have not been developed for ammonia) and ammonia and nitrate concentrations that maximise the protection of freshwater species (see Table 6.7 in the next section). In runoff from the sandy loam, nitrate guidelines for 95% freshwater species protection were exceeded not only by the surface applied 20 and 100 t ha^{-1} treatments, but also the control which had no MWOO applied-only the 50 t ha^{-1} rate would have been acceptable. For runoff from the clay soil, again the control and 20 t ha^{-1} treatments exceeded nitrate guidelines for 95% freshwater species protection-only the incorporated 50 and 100 t ha^{-1} application rates would be acceptable. For leachate, all treatments in both soils exceeded nitrate guidelines for 95% freshwater species protection. Drinking water guidelines were not exceeded by runoff or leachate.

For ammonia in runoff, guidelines for 95% protection of freshwater species were exceeded by all surface applied treatments in both soils, but not when MWOO was incorporated. For leachate, the guidelines were exceeded by all MWOO applied treatments in the sandy loam, and by the 100 t ha^{-1} surface applied treatment in the clay-only the lower 20 and 50 t ha^{-1} incorporated treatments in the clay were acceptable.

6.5.2.4 Metals and Metalloids

The metal and metalloid concentrations in runoff and leachate in this experiment were large, especially those detected in runoff samples, and we consider this to be a result of a concentration artefact of the experimental set up by using shallow trays of MWOO mixed with soil. Nevertheless, all samples were filtered to 0.45 µm and provide a valid representation of comparative dissolved metal and metalloid concentrations in leachate and runoff within this experiment. Guideline values for metal and metalloid concentrations in water in Australia are provide in Table 6.7. Comparing the results from our experiment to these guidelines provides some quantification of relative risk to water resources.

Table 6.14. Guideline values for metal and nitrogen concentrations in water with regard to freshwater species protection and healthy drinking water guidelines (ANZECC & ARMCANZ, 2000; NHMRC & NRMMC, 2011).

	Guideline values for freshwater species (% protected) (µg/L) (ANZECC & ARMCANZ, 2000)				**Drinking Water Guidelines (µg/L) (NHMRC & NRMMC, 2011)**
	99%	**95%**	**90%**	**80%**	
Arsenic					10
- **As(III)**	1	24	94	360	
- **As(V)**	0.8	13	42	140	
Cadmium	0.06	0.2	0.4	0.8	2
Chromium					50
- **Cr(III) ***	ID	ID	ID	ID	
- **Cr(VI)**	0.01	1	6	40	
Copper	1.0	1.4	1.8	2.5	2000
Nickel	8	11	13	17	20
Lead	1.0	3.4	5.6	9.4	10
Zinc	2.4	8.0	15	31	ID*
Nitrate	17	700	3400	17000	50000
Ammonia	320	900	1430	2300	ID*

* ID = insufficient data

It is evident that Cu and Zn concentrations in runoff water samples were the maximum recorded, significantly exceeding most guideline values for water resources. The other elements measured - As, , Cr, Pb and Ni - were also present in many samples above guideline concentrations but values were lower than those of Cu and Zn. Concentrations for Cd were the lowest recorded but still exceeded guidelines in some samples.

Despite these guideline exceedances, it is important to remember that this was concentrated leachate and runoff with only a small volume from a small laboratory trial so comparisons to environmental guidelines values serve only to give a comparative evaluation of relative risk. Water concentrations measured in laboratory experiments cannot directly represent field values due to dilution and attenuation processes and such data must be validated with field experiments for accurate risk assessment. Nevertheless, the results suggest there is potential for more elevated Cu and Zn concentrations in water resources with MWOO application to the two soils tested here compared with the other trace metals analysed.

Although metal fractions in soil after MWOO application have also been reported extensively in the literature (Brunori et al., 2005; Jordão et al. (2006); Tisdell & Breslin, 1995), the number of studies on metals and metalloids in runoff and leachate after MWOO application are few. Metals in runoff were examined by Glanville et al. (2004) and Martinez et al. (2003), and both studies reported that most metals were at or below detection limits, except Zn, which Glanville et al. (2004) stated increased by up to 12 times with MWOO application, and Cu, which Martinez et al. (2003) reported had concentrations in runoff of 0.01 mg/L. All Cu runoff concentrations in our experiment were higher than 0.01 mg/L.

Kaschl et al. (2002) is one of the only studies to have reported metal concentrations in leachate after MWOO application. Maximum metal concentrations reported by Kaschl et al. (2002) in leachate after MWOO application were 100 µg/L for Cu, 70 µg/L for Ni, 140 µg/L for Zn and 70 µg/L for Cr. Leachate results from our experiment recorded lower Cr concentrations in all treatments compared to Kaschl et al. (2002) but higher Cu, Ni and Zn concentrations in almost all MWOO applied treatments. The exception to this was Ni concentrations in the clay, which only exceeded the concentrations obtained by Kaschl et al. (2002) in the higher surface applied treatments.

In addition, Giusquiani et al. (1992) reported metal masses lost in leachate from an MWOO applied sandy loam and clay loam soil, following seven irrigation events 2-3 weeks apart. A comparison of the maximum results obtained from Giusquiani et al. (1992), which were generally obtained after the second or third irrigation event, with the results from our experiment, revealed that metal masses lost from our experiment were generally much lower. The exception to this was for Ni, whereby our 50 t ha^{-1} surface applied treatment and 100 t ha^{-1} incorporated treatment in the sandy loam had similar metal loss, and our 100 t ha^{-1} surface applied treatments in the clay had higher Ni metal loss.

Overall, trends for metal concentrations in runoff and leachate were that the surface applied treatments recorded higher metal concentrations than the incorporated treatments for both soils. Metal concentrations also increased with increasing application rate for both soils, and the sandy loam generally exhibited higher metal concentrations in runoff and leachate than the clay. The main difference between runoff and leachate was that metal concentrations in runoff were unaffected by incorporation of MWOO but followed the trends mentioned above for leachate.

Since runoff does not pass through the soil profile, it is likely that sorption processes may not have been as strong for runoff as leachate, particularly in the incorporated treatments. This may explain why the metal concentrations in runoff from incorporated treatments were not significantly different from the control. However, in the surface applied treatments, leachate and runoff followed the same pattern, suggesting that sorption properties had influenced metal and metalloid concentrations in runoff when MWOO was surface applied.

Initial soil tests revealed that the clay had inherently higher concentrations of all metals and metalloids except Pb, yet, results showed that the control sandy loam (and most MWOO applied treatments) had higher metal and metalloid concentrations in leachate and runoff compared with the clay. A potential reason for reduced metal and metalloid concentrations in the clay compared with the sandy loam is that the clay has higher clay content, more organic matter and hence more negative sorption sites for cationic sorption.

All of the metals and metalloids examined in this study are cations in soil and solution, except for As. Generally, adsorption of these metals and metalloids is pH dependent, with As sorption decreasing and cationic sorption increasing with increased pH (Cavallaro & McBride, 1984; Zinati et al., 2004). However in this study, pH was not significantly affected by MWOO application to the sandy loam and only the 100 t ha^{-1} surface applied treatment was impacted by MWOO application in the clay for both runoff and leachate. Despite the minimal effect of pH on the clay, any significant pH increase could increase cationic sorption, which could explain the lower runoff and leachate concentrations in the clay compared to the sandy loam (Christensen & Christensen, 2000; Temminghoff et al., 1997). However, since the pH effect in the clay was minimal and non-existent in the sandy loam, it is likely that different factors were influencing the sorption and mobility of these metals and metalloids.

One of these factors is the increase in soil organic matter following MWOO addition, which can increase the negative adsorption sites for cationic binding. This should cause a reduction in cationic metal loss through runoff and leachate, however, in our study, this was not the case. This can be explained by DOC in the runoff and leachate increasing significantly with MWOO application and the formation of complexes between DOC and cations, particularly Cd, Cu, Ni and Zn (Christensen & Christensen, 2000; Navarro-Pedreno et al., 2003). It is likely that since DOC is also negatively charged, cationic elements formed complexes with the DOC and as DOC concentrations in runoff and leachate increased, metal concentrations in runoff and leachate also increased. This was demonstrated by significant positive correlations between DOC and most metals in leachate and runoff across each application rate and each application method. Since metal mobility was often related to DOC, and DOC in runoff and leachate was consistently lower in the clay than in the sandy loam, this could be another explanation as to why the metal concentrations in runoff and leachate from the clay were generally lower than those in the sandy loam.

However, since Ni forms the strongest complexes with DOC (Christensen & Christensen, 2000), it could therefore be expected that its concentration in runoff and leachate from the sandy loam would be higher than from the clay, but this was not the case-it was the only metal with higher concentrations in clay runoff and leachate. An explanation for this may be found in the initial soil tests, which revealed that the clay had approximately nine times more Ni than the sandy loam. When MWOO was applied and additional Ni was added, the exchange in clay may have become saturated, and because Ni has been shown to have the weakest competitive adsorption, increasing Ni in the soil would have resulted in increased Ni leaching (Jalali & Jalili, 2011; Vidal et al., 2009).

In contrast to Ni, Zn forms the weakest complex with DOC, and along with Pb can be immobilised by *P* (Christensen & Christensen, 2000; Kumpiene et al., 2008). Because the clay contained more than ten times the amount of *P* than the sandy loam, this could be one reason for Zn and Pb recording lower concentrations in runoff and leachate from the clay compared to the sandy loam.

Unlike the Cd, Cu, Ni and Zn cations, Chromium is not sorbed to DOC, and its mobility depends on its speciation, which is in turn influenced by organic matter and clay content. Chromium can be found as either Cr (III), which is less toxic and relatively insoluble, and Cr (VI), which is more toxic and more soluble (Banks et al., 2006). Because organic matter is a reducing agent (Kumpiene et al., 2008), when MWOO was added to the soils, it was likely that Cr would have been present as the less mobile Cr (III).However, as with all of the metals and metalloids analysed, adding MWOO increased the total amount of metal in the soil, and since it is unlikely that all of it could sorb to the soil, Cr concentrations in runoff and leachate increased (McLean & Bledsoe, 1992). Furthermore, chromium concentrations in runoff and leachate in the clay were slightly lower than in the sandy loam, which was likely due to the clay having more organic matter and hence sorptive capacity than the sandy loam.

Arsenic, being oxyanionic in solution, would also not sorb to DOC, and as with Cr, the factors affecting sorption and mobility in soil are different. Like Cr, As may be present as two species, As (III), which is more mobile and more toxic, and As (V), which is less mobile and less toxic. The dominant species in soil is the negatively charged As (V) and its mobility in soil is pH dependent (Bissen & Frimmel, 2003; Masscheleyn et al., 1991). The pH of runoff and leachate tended to increase slightly with MWOO application, which appeared to result in increased As mobility. However, pH changes with MWOO application were generally not significant, so other soil processes may have been contributing to As mobility in runoff and leachate. It is likely that, while As would not sorb to and leach out with DOC, its sorption to the soil would have decreased with MWOO application since addition of organic matter increases the negative sorption sites in the soil (McConnell et al., 1993). This would have reduced the positive binding sites for As and as a result As concentrations in runoff and leachate increased as MWOO application rates, and hence organic matter concentration, increased. Even though the same processes are likely to occur in both soils, the clay had significantly lower As concentrations in leachate and runoff than the sandy loam. This could be explained by As being present in higher concentrations in the sandy loam before MWOO was applied, and further demonstrated by the sandy loam control appearing to have higher As concentrations in runoff and leachate compared to the control for the clay.

Overall, it appears that MWOO application to soil can result in some negative effects on the water quality of leachate and runoff. Although pH was not changed significantly, the concentration of potentially harmful parameters in concentrated leachate and runoff, such as DOC, EC, ammonia and sediment, increased significantly with MWOO application. Additionally, all metal and metalloid concentrations in the leachate and runoff increased when MWOO was applied, with some exceeding guideline levels. Based on this data we can conclude, that application of MWOO to soils has the potential to increase trace metals and metalloids in surface water and groundwater for the two NSW soils tested. Because the experiments included only one rainfall event, within a relatively short controlled experiment, evaluation of actual risk and the limitations on MWOO applications requires larger scale and field validation

6.5.3 Comparing Leachate and Runoff

For the non-metal variables, leachate generally showed higher mean results than runoff, however this was not always significant and may partly be a result of larger variability in runoff results. The higher values recorded in the leachate could also have been due to the leachate solution passing through the soil and being subject to soil sorption processes which can reduce variability. With runoff, soil processes have a reduced effect as the solution has minimal contact with the soil, and runoff can also depend on factors that were not controlled in this experiment, such as micro-topography and flow paths on the soil surface (Mayor et al., 2008).

Metal concentrations in runoff and leachate were different depending on the method of MWOO application. Runoff from the surface applied treatments contained higher concentrations of metals compared with leachate, but metal concentrations were higher in the leachate when MWOO was incorporated. However, since very different volumes of solution were collected from the leachate and runoff samples, this may not be very informative. To account for this, the total mass of each metal lost in leachate and runoff was calculated and the data showed that a significantly (in nearly all cases) larger mass of each metal was being lost in the leachate compared to the runoff, for both soils and for all metals and metalloids. . Although less than 2.5% of each metal was lost across all application rates for both soils the results suggest caution must be used for water protection close to the source, and for and repeated MWOO application.

6.6 Conclusions

The main conclusions drawn from this research are that different soil types and textures can influence metals and metalloids and the chemical constituents in runoff and leachate The MWOO application increased trace metal and metalloid concentrations in the surface water runoff and leachate of the two soils, with the sandy loam generally showing greater concentrations than the clay. In both soils, concentrations of metals and metalloids and chemical constituents in runoff and leachate tended to increase with application rate, although not always significantly. Surface application of MWOO generally resulted in significantly higher concentrations of metals and metalloids and chemical constituents in runoff and leachate, compared to incorporation of MWOO. For runoff, the control and incorporated treatments were not significantly different from each other, and variability tended to be high in the surface applied treatments. Loss of metals and metalloids tended to be higher in leachate than in runoff.

Overall, the results suggest some concern for water resources associated with application of this MWOO to soil, since metal concentrations in runoff and leachate were increased. Risk is apparently lowest with incorporation of this MWOO at a low application rate of 20 t ha^{-1} in a clay soil and higher at greater application rates especially for surface MWOO application which varies with soil type. Although dilution and attenuation processes would modify concentrations in field applications, this will depend on environmental and soil characteristics and water monitoring would be recommended to ensure water quality guidelines are not exceeded.

There is also a need for further research to examine effects across a longer time frame, with repeated and different intensity rainfall events and to consider metal and metalloid leaching from a deeper soil profile.

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7. Leaching of metals and metalloids from MWOO amended NSW soils

7.1 Summary

In this component of the work, an experiment was undertaken using a large scale column leaching experiment where leachate was collected at two depths, 15 cm and 30 cm, to assess how MWOO application using different MWOO application rates (control, 20 t ha^{-1}, 50 t ha^{-1} and 140 t ha^{-1}), different MWOO application methods (surface application and incorporation to 15 cm), different NSW soil types and textures [an acidic sandy loam (S1), a neutral clay (C1), an alkaline sandy loam (S3), a neutral silty loam (SL2) and an acidic silty loam (SL2)] and consecutive rainfall events, influenced metal and metalloid concentrations and chemical constituents in leachate. The variables that were measured for both depths included: total leachate, leachate rate, sediment yield, pH, electrical conductivity, dissolved organic carbon, nitrate, ammonia and seven metals and metalloids; arsenic, cadmium, chromium, copper, nickel, lead and zinc.

Metal and metalloid concentrations in leachate increased with application rate. The surface applied treatments typically showed higher metal and metalloid concentrations than the incorporated treatments and metal and metalloid concentrations varied depending on the leachate sampling depth. At 15 cm, metal and metalloid leachate concentrations were higher when MWOO was incorporated, however this may have been an artefact of pore water samplers being located at 15 cm and more directly in contact with the MWOO. At 30 cm, however, metal and metalloid leachate concentrations were greater when MWOO was surface applied. Generally the metal and metalloid concentrations in leachate were higher in the incorporated 15 cm samples than the surface applied 30 cm samples.

Differences in metal and metalloid concentrations in leachate between soil types were observed. In sampling event 1 metal and metalloid concentrations were generally higher in soils with high initial soil metal concentrations and the coarser textured soils. In sampling event 2 metal and metalloid concentrations in leachate were higher in soils with more DOC leaching. Despite increases in metal and metalloid concentrations with MWOO application, the total metal and metalloid lost as a percentage of what was initially present after MWOO application to soil was generally less than 1% for all metals and metalloids across all experiments.

Guideline levels for drinking water and freshwater species protection were often exceeded for the leachate collected in all treatments, especially at the higher application rates of the 50 and 140 t ha^{-1} treatments and in incorporated treatments. The leachate concentrations decreased over time suggesting an initial flush of metals in leachate from MWOO amended soils, The elements Cu, Ni and Zn generally showed a higher

percentage loss with soil leachate and at concentrations at greater % exceedance of guideline concentrations for all soils at both depths and in both sampling events.

Laboratory small-scale experiments cannot truly replicate field concentrations that may result from MWOO applications to soils due to variable environmental conditions and also dilution and attenuation processes operating. Nevertheless, the experiments here suggest some risk associated with MWOO application to certain NSW soils. The greater risk would be associated with higher MWOO application rates but the influence of MWOO on soil leachate was still evident at 20 t ha^{-1}. Consequently monitoring of leachate is recommended for MWOO applications to NSW soils. . Future research should aim to better quantify concentrations that may occur under field conditions and further explore the ecotoxicity of these concentrations in Australian soils.

7.2 Introduction

This component of the project examines the effects of MWOO application on the metal and metalloid concentration in leachate and runoff for five NSW soils with different textures. By undertaking a leaching experiment using soil columns to 30 cm depth, this research provides an understanding of how metal sorption to soil after MWOO amendment changes throughout a deep soil profile, particularly when MWOO is incorporated to the approximate plough layer of 15 cm. The differences in metal concentrations in leachate due to different MWOO application rates and different MWOO application methods were assessed. The aims of the experiment were to determine:

- the influence of MWOO addition to five different soil types on metals, metalloids and the chemical constituents in leachate
- the influence of MWOO addition on metals, metalloids and chemical constituents in leachate at different depths and with two consecutive rainfall events.

7.3 Methods

7.3.1 Soil and MWOO Characterisation

Five soils and MWOO sourced from an AWT plant in Sydney were used in this experiment. Soil types were chosen based upon differences in pH and texture. Since regulations on the use of MWOO were designed for both Minesite rehabilitation and agricultural uses (EPA, 2014) two soils used were specifically collected from a Minesite and an agricultural research station. The five soils used included a selection of those soils described in Table 2.1 comprising:

- an acidic sandy loam (referred to as the sandy loam) (S1)
- a neutral clay (referred to as the clay) (C1)
- an alkaline sandy loam (referred to as the river loam) (S3)
- a neutral silty loam (referred to as the Minesite soil) (SL2)
- an acidic silty loam (referred to as the CROA soil) (SL1).

The soils and MWOO materials used in this experiment were however, sub-sampled from the bulk samples collected at the initiation of the project. As a consequence, physical and chemical properties differ slightly, though not substantially, within each experiment. This reflects the inherent variability of the soils and materials used.

The soils were sampled, air dried and sieved to 4mm. Characterisation was undertaken for pH ($CaCl_2$), EC, texture, exchangeable cations, metals and metalloids. For pH and EC the soil was analysed using a ratio of 1:5 (soil:$CaCl_2$ for pH and soil:water for EC), shaken for 1 hour, then allowed to settle for 20-30 minutes before readings were taken using a calibrated electrode. The pH meter was a TPS model 901-CP and the EC meter was a TPS model labCHEM Conductivity- TDS- Temperature meter. Exchangeable cations were analysed by weighing 2 g of soil into a 50 mL centrifuge tube and adding 40mL of 1 M NH_4Cl before being shaken for 1 hour (Rayment & Higginson, 1992). Samples were then filtered through Whatman no. 42 filter paper and stored in a cool room until being analysed for Ca, Mg, Na and K using an ICP-OES. For metal analysis, 4.5 mL of hydrochloric acid (36%) and 1.5 mL of nitric acid (70%) were added to 0.5 g of soil, the solution was swirled and then left to pre-digest in a fume hood for 1 hour. Soils were sonified for 2 minutes and then analysed using a Milestone Ultrawave following the EPA 3051 microwave digestion method (USEPA, 2007).

7.3.2 Experimental Methods

Thirty cm lengths of polyvinyl chloride columns with an internal diameter of 9 cm and 30 cm I length were washed with phosphorus free detergent and then rinsed with deionised water. Caps for the bottom of these columns were washed using the same procedure. Five holes were drilled into the cap using a 1/8 inch drill bit and this formed the base of the column (Figure 7.1). The cap was fastened to the base of the column using packing tape with Teflon tape as a seal. Three additional holes were also drilled into the side of the column at 15 cm so that pore water samplers could be inserted into the soil. Holes were drilled at 17 cm in columns containing the clay soil to allow for expansion.

Figure 7.5. Drill holes in the caps for the bottom of the soil columns and the side of the columns for the pore water samplers.

Glass wool was placed at the bottom of the column to prevent large soil aggregates passing through the bottom of the column. The holes in the bottom of the column were initially plugged until the rainfall event occurred and leaching was required. For each column, leachate was collected at 15 cm (equivalent to MWOO incorporation depth) using three pore water samplers per column and at 30 cm using round plastic containers. Pore water samplers were assembled prior to the commencement of the experiment using hollow-fibre samplers constructed from polyacrylonitrile filter elements as outlined by Menzies and Guppy (2000) (Figure 7.2). Total leachate at 15 cm may have been limited by the capabilities of the pore water samplers, and this therefore influences any inferences and comparisons made using these results.

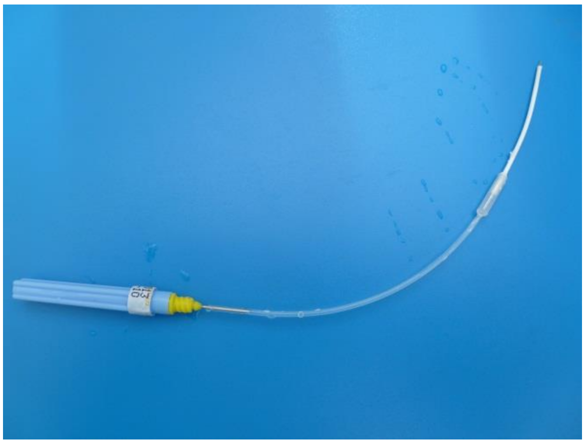


Figure 7.6. Pore water sampler.

The seven treatments applied to each soil type included three MWOO application rates (20 t ha^{-1}, 50 t ha^{-1} and 140 t ha^{-1}), two methods of application (surface applied and incorporated to 15 cm), plus a control for each soil. All treatments were set up in triplicate, giving a total of 105 soil leaching columns. Table 7.1 outlines the dry weight of MWOO required for each application rate; both application methods required the same weight of MWOO.

Table 7.13. Amount of MWOO (g) required for each of the application rates.

	MWOO (g)
Control	0
20 t ha^{-1}	12.73
50 t ha^{-1}	31.81
140 t ha^{-1}	89.08

Soil was added to 25 cm and watered (using deionised water) to 100% FC. This was achieved in stages. First, 10 cm of soil was added to the column and then the three pore water samplers were inserted. The total amount of water required to fill the columns to 100% FC was weighed and a portion of this was added to the column to ensure the pore water samplers remained wet. For the control and surface applied treatments the remaining 15 cm of soil was added, then, for the surface applied treatments the appropriate amount of MWOO was weighed and place on top of the soil column. For the incorporated treatments the remaining top 15 cm of soil required was mixed with the appropriate weight of MWOO (shaken in a bag until uniformly mixed), then added to the column. All columns were then watered using the remaining water.

Columns were left overnight and the following morning were weighed and watered to 100% FC if necessary. Columns were then left to equilibrate for 24 hours before the rainfall event. Deionised water was used for the rainfall, with 65 mL of ‘rainfall’ being added to each column. This is equivalent to a 100 mm rainfall event (BOM, 2014). After the ‘rainfall’ was added a 16 x 100 mm BD Vacutainer Urinalysis Plus Urine Tube with a 10 mL draw volume, with no additive and a yellow stopper, was attached to the pore water samplers to collect leachate (Figure 7.3).

Figure 7.7. The BD Vacutainer Urinalysis Plus Urine Tubes used to collect leachate at 15 cm from the pore water samplers.

Round plastic containers were used to collect leachate at 30 cm and smaller plastic platforms were placed inside these containers to elevate the soil columns (Figure 7.4). All containers were acid washed prior to use. Leachate was collected for 24 hours. Two sampling events were undertaken with a two week interval between, following the method of Giusquiani et al. (1992). Soil columns were maintained at 80% FC between events. Columns were brought to 100% FC before the second sampling event to be consistent with the first event. This second rainfall event and sampling followed the same methods for event 1.

Figure 7.8. Set up of one soil column with the plastic container at the bottom to collect leachate and pore water samplers at 15 cm.

7.3.3 Laboratory Methods

After 24 hours the leachate from the three pore water samplers for each sample was combined and the leachate from 15 cm and from 30 cm were placed in separate acid washed yellow topped vials and their weights recorded. For all samples, pH (TPS 901-CP pH meter) and EC (TPS labCHEM Conductivity- TDS-Temperature meter) were measured after the readings had stabilised. Dissolved organic carbon, nitrate and ammonia analysis was undertaken after samples had been diluted, to reduce surfactant interference, by weighing out 5 g of leachate and then adding 25 g of deionised water to give a dilution ratio of 1:5. Samples were stored at 4°C until analysis. Measurements for DOC were taken and recorded electronically using the Sievers InnovOx Laboratory TOC Analyzer. For nitrate and ammonia, analysis was undertaken using a San++ Automated Wet Chemistry Analyzer manufactured by Skalar.

Some leachate samples from 30 cm appeared cloudy, which indicated that suspended particles passed through the glass wool into the leachate sample. These samples were collected using a syringe then filtered through 0.45 µm filter paper. For samples to be analysed for their metal content, nitric acid was added to the samples to lower pH to less than 2 to preserve the samples. Analysis took place at ALS, Brisbane, using ICP-MS. All samples were stored at 4°C until analysis. Detection limits for metals and metalloids, as identified by ALS, were: 1 µg/L for As, Cr, Cu, Ni and Pb, 0.1 µg/L for Cd and 5 µg/L for Zn.

7.3.4 Statistical Analysis

Statistical analysis was undertaken in Rstudio using version 3.0.3 of R (R Core Team, 2014). Analysis of variance (ANOVA) was performed and statistically significant differences were indicated by p-values <0.05. Assumptions of the model, approximate normality and equal variances were checked, and where necessary the appropriate transformations were undertaken. Values identified as outliers were removed from the data if standardised residuals were less than or greater than 3 in the normal Q-Q diagnostics plot (Simkiss et al., 2014). Application rate and method were combined into one factor for statistical analysis.

If treatments recorded metal concentrations at or below detection limits for all three replicates, analysis was undertaken using a non-parametric kruskal-wallis test. All significant differences were analysed using Tukey's post-hoc comparison test. Statistical analyses were undertaken for each variable to determine significant differences between treatments for each soil at both depths for both sampling events, between depths for each soil for both sampling events, and between soil types (at 15 cm for pH, DOC and the metals and metalloids only) for both sampling events and between sampling events. Total metal lost as a mass and as a percentage of the total metal initially present in the soil/MWOO treatment was also calculated and statistical analysis was undertaken to determine differences between depths for each soil at each sampling event.

7.4 Results

7.4.1 Soil and MWOO characterisation

The soil and MWOO characterisation is provided in Table 7.2. The pH in the soils ranged from 4.44 in the sandy loam to 7.26 in the river loam. The Minesite soil had the highest mean salinity at 243.43 µS/ cm, and the river loam the lowest mean salinity of the soils used at 122.37 µS/ cm. Elevated As concentration was evident in the sandy loam and elevated Cd, Cr and Ni concentrations were recorded in the clay. The other soils had low metal concentrations.

The pH of the MWOO was neutral at just above 7 and the EC would be considered high at 6220 µS/ cm. The MWOO also had relatively high N and C content as well as high concentrations of exchangeable cations. Phosphorus was also present in MWOO at high concentrations. High Cu, Pb and Zn concentrations were also found in the MWOO.

Table 7.14. Characterisation of the sandy loam, clay, river loam, minesite and CROA soils and MWOO (mean ± SE).

	Sandy Loam (S1)	Clay (C1)	River Loam (S3)	Minesite (SL2)	CROA (SL1)	MWOO
pH	4.44 ± 0.21	6.21 ± 0.04	7.26 ± 0.09	6.19 ± 0.01	5.99 ± 0.06	7.36 ± 0.16
EC (µS/ cm)	126.37 ± 0.62	154.47 ± 6.30	122.37 ± 0.87	243.43 ± 3.98	150.50 ± 0.82	6220 ± 0.15
Clay (%)	13.2	55.0	13.7	14.3	27.6	-
Silt (%)	10.5	29.2	12.0	29.2	41.4	-
Sand (%)	76.3	56.5	74.3	56.5	30.0	-
Soil Texture	Sandy loam	Clay	Sandy loam	Silty loam	Silty loam	-
Nitrogen (%)	0.04 ± 0.00	0.25 ± 0.00	0.09 ± 0.00	0.11 ± 0.00	0.16 ± 0.01	1.46 ± 0.09
Carbon (%)	0.49 ± 0.00	3.34 ± 0.01	1.09 ± 0.00	1.69 ± 0.05	2.05 ± 0.02	24.90 ± 1.88
Ca (cmol$_+$/kg)	1.017 ± 0.014	25.55 ± 0.16	12.13 ± 0.22	4.66 ± 0.09	4.81 ± 0.12	28.014 ± 0.45
K (cmol$_+$/kg)	0.09 ± 0.00	0.85 ± 0.02	0.41 ± 0.01	0.41 ± 0.01	0.58 ± 0.01	12.45 ± 0.30
Mg (cmol$_+$/kg)	1.12 ± 0.01	24.09 ± 0.14	3.50 ± 0.05	5.81 ± 0.15	4.63 ± 0.11	7.00 ± 0.14
Na (cmol$_+$/kg)	0.24 ± 0.01	0.35 ± 0.01	0.10 ± 0.01	0.95 ± 0.03	0.30 ± 0.01	24.53 ± 0.67
P (µg/g)	6.22 ± 0.24	77.19 ± 7.59	88.49 ± 6.88	10.87 ± 0.19	17.33 ± 0.58	584.20 ± 16.36
Al (%)	1.49 ± 0.06	5.19 ± 0.09	2.34 ± 0.11	2.57 ± 0.02	3.52 ± 0.09	0.95 ± 0.01
Fe (%)	1.32 ± 0.06	6.41 ± 0.10	2.72 ± 0.11	3.24 ± 0.18	3.19 ± 0.30	0.93 ± 0.04
As (mg kg^{-1})	66.49 ± 4.89	1.29 ± 0.55	4.27 ± 0.25	6.39 ± 0.60	7.70 ± 0.82	5.64 ± 0.45
Cd (mg kg^{-1})	0.00 ± 0.00	3.73 ± 0.10	0.65 ± 0.09	0.98 ± 0.16	0.90 ± 0.23	1.10 ± 0.09
Co (mg kg^{-1})	3.01 ± 0.11	64.93 ± 1.34	11.39 ± 0.60	5.92 ± 0.16	14.87 ± 0.22	3.61 ± 0.20
Cr (mg kg^{-1})	37.61 ± 1.29	195.11 ± 4.22	50.46 ± 7.30	41.96 ± 2.40	45.14 ± 3.43	88.78 ± 18.10
Cu (mg kg^{-1})	15.50 ± 0.45	45.60 ± 0.68	21.24 ± 1.03	8.66 ± 0.04	17.05 ± 0.25	202.69 ± 52.82
Mn (mg kg^{-1})	166.11 ± 3.38	1709.67 ± 22.92	533.84 ± 28.60	323.73 ± 19.76	1129.05 ± 20.63	232.51 ± 4.78
Ni (mg kg^{-1})	15.11 ± 0.27	124.77 ± 1.61	29.85 ± 3.23	16.04 ± 0.73	14.92 ± 0.20	32.01 ± 6.68
Pb (mg kg^{-1})	9.38 ± 0.68	4.08 ± 0.22	4.75 ± 0.53	12.07 ± 0.31	21.11 ± 1.10	303.02 ± 29.80
Sb (mg kg^{-1})	2.71 ± 1.75	3.36 ± 0.76	1.00 ± 0.36	1.35 ± 0.62	1.20 ± 0.47	3.57 ± 0.43
Zn (mg kg^{-1})	21.28 ± 0.55	86.57 ± 1.56	50.64 ± 2.53	37.01 ± 0.89	32.19 ± 0.30	831.97 ± 287.15

7.4.2 Sandy Loam

In sampling event 1, leachate from the pore water samplers located at 15 cm in the sandy loam indicated significant differences between treatments for pH ($F_{6,13}$ = 11.35, *p* = 1.6x10^{-4}), EC ($F_{6,14}$ = 126.13, *p* = 2.3x10^{-11}), DOC ($F_{6,14}$ = 43.22, *p* = 3.04x10^{-8}) and ammonia ($F_{6,14}$ = 34.53, *p* = 1.31x10^{-7}). Generally, the incorporated treatments, but not the surface applied treatments, were significantly different from the control (see Table 7.3). For example, the pH in leachate from the 50 and 140 t ha^{-1} incorporated treatments was significantly lower than the control and surface applied treatments. For EC, DOC and ammonia, leachate from the incorporated treatments contained significantly higher concentrations of each compared to the control and surface applied treatments, and mean results increased as application rate increased (significantly so in most cases) (see Table 7.3). Nitrate concentrations were not significantly influenced by MWOO application despite all three incorporated treatments recording a lower mean nitrate than the control. Total leachate was also not significantly influenced by MWOO application.

The results from leachate collected at 30 cm in sampling event 1 were significantly different between treatments for pH ($F_{6,14}$ = 2.96, *p* = 0.04) and ammonia ($F_{6,14}$ = 3.40, *p* = 0.03) only. The 50 t ha^{-1} surface applied treatment recorded the lowest pH (6.57) and the 50 t ha^{-1} incorporated treatment the highest pH (7.48) (Table 7.3). This 50 t ha^{-1} incorporated treatment plus the 140 t ha^{-1} surface applied treatment (pH 7.45) were significantly higher than the lowest pH recorded for the 50 t ha^{-1} surface applied treatment. Ammonia concentrations were highest in the 140 t ha^{-1} incorporated treatment (1.82 µg/mL) and this was significantly higher than the 140 t ha^{-1} surface applied (0.32 µg/mL) and 50 t ha^{-1} incorporated treatments (0.32 µg/mL). As for the 15 cm depth leachate, EC and DOC tended to be higher in the incorporated treatments, however, in this case, they were not significantly different from the control nor the surface applied treatments. In contrast to the 15 cm depth leachate, nitrate concentrations tended to be lower in the surface applied MWOO treatments rather than the incorporated treatments, but again, significant results were not obtained. Total leachate at 30 cm tended to be lower in the incorporated MWOO application rates, however, this was also not significant.

The chemical characteristics of the leachate collected at the two different depths in sampling event 1 were significantly different for most of the variables measured. For example, pH ($F_{13,27}$ = 49.56, *p* = 2.15x10^{-15}) was significantly higher across all treatments in leachate at 30 cm compared to 15 cm and nitrate followed the same trend ($F_{13,28}$ = 10.21, *p* = 1.94x10^{-7}). Similarly, EC ($F_{13,28}$ = 35.25, *p* = 7.14x10^{-14}) and DOC ($F_{13,27}$ = 27.68, *p* = 3.12x10^{-12}) were higher at 30 cm, but in this case, only for the control and the surface applied treatments; for the incorporated treatments, these appeared higher at 15 cm. Ammonia generally showed higher mean concentrations at 15 cm, however this trend was only significant with the 50 and 140 t ha^{-1} incorporated treatments ($F_{13,28} = 26.57$, $p = 2.57 \times 10^{-12}$). Similar trends for all of these chemical characteristics were observed in sampling event 2 (Table 7.4).

The differences between the two sampling events were significant for most variables. Changes in pH between the two sampling events were evident with pH increasing in all incorporated treatments at 15 cm (from pH 5.4, 5.0 and 4.7 to 5.9, 5.9 and 6.3 for the 20, 50 and 140 t ha^{-1} treatments respectively). The pH at 15 cm for the control and surface applied 20 t ha^{-1} treatments decreased (from pH 5.5 and 5.4 to 5.0 and 5.2 respectively), the 50 t ha^{-1} surface applied remained the same (pH 5.4), and the 140 t ha^{-1} surface applied increased only slightly (pH 5.5 to 5.7). In contrast, the pH in leachate at 30 cm consistently decreased for all treatments.

For EC, the incorporated treatments tended to record a decrease in leachate EC at sampling event 2 at 15 cm, whereas the control and the surface applied treatments were very similar. Again, as for pH, EC in leachate at 30 cm decreased in nearly all treatments at sampling event 2 (only EC in the leachate from the 140 t ha^{-1} incorporated treatment increased).

All treatments recorded lower DOC concentrations at sampling event 2 for both depths, but particularly for the incorporated treatments at 15 cm (as was the case for EC and increases recorded for pH). Ammonia concentrations in leachate tended to be higher in sampling event 2 across both depths for the surface applied treatments, as it was for the control. For the incorporated treatments, leachate at sampling event 2 also increased consistently at 30 cm but at 15 cm the treatments were similar, or, in the case of the 140 t ha^{-1} treatment, leachate ammonia concentrations decreased.

Nitrate concentrations appeared similar across both sampling events in the control and surface applied treatments at 15 cm but in the incorporated treatments nitrate concentrations decreased considerably in sampling event 2 (as occurred for EC, DOC and increases in pH). For all MWOO applied treatments at 30 cm, nitrate concentrations decreased in sampling event 2.

Total leachate did not appear to differ between the two sampling events at either depth, except for both 20 t ha^{-1} treatments at 30 cm, whereby sampling event 2 recorded higher leachate volumes than sampling event 1.

Table 7.15. The sandy loam leachate results for non-metal variables from sampling event 1 (mean ± standard error).

			Surface			**Incorporated**		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	23.65 ± 2.90^{a}	26.28 ± 0.59^{a}	26.92 ± 0.45^{a}	26.48 ± 0.14^{a}	22.29 ± 2.51^{a}	26.30 ± 0.21^{a}	21.98 ± 2.29^{a}
15 cm	**pH**	5.54 ± 0.11^{b}	5.40 ± 0.02^{b}	5.44 ± 0.05^{b}	5.46 ± 0.01^{b}	5.40 ± 0.15^{b}	4.96 ± 0.08^{a}	4.73 ± 0.06^{a}
15 cm	**EC (µS/ cm)**	318.67 ± 42.06^{a}	452.67 ± 28.26^{ab}	377.67 ± 29.69^{a}	406.00 ± 25.24^{ab}	996.00 ± 146.40^{b}	1749.67 ± 162.93^{c}	4136.67 ± 235.11^{d}
15 cm	**DOC (µg/mL)**	37.63 ± 3.12^{a}	44.17 ± 3.74^{a}	43.03 ± 3.95^{a}	47.07 ± 3.57^{a}	206.00 ± 59.71^{b}	361.60 ± 64.93^{b}	1003.20 ± 216.65^{c}
15 cm	**Nitrate (µg/mL)**	26.70 ± 4.59^{a}	27.84 ± 2.31^{a}	24.86 ± 1.06^{a}	29.14 ± 2.71^{a}	21.34 ± 0.26^{a}	19.82 ± 3.94^{a}	17.96 ± 4.54^{a}
15 cm	**Ammonia (µg/mL)**	1.4 ± 0.11^{ab}	1.28 ± 0.20^{ab}	1.02 ± 0.15^{a}	1.2 ± 0.19^{a}	3.18 ± 0.96^{bc}	6.16 ± 1.27^{c}	23.55 ± 1.23^{d}
30 cm	**Total leachate (mL)**	45.71 ± 13.58^{a}	50.62 ± 10.25^{a}	49.51 ± 4.96^{a}	39.61 ± 4.63^{a}	25.11 ± 2.47^{a}	18.75 ± 5.89^{a}	25.07 ± 4.96^{a}
30 cm	**pH**	7.08 ± 0.18^{ab}	7.16 ± 0.25^{ab}	6.57 ± 0.19^{a}	7.45 ± 0.03^{b}	7.21 ± 0.06^{ab}	7.48 ± 0.19^{b}	7.02 ± 0.30^{ab}
30 cm	**EC (µS/ cm)**	928.33 ± 266.06^{a}	809.67 ± 192.03^{a}	806.67 ± 93.85^{a}	963.67 ± 104.96^{a}	1179.33 ± 180.48^{a}	1236.00 ± 94.54^{a}	1592.67 ± 283.56^{a}
30 cm	**DOC (µg/mL)**	71.93 ± 14.87^{a}	55.80 ± 6.64^{a}	60.13 ± 5.59^{a}	68.00 ± 3.70^{a}	90.93 ± 17.45^{a}	72.00 ± 1.83^{a}	88.80 ± 18.60^{a}
30 cm	**Nitrate (µg/mL)**	118.32 ± 26.83^{a}	79.34 ± 23.66^{a}	80.44 ± 16.06^{a}	98.32 ± 9.74^{a}	105.20 ± 8.20^{a}	121.68 ± 9.14^{a}	100.32 ± 9.30^{a}
30 cm	**Ammonia (µg/mL)**	0.84 ± 0.18^{ab}	0.68 ± 0.18^{ab}	0.60 ± 0.16^{ab}	0.32 ± 0.26^{a}	0.98 ± 0.37^{ab}	0.32 ± 0.17^{a}	1.82 ± 0.50^{b}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

Table 7.16. The sandy loam leachate results for non-metal variables from sampling event 2 (mean ± standard error).

			Surface			Incorporated		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	20.88 ± 2.88^{a}	21.49 ± 2.23^{a}	26.89 ± 0.09^{a}	24.12 ± 2.95^{a}	21.66 ± 2.60^{a}	17.93 ± 5.06^{a}	22.83 ± 1.67^{a}
15 cm	**pH**	5.00 ± 0.05^{a}	5.16 ± 0.08^{ab}	5.43 ± 0.04^{bc}	5.70 ± 0.07^{cd}	5.89 ± 0.03^{d}	5.89 ± 0.06^{d}	6.25 ± 0.13^{e}
15 cm	**EC (µS/ cm)**	318.33 ± 26.77^{a}	544.00 ± 29.05^{b}	389.00 ± 7.02^{ab}	460.00 ± 59.88^{ab}	583.33 ± 40.39^{b}	896.00 ± 94.05^{c}	2359.00 ± 202.16^{d}
15 cm	**DOC (µg/mL)**	28.24 ± 1.59^{ab}	35.86 ± 5.27^{ab}	21.10 ± 1.18^{a}	24.24 ± 3.21^{a}	36.28 ± 6.01^{ab}	73.14 ± 26.34^{b}	211.40 ± 12.25^{c}
15 cm	**Nitrate (µg/mL)**	30.56 ± 4.88^{b}	22.99 ± 5.12^{b}	18.12 ± 5.44^{b}	21.26 ± 9.70^{b}	19.23 ± 5.72^{b}	13.29 ± 10.06^{ab}	0.88 ± 0.52^{a}
15 cm	**Ammonia (µg/mL)**	2.47 ± 0.19^{a}	3.23 ± 0.08^{ac}	2.95 ± 0.12^{ab}	3.55 ± 0.02^{bc}	3.97 ± 0.18^{c}	5.72 ± 0.26^{d}	9.91 ± 1.61^{e}
30 cm	**Total leachate (mL)**	86.31 ± 3.85^{c}	69.90 ± 11.52^{bc}	48.17 ± 6.25^{ab}	35.02 ± 8.63^{ab}	50.14 ± 4.67^{ac}	45.91 ± 11.59^{ab}	24.44 ± 3.43^{a}
30 cm	**pH**	4.97 ± 0.04^{a}	5.21 ± 0.11^{a}	5.41 ± 0.08^{ab}	5.82 ± 0.20^{bc}	5.87 ± 0.03^{bc}	5.86 ± 0.05^{bc}	6.28 ± 0.13^{c}
30 cm	**EC (µS/ cm)**	370.00 ± 39.07^{a}	586.00 ± 92.00^{ab}	534.00 ± 111.33^{ab}	637.33 ± 182.22^{ab}	616.33 ± 39.60^{ab}	1020 ± 79.74^{b}	2293.67 ± 141.93^{c}
30 cm	**DOC (µg/mL)**	32.64 ± 2.61^{a}	33.94 ± 5.34^{a}	25.74 ± 1.42^{a}	40.02 ± 3.67^{ab}	34.76 ± 4.58^{a}	39.62 ± 6.87^{ab}	74.64 ± 15.73^{b}
30 cm	**Nitrate (µg/mL)**	36.73 ± 6.33^{a}	34.43 ± 15.92^{a}	43.06 ± 18.41^{a}	51.08 ± 20.90^{a}	28.96 ± 6.45^{a}	43.18 ± 8.03^{a}	44.86 ± 16.91^{a}
30 cm	**Ammonia (µg/mL)**	2.14 ± 0.26^{a}	2.75 ± 0.06^{ab}	2.42 ± 0.24^{ab}	1.58 ± 0.47^{a}	2.50 ± 0.28^{ab}	2.46 ± 0.35^{ab}	6.09 ± 1.40^{b}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

7.3.2.1. Metals and Metalloids

Metal results for the leachate collected from the sandy loam at 15 and 30 cm in sampling event 1 are provided in Figure 7.5. Copper and Zn recorded the highest metal concentrations at both depths in sampling event 1, followed by Ni, Cr, Pb, As and Cd. For all metals in leachate from the sandy loam at 15 cm, the surface applied MWOO treatments were not significantly different from the control. However, metal concentrations in leachate at 15 cm from the incorporated treatments were generally significantly higher than both the control and surface applied treatments, except for the 20 t ha^{-1} incorporated application rate for Cd, Pb and Zn. Within the incorporated treatments, metals and metalloid concentrations in leachate from 20 and 50 t ha^{-1} application rates were generally the same, except for Cd, but the 140 t ha^{-1} rate was consistently significantly higher than all other treatments.

In sampling event 1, the effect of MWOO application was less apparent on metal leachate concentrations collected at 30 cm. There was no significant difference in metal concentrations across application rates or application methods for any metal except Ni. For Ni, the 140 t ha^{-1} incorporated treatment was significantly higher than the 20 t ha^{-1} surface applied application rate ($F_{6,14}$ = 3.81, p = 0.018). There were no other significant differences.

For the 15 and 30 cm depths, leachate response patterns were similar across all metals. Generally, mean metal concentrations in the leachate collected from the bottom of the soil profile (30 cm) was slightly higher that that at 15 cm for both the control and the surface applied treatments. However, mean metal concentrations in leachate collected from the incorporated treatments was higher at 15 cm. When these volumes were converted into the total metal lost in leachate as a mass and as a percentage of the total metal initially in the soil/MWOO treatment, the main finding was that metal mass lost at both depths was low and the percentage of each metal lost was less than 0.1% of what was initially present in the soil/MWOO treatment.

Generally, similar patterns between treatments were seen in sampling event 2, except for Zn, Pb and Cd, which showed no significant differences between treatments or depths (Figure 7.6). This could have been due to large variability between replicates within treatments for all three, and also the number of samples at or below detection limits for Cd and Pb. The percentage of total metal loss in sampling event 2 was less than 0.1% for all metals. Further comparison between sampling event 1 and sampling event 2 for the sandy loam is outlined in section 7.4.7 in conjunction with the other soils, since differences between the two sampling events were generally consistent across soils.

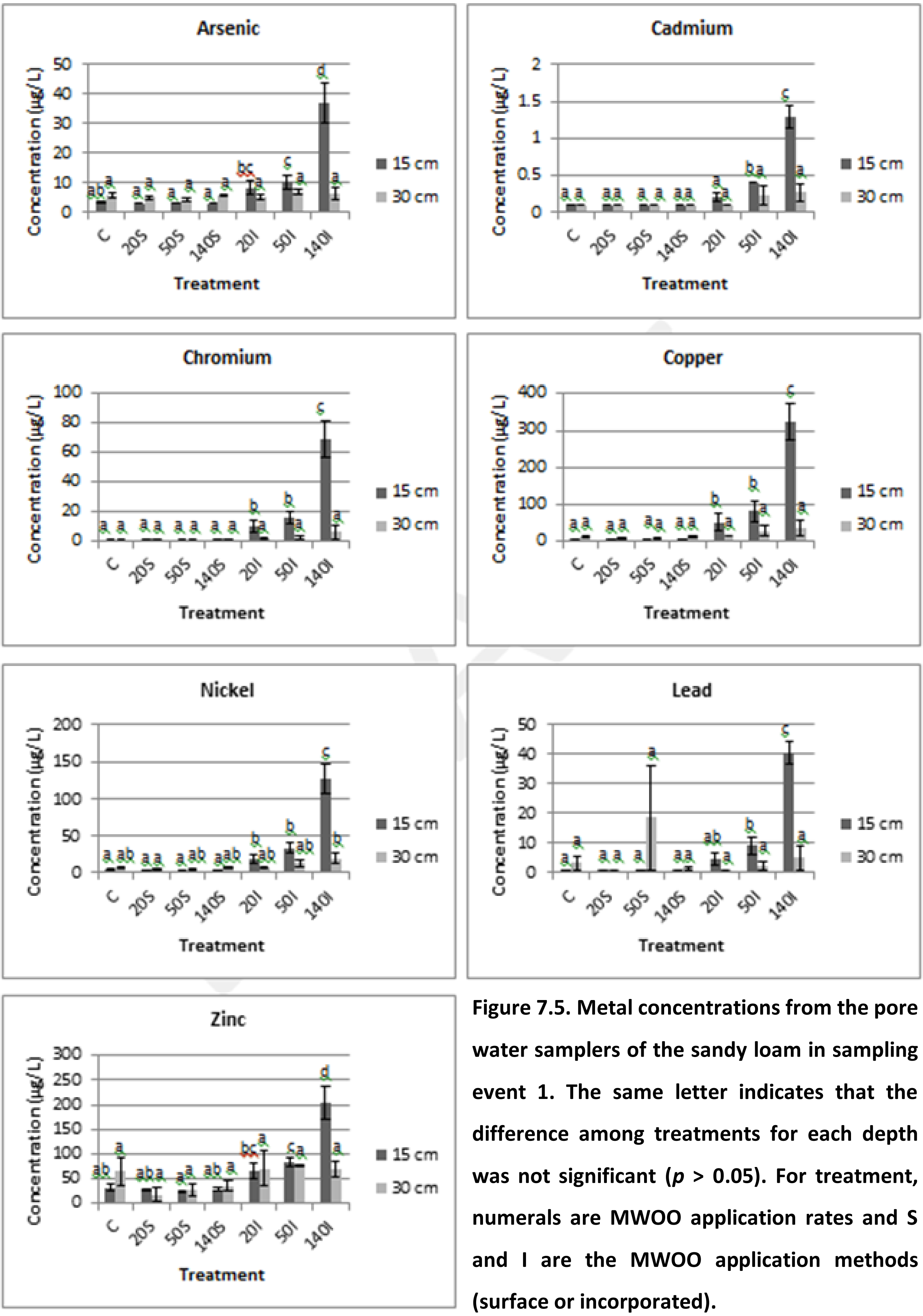
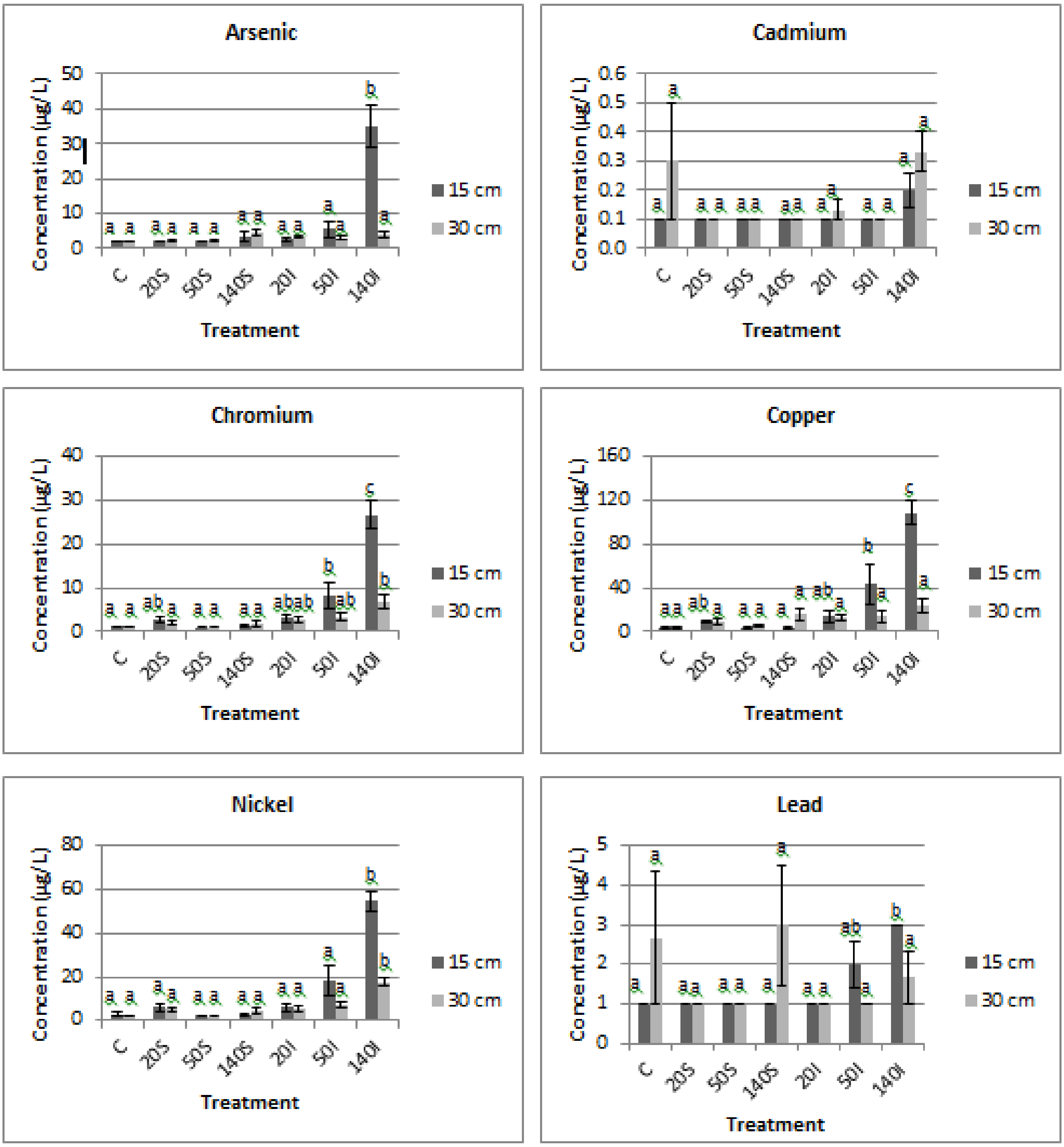


Figure 7.5. Metal concentrations from the pore water samplers of the sandy loam in sampling event 1. The same letter indicates that the difference among treatments for each depth was not significant (*p* > 0.05). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).



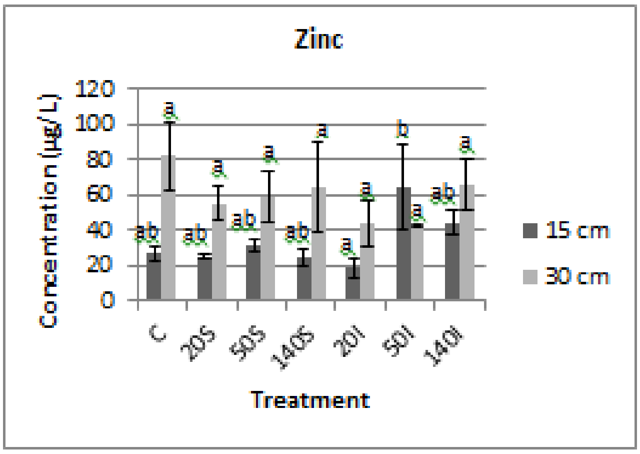


Figure 7.6. Metal concentrations from the pore water samplers of the sandy loam in sampling event 2. The same letter indicates that the difference among treatments for each depth was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

7.4.3 Clay

Results for the leachate collected at 15 cm from the clay soil with MWOO applied are provided in Table 7.5. Of the non-metal variables measured, pH ($F_{6,14} = 4.15$, $p = 0.01$) and EC ($F_{6,14} = 86.48$, $p = 2.97\text{x}10^{-10}$) in leachate at 15 cm were significantly different following MWO application. However, for pH, the difference was not with the control, which was statistically the same as all treatments, but between the 140 t ha^{-1} incorporated treatment (pH of 7.04) and the 50 and 140 t ha^{-1} surface applied treatments (pH of 6.41 and 6.49, respectively). For EC, leachate in the incorporated treatments was significantly higher than the control, and as application rate increased, so too did the mean EC. However, EC from the surface applied treatments were generally not significantly different from the control.

Total leachate tended to be lower in the incorporated treatments compared to the control, however, this was not significantly different for any treatments. Non-significant trends were also apparent with DOC and nitrate, where the control had the lowest mean DOC concentration and highest mean nitrate concentration. There were no obvious or significant trends present for ammonia, however the 50 t ha^{-1} incorporated treatment had the lowest ammonia concentration.

For most non-metal variables, MWOO application generally had no significant effect on leachate characteristics at 30 cm, except for total leachate and DOC (Table 7.5). The greatest leachate volume was collected from the bottom of the 50 t ha^{-1} surface applied treatment (74 mL) and was significantly higher than the 140 t ha^{-1} incorporated treatment, which had the lowest amount of leachate collected (42 mL) ($F_{6,14} = 3.52$, $p = 0.025$). The only significant difference in mean DOC concentrations was that the 50 t ha^{-1} incorporated treatment (314 µg/mL) had significantly higher mean DOC concentrations than the 140 t ha^{-1} incorporated treatment (165 µg/mL) ($F_{6,14} = 2.90$, $p = 0.047$). The control showed the highest mean pH and the lowest mean EC at 30 cm in the clay, however this was not significantly different from the other treatments. There was little evidence of change for nitrate or ammonia concentrations in leachate from the clay at 30 cm in sampling event 1.

A comparison of results at both depths, showed that total leachate ($F_{13,28} = 23.82$, $p = 1.01\text{x}10^{-11}$) and pH ($F_{13,28} = 7.54$, $p = 4.31\text{x}10^{-6}$) were significantly higher in the leachate at 30 cm compared with the top 15 cm for the 50 and 140 t ha^{-1} surface applied treatments for pH and for all treatments for total leachate (Table 7.5). However, total leachate at 15 cm was limited by the pore water samplers, making comparisons between depths difficult. Electrical conductivity was significantly higher at 15 cm for the 50 and 140 t ha^{-1} incorporated treatments ($F_{13,28} = 48.63$, $p = 1.05\text{x}10^{-15}$). Nitrate and DOC were not significantly different across depths, however ammonia concentrations were significantly higher at 15 cm in the 50 and 140 t ha^{-1} incorporated treatments ($F_{6,14} = 26.57$, $p = 2.57\text{x}10^{-12}$).

Similar patterns were recorded in sampling event 2 (Table 7.6), however, the differences between the two sampling events were of far greater significance than differences between treatments. Differences in pH were consistent across both depths and all treatments, with pH tending to be higher in sampling event 2 than sampling event 1. Sampling event 2 tended to show a higher EC across all treatments at both depths, except with the incorporated 15 cm treatments, which were all similar to results for sampling event 1. Concentrations of DOC were higher in sampling event 1 across both depths and all treatments, apart from the 140 t ha^{-1} incorporated treatment at 15 cm which recorded a higher DOC concentration in sampling event 2. With the exception of the 140 t ha^{-1} incorporated treatment at 30 cm, which recorded similar nitrate concentrations for both sampling events, nitrate and ammonia concentrations were otherwise much higher in sampling event 2 than sampling event 1 across both depths and all treatments-this was in contrast to DOC but similar to patterns for pH. Total leachate was generally not different between the two sampling events at either depth across all treatments.

Table 7.17. The clay leachate results for non-metal variables from sampling event 1 (mean ± standard error).

			Surface			Incorporated		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	24.58 ± 0.97^{a}	23.8 ± 0.58^{a}	25.02 ± 0.10^{a}	21.18 ± 3.63^{a}	21.29 ± 2.79^{a}	18.9 ± 2.66^{a}	17.14 ± 0.43^{a}
15 cm	**pH**	6.71 ± 0.07^{ab}	6.62 ± 0.08^{ab}	6.41 ± 0.07^{a}	6.49 ± 0.16^{a}	6.58 ± 0.15^{ab}	6.77 ± 0.06^{ab}	7.04 ± 0.05^{b}
15 cm	**EC (µS/ cm)**	386.00 ± 28.01^{a}	560.00 ± 113.97^{ab}	1058.67 ± 125.89^{b}	987.67 ± 199.47^{ab}	1165.00 ± 171.26^{b}	2434.00 ± 75.05^{c}	4020.00 ± 190.79^{d}
15 cm	**DOC (µg/mL)**	130.00 ± 5.60^{a}	166.60 ± 15.68^{a}	205.60 ± 36.79^{a}	255.20 ± 30.58^{a}	219.30 ± 33.51^{a}	189.00 ± 32.36^{a}	279.30 ± 18.30^{a}
15 cm	**Nitrate (µg/mL)**	0.88 ± 0.45^{a}	0.24 ± 0.03^{a}	0.32 ± 0.04^{a}	0.24 ± 0^{a}	0.34 ± 0.1^{a}	0.24 ± 0^{a}	0.24 ± 0^{a}
15 cm	**Ammonia (µg/mL)**	0.22 ± 0.05^{a}	0.1 ± 0.09^{a}	0.24 ± 0.24^{a}	0.14 ± 0.07^{a}	0.24 ± 0.24^{a}	0.06 ± 0.06^{a}	0.12 ± 0.07^{a}
30 cm	**Total leachate (mL)**	68.45 ± 5.36^{ab}	64.81 ± 6.41^{ab}	73.61 ± 3.74^{b}	52.50 ± 8.99^{ab}	69.97 ± 1.52^{ab}	57.57 ± 6.05^{ab}	41.65 ± 7.48^{a}
30 cm	**pH**	7.16 ± 0.09^{a}	7.08 ± 0.05^{a}	6.98 ± 0.05^{a}	7.05 ± 0.07^{a}	6.91 ± 0.04^{a}	7.03 ± 0.08^{a}	7.06 ± 0.12^{a}
30 cm	**EC (µS/ cm)**	646.67 ± 91.30^{a}	658.33 ± 17.24^{a}	751.67 ± 100.18^{a}	815.33 ± 41.01^{a}	950.33 ± 114.44^{a}	1178.33 ± 128.55^{a}	986.33 ± 272.88^{a}
30 cm	**DOC (µg/mL)**	270.30 ± 43.02^{ab}	279.60 ± 23.98^{ab}	275.73 ± 32.31^{ab}	295.00 ± 10.96^{ab}	297.60 ± 20.19^{ab}	314.20 ± 32.64^{b}	165.80 ± 28.48^{a}
30 cm	**Nitrate (µg/mL)**	0.24 ± 0^{a}	0.24 ± 0^{a}	0.24 ± 0^{a}	0.24 ± 0^{a}	0.24 ± 0^{a}	0.24 ± 0^{a}	0.24 ± 0^{a}
30 cm	**Ammonia (µg/mL)**	0.14 ± 0.11^{a}	0.00 ± 0.03^{a}	0.12 ± 0.07^{a}	0.24 ± 0.16^{a}	0.14 ± 0.05^{a}	0.06 ± 0.06^{a}	0.00 ± 0^{a}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

Table 7.18. The clay leachate results for non-metal variables from sampling event 2 (mean ± standard error).

			Surface			Incorporated		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	26.77 ± 0.16^{b}	23.72 ± 2.64^{b}	23.10 ± 2.51^{b}	22.52 ± 2.09^{ab}	23.71 ± 3.01^{b}	23.01 ± 2.91^{b}	11.29 ± 1.63^{a}
15 cm	**pH**	7.23 ± 0.06^{bc}	7.03 ± 0.04^{ab}	6.87 ± 0.04^{ab}	7.00 ± 0.12^{ab}	6.68 ± 0.11^{a}	7.05 ± 0.07^{ab}	7.36 ± 0.30^{c}
15 cm	**EC (µS/ cm)**	1000.00 ± 74.11^{ab}	1392.33 ± 35.53^{ac}	1807.00 ± 57.66^{bc}	2179.00 ± 462.81^{c}	950.33 ± 83.10^{a}	1463.67 ± 94.91^{ac}	4586.67 ± 994.99^{d}
15 cm	**DOC (µg/mL)**	29.06 ± 1.20^{a}	30.16 ± 0.82^{a}	31.41 ± 1.09^{a}	73.62 ± 34.72^{a}	40.12 ± 2.06^{a}	73.72 ± 13.64^{a}	419.40 ± 196.65^{b}
15 cm	**Nitrate (µg/mL)**	93.48 ± 7.58^{c}	103.65 ± 2.62^{c}	95.14 ± 8.23^{c}	47.48 ± 23.32^{bc}	33.25 ± 16.63^{bc}	3.12 ± 2.56^{ab}	0.13 ± 0.04^{a}
15 cm	**Ammonia (µg/mL)**	1.04 ± 0.34^{ab}	0.94 ± 0.06^{ab}	1.01 ± 0.36^{ab}	4.81 ± 1.44^{b}	0.32 ± 0.27^{a}	0.97 ± 0.87^{ab}	0.84 ± 0.60^{ab}
30 cm	**Total leachate (mL)**	84.98 ± 6.89^{d}	74.36 ± 9.22^{cd}	85.26 ± 7.66^{d}	16.65 ± 4.79^{a}	75.27 ± 2.90^{cd}	49.28 ± 6.53^{bc}	17.14 ± 4.31^{ab}
30 cm	**pH**	7.22 ± 0.07^{ac}	6.96 ± 0.06^{ab}	6.83 ± 0.04^{a}	8.03 ± 0.33^{c}	7.35 ± 0.19^{ac}	7.52 ± 0.35^{ac}	7.90 ± 0.14^{bc}
30 cm	**EC (µS/ cm)**	901.00 ± 100.72^{a}	1240.00 ± 17.56^{ab}	1531.00 ± 108.58^{b}	1378.33 ± 115.53^{ab}	1074.00 ± 77.80^{ab}	1439.67 ± 58.25^{b}	2774.67 ± 541.68^{c}
30 cm	**DOC (µg/mL)**	32.44 ± 3.73^{a}	25.36 ± 0.61^{a}	34.24 ± 3.33^{a}	147.12 ± 55.58^{a}	31.72 ± 4.41^{a}	56.84 ± 14.14^{a}	116.26 ± 40.62^{a}
30 cm	**Nitrate (µg/mL)**	73.27 ± 16.03^{bc}	103.05 ± 11.48^{c}	63.43 ± 2.07^{ac}	19.98 ± 19.66^{ab}	43.26 ± 21.84^{ac}	3.67 ± 2.29^{a}	5.16 ± 4.38^{a}
30 cm	**Ammonia (µg/mL)**	1.70 ± 0.49^{a}	1.39 ± 0.17^{a}	2.98 ± 1.07^{a}	2.50 ± 0.23^{a}	0.49 ± 0.49^{a}	1.69 ± 0.77^{a}	3.20 ± 1.41^{a}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

7.3.3.1. Metals and Metalloids

The mean metal concentrations for leachate collected at 15 and 30 cm from the clay in sampling event 1 are provided in Figure 7.7. At 15 cm, surface applied treatments and the control were generally not significantly different from each other, however incorporated treatments were generally significantly higher than both; the only exceptions being the 20 t ha^{-1} application rate for all metals and metalloids and the 50 t ha^{-1} application rate for Pb. For these incorporated treatments, the 50 t ha^{-1} rate was significantly higher than the 20 t ha^{-1} application rate for As, Cd, Cr, Ni but not Cu, Pb and Zn, and the 140 t ha^{-1} rate was higher than both the 20 and 50 t ha^{-1} rates for all metals. This increase was significant for As, Cd, Cr, Ni and Pb, but for Cu and Zn, the 50 and 140 t ha^{-1} treatments were not considered significantly different.

There were generally fewer significant effects of MWOO application to the clay soil at 30 cm in sampling event 1 (see Figure 7.7). For leachate collected from the clay at 30 cm, both As and Cd recorded concentrations at or below detection limits for all treatments, and mean metal concentrations of Ni, Pb and Zn were statistically the same across all treatments. Of the seven metals tested, only Cr ($F_{6,14} = 7.74$, $p = 8.21 \times 10^{-4}$) and Cu ($\chi^2_6 = 13.11$, $p = 0.041$) in leachate at 30 cm exhibited significant differences among treatments. For Cr, the 140 t ha^{-1} incorporated application rate showed significantly higher concentrations than the control and all surface applied treatments, and the 50 t ha^{-1} incorporated treatment was significantly higher than the control and the 20 t ha^{-1} surface applied treatment (Figure 7.7). With Cu, all incorporated rates were significantly higher than the control and the 20 and 50 t ha^{-1} surface applied application rates, but not the 140 t ha^{-1} surface applied application rate (Figure 7.7).

For the two depths at sampling event 1, metal concentrations for the surface applied treatments did not appear to differ between leachate collected at 15 cm and 30 cm. However, for the incorporated treatments, metal concentrations generally appeared higher at 15 cm compared to 30 cm, except for Pb, where the leachate at 30 cm had higher mean concentrations. Total metal loss with both depths combined was less than 0.02% across all metals and metalloids and all treatments.

Generally, fewer significant differences between metal concentrations were found in sampling event 2 (Figure 7.8) and less than 0.01% of the total metal present initially was leached. Further comparison between sampling event 1 and sampling event 2 for the clay is outlined in section 7.4.7 in conjunction with the other soils.

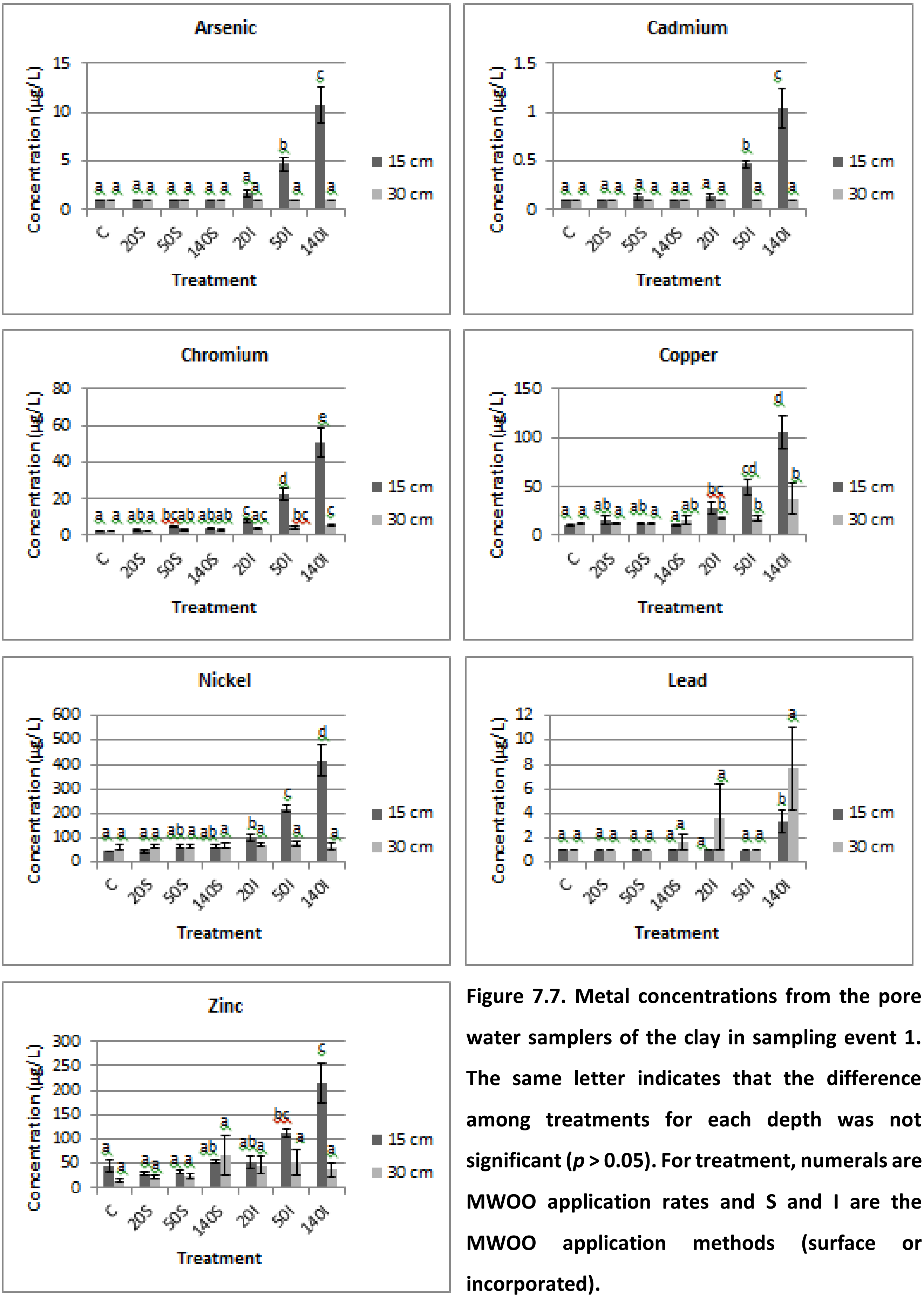
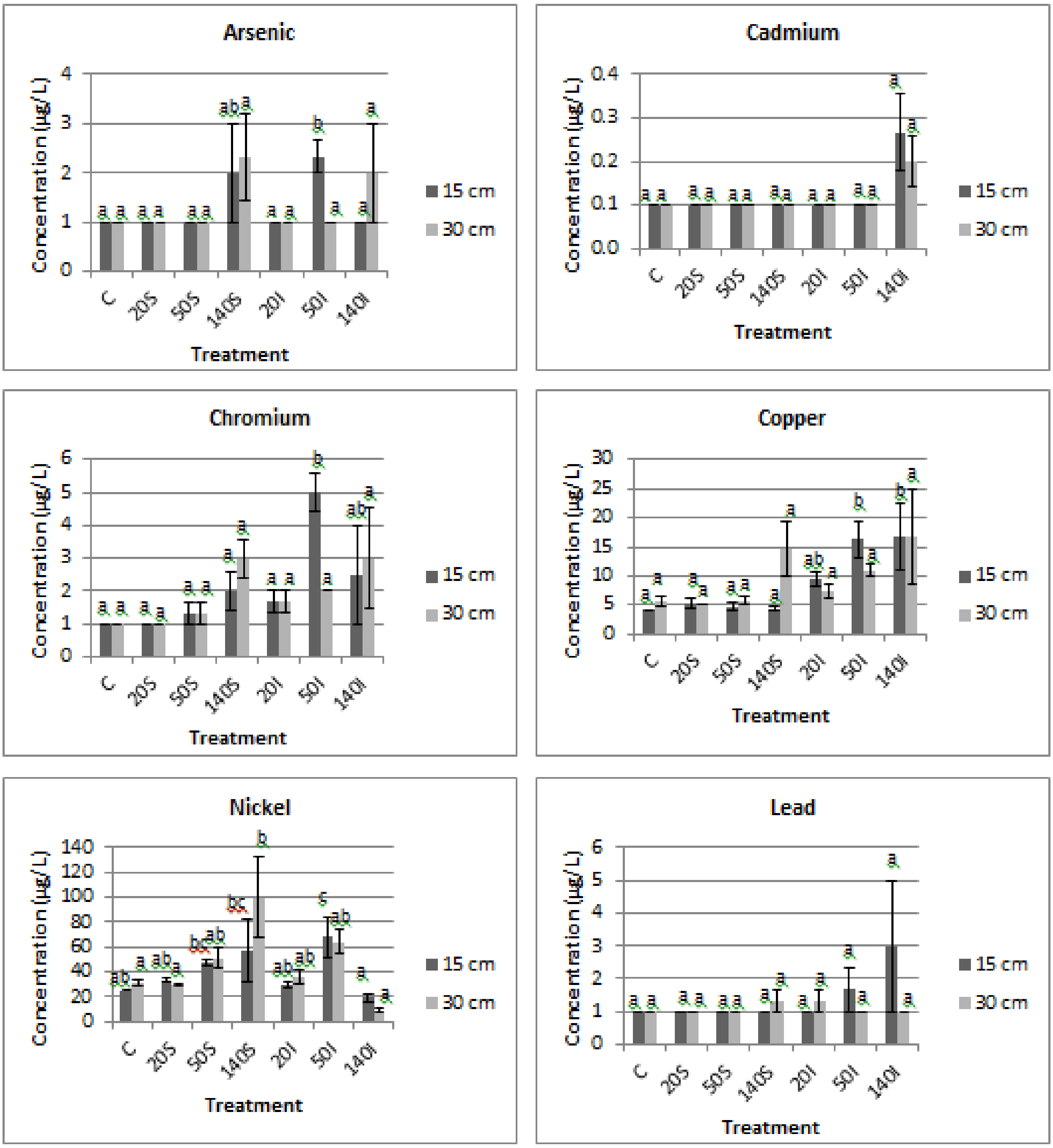


Figure 7.7. Metal concentrations from the pore water samplers of the clay in sampling event 1. The same letter indicates that the difference among treatments for each depth was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).



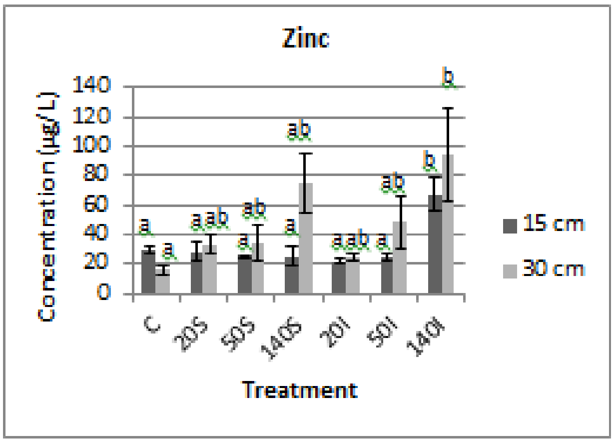


Figure 7.8. Metal concentrations from the pore water samplers of the clay in sampling event 2. The same letter indicates that the difference among treatments for each depth was not significant (*p* > 0.05). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

7.4.4 River Loam

Results for the non-metal variables in leachate from the river loam for sampling event 1 at 15 cm are provided in Table 7.7. Results show that at 15 cm EC ($F_{6,14} = 219$, $p = 5.11\text{x}10^{-13}$), DOC ($F_{6,13} = 437$, $p = 3.20\text{x}10^{-14}$), nitrate ($F_{6,14} = 9.25$, $p = 3.30\text{x}10^{-4}$) and ammonia ($\chi^2_6 = 15.60$, $p = 0.016$) were significantly different among treatments, but pH and total leachate were not. With EC and DOC, incorporation of MWOO significantly increased mean concentrations compared to surface application and the control, and as the incorporated application rate increased so too did EC and DOC (see Table 7.7). The lowest mean nitrate concentration at 0.1 µg/mL was in the 140 t ha^{-1} incorporated treatment and the highest was the 140 t ha^{-1} surface applied treatment at 103.52 µg/mL. Conversely, the 140 t ha^{-1} incorporated treatment had the highest mean ammonia concentration (5.62 µg/mL) and was significantly higher than the other application rates. Despite not being significantly influenced by MWOO application, total leachate tended to record higher mean volumes in the surface applied treatments than the control and incorporated treatments. However, because total leachate at 15 cm was collected by the pore water samplers, for which there were limitations, these results may be uninformative. There was very little variation in mean pH across the treatments whereby pH ranged from 7.71 – 7.87.

Results for leachate collected at 30 cm in sampling event 1 are provided in Table 7.7. Significant differences between treatments were observed for pH ($F_{6,13} = 12.87$, $p = 8.31\text{x}10^{-5}$), DOC ($F_{6,14} = 13.14$, $p = 4.82\text{x}10^{-5}$) and ammonia ($\chi^2_6 = 13.56$, $p = 0.035$). The 140 t ha^{-1} incorporated treatment recorded a significantly lower mean pH (7.86) than the other treatments (≥ 8.5). Increasing MWOO incorporated application rates significantly increased DOC concentrations. Ammonia concentrations were significantly decreased for most MWOO treatments compared to the control (0.27 µg/mL), except for the 20 t ha^{-1} surface applied (0.47 µg/mL) and the 140 t ha^{-1} incorporated treatment (0.88 µg/mL), although these were not significantly higher than the control due to large variability among the replicates. Total leachate, EC and nitrate were not significantly influenced by MWOO application and total leachate and EC demonstrated high variability among treatments, with total leachate ranging from 38.40 – 60.56 mL and EC ranging from 1701 – 2578 µS/ cm.

Again there were some significant differences between depths in sampling event 1. The leachate collected at 30 cm recorded a significantly higher pH than the leachate collected at 15 cm for all treatments, except the incorporated 140 t ha^{-1} application rate ($F_{13,27} = 30.11$, $p = 1.11\text{x}10^{-12}$). Electrical conductivity was significantly higher in the 30 cm leachate for the control and both 20 t ha^{-1} treatments, however, EC was significantly higher at 15 cm for the 140 t ha^{-1} incorporated application rate ($F_{13,28} = 35.68$, $p = 5.99\text{x}10^{-14}$). Similarly, DOC appeared higher in the leachate at 30 cm for the control and surface applied treatments but this was not significant; however DOC was significantly higher in the leachate at 15 cm for the 50 and 140 t ha^{-1} incorporated treatments ($F_{13,27} = 272$, $p = <2.2\text{x}10^{-16}$). Nitrate was significantly higher in the 30 cm leachate sample for all treatments ($F_{13,28}$ = 12.23, p = 2.69x10^{-8}). However there were no significant differences in ammonia concentrations with depth.

Similar trends were evident in sampling event 2 (Table 7.8), however, the differences between the two sampling events were of greater significance than differences between treatments. The pH in sampling event 2 tended to be higher than in sampling event 1 at 15 cm, but at 30 cm pH tended to be very similar to sampling event 1. At both depths for the control and surface applied treatments, EC tended to be similar for both times or slightly higher in sampling event 1, but in the incorporated treatments, EC in leachate at 15 cm EC was significantly higher in sampling event 1; at 30 cm EC was similar at both times. Sampling event 1 tended to record lower DOC concentrations in the surface applied treatments at 15 cm but higher DOC concentrations in the incorporated treatments at both depths. Nitrate concentrations tended to be higher in sampling event 1 for both depths and nearly all treatments, whereas ammonia concentrations tended to be slightly higher in sampling event 2 across both depths and all treatments (except the 140 t ha^{-1} incorporated treatment which had higher ammonia concentrations for both depths at event 1). There were generally few differences between sampling events for total leachate at both depths.

Table 7.19. The river loam leachate results for non-metal variables from sampling event 1 (mean ± standard error).

			Surface			Incorporated		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	20.80 ± 3.28^{a}	28.05 ± 0.41^{a}	25.05 ± 1.61^{a}	25.91 ± 2.17^{a}	21.25 ± 1.76^{a}	23.37 ± 2.52^{a}	20.21 ± 2.78^{a}
15 cm	**pH**	7.86 ± 0.11^{a}	7.77 ± 0.02^{a}	7.79 ± 0.02^{a}	7.87 ± 0.02^{a}	7.79 ± 0.04^{a}	7.71 ± 0.08^{a}	7.77 ± 0.10^{a}
15 cm	**EC (µS/ cm)**	965.33 ± 66.88^{a}	1072.33 ± 80.03^{a}	1060.33 ± 93.61^{a}	1164.00 ± 139.51^{a}	1753.00 ± 111.84^{b}	2491.33 ± 89.64^{c}	5010.00 ± 75.06^{d}
15 cm	**DOC (µg/mL)**	38.23 ± 0.20^{a}	41.83 ± 1.40^{a}	42.73 ± 3.85^{a}	40.47 ± 5.44^{a}	120.60 ± 5.44^{b}	290.20 ± 10.43^{c}	810.00 ± 30.00^{d}
15 cm	**Nitrate (µg/mL)**	73.90 ± 9.11^{bc}	91.28 ± 12.90^{c}	90.00 ± 8.93^{c}	103.52 ± 23.20^{c}	69.68 ± 16.07^{bc}	9.92 ± 8.39^{ab}	0.1 ± 0.07^{a}
15 cm	**Ammonia (µg/mL)**	0.42 ± 0.12^{b}	0.14 ± 0.10^{ab}	0.00 ± 0^{a}	0.00 ± 0^{a}	0.00 ± 0^{a}	0.26 ± 0.26^{ab}	5.62 ± 1.61^{c}
30 cm	**Total leachate (mL)**	46.38 ± 1.88^{a}	45.75 ± 3.94^{a}	55.58 ± 3.35^{a}	42.66 ± 10.54a	38.40 ± 9.50^{a}	60.56 ± 7.50^{a}	40.31 ± 4.39^{a}
30 cm	**pH**	8.55 ± 0.04^{b}	8.64 ± 0.01^{b}	8.64 ± 0.03^{b}	8.72 ± 0.02^{b}	8.59 ± 0.03^{b}	8.48 ± 0.20^{b}	7.85 ± 0.16^{a}
30 cm	**EC (µS/ cm)**	2036.33 ± 57.33^{a}	1981.00 ± 174.55^{a}	1701.00 ± 49.17^{a}	2101.67 ± 434.81^{a}	2217.67 ± 95.66^{a}	1996.67 ± 158.23^{a}	2578.33 ± 261.59^{a}
30 cm	**DOC (µg/mL)**	87.71 ± 1.51^{ab}	71.40 ± 2.91^{ab}	60.57 ± 3.94^{a}	69.80 ± 6.94^{ab}	81.00 ± 4.08^{ab}	95.80 ± 8.32^{b}	165.80 ± 29.18^{c}
30 cm	**Nitrate (µg/mL)**	205.63 ± 8.49^{a}	218.40 ± 23.63^{a}	178.40 ± 5.20^{a}	232.28 ± 68.04^{a}	230.40 ± 14.40^{a}	167.32 ± 19.48^{a}	189.00 ± 18.40^{a}
30 cm	**Ammonia (µg/mL)**	0.27 ± 0.04^{b}	0.42 ± 0.28^{ab}	BDL	BDL	BDL	BDL	0.88 ± 0.85^{ab}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$). BDL = Below Detection Limits.

Table 7.20. The river loam leachate results for non-metal variables from sampling event 2 (mean ± standard error).

			Surface			**Incorporated**		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	19.61 ± 5.31^{a}	27.02 ± 0.70^{a}	24.41 ± 2.60^{a}	24.65 ± 2.33^{a}	25.28 ± 1.97^{a}	24.76 ± 2.36^{a}	27.10 ± 0.33^{a}
15 cm	**pH**	8.02 ± 0.13^{a}	7.89 ± 0.02^{a}	7.97 ± 0.05^{a}	7.87 ± 0.04^{a}	7.94 ± 0.03^{a}	7.93 ± 0.05^{a}	7.78 ± 0.06^{a}
15 cm	**EC (µS/ cm)**	1095.67 ± 118.10^{a}	1268.67 ± 61.17^{ab}	1098.67 ± 27.79^{a}	1647.00 ± 166.77^{b}	1166.00 ± 90.07^{ab}	1468.33 ± 98.26^{ab}	3360.00 ± 98.49^{c}
15 cm	**DOC (µg/mL)**	25.22 ± 0.45^{a}	44.00 ± 13.10^{a}	170.80 ± 19.00^{b}	62.00 ± 38.70^{a}	33.44 ± 7.36^{a}	32.84 ± 5.11^{a}	39.24 ± 4.28^{a}
15 cm	**Nitrate (µg/mL)**	83.56 ± 17.55^{a}	52.29 ± 11.78^{a}	45.49 ± 5.96^{a}	82.53 ± 32.65^{a}	26.80 ± 14.07^{a}	9.60 ± 5.86^{a}	2.70 ± 0.52^{a}
15 cm	**Ammonia (µg/mL)**	0.60 ± 0.40^{a}	0.16 ± 0.08^{a}	0.44 ± 0.04^{a}	0.19 ± 0.19^{a}	0.09 ± 0.09^{a}	0.71 ± 0.41^{a}	0.27 ± 0.06^{a}
30 cm	**Total leachate (mL)**	69.72 ± 8.63^{b}	50.06 ± 3.01^{ab}	50.32 ± 3.25^{b}	29.13 ± 11.20^{a}	33.41 ± 8.94^{ab}	38.52 ± 0^{ab}	30.08 ± 0^{ab}
30 cm	**pH**	8.43 ± 0.01^{ab}	8.37 ± 0.09^{ab}	8.65 ± 0.05^{b}	8.44 ± 0.07^{ab}	8.47 ± 0.02^{ab}	8.41 ± 0.20^{ab}	8.08 ± 0.10^{a}
30 cm	**EC (µS/ cm)**	1447.33 ± 134.41^{a}	1657.33 ± 145.82^{a}	1686.00 ± 90.96^{a}	1756.33 ± 405.35^{a}	1982.00 ± 287.76^{a}	1971.67 ± 280.83^{a}	3238.33 ± 152.65^{b}
30 cm	**DOC (µg/mL)**	32.91 ± 0.59^{a}	36.90 ± 1.27^{a}	149.20 ± 7.60^{b}	68.88 ± 40.27^{ab}	20.68 ± 1.13^{a}	38.94 ± 14.70^{ab}	66.18 ± 17.98^{ab}
30 cm	**Nitrate (µg/mL)**	106.09 ± 15.68^{a}	112.93 ± 16.54^{a}	88.52 ± 11.14^{a}	40.72 ± 24.53^{a}	133.64 ± 36.65^{a}	80.97 ± 5.75^{a}	62.73 ± 8.89^{a}
30 cm	**Ammonia (µg/mL)**	BDL	0.17 ± 0.07^{a}	0.36 ± 0.15^{a}	0.30 ± 0.25^{a}	BDL	0.20 ± 0.10^{a}	0.72 ± 0.58^{a}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$). BDL = Below Detection Limits.

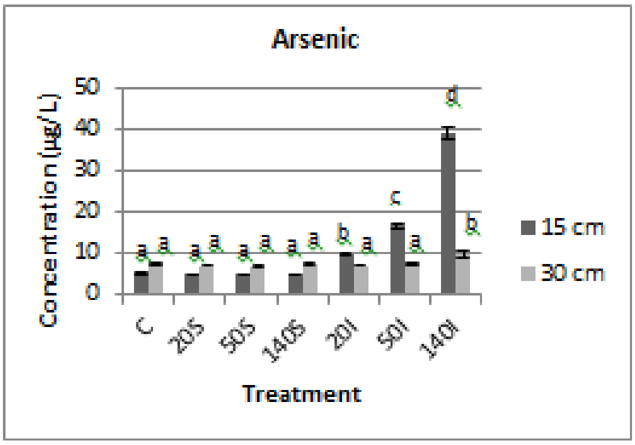
7.3.4.1. Metals and Metalloids

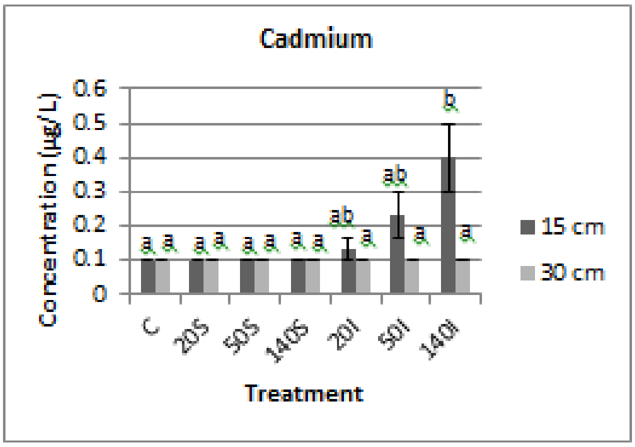
With the exception of Zn, metal and metalloid concentrations in leachate collected from the river loam at 15 cm in sampling event 1, were significantly higher in one or more incorporated treatments compared to all surface applied treatments and the control, both of which were statistically similar for all metals (Figure 7.9). For Pb ($\chi^2_6 = 17.41$, $p = 7.88\text{x}10^{-3}$), only the 140 t ha^{-1} incorporated treatment was significantly higher than the other treatments, and for Cd ($\chi^2_6 = 14.58$, $p = 0.024$) the 140 t ha^{-1} incorporated treatment was again significantly higher than the surface applied treatments and the control, but in this case, was not different to the other two incorporated treatments (see Figure 7.9). Arsenic ($\chi^2_6 = 19.12$, $p = 3.97\text{x}10^{-3}$), Cr ($\chi^2_6 = 19.09$, $p = 4.01\text{x}10^{-3}$), Cu ($F_{6,14} = 130$, $p = 1.86\text{x}10^{-11}$) and Ni ($F_{6,14} = 330$, $p = 2.96\text{x}10^{-14}$) all followed the same trend, with metal concentrations increasing significantly as the incorporated application rate also increased.

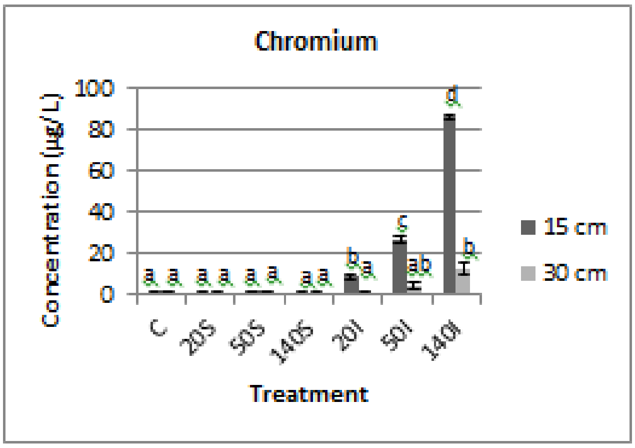
Results for leachate collected from the river sand at 30 cm in sampling event 1 are provided in Figure 7.9, and significant different results among treatments were obtained for As ($F_{6,14} = 5.66$, $p = 3.62\text{x}10^{-3}$), Cr ($\chi^2_6 = 15.47$, $p = 0.017$), Cu ($F_{6,14} = 10.94$, $p = 1.34\text{x}10^{-4}$) and Zn ($F_{6,14} = 7.76$, $p = 8.08\text{x}10^{-4}$). With As, Cr and Cu, leachate at 30 cm from the 140 t ha^{-1} incorporated treatment showed significantly higher mean metal concentrations than the other treatments, except the 50 t ha^{-1} incorporated treatment for Cr. The 140 t ha^{-1} incorporated treatment also showed the greatest mean Zn concentration and this was significantly higher than all other treatments except the 140 t ha^{-1} surface applied treatment. The 20 and 50 t ha^{-1} surface applied treatments appeared to have lower mean Zn concentrations than the control; however this was not a significant difference. Concentrations for Cd were all at or below detection limits.

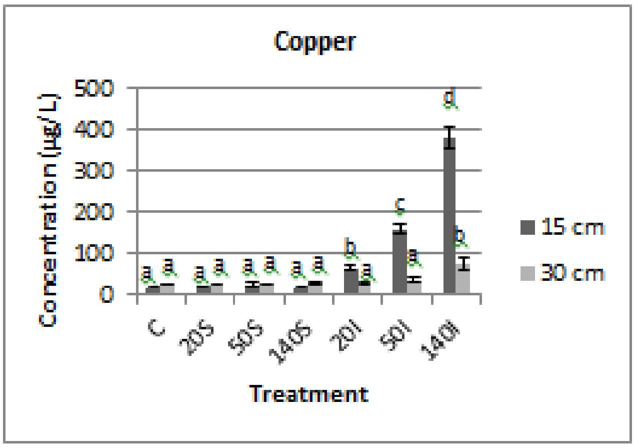
A comparison of the two different depths in sampling event 1 showed that when MWOO was surface applied, As, Cu and Ni appeared to have higher mean concentrations in leachate at 30 cm than 15 cm, whereas Cd, Cr and Pb did not change, and Zn appeared to have higher concentrations at 15 cm. Incorporation of MWOO yielded a different result, with all metals having higher mean concentrations at 15 cm compared to 30 cm, with the only exception being the 140 t ha^{-1} application rate for Pb, which had higher concentrations at 30 cm. The percentage of metal leached was less than 0.04% for all metals and metalloids across all MWOO application rates and methods.

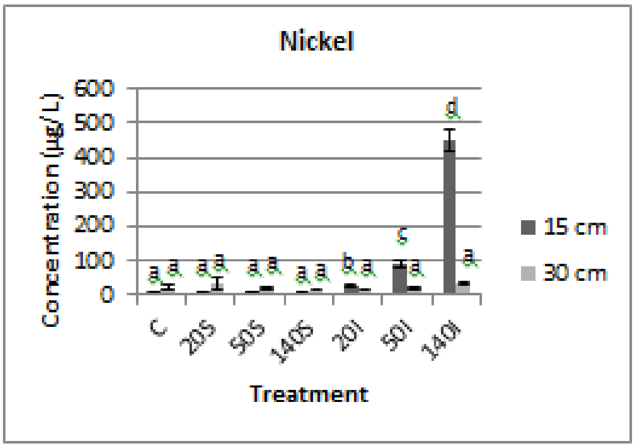
Metal concentrations in sampling event 2 (Figure 7.10) generally followed the same patterns, except that Zn and Cd recorded lower concentrations in the treatments compared to the control (significantly so for Cd in leachate at 30 cm for the surface applied treatments), and As recorded higher concentrations in the surface applied treatments compared to the control (significantly so for both depths with 140 t ha^{-1} MWOO applied). Total metal loss was less than 0.01% for each treatment and every metal. Further comparison between sampling event 1 and sampling event 2 for the river loam is outlined in section 7.4.7 in conjunction with the other soils.

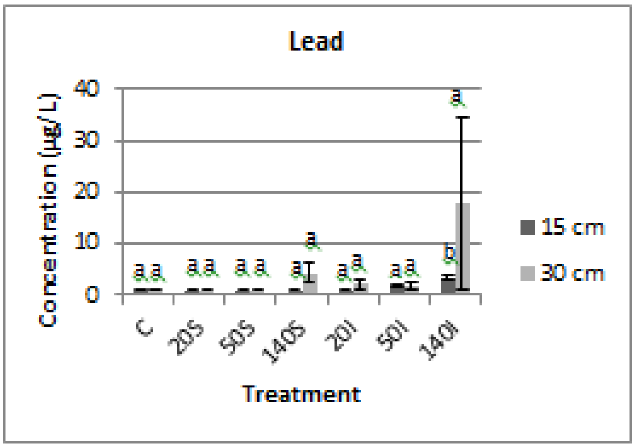












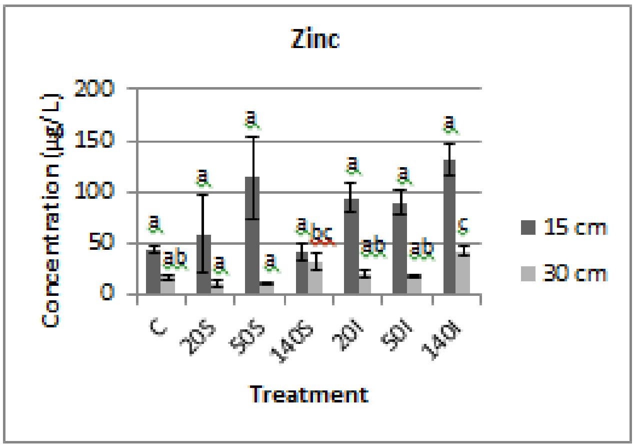


Figure 7.9. Metal concentrations from the pore water samplers of the river loam in sampling event 1. The same letter indicates that the difference among treatments for each depth was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

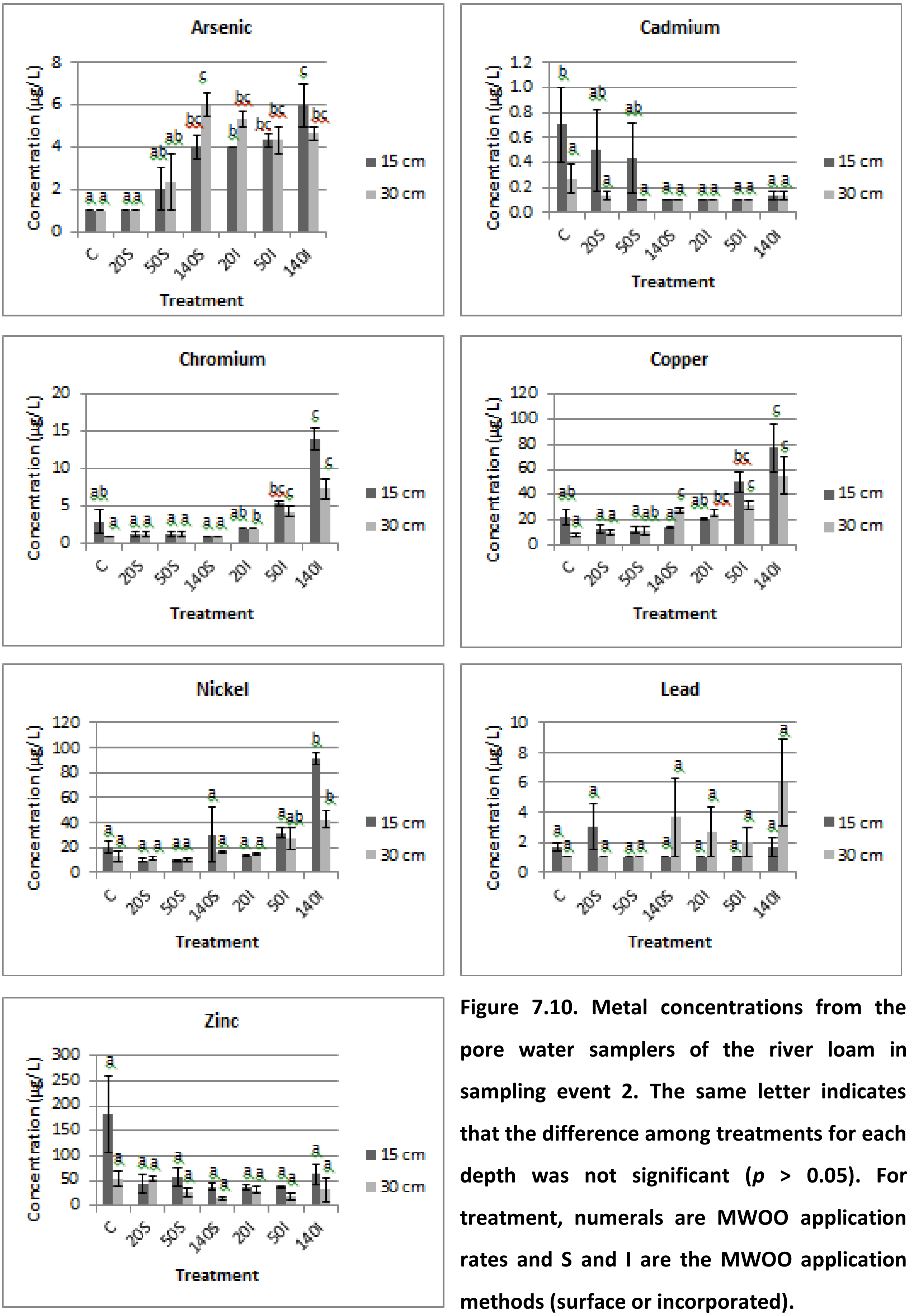


Figure 7.10. Metal concentrations from the pore water samplers of the river loam in sampling event 2. The same letter indicates that the difference among treatments for each depth was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

7.4.5 Minesite Soil

Results for the non-metal variables in leachate collected at 15 cm from the Minesite soil in sampling event 1 are provided in Table 7.9. A number of these were found to be significantly different among treatments, including EC ($F_{6,13}$ = 21.93, p = 4.30x10^{-6}), nitrate ($F_{6,13}$ = 20.11, p = 7.05x10^{-6}) and ammonia ($F_{6,12}$ = 15.49, p = 5.09x10^{-5}). For leachate EC, the 50 and 140 t ha^{-1} incorporated treatments recorded significantly higher mean values than the other treatments, and the 140 t ha^{-1} treatment (5863 µS/ cm) was significantly higher than the 50 t ha^{-1} treatment (3353 µS/ cm). For nitrate, leachate from nearly all treatments recorded significantly lower concentrations compared to the control (129.48 µg/mL), and generally the surface applied treatments exhibited significantly higher nitrate concentrations than the incorporated treatments. Nitrate concentration also decreased with increasing application rate, regardless of method, although not always significantly, meaning that nitrate was lowest in leachate from the 140 t ha^{-1} incorporated treatment. In contrast, the 140 t ha^{-1} incorporated treatment recorded the highest mean ammonia concentration (13.38 µg/mL), which was more than double the remaining treatments (4.2 - 6.1 µg/mL). Results for pH and DOC in leachate were very similar among all MWOO treatments and the control.

For leachate collected at 30 cm there were no significant difference between treatments for any of the non-metal variables, which was partly due to the large variability among replicates, despite the appearance of some trends (Table 7.9). For example, total leachate appeared to decrease with MWOO application, particularly in the incorporated treatments, however this was not significant. With pH and EC, all MWOO applied treatments showed higher mean values than the control but no significant differences were observed.

In the comparison of the results for both the 15 and 30 cm depths, all non-metal variables appeared higher in the leachate from 30 cm but this was not always significant. Leachate at 30 cm had a significantly higher pH than leachate at 15 cm for all treatments ($F_{13,27}$ = 13.48, p = 1.38x10^{-8}), and even though EC appeared to be in higher concentrations at 30 cm for all treatments, this was significant only for the surface applied and incorporated 50 t ha^{-1} treatments ($F_{13,27}$ = 6.17, p = 3.50x10^{-5}). Nitrate concentrations were also significantly higher at 30 cm than at 15 cm for most treatments, with the exception of the control and both 20 t ha^{-1} treatments (χ^2_{13} = 32.13, p = 2.30x10^{-3}). No significant differences were found between DOC and ammonia concentrations at the two depths.

Similar trends were observed in sampling event 2 (Table 7.10), however, there were fewer significant differences in the second sampling event compared to the first. As with the other soils there was generally no difference and no trend in total leachate volume between the two sampling events for either depth. Similarly, there were generally no differences in pH between the two sampling events, except in the incorporated treatments, where pH tended to increase in sampling event 2 at both depths. At both depths EC tended to be higher in sampling event 1, except for the surface applied treatments at 15 cm, where sampling event 2 recorded higher EC. Sampling event 1 recorded higher DOC concentrations than sampling event 2 across all treatments at both depths. Generally, nitrate concentrations were higher in sampling event 2 at 15 cm but higher in sampling event 1 at 30 cm across all treatments. Ammonia concentrations were higher in sampling event 1 for all treatments and at both depths.

Table 7.21. Minesite soil leachate results for non-metal variables from sampling event 1 (mean ± standard error).

			Surface			Incorporated		
		Control	20 t ha^{-1}	50 t ha^{-1}	140 t ha^{-1}	20 t ha^{-1}	50 t ha^{-1}	140 t ha^{-1}
15 cm	Total leachate (mL)	25.76 ± 0.57^{a}	25.43 ± 0.31^{a}	24.58 ± 1.21^{a}	25.20 ± 0.83^{a}	24.74 ± 0.26^{a}	23.64 ± 0.65^{a}	20.26 ± 2.27^{a}
15 cm	pH	6.58 ± 0.10^{a}	6.63 ± 0.13^{a}	6.57 ± 0.07^{a}	6.51 ± 0.01^{a}	6.70 ± 0.11^{a}	6.78 ± 0.06^{a}	6.88 ± 0.05^{a}
15 cm	EC (µS/ cm)	3275 ± 115^{ab}	2343 ± 95.05^{a}	2312.67 ± 272.19^{a}	2385 ± 206.78^{ab}	2615.33 ± 85.81^{ab}	3353.33 ± 178.92^{b}	5863.33 ± 428.34^{c}
15 cm	DOC (µg/mL)	158.20 ± 6.12^{a}	152.80 ± 7.87^{a}	161.40 ± 12.90^{a}	169.00 ± 11.33^{a}	267.60 ± 25.98^{a}	183.00 ± 39.12^{a}	193.80 ± 44.72^{a}
15 cm	Nitrate (µg/mL)	129.48 ± 9.72^{d}	87.60 ± 6.59^{cd}	78.80 ± 10.52^{c}	69.60 ± 6.12^{bc}	31.20 ± 16.10^{ab}	13.04 ± 9.64^{a}	6.30 ± 0.52^{a}
15 cm	Ammonia (µg/mL)	5.55 ± 0.39^{a}	4.82 ± 0.22^{a}	4.26 ± 0.48^{a}	4.20 ± 0.27^{a}	4.66 ± 0.49^{a}	6.10 ± 0.47^{a}	13.38 ± 1.80^{b}
30 cm	Total leachate (mL)	83.61 ± 39.23^{a}	30.14 ± 9.72^{a}	27.84 ± 10.55^{a}	40.07 ± 1.77^{a}	47.63 ± 15.74^{a}	32.67 ± 9.59^{a}	26.48 ± 10.71^{a}
30 cm	pH	6.98 ± 0.32^{a}	7.22 ± 0.14^{a}	7.44 ± 0.11^{a}	7.43 ± 0.11^{a}	7.26 ± 0.13^{a}	7.35 ± 0.05^{a}	7.35 ± 0.14^{a}
30 cm	EC (µS/ cm)	4305 ± 1789.44^{a}	5230 ± 1876.07^{a}	8007 ± 1583.21^{a}	5003 ± 319.91^{a}	5127 ± 1317.02^{a}	8510 ± 1043.12^{a}	9653 ± 1658.38^{a}
30 cm	DOC (µg/mL)	233.83 ± 51.17^{a}	263.20 ± 37.71^{a}	176.80 ± 27.72^{a}	259.80 ± 20.31^{a}	242.60 ± 23.52^{a}	286.20 ± 85.12^{a}	234.20 ± 69.24^{a}
30 cm	Nitrate (µg/mL)	368.40 ± 127.82^{a}	216.58 ± 165.59^{a}	475.60 ± 116.88^{a}	240.00 ± 22.97^{a}	262.08 ± 115.82^{a}	528.00 ± 100.76^{a}	652.80 ± 171.16^{a}
30 cm	Ammonia (µg/mL)	9.29 ± 1.25^{a}	14.74 ± 5.44^{a}	12.12 ± 3.89^{a}	8.18 ± 2.73^{a}	6.00 ± 0.93^{a}	8.34 ± 0.82^{a}	10.08 ± 0.81^{a}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

Table 7.22. Minesite soil leachate results for non-metal variables from sampling event 2 (mean ± standard error).

			Surface			Incorporated		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	26.45 ± 0.20^{a}	14.55 ± 6.69^{a}	25.73 ± 1.00^{a}	23.08 ± 2.62^{a}	25.71 ± 1.02^{a}	25.29 ± 1.28^{a}	21.97 ± 2.53^{a}
15 cm	**pH**	6.75 ± 0.23^{ab}	6.32 ± 0.19^{a}	6.51 ± 0.17^{a}	6.77 ± 0.14^{ab}	6.66 ± 0.10^{ab}	6.92 ± 0.20^{ab}	7.38 ± 0.11^{b}
15 cm	**EC (µS/ cm)**	1991.67 ± 470.79^{a}	3175.33 ± 494.52^{a}	2704.67 ± 173.01^{a}	2703.67 ± 107.64^{a}	2509.67 ± 237.26^{a}	2523.00 ± 231.51^{a}	3058.33 ± 113.42^{a}
15 cm	**DOC (µg/mL)**	45.66 ± 15.32^{ab}	25.05 ± 1.40^{a}	32.26 ± 3.02^{a}	77.80 ± 2.80^{b}	30.94 ± 1.99^{a}	40.92 ± 6.41^{a}	47.34 ± 1.56^{ab}
15 cm	**Nitrate (µg/mL)**	96.12 ± 43.34^{a}	151.63 ± 10.53^{a}	129.78 ± 25.27^{a}	58.17 ± 16.20^{a}	102.04 ± 32.76^{a}	82.44 ± 43.55^{a}	39.22 ± 15.04^{a}
15 cm	**Ammonia (µg/mL)**	1.53 ± 0.13^{a}	0.93 ± 0.47^{a}	1.55 ± 0.17^{a}	3.20 ± 0.58^{a}	0.67 ± 0.20^{a}	1.82 ± 0.79^{a}	3.55 ± 1.37^{a}
30 cm	**Total leachate (mL)**	139.09 ± 40.98^{b}	50.80 ± 23.19^{ab}	29.45 ± 15.58^{a}	9.60 ± 2.47^{a}	94.99 ± 21.76^{ab}	64.39 ± 5.50^{ab}	54.32 ± 13.03^{ab}
30 cm	**pH**	7.32 ± 0.13^{a}	7.20 ± 0.11^{a}	7.23 ± 0.10^{a}	7.59 ± 0.28^{a}	7.39 ± 0.06^{a}	7.34 ± 0.05^{a}	7.64 ± 0.07^{a}
30 cm	**EC (µS/ cm)**	2071.33 ± 549.22^{a}	1799.67 ± 401.32^{a}	2949.00 ± 539.7^{ab}	5066.67 ± 1163.12^{b}	1897.33 ± 190.92^{a}	1834.00 ± 476.36^{a}	2906.33 ± 362.52^{ab}
30 cm	**DOC (µg/mL)**	37.62 ± 6.87^{a}	33.15 ± 2.13^{a}	39.42 ± 0.20^{a}	59.34 ± 6.91^{a}	42.94 ± 2.68^{a}	41.28 ± 8.67^{a}	41.72 ± 4.16^{a}
30 cm	**Nitrate (µg/mL)**	97.04 ± 43.18^{a}	94.56 ± 0.69^{a}	139.15 ± 54.03^{a}	117.45 ± 52.86^{a}	46.06 ± 9.19^{a}	44.42 ± 15.32^{a}	90.78 ± 26.68^{a}
30 cm	**Ammonia (µg/mL)**	0.34 ± 0.07^{a}	0.77 ± 0.25^{a}	1.19 ± 0.18^{ab}	3.39 ± 0.72^{b}	1.30 ± 0.61^{ab}	0.66 ± 0.04^{a}	0.68 ± 0.38^{a}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

7.3.5.1. Metals and Metalloids

Results for metal concentrations in leachate at 15 cm from the Minesite soil from sampling event 1 are provided in Figure 7.11. These results show that, once again, the surface applied treatments were not significantly different from the control for any metals, but that the 140 t ha^{-1} incorporated application was significantly higher than all other treatments across all metals and metalloids. For the 20 t ha^{-1} incorporated application rate, Cd, Pb and Zn in leachate were not significantly different from the control or surface applied treatments, and neither was Pb for the 50 t ha^{-1} incorporated application rate. However, Cd ($F_{6,14}$ = 25.31, p = 9.43x10^{-7}) and Zn ($F_{6,14}$ = 43.55, p = 2.89x10^{-8}) in leachate from the 50 t ha^{-1} incorporated treatment was significantly higher the 20 t ha^{-1} treatment and as well as the control and surface applied treatments. For the remaining metals, As, Cr, Cu and Ni, concentrations in leachate from both the 20 and 50 t ha^{-1} incorporated rates were similar and were significantly higher than the control and the surface applied application rates: As ($F_{6,14}$ = 38.84, p = 6.10x10^{-8}), Cr ($F_{6,14}$ = 87.95, p = 2.65x10^{-10}), Cu ($F_{6,13}$ = 72.72, p = 3.02x10^{-9}) and Ni ($F_{6,13}$ = 82.78, p = 1.34x10^{-9}).

In contrast to results for leachate from the minesite soil at 15 cm, results for metal concentrations at 30 cm in sampling event 1 showed no significant differences between treatments for any metals or metalloids, even though the 20 t ha^{-1} surface applied treatment showed the greatest mean concentration for all (Figure 7.11). This was due to the high variability within treatments.

A comparison of leachate from 15 cm with leachate from 30 cm shows the leachate at 30 cm contained higher mean metal concentrations for the surface applied treatments, but generally lower metal concentrations for the incorporated treatments (except for Zn and the 20 and 50 t ha^{-1} application rates with Pb, which were higher in the 30 cm leachate). The total metal lost from the soil after MWOO application was less than 0.05% of what was initially present for each metal and metalloid and each treatment.

In sampling event 2, metal concentrations in leachate tended to increase as MWOO application rate increased for both application methods, and metal concentrations were generally higher in leachate from 30 cm, particularly in the surface applied treatments (Figure 7.12). The maximum metal content leached from any treatment for all metals was 0.05% of the initial metal content, and was for Cu leached from the 140 t ha^{-1} incorporated treatment. Further comparison between sampling event 1 and sampling event 2 for the Minesite soil is outlined in section 7.4.7 in conjunction with the other soils.

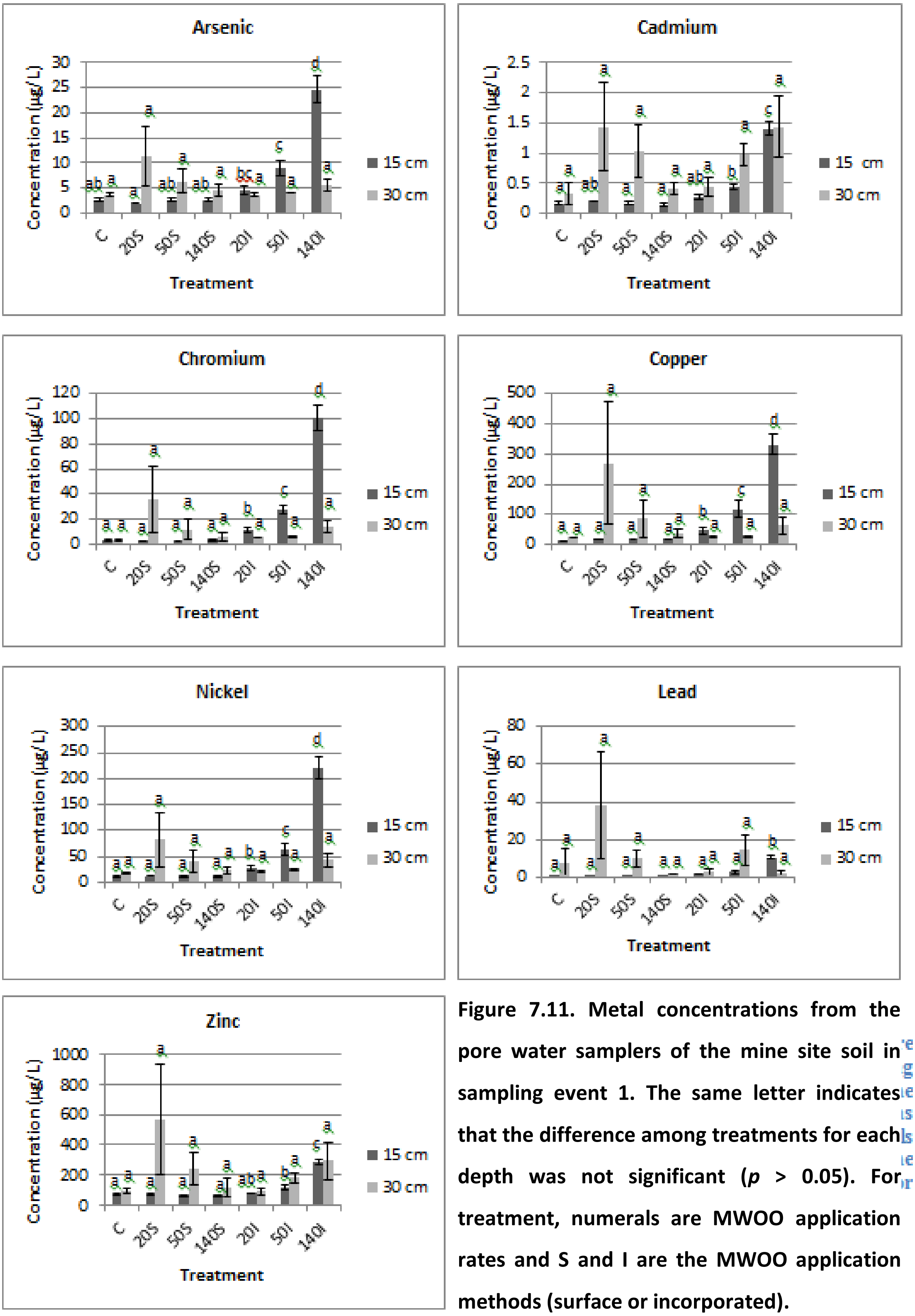


Figure 7.11. Metal concentrations from the pore water samplers of the mine site soil in sampling event 1. The same letter indicates that the difference among treatments for each depth was not significant (*p* > 0.05). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

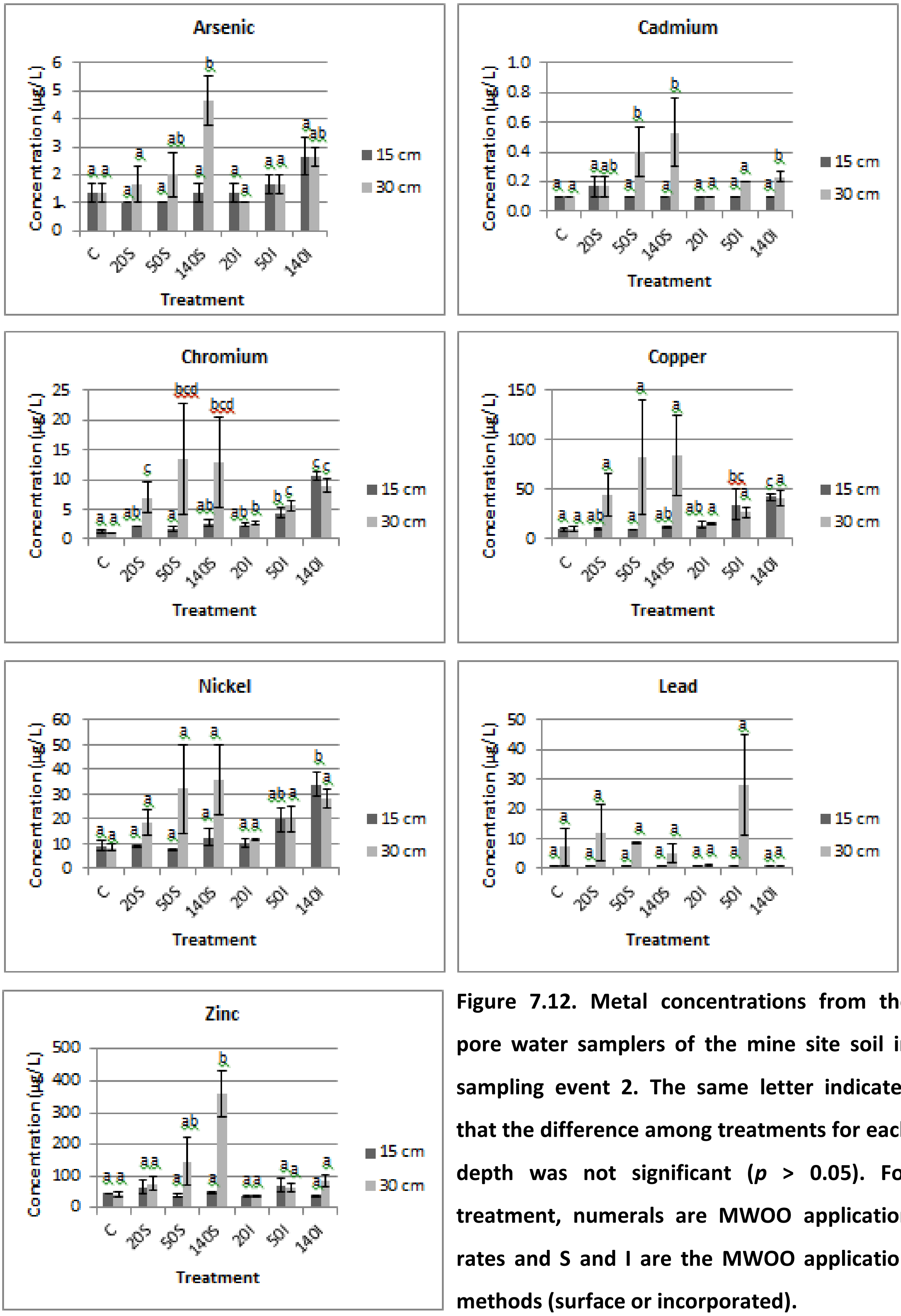


Figure 7.12. Metal concentrations from the pore water samplers of the mine site soil in sampling event 2. The same letter indicates that the difference among treatments for each depth was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

7.4.6 CROA Soil

Results for the non-metal variables in leachate collected at 15 cm from the CROA soil in sampling event 1 are provided in Table 7.11. A significant difference at 15 cm was found between MWOO treatments for pH ($F_{6,14}$ = 10.12, p = 2.05x10^{-4}), EC ($F_{6,14}$ = 118, p = 3.57x10^{-11}), DOC ($F_{6,13}$ = 104, p = 3.22x10^{-10}) and ammonia ($F_{6,14}$ = 34.34, p = 1.36x10^{-7}). With pH, only the 140 t ha^{-1} incorporated treatment was significantly higher than control and all other MWOO treatments, but for EC and DOC, all incorporated treatments were higher than the control and surface applied treatments. For EC, as incorporated application rate increased, so too did EC, but for DOC, concentrations in leachate from the 20 and 140 t ha^{-1} incorporated rates were significantly higher than the 50 t ha^{-1} rate. For ammonia, the 140 t ha^{-1} incorporated treatment recorded the greatest concentration (21.88 µg/mL) and this was significantly more than all other treatments, including the 140 t ha^{-1} surface applied treatment which recorded the lowest ammonia concentration at zero. Nitrate concentrations did not change significantly with MWOO application but as was the trend with other soils, the 140 t ha^{-1} incorporated treatment showed the lowest mean nitrate concentration along with the highest mean ammonia concentration.

Results for leachate collected at 30 cm in sampling event 1 are provided in Table 7.11. Of the non-metal variables measures, pH ($F_{6,13}$ = 4.53, p = 0.011) and ammonia ($F_{6,13}$ = 10.11, p = 2.90x10^{-4}) were shown to have significant differences among treatments. For pH, surface application of MWOO appeared to reduce pH and incorporation of MWOO appeared to increase pH. The 140 t ha^{-1} surface applied treatment recorded the lowest pH at 6.68, and the 140 t ha^{-1} incorporated treatment the highest at 7.36 (these were shown to be significantly different). For ammonia, concentrations in leachate at 30 cm tended to be lower in response to MWOO application regardless of application method, with the 140 t ha^{-1} surface applied treatment recording the lowest ammonia concentration (0.46 µg/mL compared to 3.16 µg/mL from the control) of all treatments. Nitrate concentrations were not significantly different among the treatments and did not appear to follow any trend. Even though EC and DOC concentrations in leachate also showed no significant change in response to MWOO application, all three incorporated treatments recorded a lower mean EC and a mean DOC higher than the control.

A comparison of the leachate results for both the 15 and 30 cm depths in sampling event 1 revealed that EC ($F_{13,27}$ = 15.44, p = 3.03x10^{-9}) and nitrate ($F_{13,27}$ = 9.28, p = 7.18x10^{-7}) were significantly higher in leachate at the 30 cm depth for the control and the 20 and 140 t ha^{-1} surface applied treatments. Leachate at 30 cm had a significantly higher pH (of approximately 1 unit) than leachate at 15 cm for all treatments ($F_{13,27}$ = 22.27, p = 4.30x10^{-11}), but this was also the case for the control. Leachate at 15 cm showed significantly higher DOC concentrations with the incorporated 50 t ha^{-1} treatment ($F_{13,26}$ = 19.33, p = 4.06x10^{-10}) and significantly higher ammonia concentrations with the incorporated 140 t ha^{-1} treatment ($F_{13,27}$ = 20.50, p = 1.15x10^{-10}) than leachate at 30 cm.

In sampling event 2, trends between treatments and depths generally followed the same pattern as in sampling event 1, but with fewer significant differences (Table 7.12). Total leachate was very similar across sampling events at 15 cm, due to the limitation of the pore water samplers, but at 30 cm, sampling event 2 tended to show a higher total leachate volume than sampling event 1, except at both 140 t ha^{-1} application rates. Sampling event 1 recorded a higher pH than sampling event 2 at both depths and across nearly all treatments. Leachate from the surface applied treatments at 15 cm tended to record a similar EC for both sampling events but the incorporated treatments recorded a higher EC in sampling event 1. Electrical conductivity in leachate from 30 cm was also higher for all treatments in sampling event 1. Sampling event 1 also tended to show higher DOC concentrations than sampling event 2 across almost all treatments at 15 cm and at 30 cm. Nitrate concentrations were very similar for both sampling events at 15 cm, but at 30 cm nitrate concentrations were consistently higher in sampling event 1. Ammonia concentrations were higher in sampling event 1 than sampling event 2 at both depths and across almost all treatments.

Table 7.23. The CROA soil leachate results for non-metal variables from sampling event 1 (mean ± standard error).

			Surface			Incorporated		
		Control	20 t ha^{-1}	50 t ha^{-1}	140 t ha^{-1}	20 t ha^{-1}	50 t ha^{-1}	140 t ha^{-1}
15 cm	**Total leachate (mL)**	24.67 ± 2.05^{a}	20.60 ± 2.71^{a}	25.30 ± 0.92^{a}	17.91 ± 4.63^{a}	20.62 ± 2.43^{a}	23.80 ± 1.27^{a}	15.73 ± 1.98^{a}
15 cm	**pH**	5.90 ± 0.06^{a}	6.01 ± 0.10^{a}	5.83 ± 0.06^{a}	6.09 ± 0.13^{a}	6.01 ± 0.26^{a}	5.77 ± 0.05^{a}	6.96 ± 0.11^{b}
15 cm	**EC (µS/ cm)**	1164.67 ± 78.34^{a}	1237 ± 168.05^{a}	1372.67 ± 75.69^{a}	1211 ± 118.75^{a}	1849 ± 264.72^{a}	2841 ± 199.34^{b}	5960 ± 115.04^{c}
15 cm	**DOC (µg/mL)**	61.02 ± 2.95^{a}	65.38 ± 5.62^{a}	73.40 ± 5.38^{a}	83.80 ± 13.65^{a}	189.60 ± 3.62^{b}	366.00 ± 4.80^{c}	165.00 ± 18.01^{b}
15 cm	**Nitrate (µg/mL)**	99.92 ± 7.09^{a}	100.40 ± 17.23^{a}	111.60 ± 11.82^{a}	90.00 ± 21.68^{a}	83.98 ± 39.26^{a}	101.63 ± 20.63^{a}	67.08 ± 14.72^{a}
15 cm	**Ammonia (µg/mL)**	3.26 ± 0.02^{bc}	2.80 ± 0.52^{bc}	1.36 ± 0.66^{ab}	0.00 ± 0^{a}	1.06 ± 0.34^{ab}	5.28 ± 1.20^{c}	21.88 ± 3.02^{d}
30 cm	**Total leachate (mL)**	33.31 ± 5.32^{a}	36.01 ± 6.25^{a}	43.91 ± 1.71^{a}	32.75 ± 7.11^{a}	36.21 ± 11.23^{a}	25.28 ± 5.66^{a}	37.03 ± 1.82^{a}
30 cm	**pH**	7.07 ± 0.17^{ab}	6.86 ± 0.04^{ab}	6.89 ± 0.09^{ab}	6.68 ± 0.05^{a}	6.87 ± 0.06^{ab}	7.29 ± 0.19^{b}	7.36 ± 0.00^{b}
30 cm	**EC (µS/ cm)**	3249 ± 263.54^{a}	3417 ± 274.31^{a}	2354 ± 180.23^{a}	3854 ± 582.40^{a}	2897 ± 323.73^{a}	2651 ± 663.71^{a}	2994 ± 740.15^{a}
30 cm	**DOC (µg/mL)**	130.78 ± 15.15^{a}	130.80 ± 10.34^{a}	97.00 ± 6.55^{a}	150.40 ± 21.69^{a}	139.00 ± 18.76^{a}	139.20 ± 34.00^{a}	138.30 ± 10.04^{a}
30 cm	**Nitrate (µg/mL)**	371.00 ± 41.84^{a}	387.60 ± 36.05^{a}	270.00 ± 33.41^{a}	466.00 ± 85.58^{a}	308.00 ± 36.39^{a}	267.60 ± 74.87^{a}	282.00 ± 93.08^{a}
30 cm	**Ammonia (µg/mL)**	3.16 ± 0.16^{d}	2.84 ± 0.40^{cd}	0.92 ± 0.50^{ab}	0.46 ± 0.24^{a}	1.14 ± 0.33^{abc}	2.36 ± 0.23^{bd}	2.76 ± 0.69^{bd}

In each row, the same letter indicates that the difference among treatments was not significant ($p>0.05$).

Table 7.24. The CROA soil leachate results for non-metal variables from sampling event 2 (mean ± standard error).

			Surface			**Incorporated**		
		Control	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**	**20 t ha^{-1}**	**50 t ha^{-1}**	**140 t ha^{-1}**
15 cm	**Total leachate (mL)**	26.70 ± 0.17^{a}	24.36 ± 2.19^{a}	23.18 ± 1.91^{a}	15.40 ± 5.73^{a}	21.66 ± 2.27^{a}	25.22 ± 1.53^{a}	18.97 ± 2.81^{a}
15 cm	**pH**	5.31 ± 0.07^{a}	5.18 ± 0.16^{a}	5.67 ± 0.22^{a}	5.84 ± 0.43^{ab}	5.77 ± 0.39^{a}	5.64 ± 0.13^{a}	6.98 ± 0.04^{b}
15 cm	**EC (µS/ cm)**	1263.67 ± 97.20^{a}	1364.00 ± 110.99^{a}	1181.33 ± 223.52^{a}	1264.00 ± 399.70^{a}	1077.67 ± 15.19^{a}	1467.3 ± 226.32^{ab}	2371.33 ± 103.65^{b}
15 cm	**DOC (µg/mL)**	73.96 ± 25.44^{a}	123.00 ± 21.90^{a}	91.80 ± 9.70^{a}	75.60 ± 0^{a}	116.20 ± 21.94^{a}	111.00 ± 11.45^{a}	112.30 ± 24.65^{a}
15 cm	**Nitrate (µg/mL)**	124.88 ± 8.13^{a}	121.44 ± 14.54^{a}	82.77 ± 32.11^{a}	116.48 ± 0^{a}	80.76 ± 7.45^{a}	106.19 ± 24.53^{a}	90.02 ± 27.40^{a}
15 cm	**Ammonia (µg/mL)**	0.61 ± 0.19^{a}	0.43 ± 0.21^{a}	1.31 ± 0.57^{ab}	0.74 ± 0^{ab}	0.52 ± 0.04^{a}	0.76 ± 0.51^{a}	3.01 ± 0.22^{b}
30 cm	**Total leachate (mL)**	74.2 ± 5.65^{c}	56.96 ± 2.25^{bc}	54.22 ± 14.40^{ac}	23.12 ± 7.08^{a}	63.33 ±6.83^{bc}	32.90 ± 1.06^{ac}	24.32 ± 10.80^{ab}
30 cm	**pH**	6.41 ± 0.17^{a}	6.08 ± 0.03^{a}	6.28 ± 0.09^{ab}	6.63 ± 0.15^{ab}	6.38 ± 0.10^{ab}	6.56 ± 0.12^{ab}	6.95 ± 0.17^{b}
30 cm	**EC (µS/ cm)**	1292.33 ± 177.45^{ab}	1226.00 ± 76.79^{ab}	1388.00 ± 80.54^{ab}	2109.67 ± 565.33^{b}	957.67 ± 84.37^{a}	945.67 ± 23.34^{a}	2219.50 ± 521.82^{b}
30 cm	**DOC (µg/mL)**	68.00 ± 3.64^{a}	78.40 ± 2.00^{a}	90.60 ± 5.40^{a}	98.80 ± 5.37^{a}	210.00 ± 17.02^{b}	100.80 ± 14.48^{a}	112.20 ± 22.95^{a}
30 cm	**Nitrate (µg/mL)**	128.30 ± 20.66^{a}	97.71 ± 12.65^{a}	90.70 ± 19.23^{a}	180.12 ± 64.98^{a}	63.18 ± 10.95^{a}	54.54 ± 5.27^{a}	128.01 ± 54.86^{a}
30 cm	**Ammonia (µg/mL)**	0.38 ± 0.10^{a}	0.49 ± 0.11^{a}	0.88 ± 0.31^{a}	0.38 ± 0.12^{a}	0.52 ± 0.04^{a}	0.51 ± 0.10^{a}	0.33 ± 0.14^{a}

In each row, the same letter indicates that the difference among treatments was not significant (p>0.05).

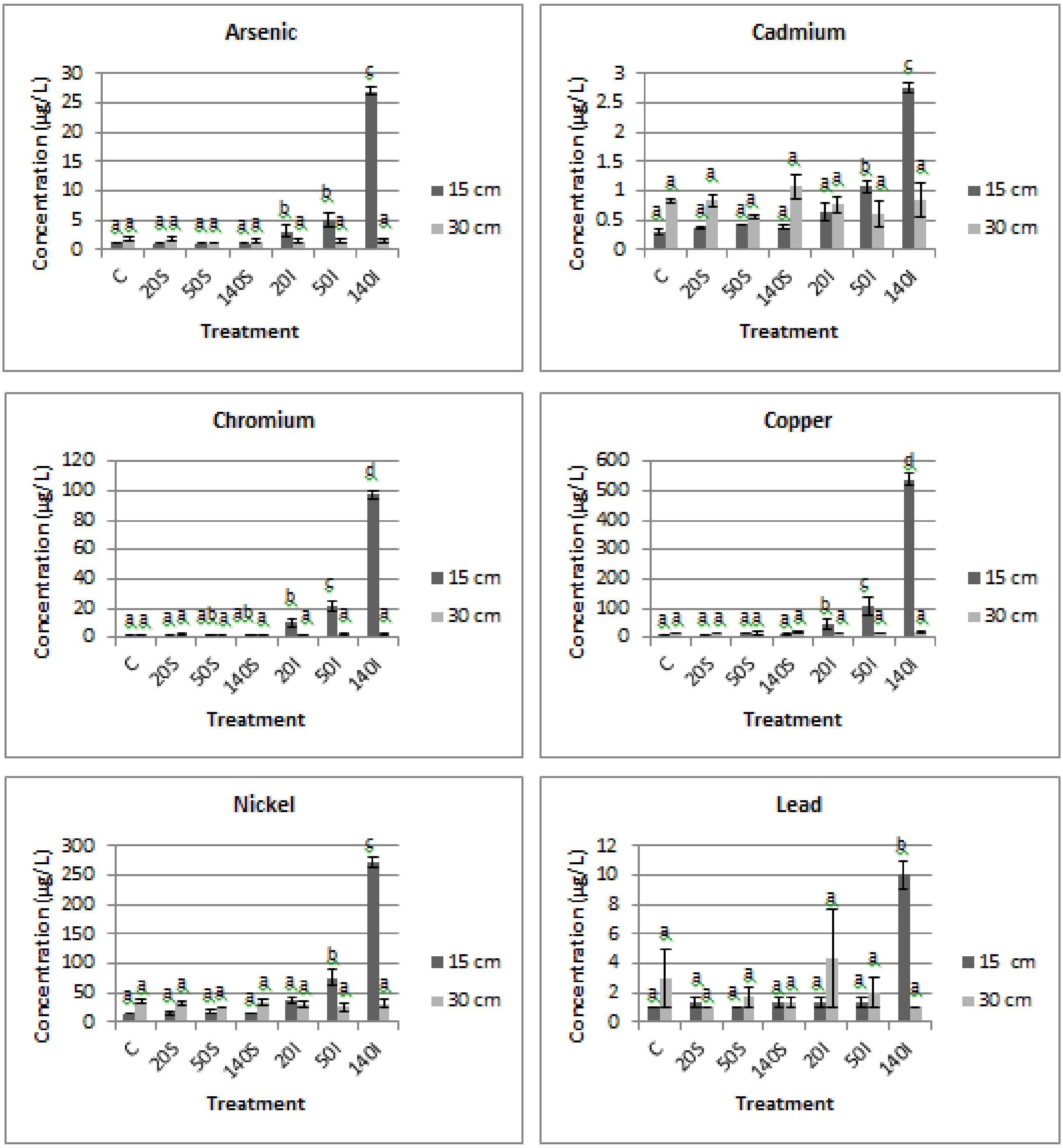
7.3.6.1. Metals and Metalloids

Metal concentrations in leachate collected from the CROA soil at 15 cm in sampling event 1 are illustrated in Figure 7.13. Surface applied treatments were not significantly different from the controls for any metal or metalloid, but the incorporated treatments were generally significantly higher than the control and the surface applied treatments. For all metals and metalloids, the 140 t ha^{-1} incorporated application rate recorded the greatest mean concentration by a significant margin. At 15 cm, the 20 t ha^{-1} incorporated treatment was not significantly different from the surface applied treatments or the control for Cd ($F_{6,14}$ = 131, p = 1.76x10^{-11}), Ni ($F_{6,14}$ = 224, p = 4.34x10^{-13}), Pb (χ^2_6 = 11.90, p = 0.06) or Zn ($F_{6,14}$ = 16.69, p = 1.20x10^{-5}), and this was the same for Pb and Zn for the 50 t ha^{-1} incorporated rate. For As, the 20 and 50 t ha^{-1} incorporated application rates were similar but were higher than the control and surface applied treatments (χ^2_6 = 19.60, p = 3.26x10^{-3}). For Cr ($F_{6,14}$ = 388, p = 9.63x10^{-15}) and Cu ($F_{6,14}$ = 69.76, p = 1.27x10^{-9}) metal concentrations for all the incorporated treatments were higher than the control and the surface applied treatments, and increased significantly as application rate increased.

As with the Minesite soil, results for metal concentrations in leachate from the CROA were very different at 30 cm compared to 15 cm in sampling event 1, with no significant differences between treatments for any metals or metalloids at 30 cm, being partly due to the high variability among replicates within a treatment (Figure 7.13).

In the comparison of the 15 cm and 30 cm depths in sampling event 1, the surface applied treatments generally appeared to have higher metal concentrations at 30 cm, but the incorporated treatments generally had higher metal concentrations in leachate at 15 cm. Metal losses were low at less than 0.04% of the total metal that was initially present in the soil/MWOO treatment.

In sampling event 2, many metal concentrations were at or below detection limits across multiple treatments (Figure 7.14). As a result, fewer significant differences were observed between treatments, but the 140 t ha^{-1} incorporated application rate was still generally the treatment with the greatest metal concentrations. Total metal loss for each treatment was generally less than 0.02% of the total metal initially present after MWOO addition to the soil for all metals. Further comparison between sampling event 1 and sampling event 2 for the CROA soil is outlined in section 7.4.7 in conjunction with the other soils.



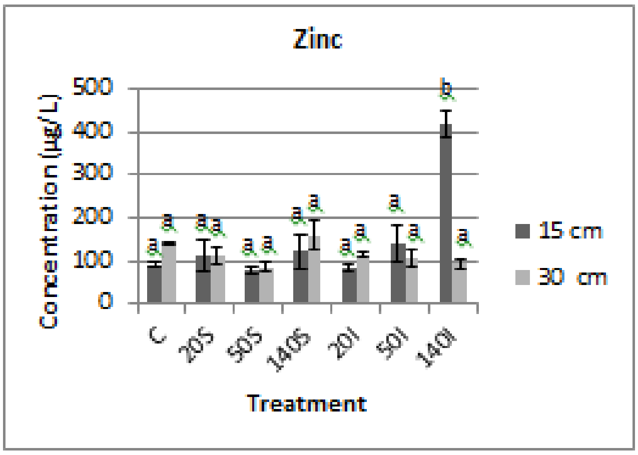


Figure 7.13. Metal concentrations from the pore water samplers of the CROA soil in Sampling Event 1. The same letter indicates that the difference among treatments for each depth was not significant (*p* > 0.05). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

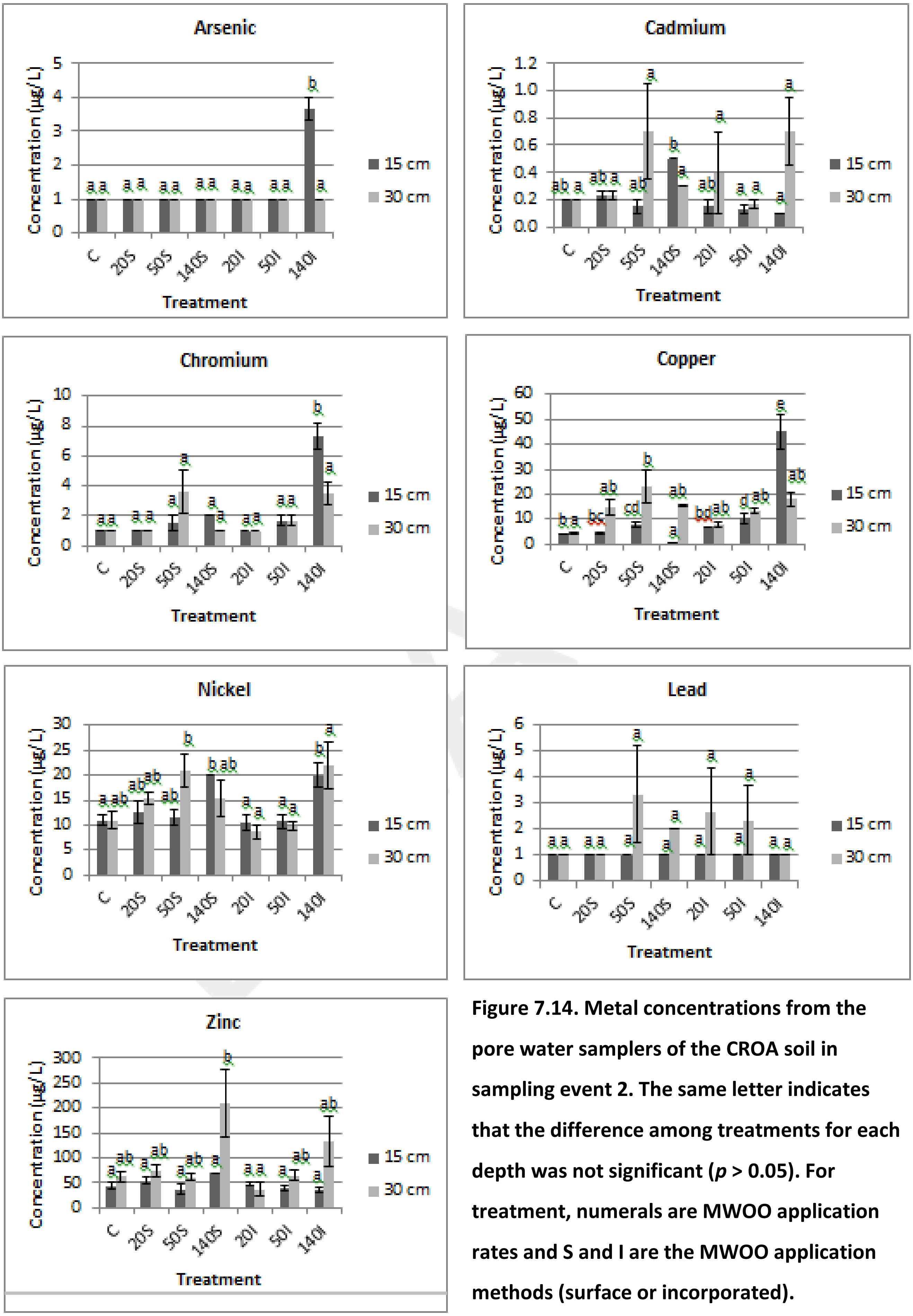


Figure 7.14. Metal concentrations from the pore water samplers of the CROA soil in sampling event 2. The same letter indicates that the difference among treatments for each depth was not significant ($p > 0.05$). For treatment, numerals are MWOO application rates and S and I are the MWOO application methods (surface or incorporated).

7.4.7 Comparing Sampling Events for Metals and Metalloids

Differences between the two sampling events for metals and metalloids were generally consistent across all soils. Surface applied treatments generally had very similar metal concentrations for both sampling events at 15 cm for all soils. By contrast, in the incorporated treatments, nearly all metals and metalloid concentrations at 15 cm decreased from sampling event 1 to sampling event 2, particularly for the 140 t ha^{-1} application rate, for all soils. The exceptions to this were both in the river loam, with Cd and Zn concentrations being higher in the control in sampling event 2 and Cd again in the 20 and 50 t ha^{-1} surface applied treatments.

There were some differences between sampling events with the different soils at 30 cm, however, the general trend was that metal concentrations also decreased at 30 cm. An exception was Zn concentrations in all soils except the Minesite soil. In the sandy loam, Zn concentrations tended to increase in all the surface applied treatments in sampling event 2; in the clay concentrations increased in all surface applied treatments as well as the 140 t ha^{-1} incorporated treatment; in the river loam Zn concentrations were higher in the 20 and 50 t ha^{-1} surface applied application rates only, and; in the CROA soil Zn concentrations increased for both 140 t ha^{-1} application rates. Additionally, Ni concentrations in the river loam tended to increase in the incorporated treatments in sampling event 2.

7.4.8 Comparing Soils

7.3.8.1. pH and DOC

A comparison between soils was undertaken for leachate collected at 15 cm in sampling event 1 for the two variables most likely to influence metal mobility and sorption-pH and DOC. For pH The differences between soils were consistent for the control ($F_{4,10}$ = 93.11, p = 37.17x10^{-8}), the surface applied treatments ($F_{14,29}$ = 68.58, p = <2.2x10^{-16}) and the incorporated treatments ($F_{14,30}$ = 109, p = <2.2x10^{-16}) with the river loam > Minesite soil > clay > CROA soil > sandy loam.

For DOC, results for concentrations in leachate at 15 cm were not consistent across treatments, indicating that MWOO application had an effect. There was no significant difference between any of the soil types with the incorporated treatments, however, in the controls differences in DOC concentrations were evident according to the following order: Minesite soil > clay > CROA soil > sandy loam ≈ river loam ($F_{4,10}$ = 177, p = 3.08x10^{-9}). Furthermore, changes to this order were evident in the surface applied treatments with clay ≈ Minesite soil > CROA ≈ river loam ≈ sandy loam ($F_{14,30}$ = 23.07, p = 2.88x10^{-12}). In the surface applied treatments, DOC concentrations in the 140 t ha^{-1} application rate in sampling event 1 showed a higher relative increase in the clay than the Minesite soil.

7.3.8.2. Metals and Metalloids

For the metals and metalloids in leachate from 15 cm in sampling event 1 the differences between soil types were not consistent. For concentrations of As in leachate, significant differences between the five soil types were mostly consistent for the control ($F_{4,10} = 49.17$, $p = 1.53 \times 10^{-6}$) and surface applied treatments ($\chi^2_{14} = 43.31$, $p = 7.65 \times 10^{-5}$), following the order: river loam > sandy loam ≈ Minesite soil > CROA soil ≈ clay. However, with the incorporated treatments, there was no obvious significant difference between soil types, except for the 140 t ha^{-1} application rate whereby similar trends as the control were evident ($F_{14,30} = 30.76$, $p = 5.99 \times 10^{-14}$).

For Cd, the control ($\chi^2_4 = 11.65$, $p = 0.02$) and the surface applied treatments ($\chi^2_{14} = 38.60$, $p = 4.20 \times 10^{-4}$) followed the same pattern, with CROA soil > Minesite soil > river loam ≈ clay ≈ sandy loam. The incorporated treatments generally followed the same pattern, however the differences between soils were not as strong, to the point where for the 20 t ha^{-1} application rate there were no significant differences between soils ($F_{14,30} = 54.17$, $p = <2.2 \times 10^{-16}$).

Again, the control ($\chi^2_4 = 13.58$, $p = 8.80 \times 10^{-3}$) and surface applied treatments ($F_{14,30} = 9.35$, $p = 1.96 \times 10^{-7}$) generally followed the same pattern for Cr, with clay > sandy loam ≈ river loam ≈ Minesite soil ≈ CROA soil. This trend was not present in the incorporated treatments, and there was no significant difference between soils for the 20 and 50 t ha^{-1} application rates. However, at the 140 t ha^{-1} incorporated application rate, Cr concentrations were different for the soil types according to the order: CROA soil ≈ Minesite soil > river loam > sandy loam ≈ clay ($F_{14,30} = 42.41$, $p = 7.01 \times 10^{-16}$). This indicates that Cr concentrations in leachate from the CROA soil and Minesite soil had significantly increased relative to the control after incorporation of MWOO.

Differences in Cu concentrations between soil types were evident with MWOO application. In the control the pattern for Cu concentrations was: river loam > CROA soil ≈ clay ≈ Minesite soil > sandy loam ($F_{4,9} = 33.72$, $p = 2.00 \times 10^{-5}$). However, there was generally no significant difference in soil types between the surface applied treatments, except for the 20 and 50 t ha^{-1} application rates where, like the control, the river loam had significantly higher Cu concentrations ($F_{14,30} = 4.97$, $p = 1.14 \times 10^{-4}$). There was no significant difference between soils with the incorporated treatments, except for the 140 t ha^{-1} application rate. In this case, although Cu concentrations increased in response to MWOO application in all soils, concentrations in the CROA soil appeared to have increased the greatest relative to the control, and in the clay, appeared to have increased the lowest relative to the control ($F_{14,30} = 42.78$, $p = 6.21 \times 10^{-16}$).

Concentrations of Ni in leachate from the surface applied ($F_{14,30}$ = 90.80, p = <2.2×10^{-16}), incorporated ($F_{14,30}$ = 37.06, p = 4.61×10^{-15}), and the control ($F_{4,9}$ = 48.83, p = 4.19×10^{-6}) treatments generally followed the same pattern, whereby clay > Minesite soil ≈ CROA soil ≈ river loam > sandy loam.

For Pb, there was generally no difference between soil types due to the high occurrence of values at or below detection limits across all application rates. However, in the incorporated MWOO treatments, differences in Pb concentrations between soil types were evident, according to the following pattern: sandy loam > Minesite soil ≈ CROA soil ≈ river loam ≈ clay ($F_{14,30}$ = 17.82, p = 8.33×10^{-11}).

Differences in Zn concentrations in leachate between soil types in sampling event 1 were influenced by MWOO application, with the following pattern being consistent for the control ($F_{4,10}$ = 10.34, p = 1.41×10^{-3}), surface applied treatments ($F_{14,29}$ = 7.13, p = 4.54×10^{-6}) and incorporated treatments ($F_{14,29}$ = 19.92, p = 3.51×10^{-11}): CROA soil > river loam ≈ clay ≈ Minesite soil > sandy loam. For the sandy loam, Zn concentrations tended to increase more significantly in the incorporated treatments, relative to the control, than for the surface applied treatments.

Results from sampling event 2 tended to record less significant results, as was also the case for the control, and trends were not always consistent with sampling event 1. For DOC, concentrations tended to increase in the CROA soil and the river loam relative to the control when MWOO was surface applied, but when MWOO was incorporated, increases occurred in the clay as well as the CROA soil. Differences between soils for the pH of leachate in sampling event 2 were the same as sampling event 1.

For the metal and metalloid concentrations in sampling event 2, differences between the soils for As and Cr were similar to sampling event 1. Conversely, MWOO had a stronger effect on Cd in leachate from all soils during sampling event 2. Differences in Cr concentrations between soils were not significantly different from sampling event 1 to 2, for the control or either of the MWOO application rates. Any MWOO effect on Cu concentrations between the soils in sampling event 1 was absent by sampling event 2, with differences between the soil types being consistent for the controls and MWOO application rates. The effect of MWOO on Ni concentrations between soil types became more evident in the incorporated treatments in sampling event 2, despite the control and the surface applied treatments following the same trends. Generally, Pb concentrations did not change significantly from sampling event 1 to2, across all treatments, except when MWOO was incorporated into the sandy loam at 140 t ha^{-1} Pb concentrations increased significantly relative to the control. There were generally no significant differences between soil types for Zn concentrations in sampling event 2.

7.5 Discussion

7.5.1 Effects of MWOO application rates and methods on leachate

Some general trends in effects of MWOO application on leachate variables were seen across all soils, across both depths and across both sampling events, although there were some exceptions to these trends, as outlined in the results above. Generally, few significant differences in total leachate were seen. While not always significant, the 140 t ha^{-1} incorporated treatment generally had the lowest mean volume of leachate collected. This suggests that incorporating MWOO into the soil at high application rates resulted in an increase in the water holding capacity of the soil. This was also noticed when maintaining the columns at 80% FC, where less water was generally lost from these treatments in all soils, but particularly in the clay and the Minesite soil.

Such observations are supported by McConnell et al. (1993) who reported that application of MWOO at rates of approximately 360 t ha^{-1} can increase water holding capacity by up to 43%. Similarly, a review of the use of MWOO in agriculture by Hargreaves et al. (2008) revealed that due to its high organic matter content, MWOO had a high water holding capacity and its application to soil subsequently increased soil water holding capacity. Increasing water holding capacity of soils is important both agriculturally and environmentally because soils that hold larger volumes of water will require less irrigation to optimise plant growth and will not be as susceptible to nutrient, metal or chemical leaching (DEPI, 2014). As a result MWOO application to NSW soils, particularly when incorporated at high application rates, would appear to have positive effects on soil water holding capacity.

There was generally more variability in pH in leachate among the various soil types, depths and sampling events, however, it tended to increase with MWOO application for most soils (but not the sandy loam), and more so in leachate from the incorporated treatments. Leachate pH from the 140 t ha^{-1} incorporated application rate was generally the most significantly affected. Increases in soil pH by up to 1.4 pH units with MWOO application have been reported in much of the literature (Hargreaves et al., 2008; McConnell et al., 1993; Weber et al., 2007). This increase in pH with MWOO application has been attributed to the increase in negatively charged organic matter binding with and decreasing the hydrogen ions in the soil and solution, which can have implications for metal sorption and mobility.

There has been little investigation into pH values in leachate after MWOO application, however, one study has reported that pH in leachate from a loamy soil was unchanged after MWOO application, and decreased only slightly for a sandy soil after MWOO application (Kaschl et al., 2002). This is consistent with our findings for the sandy loam which tended to decrease at the higher incorporated application rates (as opposed to increase for most other soils), possibly due to the sandy loam having a low buffering capacity. This decrease in pH may also be attributed to the breakdown of organic matter by microbes reducing the negative ions (Kaschl et al., 2002), which would be more likely in the higher incorporated application rates since a larger surface area of MWOO would be available for microbial activity. Regardless of the trend, monitoring pH in both soil and water is important because changes in soil pH can influence plant growth and nutrient availability, and changes in water pH can have adverse effects on aquatic ecosystems and organisms (Boyd, 1998; Shiralipour et al., 1992).

Patterns in EC were generally consistent across all soils at both depths and in both sampling events. Application of MWOO tended to increase EC in leachate although not always significantly. There was a general trend of increasing EC with increasing MWOO application rates, however, it was mostly the incorporated treatments that showed a significantly higher EC than the control. Increases in EC with MWOO application are supported by the literature and are attributed to the high EC of MWOO (Hargreaves et al., 2008; Iglesias-Jimenez & Alvarez, 1993). The MWOO used in our research recorded a high EC and its addition to soil could have induced the downward movement of salt ions into the leachate. Another explanation is that high concentrations of salt forming ions, such as Na and Ca, are usually present in MWOO and application of these to soil may have also increased the EC of the leachate.

Increasing EC can cause salinity problems for both soil and waterways. Saline soils can inhibit seed germination and plant growth, and increased salinity in water ways can have potentially lethal effects on aquatic organisms (Hargreaves et al., 2008; Hart et al., 1991). As a result, guideline trigger values for EC were developed for freshwater systems in Australia, with the highest trigger value being 2200 µS/ cm in lowland rivers (ANZECC & ARMCANZ, 2000). This trigger value was almost always exceeded by the 140 t ha^{-1} incorporated treatments and also occasionally by the 50 t ha^{-1} incorporated treatment and 140 t ha^{-1} surface applied treatment. This indicates that MWOO addition to soils at high application rates, regardless of application method, would increase the potential for salinity to occur in soil and groundwater and hence have negative impacts on the organisms utilising these systems.

Consistent trends were also seen with DOC, whereby leachate from all MWOO applied treatments generally recorded higher mean DOC concentrations than the control. However, the incorporated treatments tended to have higher mean concentrations than the surface applied treatments. Additionally, DOC concentrations in leachate generally increased as MWOO application rate increased, with the 140 t ha^{-1} incorporated treatment almost always recording the greatest mean DOC concentration. Again, these findings are consistent with the literature (McConnell et al., 1993; Shiralipour et al., 1992). An increase in DOC concentrations with MWOO application can be explained by increased negative sorption surfaces in the soil through organic matter addition, which can increase the mobility of DOC. This increase can have negative impacts on the environment because DOC itself can be considered a contaminant as it can cause eutrophication of water ways and water colour, odour and taste issues (Kalbitz et al., 2000; Orica, 2012). It can also form complexes with cationic metals, and therefore, increased DOC concentrations in leachate can result in increased metal and metalloid concentrations in leachate, as was demonstrated in our research.

Nitrate concentrations in leachate were generally not significantly influenced by MWOO application, although a trend of decreasing concentrations in response to increasing MWOO application rates was observed, and this was significant for the 140 t ha^{-1} incorporated application rate. Ammonia concentrations in leachate tended to be lower in the surface applied treatments than the control, but increased in the incorporated treatments, particularly at 140 t ha^{-1}. This trend was often not significant and was inconsistent across all soils, depths and sampling events. The lower ammonia concentrations in leachate following surface application of MWOO, was also not consistent with the literature, which generally reports that MWOO application increases both ammonia and nitrate concentrations (Krogmann & Woyczechowski, 2000; Mamo et al., 1999). Possible reasons for the trends found in our research include immobilisation and nitrification of N.

Immobilisation is the conversion of inorganic forms of nitrogen, such as nitrate and ammonia, back to organic forms by microorganisms and usually occurs in high C:N ratio environments (>25) (Iglesias-Jimenez & Alvarez, 1993). The C:N ratio of the MWOO used in this experiment was only 17, however Iglesias-Jimenez and Alvarez (1993) reported that mature composts with C:N ratios less than 12 can still undergo N immobilisation. However, leachate nitrate concentrations were generally higher than ammonia concentrations, except in the 140 t ha^{-1} incorporated application rate, which could be explained by nitrification. Nitrification is the conversion of ammonia to nitrate and can be inhibited by metals and metalloids (Tomlinson et al., 1966; You et al., 2009). Since MWOO has high metal concentrations present there are likely to be more metals and metalloids at the higher MWOO application rates, which means that at 140 t ha^{-1} ammonia may not have undergone conversion to nitrate. Both nitrification and immobilisation are undertaken by microorganisms and since the soil columns were in place for 48 hours only before leachate was collected during the first sampling event, some doubt remains as to whether these processes would occur within this timeframe.

Patterns between metal and metalloid concentrations are also consistent between metals, soils, depths and, to a lesser extent, sampling events. Generally there were no significant differences in metal and metalloid concentrations between the surface applied treatments and the control for all metals and metalloids. Metal and metalloid concentrations tended to increase as incorporated MWOO application rate increased, with the 140 t ha^{-1} incorporated treatment almost always having significantly higher metal concentrations than the other treatments across all metals and metalloids. This trend is supported by the literature, whereby increases in metal concentrations in both soil and leachate of up to 500% after MWOO application are reported (Giusquiani et al., 1992; Kaschl et al., 2002; McConnell et al., 1993; Smith, 2009). For example, the column leaching experiment undertaken by Kaschl et al. (2002) with MWOO applied at a rate of 65 t ha^{-1} to a sandy soil (irrigated once a week with 200 mL of deionised water), revealed Cu, Ni and Zn had leached with maximum concentrations of 100 µg/L, 70 µg/L and 140 µg/L, respectively, well above the guidelines for freshwater systems in Australia. Maximum concentrations from our research exceeded these concentrations obtained by Kaschl et al. (2002) in sampling event 1 but not sampling event 2. Similarly, Giusquiani et al. (1992) undertook a soil column leaching experiment applying 90 t ha^{-1} of MWOO to a clay loam and a sandy loam with 650 mL irrigations for the clay loam and 550 mL irrigations for the sandy loam. Leachate was analysed for Cd, Cr, Cu, Ni, Pb and Zn and results reported as the mass of metal leached. The maximum metal mass leached in this study by Giusquiani et al. (1992) was much higher than the metal mass leached in our research, for all metals, all soils, both depths and both sampling events. The reason for this could be due to Giusquiani et al. (1992) applying much more water for the irrigation event, which would have resulted in a larger metal mass leaching compared to our experiment. The difference in results were not likely due to Giusquiani et al. (1992) oversaturating the soil with MWOO since both the soil and MWOO used in both experiments were similar.

Two main reasons have been attributed to the increase in metal concentrations with MWOO application. The first reason is that the metals are forming complexes with DOC, and as Christensen and Christensen (2000) and Navarro-Pedreno et al. (2003) state, these organic-metal complexes are formed primarily with Cd, Cu, Ni and Zn. It is likely, therefore, that as DOC concentrations in the leachate increase the metal concentrations will also increase. This is supported by findings from our research and also by Kaschl et al. (2002,) who reported that after applying MWOO to soil, metal concentrations increased due to increased organic matter in solution.

The second reason is that applying MWOO to soil can oversaturate the soil with metals, causing them to leach. Various studies in the literature report that this occurs, particularly when soil exchange sites are taken up by available Ca, K and Mg ions, which are also increased with MWOO application (Achiba et al., 2009; Weber et al., 2007). Giusquiani et al. (1992) reported that total metal content in leachate from MWOO amended soils was due to the increase in soluble metal ions from MWOO application. It is likely that in our research, both DOC-metal complex formation and oversaturation of the soil exchange would have occurred simultaneously, particularly as not all of the metals and metalloids analysed are influenced by DOC complex formation but were still leached with MWOO application.

Overall, it appears that incorporation of MWOO resulted in higher metal concentrations in leachate than surface application of MWOO. Applying MWOO at high rates of 140 t ha^{-1} also increased the metal and metalloid concentrations in leachate compared to the control and the lower application rates. There were often no significant differences in leachate metal and metalloid concentrations for the 20 and 50 t ha^{-1} application rates, however, EC, DOC and ammonia concentrations in leachate were often significantly higher at 50 t ha^{-1} than 20 t ha^{-1}. As a result, the 20 t ha^{-1} surface applied application rate appears to be the MWOO treatment with the least risks in terms of potential metal and metalloid contamination and salinity problems.

7.5.2 Effects of MWOO application on leachate at different depths

General trends between the two leachate depths for each variable were observed for all soil types and across both sampling events. In part, however, our results may have been due to the design of the pore water samplers used to collect soil solution (leachate) mid-way through a soil column at 15 cm using a vacuum. Hence, these samples were not expected to collect as much solution as at 30 cm, where leachate was freely dripping out the bottom of the soil column. Litaor (1988) reported that soil solution volumes collected from soil solution samplers may be low and will vary. In our research, the volume of soil solution collected was less significant at the higher application rates, particularly in the incorporated treatments, which may indicate that the larger amounts of MWOO retained more water, which is supported by the literature (McConnell et al., 1993; Weber et al., 2007).

A similar trend was noticed with pH, whereby pH in leachate was generally higher at 30 cm than at 15 cm, and this was less significant at the higher application rates. These results were variable across soils and sampling events, however, and a decrease in pH at 30 cm compared to at 15 cm was actually more evident with the sandy loam and the clay in sampling event 2. Achiba et al. (2009) also report increased pH with depth following MWOO application (at rates of 40, 80 and 120 t ha^{-1}), although their results were for soil rather than leachate. In addition, Kaschl et al. (2002), who did investigate leachate pH after MWOO application (65 t ha^{-1}), found that the pH in leachate was also generally higher with depth; at 50 cm compared to 25. These changes could be due to an increased interaction between negatively charged soil particles and positively charged hydrogen ions in a deeper soil profile, allowing for higher sorption of hydrogen ions and a reduction in pH in leachate at depth.

Electrical conductivity was generally higher at 30 cm when MWOO was surface applied, but appeared to be higher at 15 cm when MWOO was incorporated. Again, these results were more variable across soils and sampling events, but generally they were more significant at higher MWOO application rates. These EC results are supported by the work of Kaschl et al. (2002) and Achiba et al. (2009). The study by Kaschl et al. (2002) used MWOO mixed with the first 2 cm of soil, and could be therefore considered surface applied, compared to Achiba et al. (2009) who incorporated MWOO to 15 cm. Results from Kaschl et al. (2002) show that EC in leachate was higher at 50 cm compared to 25 cm (similar to our results for the surface applied treatments), but the results from Achiba et al. (2009) show that EC in the incorporated MWOO soil (a surrogate for leachate) was higher in the top 20 cm compared to 20-40 cm (similar to our incorporated treatments). However, because the EC of the MWOO used in our research was high (6220 µS/ cm) and the pore water samplers at 15 cm in the incorporated treatments were likely to come into direct contact with the MWOO; as opposed to those in samples with MWOO surface applied and for leachate collected at 30 cm, this could have resulted in higher leachate EC being recorded in samples at 15 cm.

The same trend for EC was seen with DOC, whereby concentrations were generally higher at 30 cm when MWOO was surface applied, but appeared to be higher at 15 cm when MWOO was incorporated. Of these two results, the DOC at 15 cm for the incorporated treatments was usually the highest. These trends was less evident in sampling event 2. In contrast to our results, Kaschl et al. (2002) found that organic matter in solution was higher at 25 cm than 50 cm when MWOO was surface applied to a sandy soil. However, it is important to note that the depths compared in our research were shallower than the depths compared by Kaschl et al. (2002), and this can make comparisons difficult. The reason for the results obtained in our research could be the same as was explained for EC, whereby the pore water samplers at 15 cm in direct contact with MWOO (incorporated treatments) would have higher DOC concentrations than when the pore water samplers were not in direct contact with MWOO (surface applied treatments).

Nitrate concentrations were almost always higher at 30 cm than 15 cm, whereas ammonia concentrations were higher at 15 cm. Kaschl et al. (2002) reported that nitrate concentrations in leachate their study increased with MWOO application and that the concentration from the sandy soil at 50 cm, following 65 t ha^{-1} MWOO application, was 135 mg/L. (Unfortunately Kaschl et al. (2002) did not report the concentration for the upper depth so a comparison of depths could not be made). The concentration reported by Kaschl et al. (2002) for 50 cm, however, was generally only exceeded in our research by nitrate concentrations at 30 cm in sampling event 1. Li et al. (1997) also undertook a small scale column leaching experiment using a sandy soil and applying MWOO at a rate of 100 t ha^{-1}. They reported that the maximum nitrate concentration in leachate was 246 mg/L, which was also less than nitrate concentrations collected at 30 cm in sampling event 1 of this experiment. Li et al. (1997) also reported maximum ammonia concentrations of 29 mg/L, which was higher than all ammonia concentrations across all soils and sampling events recorded in this experiment.

Metal and metalloid concentrations in leachate were generally similar at both depths for all surface applied treatments, but for the incorporated treatments, concentrations were generally higher at 15 cm, and most significant at the 140 t ha^{-1} application rate. Fewer significant differences were seen in sampling event 2. A likely explanation for the higher metal and metalloid concentrations in leachate at 15 cm when MWOO is incorporated, is that the pore water samplers are in direct contact with MWOO amended soil. Since metal concentrations in MWOO are high the metal concentrations in leachate at 15 cm when MWOO is incorporated are consequently higher than when pore water samplers are not in direct contact with MWOO, such as in the surface applied treatments.

Effects of MWOO on metal and metalloid concentrations in leachate were less easily determined at 30 cm due to increased sorption processes with depth. Kaschl et al. (2002) similarly reported higher metal concentrations in leachate at 25 cm than at 50 cm. Since the MWOO was surface applied by Kaschl et al. (2002), this is unlikely to be due to leachate samplers being in contact with the MWOO as occurred in our incorporated treatments. An alternative explanation for these results could be that sorption processes are taking place within the soil profile at depth. Since metals and metalloids from MWOO were still present at 15 cm in our research, it is likely that this occurred at depths below this. Achiba et al. (2009) also reported metal concentrations in soil after MWOO application were lower deeper down the soil profile, and they attributed this to the increase in soil cation exchange capacity after MWOO application. This increase in cation exchange capacity and hence increased metal sorption deeper in the soil profile is supported by McConnell et al. (1993) who reported a 31-94% increase in cation exchange capacity with MWOO application.

Overall it appears that negative effects associated with MWOO application, such as salinity issues and DOC and nitrate contamination, are of greater concern in leachate with increasing depth. However, metal leaching appeared to decrease with depth and at the lowest application rate of 20 t ha^{-1} was often similar to the control. These trends were generally consistent across all metals and metalloids.

7.5.3 Effects of MWOO application on leachate in different soils

Differences in leachate for the five soils were analysed for pH, DOC and metals and metalloids. The results for pH differences between soils in both sampling events revealed that the river loam had the highest pH in leachate and the sandy loam had the lowest. However, because results were consistent across the control and the MWOO applied treatments for the two soils, this indicates that MWOO application did not significantly influence their pH differences. Similarly, Kaschl et al. (2002) also found that a loamy soil had a higher leachate pH than a sandy soil, which was again consistent for the control and MWOO applied treatments.

However, the differences in leachate pH between the river loam and the sandy loam may have been associated with As leachate concentrations in leachate from the two soil types. The river loam and the sandy loam generally had the highest As concentrations in leachate, however, the river loam, did not have a high initial As concentration. The high As concentration in leachate from the river loam was likely due to the addition of As from MWOO and the high initial pH of the soil and soil solution (leachate), which would increase the negative sorption sites in the soil and hence mobilise additional As (Bissen & Frimmel, 2003). Conversely, the sandy soil recorded the lowest pH in leachate yet As was also mobile. This could be due to the sandy loam having a high As concentration initially and addition of MWOO may have saturated the exchange, causing As to leach. McLean and Bledsoe (1992) support this reasoning, reporting that increasing metal concentrations can saturate exchange sites and cause increased metal mobility. This is the most likely reason for increased As concentrations in the river loam and sandy loam after MWOO application since the differences in leachate pH between soils were not due to MWOO application.

Differences between soils for DOC concentrations and the remaining metals are generally less consistent between treatments and sampling events. For example, DOC concentrations in sampling event 1 were generally highest in the Minesite soil and lowest in the sandy loam across all treatments. When MWOO was surface applied at a higher rate DOC from the clay also increased relative to the control, but when MWOO was incorporated there were no significant differences between the soils. This indicates that incorporated application of MWOO may have added enough soluble organic carbon to negate the effect of soil type.

In sampling event 2, the application rate and method appeared to more greatly influence which soil had the highest DOC concentration. The controls showed no significant differences between soil types, indicating that any differences between soil types for the remaining treatments was due to MWOO application. For both the surface applied and incorporated 20 t ha^{-1} treatments leachate from the CROA soil had the higher DOC concentration, for the 50 t ha^{-1} surface applied treatment the river loam had the highest DOC concentration, and for the 140 t ha^{-1} incorporated treatment the clay had the highest DOC concentration. These results are in contrast with Kaschl et al. (2002), who reported that DOC concentrations in leachate were higher in a sandy soil compared to a loamy soil, which they suggested indicates that heavier soils show less DOC leaching. However, in our research, MWOO addition at high application rates resulted in higher DOC leaching from the heavier clay soil. Kaschl et al. (2002) also reported that DOC solubility can be increased by high pH. This may support the results shown in sampling event 2 since pH was highest in the river loam and the clay and generally increased significantly in the clay at high application rates. This indicates that the differences in DOC between soil types may also be due to a pH effect.

For Cd, Cu, Ni, and Pb, differences between soils for the two sampling events were similar, whereby: leachate from the CROA soil generally recorded the highest concentrations of Cd; the river loam demonstrated the highest concentrations of Cu; the clay showed the highest concentrations of Ni; and the sandy loam was generally highest for Pb. For Cr and Zn there were generally no differences between soils in sampling event 2, but there were in sampling event 1. In this case, the leachate from clay exhibited the highest Cr concentration when MWOO was surface applied but the lowest when it was incorporated, and for Zn leachate from the CROA soil generally showed the highest Zn concentrations in all treatments. Generally, patterns in metal concentrations in leachate observed between soils were similar for the control and the surface applied treatments, indicating that surface application of MWOO was not influencing differences in metal concentrations between soils. The main influence of MWOO application appeared in the incorporated treatments, where the addition of metals by MWOO may have saturated the exchange and resulted in more uniform metal leaching across all soils.

In sampling event 1, the differences in leachate metal and metalloid concentrations between soils actually appeared to be due to initial soil metal concentrations and soil texture. Generally metal leaching in sampling event 1 was greatest in soils that had high initial metal concentrations and in soils with a coarse texture. As mentioned above, addition of metals to soil, which would have occurred with MWOO application, can saturate the soil exchange and increase metal leaching (McLean & Bledsoe, 1992). However, another explanation for these results is soil texture. Giusquiani et al. (1992) also reported that increases in total metal mass leached after MWOO application were higher in a coarse textured sandy loam compared to a fine textured clay loam, which they attributed to the increase in soluble metal ions from MWOO application. Soils with a heavier texture, such as the clay, generally experience higher metal sorption, and soils with a coarser texture, such as the sandy loam and river loam, generally exhibit higher metal leaching (Giusquiani et al., 1992; McLean & Bledsoe, 1992). Despite this general texture classification, our clay soil recorded the greatest Cr leaching in sampling event 1, which can be attributed to the clay having initial Cr concentrations approximately four times higher than the other soils.

In sampling event 2 the differences between soils can be primarily attributed to DOC-metal complex formation. Cationic metal leaching was generally highest in the soils that had the highest DOC leaching, which were the CROA soil, the river loam and the clay. Numerous papers report the likelihood of increased leaching from DOC-metal complex formation, particularly with organic amendment (Christensen & Christensen, 2000; Christensen et al., 1996; Temminghoff et al., 1997). Even though our results oppose the idea that metal leaching is more likely to occur in a sandy soil than a clay soil (McLean & Bledsoe, 1992), because cations can form strong DOC-metal complexes increased metal leaching can occur regardless of soil texture, particularly so for Cd, Cu, Ni and Zn (Christensen & Christensen, 2000). Kaschl et al. (2002) demonstrated this, where metal concentrations in leachate were higher in a sandy soil compared to a loamy soil, which they attributed to organic matter in solution (DOC) being higher in the sandy soil and increased formation of DOC-metal complexes in the sandy soil. This result, therefore, was an effect of DOC-metal complex formation rather than soil texture.

Overall it appears that the risk associated with metal leaching from MWOO amendment is relatively dependent on background metal concentrations of the amended soil and DOC leachability rather than soil texture alone. While the paper by Kaschl et al. (2002) demonstrated that DOC leaching is more likely in a sandy soil than a loamy soil, this experiment conflicted with those findings.

7.5.4 Effects of MWOO application on leachate over time and with consecutive rainfall events

General trends between the two sampling events were seen between the different soil types, with some differences between depths. Total leachate did not change with the two sampling events due to soils being filled to 100% FC prior to the rainfall event and the rainfall event being 65 mL for both sampling events. Increases in leachate pH in sampling event 2 were observed for all soils except the sandy loam and the CROA soil, which decreased in sampling event 2. These findings are supported by Kaschl et al. (2002) who found that leachate pH in a sandy soil decreased with time and pH in the loamy soil increased with time.

Decreases in EC, DOC and nitrate in leachate were observed in sampling event 2 for all soils except the clay. These findings were generally supported by Kaschl et al. (2002), who reported decreases in EC and organic matter in solution after an initial flush. Similarly, Hargreaves et al. (2008) reported that the magnitude of increased EC with MWOO application decreases with time. Mamo et al. (1999) reported that initial increases in nitrate leaching after MWOO application also decreased with time. The link between all of these studies is that the soils used were either of a sandy or loamy texture, and little remains known about the longer term effects of MWOO application to clay.

It is surmised that the increases in EC, DOC and nitrate in leachate from the clay soil in our research could be due to water movement through clay being slower than the other soils. Despite obtaining the same leachate volume, much of the deionised water used to fill the soils to 100% FC initially, may not have passed through the MWOO before been leached in sampling event 1. For the other soils with higher pore spaces, water that had been contaminated with EC, DOC and nitrate may have passed through that deionised water in the equilibration period before the first sampling event. In addition, the clay soil is prone to cracking and cracks would have formed in the two weeks between sampling events, allowing for preferential flow through the cracks in the second sampling event, which may have increased EC, DOC and nitrate concentrations in leachate from the clay.

Ammonia behaved differently in the two sampling events depending on soil type. Ammonia concentrations were higher in sampling event 2 for the sandy loam, the clay and the river loam but were higher in sampling event 1 for the Minesite soil and the CROA soil. Few studies report the effect of MWOO application on ammonia in leachate, however Iglesias-Jimenez and Alvarez (1993) and Hargreaves et al. (2008) both reported that addition of MWOO increased ammonia adsorption to soil due to an increased cation exchange capacity. This would suggest that ammonia concentrations in leachate should decrease with MWOO application through time, which was the case for the Minesite soil and the CROA soil but not the sandy loam, the clay or the river loam.

Metal concentrations in leachate generally decreased in sampling event 2 compared to sampling event 1. Primary concerns are for Cu, Cd, Ni and Zn because in sampling event 2 their concentrations in leachate were sometimes higher than the control and concentrations recorded in sampling event 1. This is supported in the literature by Kaschl et al. (2002,) who found that, in general, Cu, Ni, Zn and Cr concentrations decreased through time in an MWOO amended sandy soil and loamy soil compared to a control. Giusquiani et al. (1992) also found that with subsequent irrigation events total metal mass eventually decreased in an MWOO amended clay loam and a sandy loam.

Similar findings have been reported for metal concentrations in soil years after MWOO application, rather than just consecutive rainfall events. Weber et al. (2007) reported metal concentrations in a sandy soil each year for three years after MWOO application and the general trend was that metal concentrations decreased each year. Achiba et al. (2009) reported metal concentrations in a clayey loam 5 years after MWOO application, and although concentrations immediately after MWOO application were not reported, metal concentrations (with the exception of Cu) in soil at a low application rate of 40 t ha^{-1} were not significantly different from the control after 5 years. Conversely, Ayari et al. (2010) reported metal concentrations in a loamy clay soil each year for four years with annual MWOO application. They reported that at application rates of 40 and 80 t ha^{-1} metal concentrations in soil increased in each subsequent year, with the fourth year having significantly higher metal concentrations in soil than the first year at the same application rate.

Even though, in general, metal concentrations in soil and leachate decrease with time and with subsequent rainfall events, the study by Ayari et al. (2010) demonstrates that risks are associated with repeated MWOO application.

7.5.5 Comparing metal and metalloid concentrations to guideline levels

The limitations in comparing trace metal and metalloid leachate concentrations obtained from controlled laboratory experiments to environmental guidelines for the purposes of risk assessment is discussed in Chapter 6. Such comparisons can only serve to give a measure of relative potential risk. With this in mind and examining the results obtained in this experiment against the guidelines available for 95% freshwater species protection and drinking water guidelines (Table 6.7) it is apparent that these guideline values were exceeded in samples from many treatments for most elements Nevertheless, some general trends are evident across soils, and between sampling events. The lower application rates, especially 20 t ha^{-1}, recorded fewer guideline exceedances. The maximum % exceedance was for the metals Cu, Zn and Ni but this declined with subsequent sampling events and was modified by the background soil trace element concentration. In light of the quite elevated measured soil pore water concentrations observed in this experiment, leachate monitoring is recommended where MWOO is applied to NSW soils. Extension of this experiment to field conditions and scale would provide an improved assessment of actual risk particularly with consideration of ecotoxicological implications in Australian soils.

7.6 Conclusions

Overall, pH in leachate demonstrated few significant differences across all soils, depths and sampling events but EC, DOC and metal concentrations in leachate increased after MWOO was applied to soil. These variables typically showed the highest concentrations in leachate for the 140 t ha^{-1} treatments. Incorporation of MWOO tended to significantly increase the concentrations of these negative variables, in contrast to surface application, and the effect of MWOO was evident at both 15 cm and 30 cm depth. The concentrations of variables in leachate at both sampling depths generally decreased with time and with consecutive rainfall events.. For both sampling events, differences in metal concentrations between soils was variable, although the MWOO application to the Minesite soil, with a silty loam texture and almost neutral pH, showed generally a smaller influence on trace metal and metalloid concentrations at either depth or in either sampling event.

This research has highlighted the metals of most concern for leaching in the soils tested were Cu, Ni and Zn. These elements were present in leachate in the highest concentrations, generally showed the greatest total metal mass in leachate for all soil treatments comparted to the untreated control at both depths and in both sampling events, and showed the maximum exceedance compared to guideline values . Other metals were also elevated in treatments with MWOO additions but this was restricted to certain soil types, depths or sampling events.

In light of the influence of MWOO on soil pore water concentrations observed in this experiment, leachate monitoring is recommended where MWOO is applied to NSW soils. Extension of this experiment to field conditions and scale would provide an improved assessment of actual risk for MWOO applications to NSW soils particularly with consideration of ecotoxicological implications for these soils.

7.7 References

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8. Appendices

APPENDIX A: MSc Thesis

APPENDIX B: MSc Thesis

APPENDIC C: Draft PhD Thesis