



# **Incorporation of Melton Biosolids into an Energy cropping system**

## **Final report to Smart Water**

**Firew Beshah , Barry Meehan and Nichola Porter: RMIT University, Melbourne  
Roger Wrigley: University of Melbourne**

## Acknowledgments

The authors of this report particularly want to thank William Rajendram, Rodney Curtis and the staff at the Surbiton Park Western Water treatment plant for their very generous technical support, their financial support and their enthusiastic camaraderie.

Thanks are also extended to PineGro for providing composted biosolids, to Mr. Nick Kelly for his helpful ideas in crop pest and weed management, and Mr. George Croato and Mr. John Cauduro for their collaboration in laboratory analyses of some of the samples at the Department of Primary Industry, State Chemistry Laboratory (Werribee).

Firew Beshah would also like to thank the technical staff at RMIT for their constant support, and financial support for his studies from the Australian Government (with the award of an Australian Postgraduate scholarship) and from Smart Water.

Most importantly we would like to thank Smart Water for funding this project and providing opportunities to meet others involved in similar work around the country.

## Table of Contents

|  |    |
|--|----|
| Acknowledgments.....   | 2  |
| Executive Summary .....  | 8  |
| Background.....  | 9  |
| Summary of Results .....   | 13 |
| Yield parameters .....   | 13 |
| Germination rates.....   | 17 |
| Seed analysis.....   | 18 |
| Contaminants: Heavy Metals.....                                    | 18 |
| Total metals:.....   | 18 |
| DTPA Extractable metals: .....                                     | 25 |
| Plant uptake of Metals: .....                                      | 33 |
| Soil pH: .....   | 36 |
| Nutrients.....   | 37 |
| Nitrogen -Total N.....   | 37 |
| Nitrogen N-nitrate N.....  | 38 |
| Leaching of NO <sub>3</sub> -N down the soil profile.....          | 40 |
| N uptake in plants .....   | 41 |
| Nitrogen mass balance .....  | 42 |
| Phosphorus:Total and Olsen- extractable .....                      | 43 |
| P uptake in Plants.....  | 47 |
| Microorganisms .....   | 49 |
| Economic valuation .....   | 50 |
| References.....  | 53 |
| Appendix: Methods.....   | 55 |
| Measurement of soil moisture, pH and electrical conductivity ..... | 55 |

|   |    |
|---|----|
| Determination of exchangeable bases ( $K^+$ , $Ca^{2+}$ , $Mg^{2+}$ and $Na^+$ ) and cation exchange capacity ..... | 55 |
| Determination of the bioavailable forms of heavy metals in soil and biosolids.....                                  | 55 |
| $HNO_3/H_2O_2$ extractable heavy metals in soil, biosolids and plant matter .....                                   | 56 |
| Determination of total metals in soil and biosolids using X-Ray Fluorescence Spectrometry .....                     | 56 |
| Total nitrogen and carbon in soil and total nitrogen in plant matter.....   | 57 |
| $NO_3-N$ in soil, biosolids and in plant matter.....  | 57 |
| Determination of Olsen-extractable P in soil and biosolids.....   | 58 |
| Acetic acid extractable phosphorus in plant matter .....  | 58 |
| Seed analysis.....  | 58 |

## Table of Tables

|  |    |
|--|----|
| Table 1 Concentrations of total heavy metals in soil, dewatered and composted biosolids determined by X-ray fluorescence expressed in mg/kg.....                                 | 19 |
| Table 2 Residual concentrations of total heavy metals in dewatered biosolids amended soil in canola plots in 2006 and 2007 determined by XRF expressed in mg/kg.....             | 20 |
| Table 3 Residual concentrations of total heavy metals in dewatered biosolids amended soil in oat plots in 2006 and 2007 determined by XRF expressed in mg/kg.....                | 21 |
| Table 4 Changes in XRF determined total soil heavy metals in dewatered biosolids amended canola and oats plots in 2006 and 2007 experiments.....                                 | 22 |
| Table 5 Residual concentrations of total heavy metals in composted biosolids amended soil in canola plots in 2006 and 2007 determined by XRF expressed in mg/kg.....             | 23 |
| Table 6 Residual concentrations of total heavy metals in composted biosolids amended soil in oat plots in 2006 and 2007 determined by XRF expressed in mg/kg.....                | 24 |
| Table 7 Changes in XRF determined total soil heavy metals in composted biosolids amended canola and oats plots in 2006 and 2007 experiments.....                                 | 24 |
| Table 8 Results of concentrations of DTPA extractable heavy metals in soil, dewatered and composted biosolids.....   | 26 |
| Table 9 Effect of various dewatered and composted biosolids application rates on concentrations of heavy metals in canola and oats leaves in 2006 field experiment (mg/kg).....  | 33 |
| Table 10 Effect of various dewatered and composted biosolids application rates on concentrations of heavy metals in canola and oats leaves in 2007 field experiment (mg/kg)..... | 35 |
| Table 11 Concentrations of total and extractable N and P in soil and in the two biosolids. ....  | 37 |
| Table 12 The effect of dewatered and composted biosolids applications on the levels of TN (%) in canola and oat leaves in 2006 and 2007 field experiments.....                   | 42 |
| Table 13 Mass balance of total nitrogen in dewatered biosolids amended canola and oats plots in the 2006 and 2007 field experiments.....   | 43 |
| Table 14 Concentrations of total and extractable P in soil and biosolids used for the experiments (µg/g). ....   | 44 |

|   |    |
|---|----|
| Table 15 The effect of dewatered and composted biosolids applications on acetic acid extractable phosphorus in canola and oats leaf in the 2006 and 2007 field experiments.....                               | 47 |
| Table 16 Mass balance of total phosphorus in dewatered biosolids amended canola and oats plots in the 2006 and 2007 experiments. ....   | 48 |
| Table 17 Survival of pathogens in biosolids and biosolids amended soil after 12 months of biosolids application in 2006 and 2007 at Surbiton Park. ....   | 49 |
| Table 18 Average quantity of plant available nutrients (NPK) contained in dewatered biosolids (kg/t) and the price/kg and the nutrient values of dewatered biosolids price/tonne of biosolids (in Aus\$)..... | 50 |
| Table 19 Average quantity of plant available nutrients (NPK) contained in composted biosolids (kg/t) and the price/kg and the nutrient values of composted biosolids price/tonne of biosolids (in Aus\$)..... | 51 |

## Table of Figures

|   |    |
|---|----|
| Figure 1 Canola grown using dewatered and composted biosolids.....  | 13 |
| Figure 2 Oats grown using dewatered and composted biosolids.....  | 14 |
| Figure 3 Canola seed, dry biomass yields and yield components versus biosolids application rates (2006).....  | 15 |
| Figure 4 Oats seed, dry biomass yields and yield components versus biosolids application rates (2006).....  | 16 |
| Figure 5 Effect of dewatered and composted biosolids application rates on soil temperature and canola seed germination in the 2007 field experiment. ....   | 17 |
| Figure 6 Effect of dewatered biosolids on the residual DTPA extractable metals continued. ....  | 29 |
| Figure 7 Effect of Composted biosolids on the residual DTPA extractable metals continued.....   | 31 |
| Figure 8 The change in the mean values of soil TN in canola and oats plots under increasing applications rates of dewatered and composted biosolids in 2006-2007 field experiments. ....          | 38 |
| Figure 9 The effect of dewatered and composted biosolids application rates on the level of NO <sub>3</sub> -N accumulated in the top 10 cm of soil in canola and oats plots in 2006 and 2007..... | 39 |
| Figure 10 The effect of dewatered and composted biosolids application rates on the level of total phosphorus in amended soil in 2006 and 2007 field experiments.....                              | 45 |
| Figure 11 The effect of dewatered and composted biosolids application rates on the level of Olsen- extractable phosphorus in amended soil in 2006 and 2007 field experiments.....                 | 46 |

## Executive Summary

This report presents the findings of an investigation into land application of biosolids on the basis of two years of field trials. These trials took place during 2006 and 2007 at the Western Water Recycled Water Plant (WWRWP) at Surbiton Park in Melton Victoria, to determine the economic and environmental feasibility of utilising the biosolids produced at the plant as a fertiliser to produce canola and oats for energy and fodder production respectively. Growing the crops on-site would provide Western Water with a disposal option for their biosolids with the added benefit of producing cash crops. The aim was to provide information that would allow Western Water to apply biosolids at the highest rate possible without either compromising crop production or contaminating the land on which they are applied. The results were compared with crops grown using both conventional fertilizers and composted Western Water biosolids.

In each experiment a range of application rates was employed and crop production was measured. The soil and crops were analysed for residual metals and nutrients, and biosolids and biosolids-amended soils were analysed for survival of pathogens. The economic value of the biosolids themselves was also evaluated.

By determining the rate of change in the seed yield of canola per unit of biosolids applied, it was determined that the optimal rate of application, was 54 t/ha dewatered biosolids, which would provide 4.5 t/ha of seed. The energy value of the canola oil produced per hectare of land using this rate was estimated to be 71,600 MJ/ha. However, this high rate of application would lead to nutrient and metal residuals which would need remediation after a few years.

Using the farm-gate price of canola seed in the Melton area of A\$ 470/tonne of canola seed, the value of one tonne of dewatered biosolids was calculated to be A\$39. The value of composted biosolids calculated in this way was A\$13.5/ tonne of composted biosolids. The calculated value based on nutrient content was A\$20.54 per tonne of dewatered biosolids, and A\$13.25 for composted biosolids.

The results of this field investigation have shown that biosolids produced at Surbiton Park can be used as a source of plant nutrients without significantly contaminating the receiving soil with heavy metals and pathogens, in the short term. Longer periods of application, particularly at higher rates, could create problems with nitrogen leaching and contamination with copper (Cu)



and zinc (Zn). Crop rotation showed potential as an effective means of controlling excessive build up of nitrogen (N) and phosphorus (P) in receiving soils and could also be employed with appropriate plant choices to control heavy metal build-up. In addition the results of the study could be used as inputs to refine and update the Victorian guidelines for land application of biosolids with due emphasis given on site specific biosolids applications which take into account soil physical and chemical properties. Finally, the study may assist to improving the image of biosolids and increasing the use of biosolids in the farming communities, and has demonstrated the effectiveness of collaborative practical research between industry, local communities and research institutions.,

## **Background**

Biosolids are treated or stabilized sewage sludge produced during the biological treatment of sewage. Gale (2007) estimated that approximately 360,000 dry tonnes of biosolids were produced annually in Australia in 2007 at a cost of \$300/dry tonne of biosolids. This is typically 40 to 50 percent of the total capital and operating cost for wastewater treatment facilities. In Victoria, most of this is stockpiled, which both the government and the water industry have agreed is not a sustainable management option. Since biosolids contain a high nutrient and carbon content, application to land could reduce stockpiles, reduce the amount of chemical fertiliser used in farming systems and improve soil quality. However, contaminants and nutrients need to be controlled for sustainable use of this resource.

Western Water is one of Victoria's 13 regional urban water corporations which provide water and sewage services to over 53,000 properties and 134,810 people in an area of 3000 km<sup>2</sup>. The region extends from Lancefield in the north to Melton and Rockbank in the South, and from Myrning in the West to Bulla in the East. It incorporates parts of Hume City Council and Melton, Moorabool and Macedon Ranges Shire Councils. Therefore, Western Water's region is a combination of urban and rural living with a significant proportion of the land devoted to agricultural uses, particularly grazing and cropping. This makes the area an ideal site on which to trial land application of biosolids to grow crops.

The current project was designed to investigate environmentally and economically sustainable management options for the biosolids generated at WWRWP through incorporating these biosolids into a cropping system using canola and oats under a rotation regime on a clay loam soil at Melton, Victoria. Canola and oats were chosen as they are successfully grown around the Melton area and are suitable for energy production and livestock fodder respectively. In particular the experiments were designed to investigate:

- crop responses to dewatered and composted biosolids
- the effects of increasing application rates on yields
- nutrient and heavy metal accumulation in receiving soils
- the effect of crop rotation on nutrient and heavy metal accumulations
- the survival of pathogens on amended soils
- the economic value of the biosolids in this system
- the potential for project outcomes to be incorporated into the biosolids management strategy at WWRWP

The following sections of this report present a summary of the findings from the field experiments in which canola and oats were produced for energy and fodder production respectively. More details of the experimental design and results are described in the PhD thesis manuscript (Beshah, 2010) which resulted from this work, as well as previous Milestone reports. (The PhD thesis will be available to Smart Water in June 2010.)

### **Description of the field experiment**

The project involved an innovative beneficial use of biosolids generated by WWRWP at Surbiton Park, Victoria. Two types of biosolids at various application rates were incorporated on a 40×37 m<sup>2</sup> plot of land at WWRWP. The biosolids used were dewatered biosolids from Western Water and composted Western Water biosolids supplied by Pinegro (Deer Park).

Seed and plant biomass responses of canola and oats to the applications of biosolids were evaluated under field conditions. Nutrients and heavy metals in soil and plants and pathogens in the amended soil were measured; leaching potential of NO<sub>3</sub>-N was also investigated.

The effect of cropping sequence on nutrients and heavy metal residues accumulated after two years of successive applications of biosolids was also examined.

The 37 x 40 m<sup>2</sup> plot of land at WWRWP was cleared of weeds, treated with gypsum to improve the soil structure, tilled with a rotary hoe and divided into 72 plots, each 6 x 2 m<sup>2</sup>.

Before establishing the first crop, composite soil samples from the experimental site, dewatered biosolids from the RWP and composted biosolids from Pinegro (Deer Park) were sampled and analysed for pH, EC, CEC, total N, P, S & C and total and extractable metals.

Dewatered and composted biosolids application rates were calculated based on the NLBAR (nitrogen limited biosolids application rates) for canola and oats, and incorporated into the top 10 cm of the soil depth.

Six treatments, which consisted of 4 biosolids application rates (5, 25, 45, 65 t/ha of anaerobically digested dewatered biosolids and 10, 30, 50, 70 t/ha of composted biosolids), a conventional fertilizer control (100:20:50 kg/ha for N: P: K) and an untreated control, were arranged in triplicate in a randomized block design.

Canola and oats seeds were sown at seeding rates of 5 and 100 kg/ha respectively, watered twice a week and harvested after six months.

In the second year of the trial, biosolids were reapplied at the same rates to each of the plots as in 2006, but the crops were rotated to reduce the potential for disease and gauge the effect of crop rotation parameters measured.

To monitor for residual contaminants in soil, biosolids amended soil samples (top 10 cm) were taken after the end of each experiment and analysed for pH, EC, total and extractable forms of N, P and heavy metals. To assess plant uptake of nutrients and heavy metals, canola and oats leaf samples were taken and analysed for N, P and heavy metals. Concentrations of N, P, S and oil in the canola seeds were also determined.

Whole plant samples were taken after the end of the first and second years of the experiments to determine yields and yield components of both plants.

At the end of each harvest, biosolids amended soil samples were analysed for the survival of pathogens as per Australian Standards: AS5013.15 2004 for *E.coli*, AS5013.16, 2004 for *Clostridium perfringens* and F0677 (37<sup>0</sup>C/16-20 hr) for *Salmonella*.

To examine the effect of biosolids applications on soil temperature and seed germinations, soil temperature data were recorded from biosolids treated and untreated control plots of canola and oat crops (10 readings per plot) in the top 10 cm. The numbers of canola and oats seedlings were counted by taking a 1 m<sup>2</sup> quadrant from the centre of each plot.

The economic value of dewatered and composted biosolids was assessed using two scenarios. The first scenario examined the increase in canola seed production following the use of biosolids, while the second used their nutrient value (N, P, and K).

## Summary of Results

### *Yield parameters*

Canola produced its highest seed yield with an average of 4.6 t/ha using 65 t/ha of dewatered biosolids and 2.5 t/ha using 70t/ha of composted biosolids ( $p < 0.05$ ). Oats produced the highest seed yield with an average 6.3 and 6.4 t/ha at the 45 and 70 t/ha dewatered and composted biosolids application rates respectively ( $p < 0.05$  and  $p < 0.01$ ). These results were significantly better than those achieved with conventional fertilisers, with the dewatered biosolids giving the best results. Figures 1 and 2 show the healthy growth of crops grown on biosolids with the tallest crops being grown on the highest application rate of dewatered biosolids.

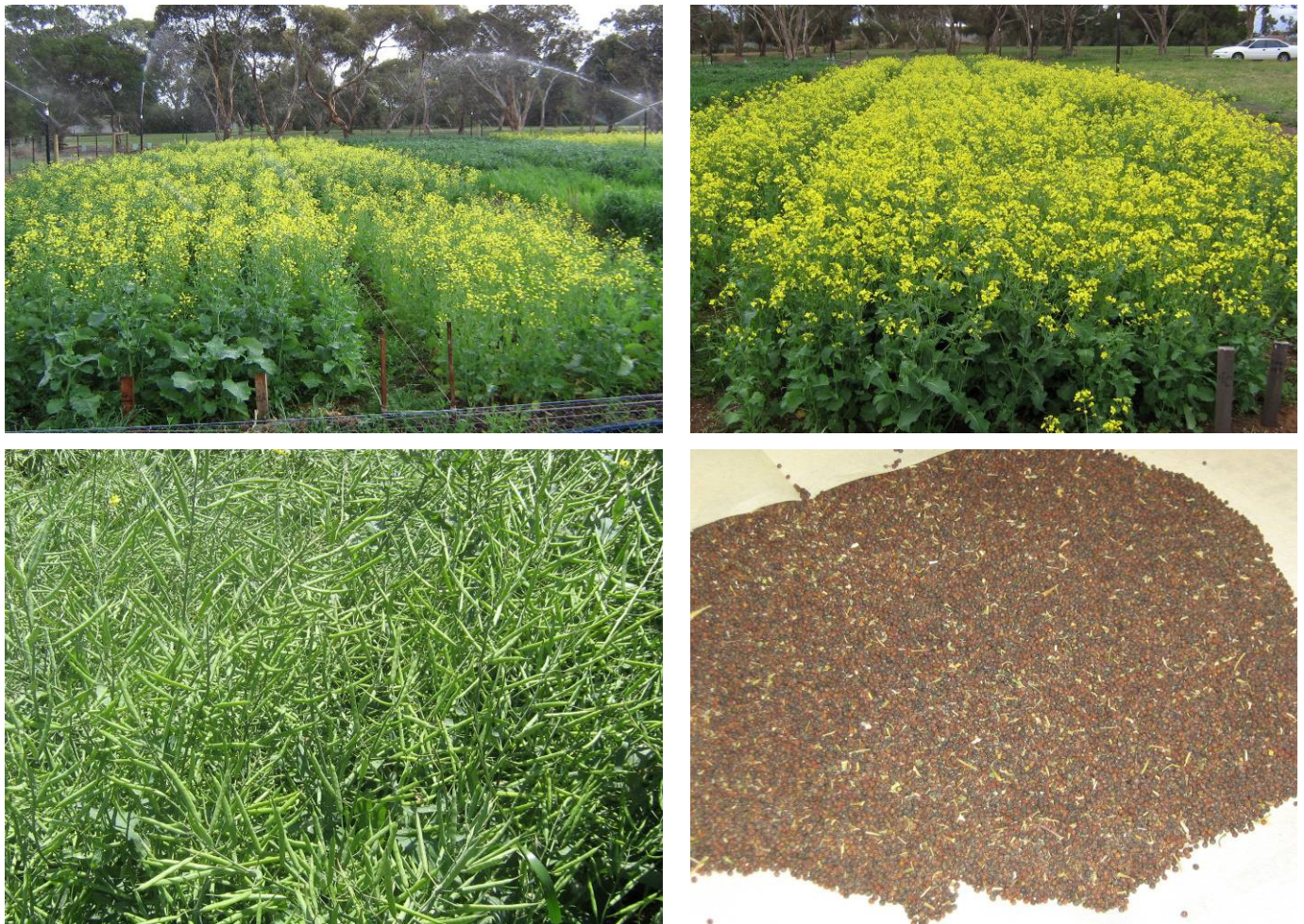


Figure 1 Canola grown using dewatered and composted biosolids





Figure 2 Oats grown using dewatered and composted biosolids

Figures 3 and 4 show the response of the various yield parameters measured versus the biosolid application rates in 2006. A similar, slightly better response was observed in 2007 due to the increased nutrient availability assumed to have resulted from the continued nitrogen mineralisation occurring from the biosolids applied in the first year. However, the oat crop was compromised in the second year due to a rust infestation.

Since neither the seed per pod (in the case of canola) nor grain per panicle ( in the case of oats) changed significantly, it is clear that the main reason for increased seed yield was due to the enhanced height and branch growth when biosolids were applied.

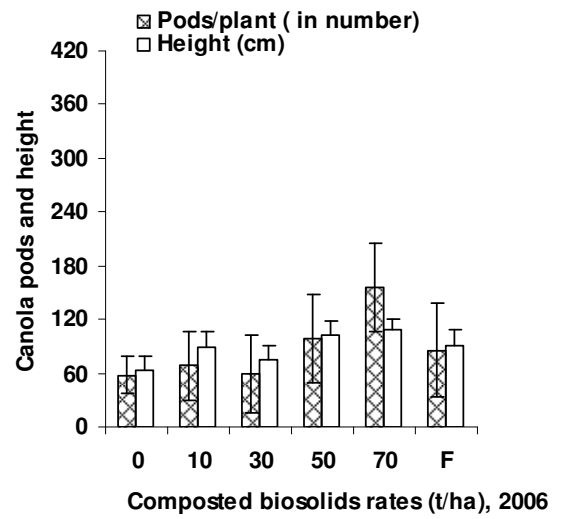
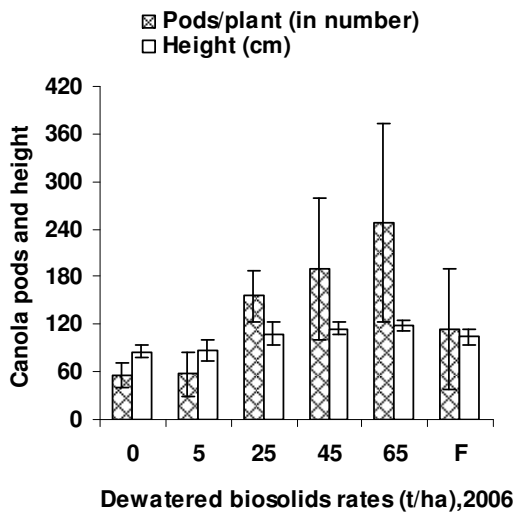
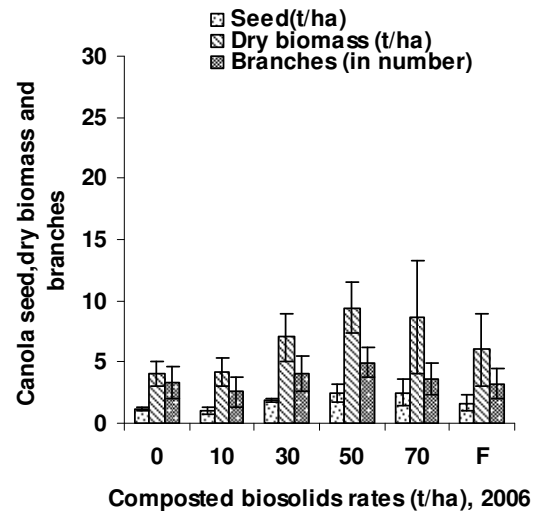
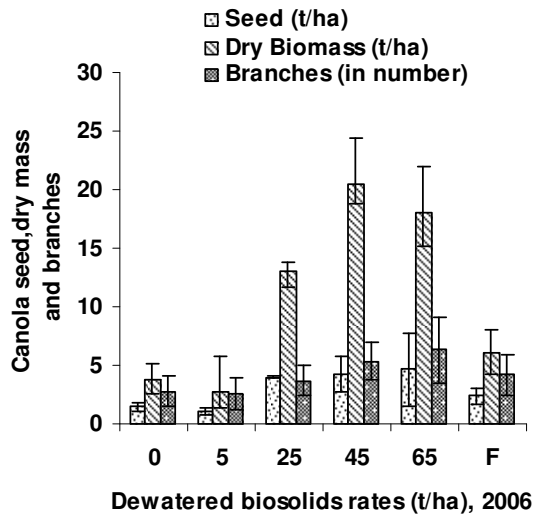


Figure 3 Canola seed, dry biomass yields and yield components versus biosolids application rates (2006).

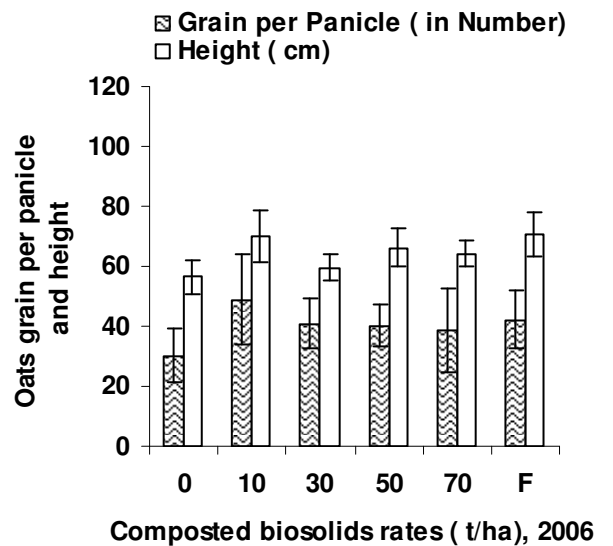
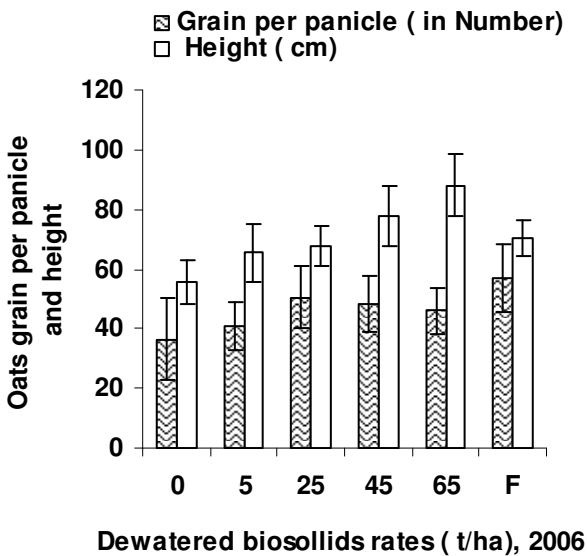
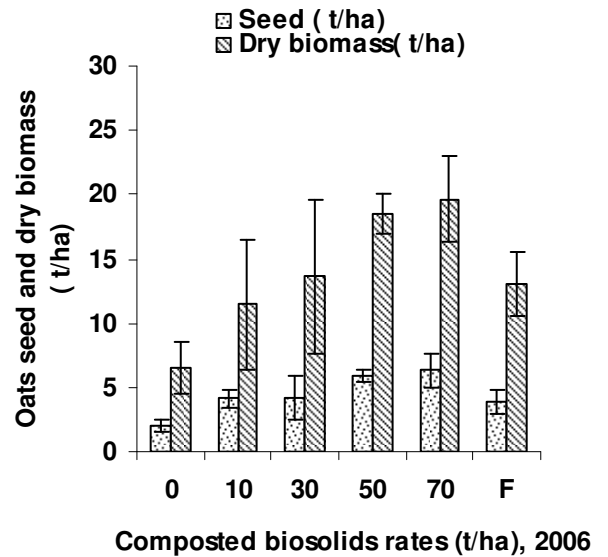
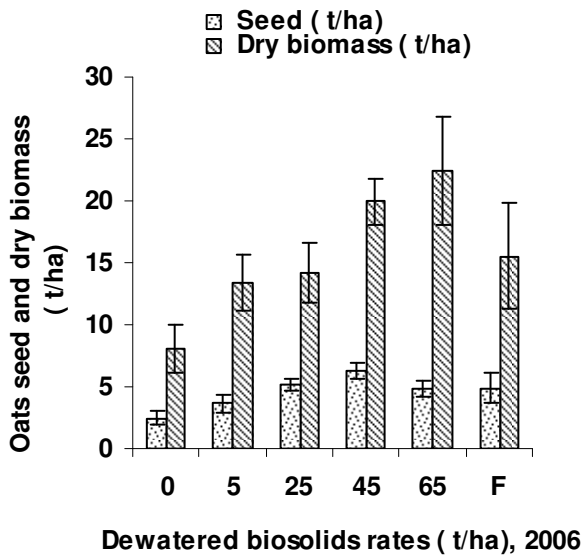


Figure 4 Oats seed, dry biomass yields and yield components versus biosolids application rates (2006).



### Germination rates

In the second year of the field trial, the seeds were planted earlier than in the first year, and as a result, the morning temperatures were lower. This affected the germination of the canola crop. Oats were unaffected as they can tolerate lower temperatures (Welch, 1995). It was noticed that the application of biosolids increased soil temperature which was positively correlated with germination rates of canola seedlings. This positive correlation can be seen in Figure 5. It can also be seen that at the highest application of both types of biosolids, germination of canola was close to 100% in these soils.

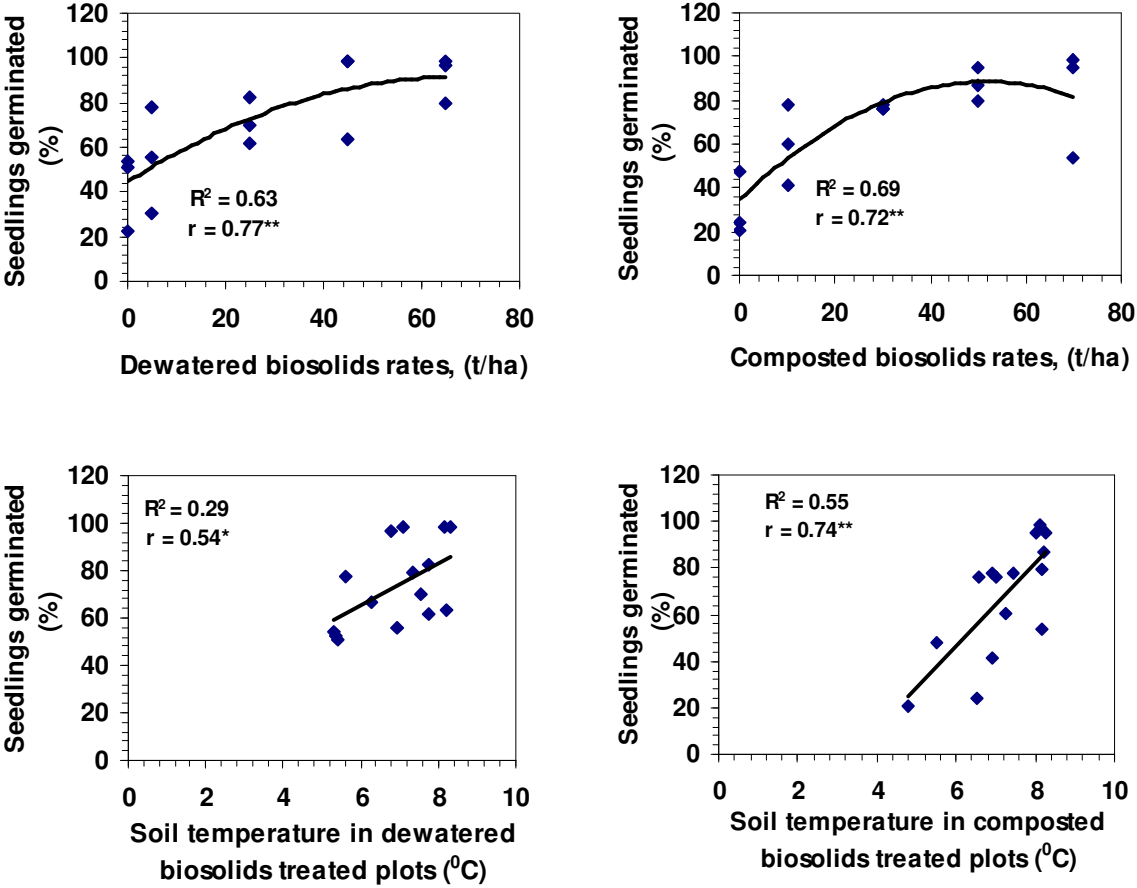


Figure 5 Effect of dewatered and composted biosolids application rates on soil temperature and canola seed germination in the 2007 field experiment.

## ***Seed analysis***

Analysis of canola seed oil showed that the levels of total N, P and S in canola seed oil increased with increasing application rates of biosolids. Conversely, seed oil concentration decreased and was negatively correlated with concentrations of total nitrogen (TN) in the seed. This has been observed by others (Pritchard et al., 2000; Norton, 1993 ). Nevertheless, since the quantity of seed was much higher with the application of dewatered biosolids, the overall oil yield was highest with the highest application rates of dewatered biosolids.

## ***Contaminants: Heavy Metals***

Total copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), nickel (Ni) and lead (Pb) were measured in the biosolids and in the soils before and after application of biosolids, by X-ray fluorescence. Bioavailable metal concentrations were determined with the use of a DTPA extraction method (details of methods are in the Appendix).

### **Total metals:**

Table 1 shows the total metals concentrations in the soil and biosolids. Cu and Zn were the metals with the highest concentrations in the two biosolids, with concentrations significantly higher than the receiving soils. Dewatered biosolids had highest concentrations of the two metals.

Concentrations of Fe and Mn in the biosolids were similar to those in the soils, and Ni, Co and Pb were very low.

Table 1 Concentrations of total heavy metals in soil, dewatered and composted biosolids determined by X-ray fluorescence expressed in mg/kg

| Analytes | Soil         | Dewatered biosolids | Composted biosolids |
|----------|--------------|---------------------|---------------------|
| Cu       | 17.0 ± 0.8   | 648 ± 1             | 210 ± 4             |
| Zn       | 37 ± 2       | 1062 ± 1            | 813 ± 8             |
| Mn       | 246 ± 2      | 213 ± 1             | 299 ± 6             |
| Fe       | 26000 ± 1000 | 14000 ± 100         | 25000 ± 1000        |
| Ni       | 23.3 ± 0.4   | 27.17 ± 0.01        | 21.2 ± 0.4          |
| Pb       | 11.4 ± 0.2   | 28 ± 3              | 47 ± 1              |

---

Values indicate mean ± standard deviations of triplicate measurements

The residual concentrations after the application of dewatered biosolids are shown in tables 2 & 3 and table 4 shows the increase at the highest loading rates. Tables 5 – 7 show the same information for composted biosolids.

Table 2 Residual concentrations of total heavy metals in dewatered biosolids amended soil in canola plots in 2006 and 2007 determined by XRF expressed in mg/kg

| Biosolids rates (t/ha) 2006 | Cu     | Zn     |
|-----------------------------|--------|--------|
| 0                           | 14 ± 2 | 30 ± 1 |
| 5                           | 15 ± 1 | 33 ± 1 |
| 25                          | 19 ± 1 | 37 ± 1 |
| 45                          | 26 ± 3 | 51 ± 4 |
| 65                          | 30 ± 2 | 55 ± 1 |
| F                           | 16 ± 1 | 30 ± 1 |
| LSD <sub>0.05</sub>         | 3***   | 4***   |
| Biosolids rates (t/ha) 2007 | Cu     | Zn     |
| 0                           | 19 ± 1 | 40 ± 1 |
| 5                           | 23 ± 1 | 45 ± 3 |
| 25                          | 33 ± 3 | 63 ± 7 |
| 45                          | 43 ± 4 | 74 ± 6 |
| 65                          | 45 ± 5 | 82 ± 8 |
| Fertilized                  | 20 ± 3 | 41 ± 4 |
| LSD <sub>0.05</sub>         | 7***   | 12***  |

The superscripts \*\*\*, \* and ns refers to significant treatment effects using ANOVA (F-test) at  $p < 0.001$ ,  $p < 0.05$  and not significant respectively, LSD 0.05 is the least significant difference (t-test) between mean values at  $p < 0.05$  level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Table 3 Residual concentrations of total heavy metals in dewatered biosolids amended soil in oat plots in 2006 and 2007 determined by XRF expressed in mg/kg

| Biosolids rates (t/ha) | Cu     | Zn     |
|------------------------|--------|--------|
| 2006                   |        |        |
| 0                      | 14 ± 1 | 31 ± 1 |
| 5                      | 15 ± 3 | 32 ± 2 |
| 25                     | 21 ± 3 | 42 ± 7 |
| 45                     | 25 ± 2 | 46 ± 2 |
| 65                     | 25 ± 1 | 47 ± 5 |
| F                      | 15 ± 2 | 31 ± 1 |
| LSD <sub>0.05</sub>    | 4***   | 8**    |
| Biosolids rates (t/ha) | Cu     | Zn     |
| 2007                   |        |        |
| 0                      | 18 ± 2 | 37 ± 1 |
| 5                      | 23 ± 2 | 46 ± 4 |
| 25                     | 30 ± 4 | 59 ± 8 |
| 45                     | 47 ± 2 | 88 ± 3 |
| 65                     | 49 ± 7 | 90 ± 9 |
| Fertilized             | 19 ± 3 | 39 ± 3 |
| LSD <sub>0.05</sub>    | 7***   | 11***  |

The superscripts \*\*\*, \* and ns refers to significant treatment effects in ANOVA (F-test) at  $p < 0.001$ ,  $p < 0.05$  and not significant respectively, LSD 0.05 stands for least significant difference (t-test) between mean values at  $p < 0.05$  level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Table 4 Changes in XRF determined total soil heavy metals in dewatered biosolids amended canola and oats plots in 2006 and 2007 experiments

| Dewatered biosolids treated canola plots |                      |                          |                  | Dewatered biosolids treated oat plots |                          |                  |    |
|--|----------------------|--------------------------|------------------|---------------------------------------|--------------------------|------------------|----|
| Heavy metals                             | Unamended plot mg/kg | Amended at 65 t/ha mg/kg | Increase (mg/kg) | Unamended plot mg/kg                  | Amended at 65 t/ha mg/kg | Increase (mg/kg) |    |
| Cu                                       | Yr 1                 | 14                       | 30               | 16                                    | 14                       | 25               | 11 |
|  | Yr 2                 | 19                       | 45               | 26                                    | 18                       | 49               | 31 |
| Zn                                       | Yr 1                 | 30                       | 55               | 25                                    | 31                       | 47               | 16 |
|  | Yr 2                 | 40                       | 82               | 42                                    | 37                       | 90               | 53 |

Table 5 Residual concentrations of total heavy metals in composted biosolids amended soil in canola plots in 2006 and 2007 determined by XRF expressed in mg/kg.

| Biosolids rates (t/ha) | Cu         | Zn      |
|------------------------|------------|---------|
| 2006                   |            |         |
| 0                      | 15 ± 2     | 33 ± 1  |
| 10                     | 15 ± 3     | 34 ± 4  |
| 30                     | 18 ± 2     | 44 ± 4  |
| 50                     | 23.6 ± 0.3 | 55 ± 4  |
| 70                     | 24.2 ± 0.5 | 59 ± 2  |
| Fertilized             | 14 ± 1     | 30 ± 1  |
| LSD <sub>0.05</sub>    | 3***       | 7***    |
| Biosolids rates (t/ha) | Cu         | Zn      |
| 2007                   |            |         |
| 0                      | 19 ± 1     | 41 ± 2  |
| 10                     | 22 ± 5     | 51 ± 8  |
| 30                     | 30 ± 2     | 71 ± 4  |
| 50                     | 40 ± 2     | 98 ± 4  |
| 70                     | 48 ± 3     | 121 ± 4 |
| Fertilized             | 19 ± 3     | 40 ± 1  |
| LSD <sub>0.05</sub>    | 6***       | 10***   |

The superscripts \*\*\*, \* and ns refers to significant treatment effects in ANOVA (F-test) at  $p < 0.001$ ,  $p < 0.05$  and not significant respectively, LSD<sub>0.05</sub> stands for least significant difference (t-test) between mean values at  $p < 0.05$  level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Table 6 Residual concentrations of total heavy metals in composted biosolids amended soil in oat plots in 2006 and 2007 determined by XRF expressed in mg/kg.

| Biosolids rates (t/ha) 2006 | Cu     | Zn      |
|-----------------------------|--------|---------|
| 0                           | 17 ± 3 | 38 ± 3  |
| 10                          | 19 ± 1 | 44 ± 2  |
| 30                          | 24 ± 3 | 60 ± 13 |
| 50                          | 28 ± 3 | 73 ± 15 |
| 70                          | 34 ± 8 | 91 ± 24 |
| Fertilized                  | 16 ± 4 | 36 ± 5  |
| LSD <sub>0.05</sub>         | 5.8*** | 22***   |
| Biosolids rates (t/ha) 2007 | Cu     | Zn      |
| 0                           | 15 ± 1 | 32 ± 3  |
| 10                          | 21 ± 2 | 52 ± 2  |
| 30                          | 30 ± 1 | 71 ± 8  |
| 50                          | 42 ± 1 | 112 ± 3 |
| 70                          | 51 ± 1 | 139 ± 5 |
| Fertilized                  | 18 ± 1 | 38 ± 1  |
| LSD <sub>0.05</sub>         | 2.0*** | 9.9***  |

The superscripts \*\*\*, \* and ns refers to significant treatment effects in ANOVA (F-test) at p < 0.001, p < 0.05 and not significant respectively, LSD<sub>0.05</sub> stands for least significant difference (t-test) between mean values at p < 0.05 level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Table 7 Changes in XRF determined total soil heavy metals in composted biosolids amended canola and oats plots in 2006 and 2007 experiments.

| Composted biosolids treated canola plots |                        |                    |                   | Composted biosolids treated oats plots |                    |                   |
|--|------------------------|--------------------|-------------------|--|--------------------|-------------------|
| Heavy metals                             | Unamended plot (mg/kg) | Amended at 65 t/ha | Increases (mg/kg) | Unamended plot                         | Amended at 65 t/ha | Increases (mg/kg) |
| Cu Yr 1                                  | 15                     | 24                 | 9                 | 17                                     | 34                 | 17                |
| Yr 2                                     | 19                     | 48                 | 29                | 15                                     | 51                 | 36                |
| Zn Yr 1                                  | 33                     | 59                 | 26                | 38                                     | 91                 | 53                |
| Yr 2                                     | 41                     | 121                | 80                | 32                                     | 139                | 107               |

There were no significant changes in total Ni, Mn, Pb Fe and Co for either biosolids with either crop. However, a significant increase in total Cu and Zn occurred, with the highest



concentrations of total Cu and Zn recorded in the plots treated with 65 and 70 t/ha dewatered and composted biosolids treated plots respectively. Nevertheless, they did not exceed the maximum EPA Victoria ceiling limits (300 and 250 mg/kg) for Cu and Zn respectively, for soils receiving biosolids for crop production (EPA Vic, 2004). Calculations based on the increase in residual metals over the two years of the experiment indicate that it would take about 9-10 years at the highest application rate to reach the ceiling limits.

Despite the dewatered biosolids having the highest concentrations of Zn and Cu, it was the treatment with composted biosolids which left the highest residuals. This suggests that these metals in dewatered biosolids were in a form which was more readily taken up by the plants. This was further investigated by studying the DTPA extractable fractions of treated soils.

### **DTPA Extractable metals:**

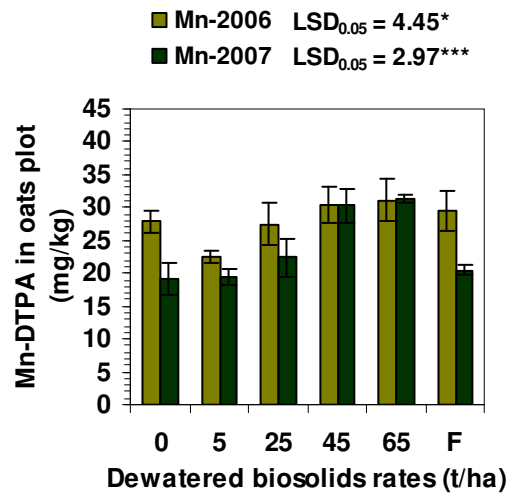
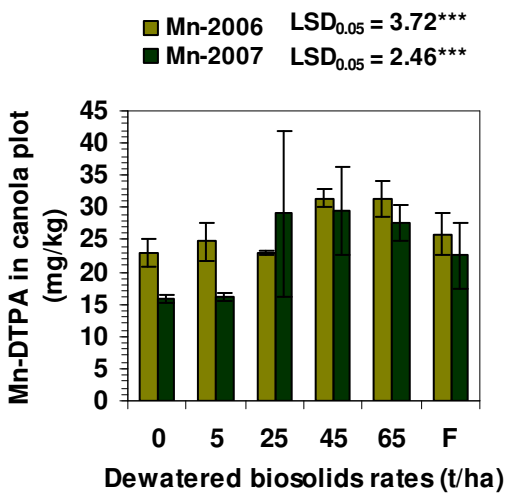
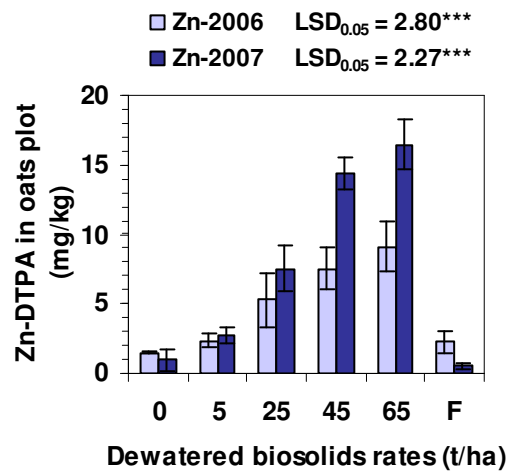
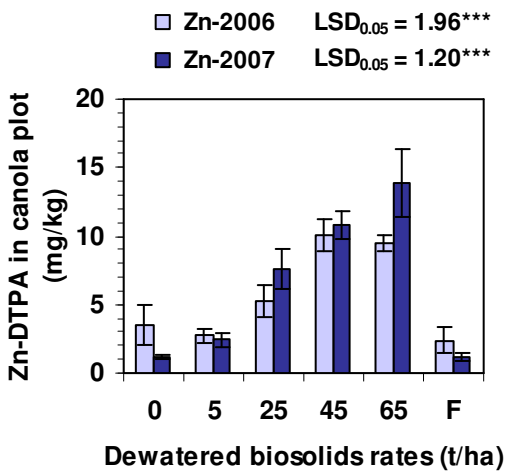
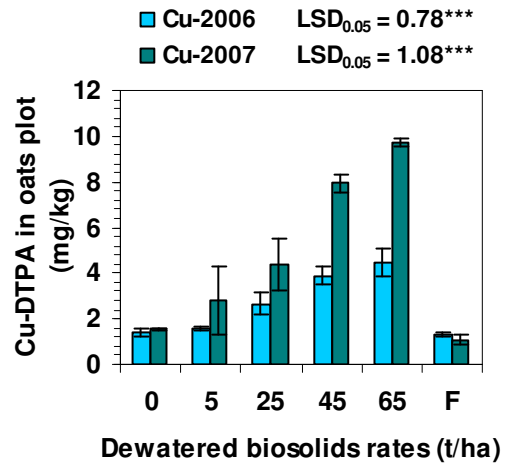
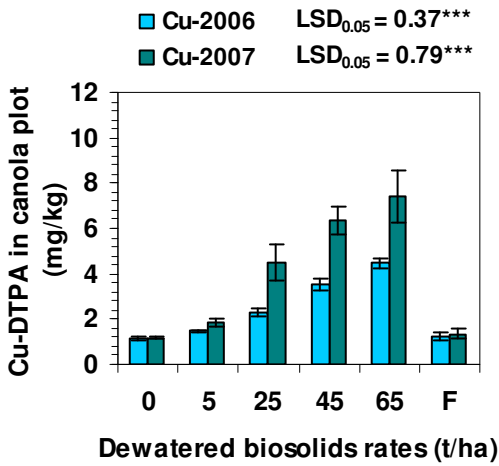
DTPA is a strong organic complexing agent which forms metal chelates. Consequently it is used to complex metals such as Cu, Zn, Mn, Fe and Ni in soils to provide an indication of readily bioavailable metals. Experiments conducted on conventionally fertilised plots show a good correlation between DTPA extractable metals and plant uptake (Bidwell and Dowdy, 1987; Sommers et al., 1991; Hooda and Alloway, 1994).

At the beginning of the experiment, soil and biosolids samples were analysed for DTPA extractable heavy metal concentrations. As shown in Table 8 the levels of DTPA extractable Cu, Zn, Mn and Ni in dewatered biosolids were significantly higher than the levels found in composted biosolids; however, the concentrations of Fe and Pb in composted biosolids were slightly higher than those found in dewatered biosolids. For all of the extractable heavy metal concentrations, the soil background concentrations were lower than the biosolids with the exception of Co and Pb.

Table 8 Results of concentrations of DTPA extractable heavy metals in soil, dewatered and composted biosolids.

| Analytes  | Soil         | Dewatered biosolids | Composted biosolids |
|---|--------------|---------------------|---------------------|
| Heavy metals extracted using DTPA and analysed by ICP-MS (mg/kg). |              |                     |                     |
| Cu  | 1.1 ± 0.1    | 185 ± 32            | 22 ± 4              |
| Zn  | 0.9 ± 0.3    | 368 ± 3             | 271 ± 57            |
| Mn  | 12 ± 1       | 98 ± 3              | 28 ± 5              |
| Fe  | 82.00 ± 0.01 | 208 ± 3             | 270 ± 62            |
| Co  | 0.20 ± 0.02  | 0.70 ± 0.03         | 0.24 ± 0.06         |
| Ni  | 0.94 ± 0.06  | 4.8 ± 0.3           | 1.6 ± 0.4           |
| Pb  | 0.5 ± 0.3    | 4.9 ± 0.01          | 9 ± 2               |

Figures 6 and 7 show the increase in DTPA extractable metals after the application of both dewatered and composted biosolids respectively. There are clearly increases in Cu and Zn and there were also increases in the concentrations of extractable Fe and Mn with increasing application rate. At first glance this might seem surprising since total Fe and Mn in the biosolids were either similar to or less than the receiving soil. However, it can be seen in Table 8, that the amount of DTPA extractable Fe and Mn is actually considerably higher in the biosolids than the receiving soil and are therefore likely to be in an organically bound form in the biosolids.



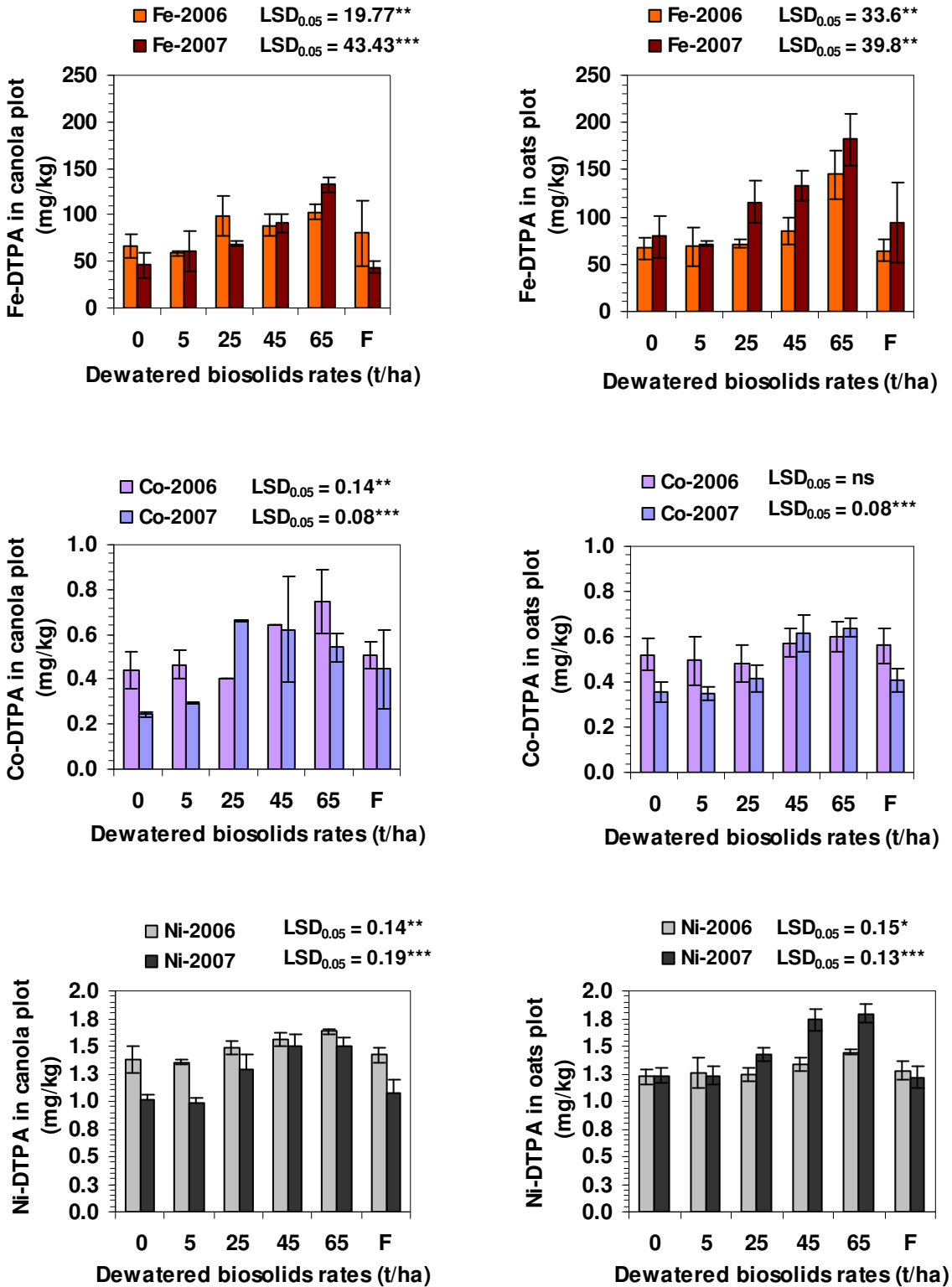


Figure 6 Effect of dewatered biosolids on the residual DTPA extractable metals

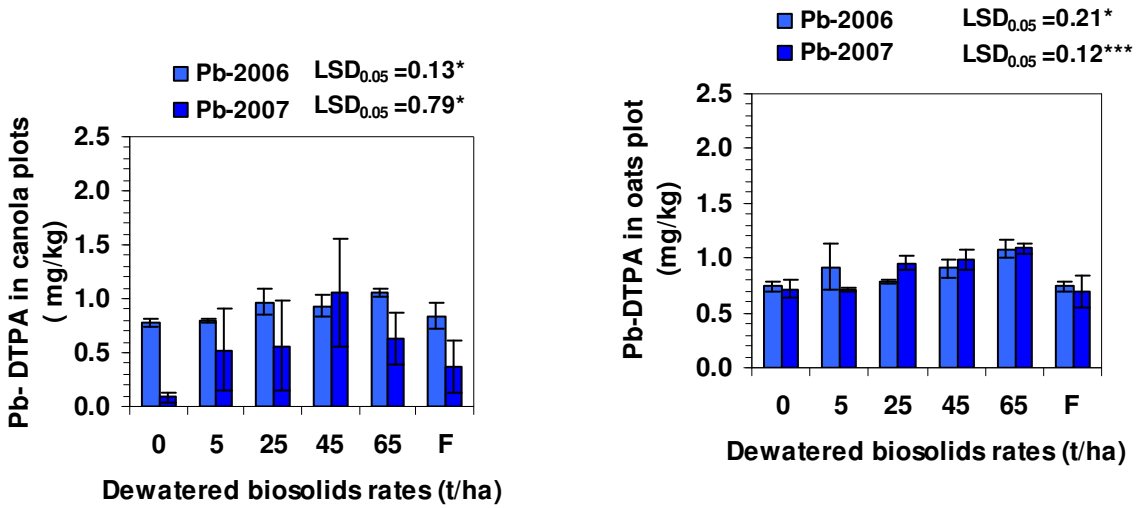


Figure 6 Effect of dewatered biosolids on the residual DTPA extractable metals continued.

The error bars indicate standard deviations of triplicate measurements, whereas The LSD 0.05 refers to the least significant difference (t-test) between mean values at 5 % probability level, whereas, \*, \*\*, \*\*\* and ns refers to significant treatment effects in ANOVA (F-test) at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  and not significant respectively ( $n = 3$ ).

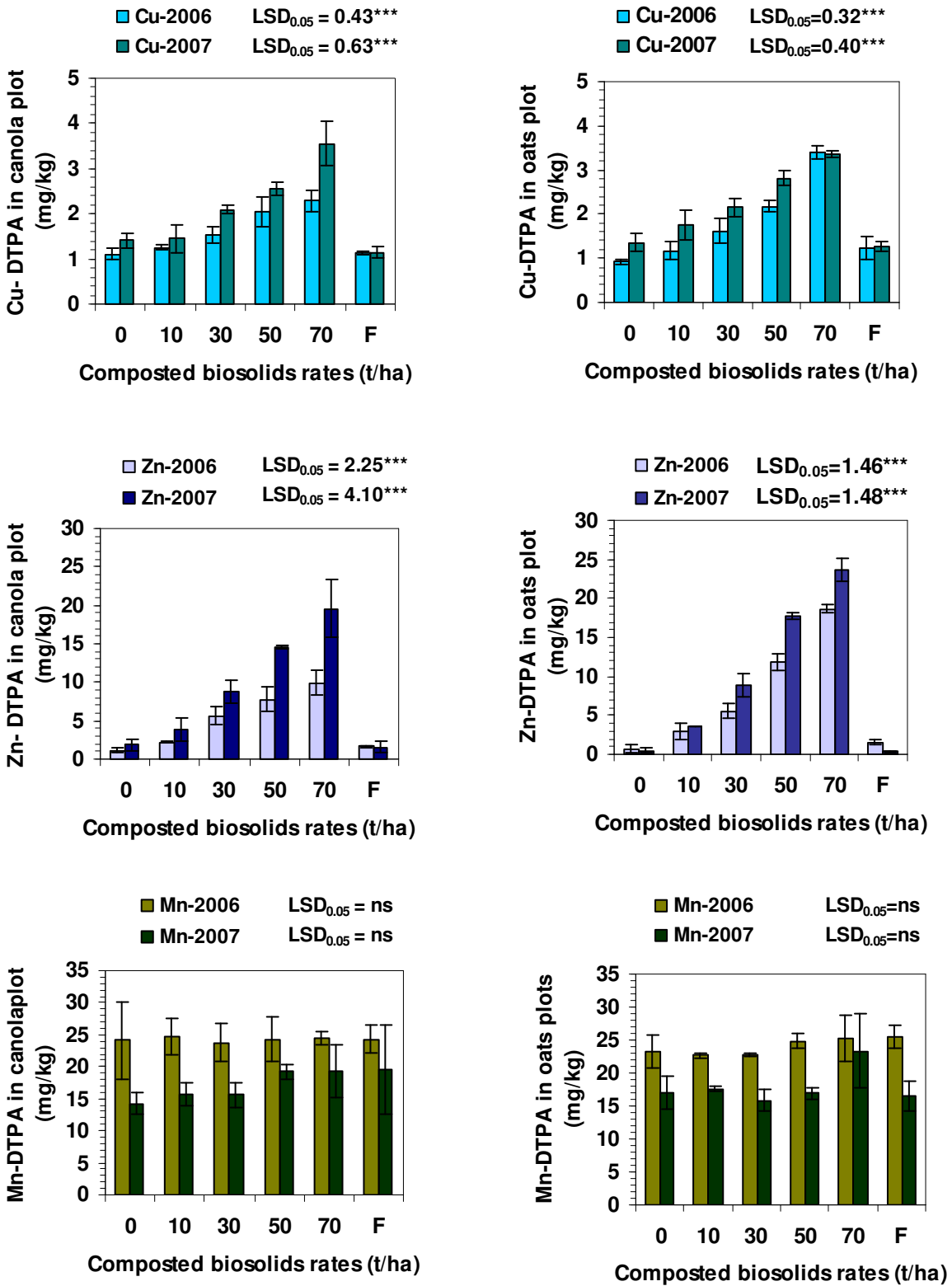


Figure 7 Effect of Composted biosolids on the residual DTPA extractable metals

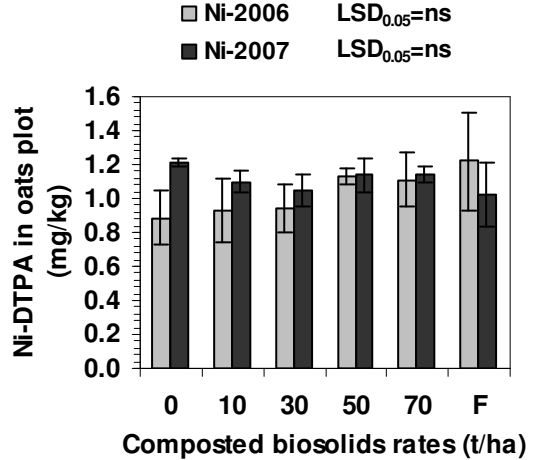
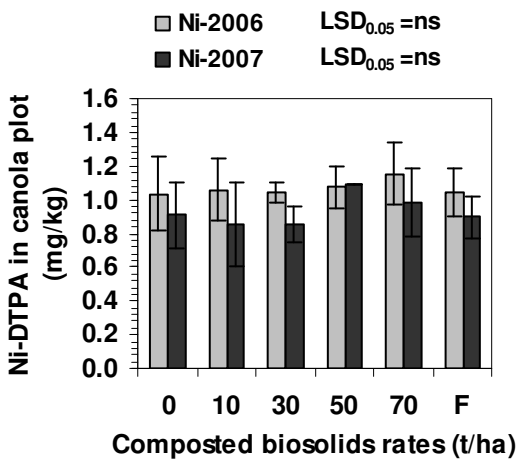
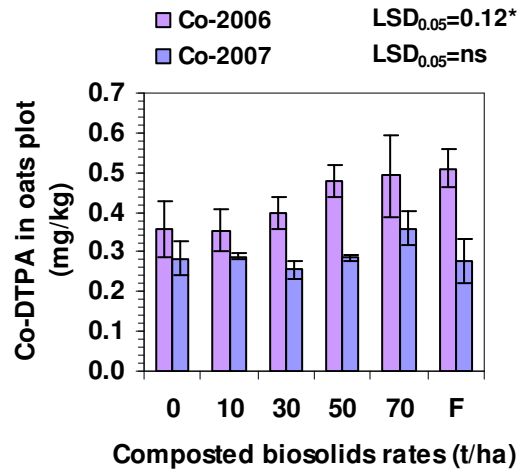
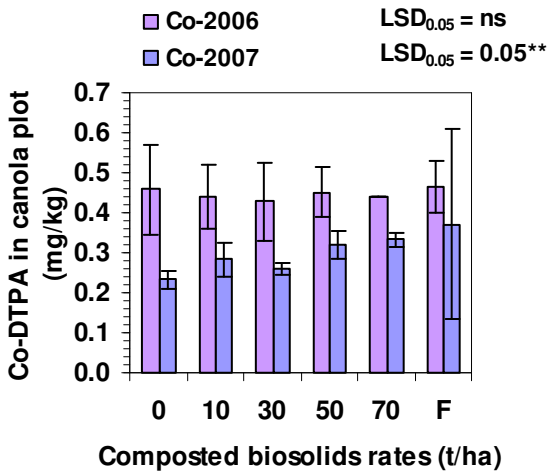
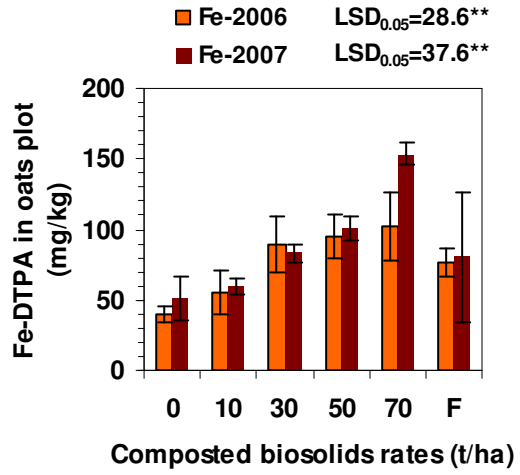
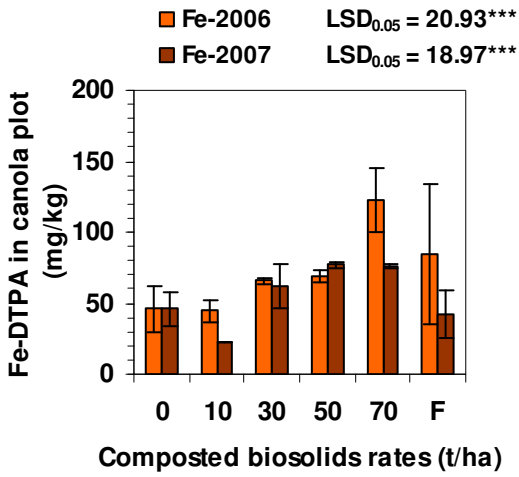


Figure 7 Effect of Composted biosolids on the residual DTPA extractable metals continued

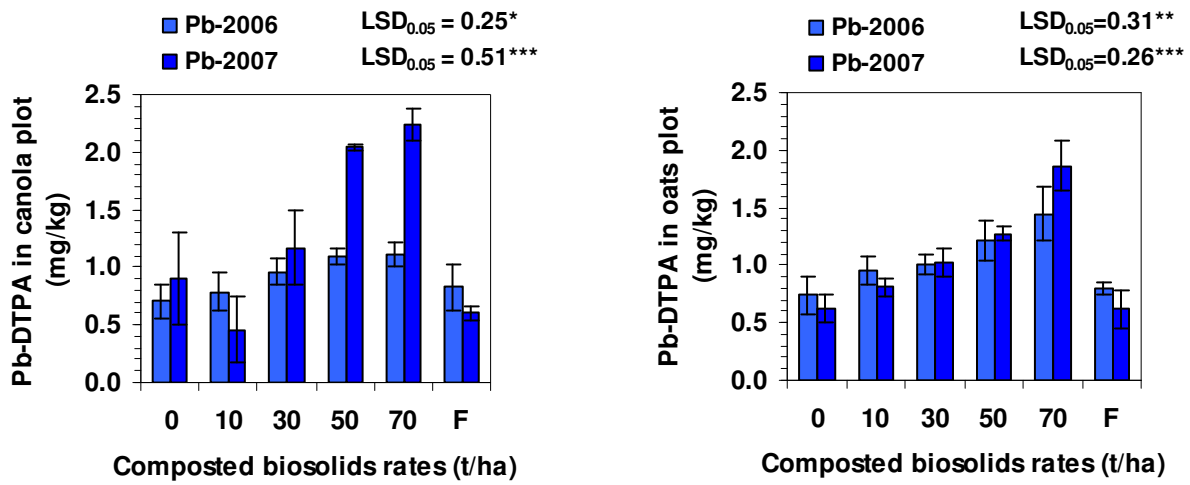


Figure 7 Effect of Composted biosolids on the residual DTPA extractable metals continued.

The error bars indicate standard deviations of triplicate measurements. LSD 0.05 refers to the least significant difference (t-test) between mean values at 5% probability level. Whereas, \*, \*\*, \*\*\* and ns refers to significant treatment effects in ANOVA (F-test) at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  and not significant respectively ( $n = 3$ ). F= fertiliser.



## Plant uptake of Metals:

The metal concentrations in the plants were determined by using a nitric acid/ peroxide digestion followed by analysis with ICPM. These results are shown in Tables 9 & 10

While the uptake by the plants increased with increasing biosolids application rates, the amounts were minimal. Nevertheless it was observed the uptake of Cu and Zn by the canola leaves was substantially higher than the corresponding concentrations observed in oats leaves. It is also apparent that canola takes up more Zn from dewatered biosolids than from composted biosolids.

Table 9 Effect of various dewatered and composted biosolids application rates on concentrations of heavy metals in canola and oats leaves in 2006 field experiment (mg/kg)

| Plant samples taken from dewatered biosolids treated canola and oats plots, 2006 |  |          |       |  |          |           |
|--|--|----------|-------|--|----------|-----------|
| Dewatered biosolids rates(t/ha)  | Concentrations of heavy metals in canola leaf expressed in mg/kg, 2006 |          |       | Concentrations of heavy metals in oats leaf expressed in mg/kg, 2006 |          |           |
|  | Cu   | Zn       | Mn    | Cu   | Zn       | Mn        |
| 0  | 10 ± 2   | 64±1     | 35±8  | 4±1  | 16±3     | 75±4      |
| 5  | 10 ± 1   | 61±1     | 48 ±8 | 4.4±0.4  | 37±4     | 97±6      |
| 25   | 10 ± 2   | 78±15    | 52±10 | 5.5±0.4  | 48±3     | 67±10     |
| 45   | 11 ± 4   | 82±12    | 45 ±5 | 6.9±0.1  | 57±2     | 56±7      |
| 65   | 16 ± 4   | 115±4    | 54±6  | 7.0±0.4  | 61±9     | 77 ±4     |
| LSD <sub>0.05</sub>  | 8.3*   | 16.4*    | 12.4* | 0.8***   | 8.9***   | 12**      |
| Measured   | 3.4±1.6  | 23.5±0.4 | 197±7 | 5.7±0.9  | 21.5±0.9 | 181.7±0.6 |
| Certified  | 4.7  | 30.9     | 246   | 4.7  | 30.9     | 246       |
| Recovery (%)   | 72   | 76       | 80    | 121  | 70       | 74        |

| Plant samples taken from composted biosolids treated canola and oats plots, 2006 |  |         |          |  |            |             |
|--|--|---------|----------|--|------------|-------------|
| Composted biosolids rates (t/ha)   | Concentrations of heavy metals in canola leaf expressed in mg/kg, 2006 |         |          | Concentrations of heavy metals in oats leaf expressed in mg/kg, 2006 |            |             |
|  | Cu   | Zn      | Mn       | Cu   | Zn         | Mn          |
| 0  | 8 ± 1  | 46 ± 9  | 34 ± 3   | 3.8 ± 0.3  | 18.3 ± 0.3 | 57 ± 4      |
| 10   | 10.5 ± 0.2   | 34 ± 1  | 46 ± 11  | 4.3 ± 0.6  | 22 ± 5     | 41 ± 3      |
| 30   | 10.2 ± 1.0   | 51 ± 6  | 39 ± 3   | 4.6 ± 0.7  | 41 ± 5     | 43 ± 11     |
| 50   | 9.1 ± 0.7  | 83 ± 21 | 36 ± 4   | 5.1 ± 0.7  | 42 ± 11    | 31 ± 5      |
| 70   | 12 ± 1   | 53 ± 20 | 39 ± 3   | 5.5 ± 0.5  | 46 ± 12    | 28 ± 4      |
| LSD <sub>0.05</sub>  | 1.6**  | 26*     | ns       | 1.0*   | 14**       | 11***       |
| Measured   | 3.05 ± 0.21  | 25 ± 8  | 197 ± 21 | 3.2 ± 0.7  | 19 ± 6     | 180.0 ± 0.5 |
| Certified  | 4.7  | 30.9    | 246      | 4.7  | 30.9       | 246         |
| Recovery   | 65   | 81      | 80       | 68   | 62         | 73          |

Values indicate means of triplicate measurements, the superscripts \*, \*\*, \*\*\* and ns refers to significant treatment effects in ANOVA ( F-test) at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  and not significant respectively, whereas the recoveries for the measured and certified values stand for NIST standard reference material SRM 1573 a ( tomato leaves). The LSD indicates the least significant difference ( t-test at  $p < 0.05$  levels) between the mean values for Cu, Zn and Mn.

Table 10 Effect of various dewatered and composted biosolids application rates on concentrations of heavy metals in canola and oats leaves in 2007 field experiment (mg/kg).

| Plant samples taken from dewatered biosolids treated canola and oats plots, 2007 |  |              |            |  |              |         |
|--|--|--------------|------------|--|--------------|---------|
| Dewatered biosolids rates(t/ha)  | Concentrations of heavy metals in canola leaf expressed in mg/kg, 2007 |              |            | Concentrations of heavy metals in oats leaf expressed in mg/kg, 2007 |              |         |
|  | Cu   | Zn           | Mn         | Cu   | Zn           | Mn      |
| 0  | 7.8 ± 1.0  | 103 ± 70     | 31 ± 4     | 5.2 ± 0.4  | 48 ± 5       | 32 ± 3  |
| 5  | 6.9 ± 0.8  | 95 ± 5       | 32 ± 4     | 5.0 ± 0.5  | 28 ± 8       | 32 ± 1  |
| 25   | 6.5 ± 0.1  | 177 ± 137    | 37 ± 4     | 5.0 ± 0.4  | 56 ± 14      | 34 ± 4  |
| 45   | 6.7 ± 0.3  | 71 ± 3       | 60 ± 15    | 6.0 ± 0.1  | 44 ± 6       | 39 ± 4  |
| 65   | 7.1 ± 0.6  | 167 ± 54     | 118 ± 31   | 7.0 ± 0.3  | 53 ± 12      | 55 ± 5  |
| LSD <sub>0.05</sub>  | ns   | ns           | 28.9*      | 0.7*   | ns           | 6.8***  |
| Measured   | 3.33 ± 0.04  | 26.00 ± 0.01 | 197 ± 1    | 3.1 ± 0.2  | 36.85 ± 0.01 | 177 ± 1 |
| Certified  | 4.7  | 30.9         | 246        | 4.7  | 30.9         | 246     |
| Recovery (%)   | 69   | 84           | 80         | 65   | 119          | 72      |
| Plant samples taken from composted biosolids treated canola and oats plots, 2007 |  |              |            |  |              |         |
| Composted biosolids rates (t/ha)   | Concentrations of heavy metals in canola leaf expressed in mg/kg, 2007 |              |            | Concentrations of heavy metals in oats leaf expressed in mg/kg, 2007 |              |         |
|  | Cu   | Zn           | Mn         | Cu   | Zn           | Mn      |
| 0  | 6.4 ± 0.2  | 63 ± 18      | 33 ± 5     | 6.4 ± 1.1  | nr           | 31 ± 3  |
| 10   | 7.4 ± 0.5  | 97.5 ± 0.1   | 33.0 ± 0.1 | 5.6 ± 0.1  | nr           | 30 ± 4  |
| 30   | 6.6 ± 0.1  | 61 ± 13      | 43 ± 8     | 5.17 ± 0.03  | nr           | 24 ± 1  |
| 50   | 5.8 ± 0.3  | 60 ± 5       | 44 ± 9     | 7.5 ± 1.9  | nr           | 24 ± 2  |
| 70   | 5.6 ± 0.6  | 103 ± 24     | 33 ± 4     | 5.6 ± 0.5  | nr           | 23 ± 4  |
| LSD <sub>0.05</sub>  | 0.7**  | 27*          | ns         | ns   | nr           | 5.3*    |
| Measured   | 4.08 ± 0.11  | 45 ± 6       | 201 ± 0.8  | 4.1 ± 0.9  | -            | 168 ± 8 |
| Certified  | 4.7  | 30.9         | 246        | 4.7  | -            | 246     |
| Recovery   | 87   | 120          | 82         | 86   | -            | 68      |

Values indicate means of triplicate measurements, the superscripts \*, \*\*, \*\*\* ,ns refers to significant treatment effects in ANOVA ( F-test) at p < 0.05, p < 0.01, p < 0.001 and not significant respectively, whereas the recoveries for the measured and certified values stand for NIST standard reference material SRM 1573 a ( tomato leaves). The LSD indicates the least significant difference ( t-test at p < 0.05 levels) between the mean values for Cu, Zn and Mn. nr means “no result”.

**Soil pH:**

Biosolid applications significantly affected the soil pH, with dewatered biosolids decreasing and composted biosolids increasing the soil pH. This would have had an impact upon the forms of metals in the soils by increasing their solubility at low pH.

## Nutrients

### Nitrogen -Total N

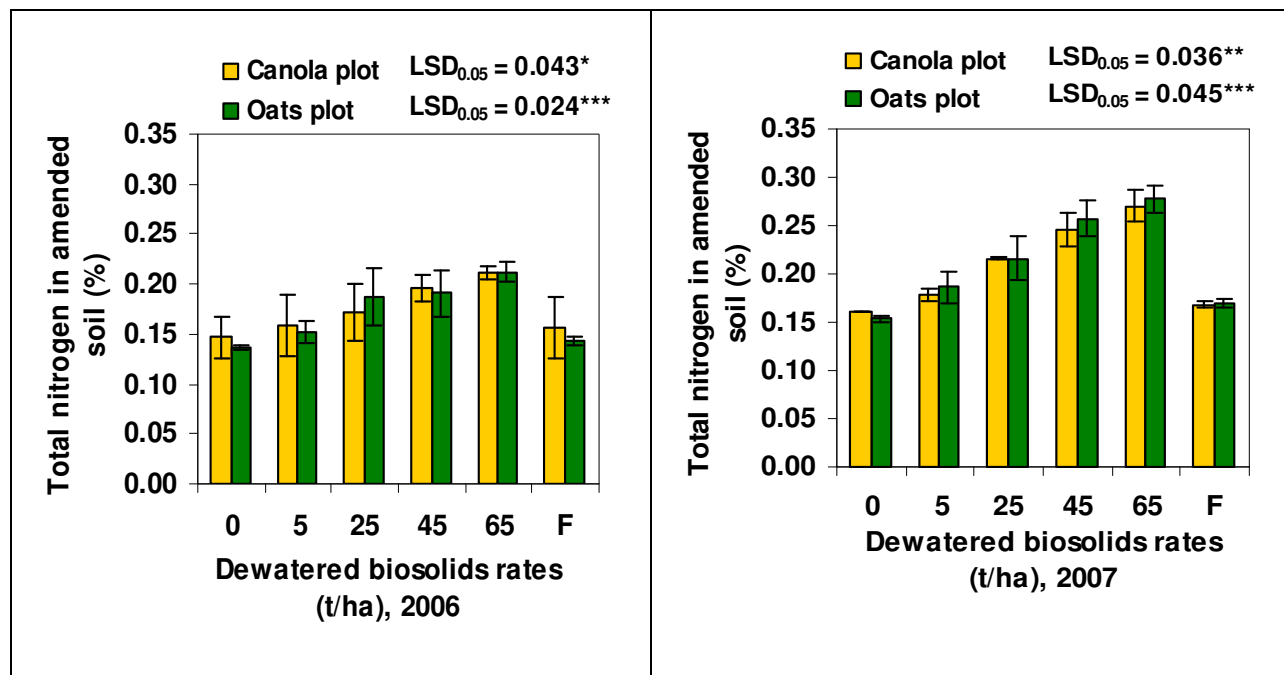
Table 11 provides the nitrogen analyses for the receiving soil and the two biosolids. It is clear that the dewatered biosolids contained a significantly higher amount of nitrogen, with a substantial amount of which is readily available in the form of ammonium and nitrate.

Table 11 Concentrations of total and extractable N and P in soil and in the two biosolids.

| Analytes                  | Soil         | Dewatered biosolids | Composted biosolids |
|---------------------------|--------------|---------------------|---------------------|
| Total N %                 | 0.17 ± 0.002 | 4.22 ± 0.005        | 1.44 ± 0.003        |
| NH <sub>4</sub> -N(μg/g)  | 5.1          | 3740                | not available       |
| NO <sub>3</sub> -N (μg/g) | 2.9          | 830                 | 1864                |

Total N was determined with a Leco FP auto carbon and nitrogen analyzer, whereas NO<sub>3</sub>-N and NH<sub>4</sub>-N were analyzed by flow injection analyzer (FIA). Soil and biosolids samples were analyzed in triplicates.

The changes in total N with biosolids application rate are shown in Figure 8.



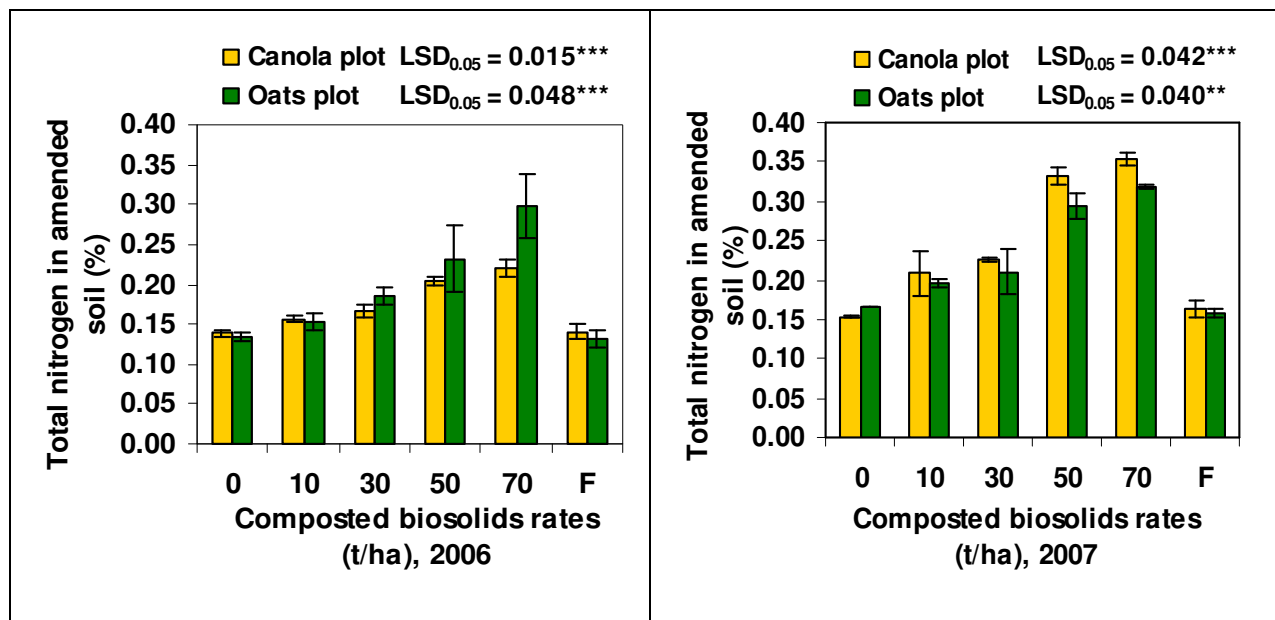


Figure 8 The change in the mean values of soil TN in canola and oats plots under increasing applications rates of dewatered and composted biosolids in 2006-2007 field experiments.

The error bars represent standard deviations of triplicate measurements. The LSD values refers to the least significant difference (t-test) for the mean TN values at 5% probability, whereas\*, \*\* and \*\*\* refers to significant treatment effects in ANOVA (F-test) at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  levels respectively

It can be seen that the levels of total N in both dewatered and composted biosolids amended soils increased with application rate and were significantly higher at the maximum application rates than the conventionally fertilized plots ( $p < 0.05$ ). In the dewatered biosolid amended plots, the residue of N after applying 5 t/ha of biosolids was similar to that of the plots receiving conventional fertiliser. The lowest application rate of the composted biosolids (10t/ha) left N residues that were slightly elevated over the conventionally fertilised plots.

### Nitrogen N-nitrate N

The residual nitrate levels are shown in Figure 9 and again, biosolids had a significant influence, with the residual levels increasing substantially after the second application. There was also a substantial increase after the second application of fertiliser.

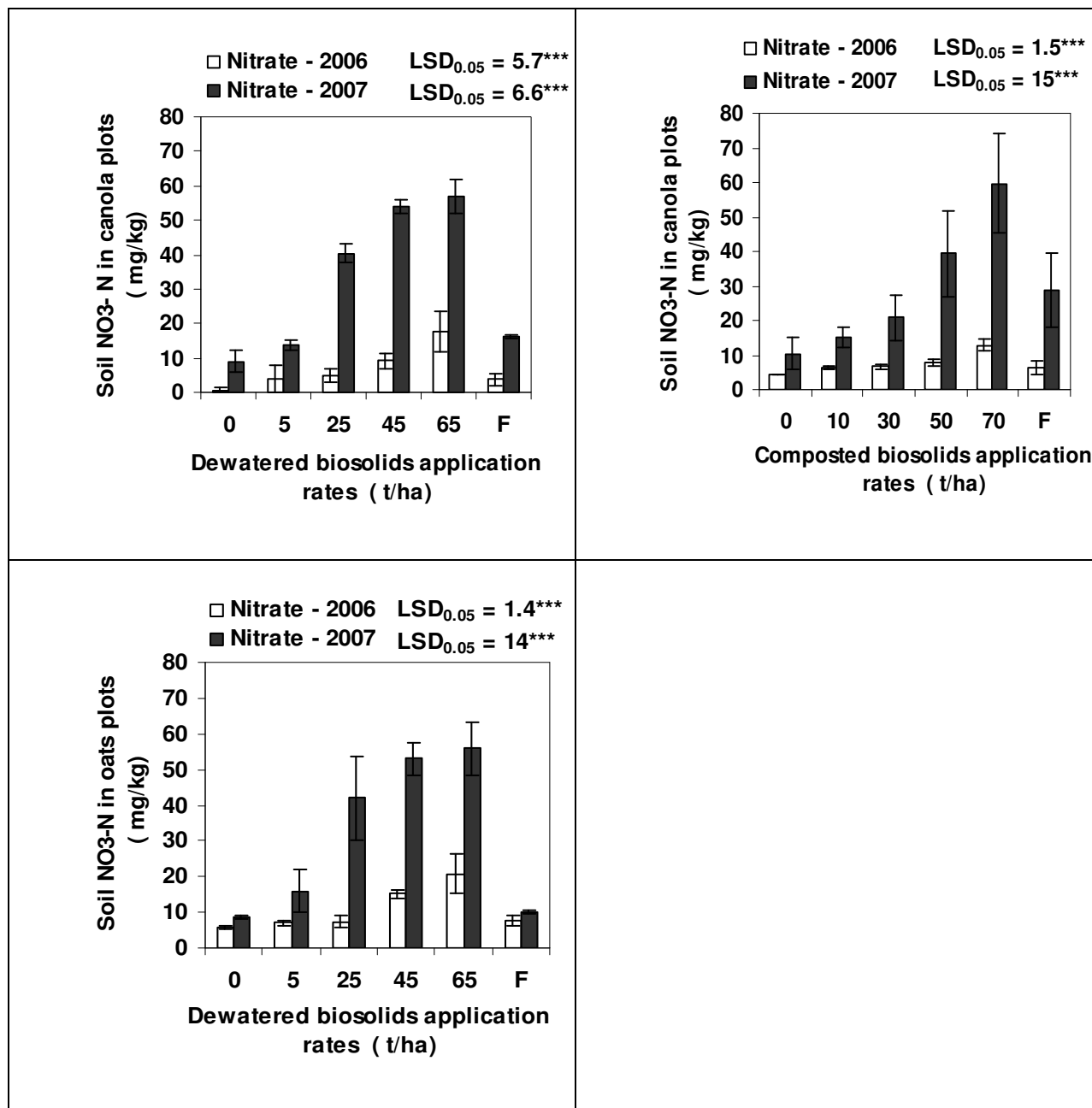


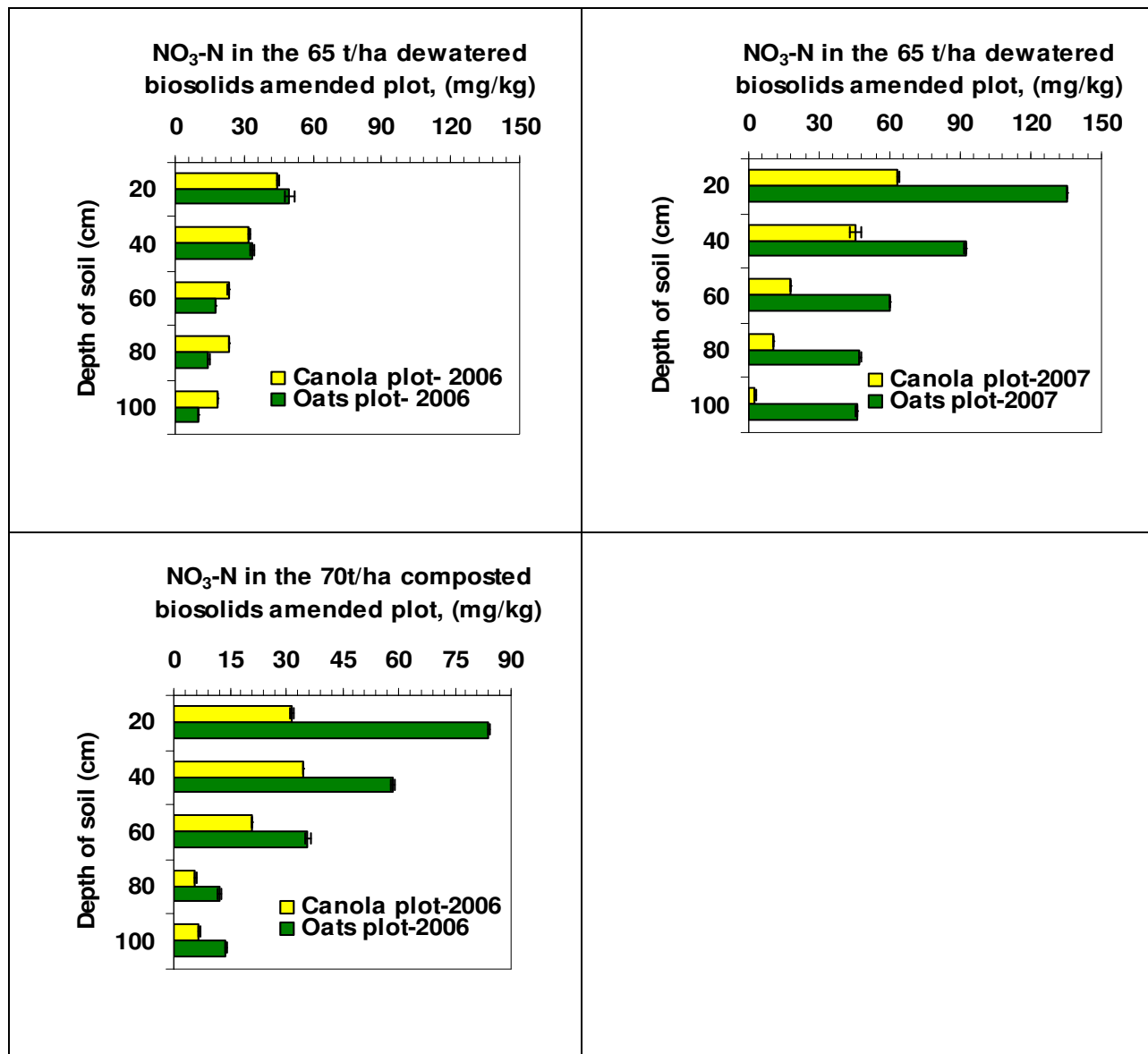
Figure 9 The effect of dewatered and composted biosolids application rates on the level of NO<sub>3</sub>-N accumulated in the top 10 cm of soil in canola and oats plots in 2006 and 2007. The LSD values refers to the least significant difference (t-test) between the mean values of NO<sub>3</sub>-N at 5 % probability, whereas \*\* and \*\*\* refers to significant treatment effects in ANOVA (F-test) at p < 0.01 and p < 0.001 levels respectively.

## Leaching of NO<sub>3</sub>-N down the soil profile

Leaching of NO<sub>3</sub>-N following the application of biosolids has the potential to contaminate the ground water. Hence NO<sub>3</sub>-N leaching can be a limiting factor for long term feasibility of agricultural land application of biosolids (Polgase and Robinson, 1996).

Comparisons of NO<sub>3</sub>-N levels at various soil depths were made between the unamended control and the highest (65 and 70 t/ha) dewatered and composted biosolids receiving plots.

Comparisons were also made between biosolids and crop types during the two years of the field





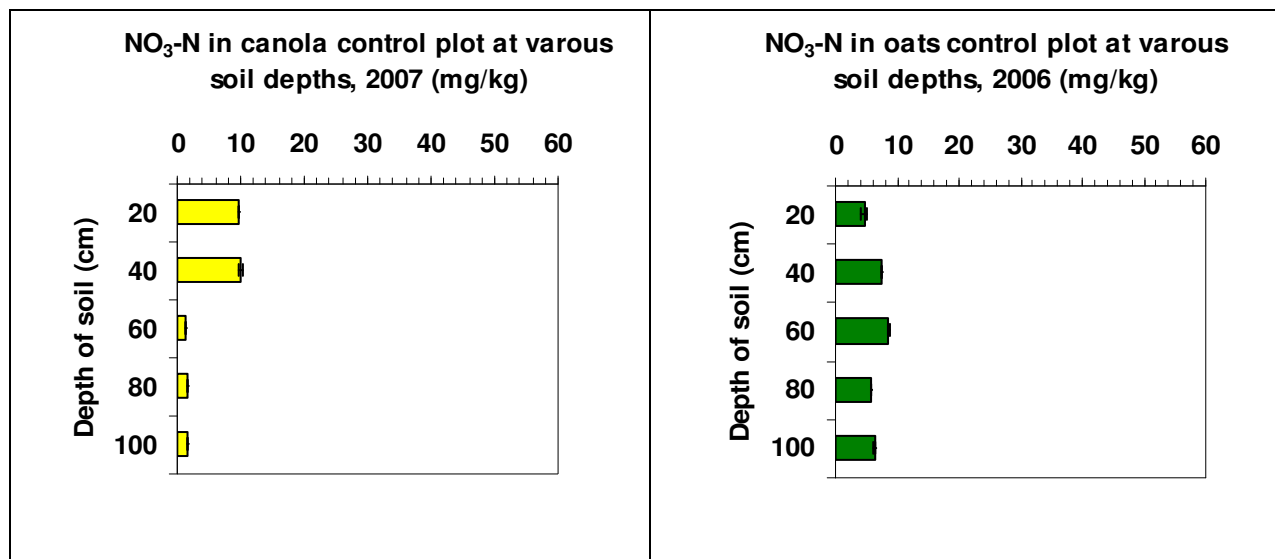


Figure 10 NO<sub>3</sub>-N leaching down the soil profile

These results show that as expected, nitrate increased with successive applications of biosolids. The residuals were higher in plots receiving dewatered biosolids which is consistent with the higher level of nitrate in the dewatered biosolids. They were also higher in plots growing oats, which is consistent with the known ability of canola to uptake more nutrients than oats. The difference between the crops can also be seen in the difference between the controls in which canola took up much more of the original nitrate in the soil than did oats. These plots were watered regularly, so the leaching of these nutrients is higher than would be expected to occur under normal farming practices.

### N uptake in plants

Plant uptake of nitrogen with increasing application of biosolids is shown in Table 12. The concentration of nitrogen in the plant tissue appears to reach similar levels to those on which conventional fertiliser was applied. However, given that the total biomass from the plots with biosolids applied was much higher, the total amount of nitrogen taken up by the crop is highest in the canola crops treated with dewatered biosolids.

Table 12 The effect of dewatered and composted biosolids applications on the levels of TN (%) in canola and oat leaves in 2006 and 2007 field experiments.

| Dewatered biosolids<br>( t/ha) | 2006              |                 | 2007              |                 |
|--------------------------------|-------------------|-----------------|-------------------|-----------------|
|                                | TN in canola leaf | TN in oats leaf | TN in canola leaf | TN in oats leaf |
| 0                              | 5.7 ± 0.3         | 2.2 ± 0.3       | 5.0 ± 0.7         | 3.3 ± 0.3       |
| 5                              | 5.8 ± 0.4         | 2.9 ± 0.6       | 5.7 ± 0.4         | 3.0 ± 0.3       |
| 25                             | 6.6 ± 0.3         | 3.0 ± 0.2       | 6.4 ± 0.1         | 3.9 ± 0.3       |
| 45                             | 6.9 ± 0.1         | 3.6 ± 0.3       | 6.8 ± 0.1         | 4.3 ± 0.1       |
| 65                             | 7.1 ± 0.1         | 4.9 ± 0.3       | 7.2 ± 0.3         | 4.1 ± 0.2       |
| Fertilized                     | 6.6 ± 0.4         | 3.2 ± 0.1       | 6.4 ± 0.4         | 3.7 ± 0.3       |
| LSD <sub>0.05</sub>            | 0.42**            | 0.63**          | 0.70**            | 0.54**          |

| Composted<br>biosolids ( t/ha) | 2006              |                 | 2007              |                 |
|--------------------------------|-------------------|-----------------|-------------------|-----------------|
|                                | TN in canola leaf | TN in oats leaf | TN in canola leaf | TN in oats leaf |
| 0                              | 5.4 ± 0.4         | 2.4 ± 0.2       | 5.1 ± 0.2         | 3.4 ± 0.4       |
| 10                             | 5.0 ± 0.1         | 2.6 ± 0.5       | 5.7 ± 0.5         | 3.3 ± 0.4       |
| 30                             | 5.4 ± 0.3         | 2.9 ± 0.9       | 6.4 ± 0.1         | 3.7 ± 0.2       |
| 50                             | 5.6 ± 0.5         | 3.2 ± 0.3       | 6.4 ± 0.3         | 3.9 ± 0.2       |
| 70                             | 5.4 ± 0.5         | 3.3 ± 0.2       | 6.8 ± 0.6         | 4.2 ± 0.4       |
| Fertilized                     | 5.5 ± 0.2         | 3.6 ± 0.9       | 6.2 ± 0.4         | 4.4 ± 0.3       |
| LSD <sub>0.05</sub>            | ns                | ns              | 0.62**            | ns              |

Values indicate means of triplicate measurements, whereas LSD values refers to the least significant difference (t-test) between the mean values of TN in plant matter at 5% probability, whereas the superscripts \*, \*\*, \*\*\* and ns refers to significant treatment effects in ANOVA (F-test) at  $p < 0.01$  and  $p < 0.001$  levels and not significant respectively.

### Nitrogen mass balance

To examine the fate of N from land applied biosolids, concentrations of total N in biosolids were used to calculate the quantity of total N applied to the soil. Total N in biosolids amended soils, in leaves and seeds, and the total plant biomass data were used to estimate a nitrogen mass balance in kg N/ha of land for the dewatered biosolids. Soil total N analytical data (mg/kg) were converted into kg/ha using  $1.24 \text{ gm/cm}^3$  as the soil bulk density at a 10 cm sampling depth.

Table 13 Mass balance of total nitrogen in dewatered biosolids amended canola and oats plots in the 2006 and 2007 field experiments.

| Dewatered biosolids rates ( t/ha) | Total-N applied (kg/ha) | Total-N residue remained in soil (kg/ha) | Plant uptake of N (kg/ha) | Total- N recovered (kg/ha) | Unaccounted N balance (kg/ha) | Unaccounted N balance in (%) |
|-----------------------------------|-------------------------|--|---------------------------|----------------------------|-------------------------------|------------------------------|
| <b>Canola-oats rotation</b>       |                         |  |                           |                            |                               |                              |
| 25                                | 2110                    | 868                                      | 113                       | 981                        | 1129                          | 53                           |
| 45                                | 3798                    | 1364                                     | 269                       | 1633                       | 2165                          | 57                           |
| 65                                | 5486                    | 1612                                     | 280                       | 1892                       | 3594                          | 66                           |
| <b>Oats-canola rotation</b>       |                         |  |                           |                            |                               |                              |
| 25                                | 2110                    | 744                                      | 127                       | 871                        | 1239                          | 59                           |
| 45                                | 3798                    | 1240                                     | 334                       | 1574                       | 2224                          | 59                           |
| 65                                | 5486                    | 1488                                     | 652                       | 2140                       | 3346                          | 61                           |

The unaccounted N balance in both cropping sequence was reasonably constant ranging from to 53 to 66 % which indicates that more than 50 % of the total N applied into the soil was lost through various routes such as leaching and volatilisation.

These results are similar to the observations from laboratory experiments conducted by NBRP in southern Queensland (Bell et al, 2007) in which they showed that 30 % of the applied N was lost through denitrification during a three months period of study, and suggested that high loses of N through denitrification may well be greater under wet soil conditions. Since the crops were watered twice a week using a sprinkler system, it is expected that most of the mineralized N from the applied biosolids was lost as through denitrification and leaching. Ammonia volatilization is also possible, particularly in the dewatered biosolids.

### **Phosphorus:Total and Olsen- extractable**

Phosphorus is a major plant nutrient for the production of crops, and most biosolids contain high concentrations of P; however, when biosolids are applied at a rate to satisfy the N requirement of

a crop based on nitrogen limited biosolids application rates (NLBAR), P may be present in excessive amounts and accumulate in the soil, creating a significant risk of off-site movement through run off (Warne et al.,2007).

To determine if this would be the case with the biosolids generated by Western Water, the total and Olsen- P concentrations in the biosolid amended soils were analysed following the harvesting of canola and oats for two consecutive years. The results are presented below. Table 14 show the P concentrations for the receiving soil and the two biosolids.

Table 14 Concentrations of total and extractable P in soil and biosolids used for the experiments ( $\mu\text{g/g}$ ).

| Analytes        | Soil        | Dewatered biosolids | Composted biosolids |
|-----------------|-------------|---------------------|---------------------|
| Total-P         | 855 $\pm$ 3 | 15003 $\pm$ 4       | 21290 $\pm$ 566     |
| Olsen-P         | 15 $\pm$ 2  | 691 $\pm$ 5         | 762 $\pm$ 7         |
| (Olsen/XRF)*100 | 1.7 %       | 4.6 %               | 3.6 %               |

Total P and S were analyzed using WD- X-ray fluorescence spectrometry, whereas Olsen-P was determined by flow injection analysis (FIA). Soil and biosolids samples were analyzed in triplicates.

It can be seen from table 14 that Total P is much higher in both biosolids than the receiving soil and the Olsen P, which represents the more readily available P, is considerably higher in the biosolids. The accumulation in the soil of Total P is shown in Figure 11 and Oslen P in Figure 12. The accumulation of both Total P and Olsen P is significantly higher on the sites with composted biosolids. It is also noticeable that the residual P (both forms) is higher on plots which grew oats, particularly on the sites with composted biosolids.

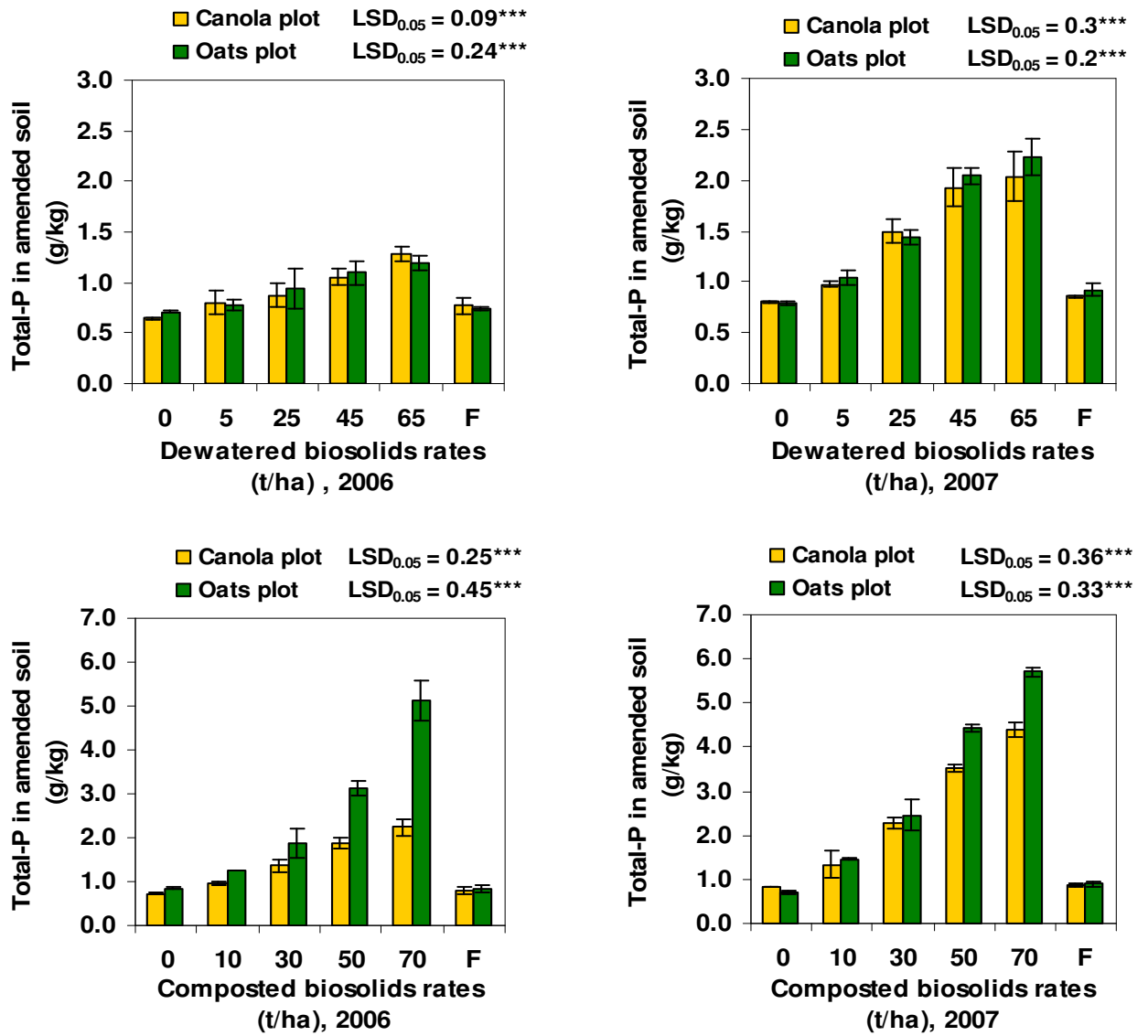


Figure 10 The effect of dewatered and composted biosolids application rates on the level of total phosphorus in amended soil in 2006 and 2007 field experiments.

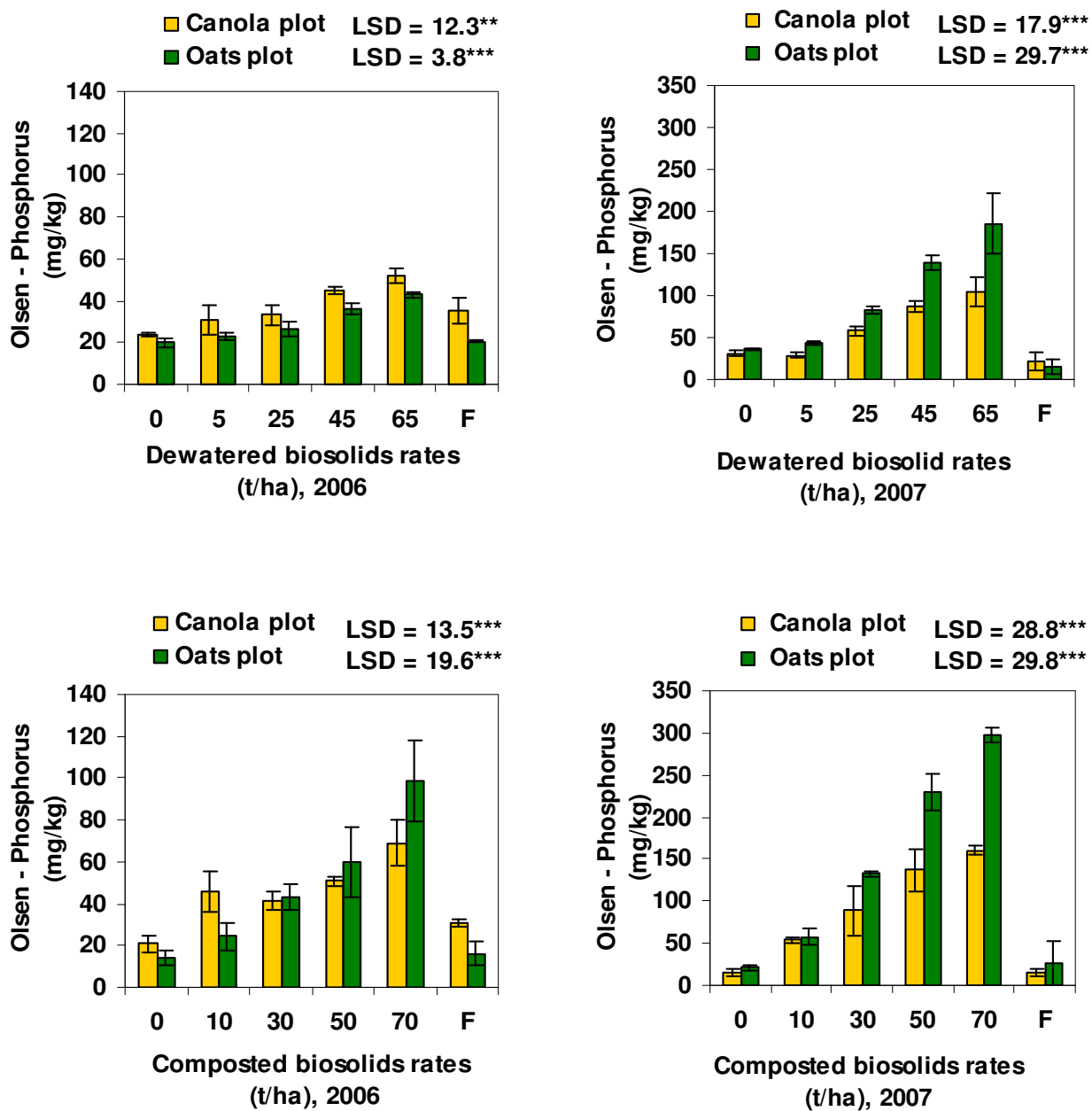


Figure 11 The effect of dewatered and composted biosolids application rates on the level of Olsen-extractable phosphorus in amended soil in 2006 and 2007 field experiments.

## P uptake in Plants

Phosphorus in the plant tissue was estimated using an acetic acid extraction technique as described in the Appendix. These are shown in Table 15.

Table 15 The effect of dewatered and composted biosolids applications on acetic acid extractable phosphorus in canola and oats leaf in the 2006 and 2007 field experiments

| Dewatered biosolids rates (t/ha) | 2006                    |                       | 2007                    |                       |
|----------------------------------|-------------------------|-----------------------|-------------------------|-----------------------|
|                                  | P in canola leaf (g/kg) | P in oats leaf (g/kg) | P in canola leaf (g/kg) | P in oats leaf (g/kg) |
| 0                                | 4.7 ± 0.4               | 2.0 ± 0.6             | 1.9 ± 0.2               | 0.13 ± 0.07           |
| 5                                | 5.2 ± 0.7               | 2.1 ± 0.6             | 2.8 ± 0.3               | 0.30 ± 0.3            |
| 25                               | 5.6 ± 0.7               | 3.9 ± 0.5             | 3.6 ± 0.2               | 0.47 ± 0.6            |
| 45                               | 5.9 ± 0.1               | 4.0 ± 0.8             | 4.3 ± 0.5               | 0.70 ± 0.8            |
| 65                               | 6.5 ± 0.6               | 4.40 ± 0.1            | 5.1 ± 0.2               | 0.80 ± 0.2            |
| Fertilized                       | 5.1 ± 0.3               | 1.6 ± 0.1             | 1.7 ± 0.2               | 0.37 ± 0.3            |
| LSD <sub>0.05</sub>              | 1.04*                   | 0.91***               | 0.40***                 | 0.30***               |
| Composted biosolids rates (t/ha) |                         |                       |                         |                       |
| 0                                | 4.5 ± 0.6               | 2.5 ± 0.2             | 3.1 ± 0.2               | 0.2 ± 0.1             |
| 10                               | 4.6 ± 0.53              | 3.2 ± 0.7             | 4.2 ± 0.4               | 0.4 ± 0.3             |
| 30                               | 5.7 ± 0.83              | 3.3 ± 0.9             | 4.4 ± 0.1               | 0.8 ± 0.2             |
| 50                               | 5.5 ± 0.4               | 4.3 ± 0.4             | 3.8 ± 0.1               | 0.8 ± 0.1             |
| 70                               | 5.2 ± 0.8               | 4.4 ± 1.2             | 4.2 ± 1.3               | 1.2 ± 0.4             |
| Fertilized                       | 4.60 ± 0.04             | 2.1 ± 0.3             | 3.1 ± 0.2               | 0.4 ± 0.2             |
| LSD <sub>0.05</sub>              | ns                      | ns                    | 1.0*                    | 0.52**                |

The superscripts \*\*\*, \*\*, \* and ns refers to significant treatment effects in ANOVA (F-test) at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  and not significant respectively, figures in parenthesis indicate standard deviations of triplicate measurements.

It can be seen that the addition of biosolids enhances the concentration of P in the plant tissue. The results for oats in 2007 may have been compromised by the rust infestation.

Using the data from the P in biosolids applied, the residuals in the soil and the amount calculated in the plant tissue, a mass balance was determined. This is shown in Table 16.

Table 16 Mass balance of total phosphorus in dewatered biosolids amended canola and oats plots in the 2006 and 2007 experiments.

| Dewatered biosolids rates ( t/ha) | Total-P applied (kg/ha) | Total-P residue in soil (kg/ha) | Plant uptake of P (kg/ha) | Total-P recovered (kg/ha) | Unaccounted P balance (kg/ha) | Unaccounted P balance in (%) |
|-----------------------------------|-------------------------|---------------------------------|---------------------------|---------------------------|-------------------------------|------------------------------|
| <b>Canola- oats rotation</b>      |                         |                                 |                           |                           |                               |                              |
| 25                                | 750                     | 637                             | 14                        | 651                       | 99                            | 13                           |
| 45                                | 1350                    | 1334                            | 27                        | 1361                      | -11                           | -0.8                         |
| 65                                | 1950                    | 1476                            | 35                        | 1511                      | 439                           | 22                           |
| <b>Oats- canola rotation</b>      |                         |                                 |                           |                           |                               |                              |
| 25                                | 750                     | 649                             | 24                        | 673                       | 77                            | 10                           |
| 45                                | 1350                    | 1090                            | 51                        | 1141                      | 209                           | 15                           |
| 65                                | 1950                    | 1169                            | 78                        | 1247                      | 703                           | 36                           |

For the computation of the mass balance for P in dewatered biosolids amended soil, the soil total P background concentration before the start of the experiment (855 mg/kg) was used and subtracted from the total P residuals remaining at the 25, 45 and 65 t/ha application rates at the end of the two year experiment. Using a value of 1.24 gm/cm<sup>3</sup> for the soil bulk density at a 10 cm depth, the concentrations were converted into kg/ha. For the calculations of P uptake (kg/ha), concentrations of P in the plant tissue were multiplied by the total seed and plant biomass

Here, the unaccounted amount is much less than that for nitrogen. This is to be expected because the potential to lose P is much less than N since it does not undergo the same oxidation processes and has a tendency to be adsorbed to the soil. Given that the plots grew weeds as well as the desired crops (despite several applications of herbicide) and it was assumed that the concentration of P in the whole plant was the same as that of the leaves, the mass balance appears to show only a limited loss.



## Microorganisms

Laboratory analyses of biosolids amended soil indicated that the concentrations of *E.coli* and *Salmonella* in dewatered and composted biosolids treated plots were below detection limits. However, the concentrations of *Clostridium perfringens* were higher in the plots treated with dewatered biosolids than either the control or composted biosolid amended soils in the first year. However, while the concentrations were elevated, they did not exceed EPA guidelines for land application.

Table 17 Survival of pathogens in biosolids and biosolids amended soil after 12 months of biosolids application in 2006 and 2007 at Surbiton Park.

| Dewatered biosolids rates | Mean E.coli/g      |      | Mean C.perfringens cfu/g |      | Mean Salmonella spp/25g |      |
|---------------------------|--------------------|------|--------------------------|------|-------------------------|------|
|                           | 2006               | 2007 | 2006                     | 2007 | 2006                    | 2007 |
| Control                   | <0.3               | <0.3 | <10 (0)                  | <10  | nd                      | nd   |
| Fertilized plots          | <0.3               | <0.3 | 126 (203)                | <10  | nd                      | nd   |
| 5                         | <0.3               | <0.3 | 430 (118)                | <10  | nd                      | nd   |
| 25                        | <0.3               | <0.3 | 423 (113)                | 370  | nd                      | nd   |
| 45                        | <0.3               | <0.3 | 333 (117)                | 530  | nd                      | nd   |
| 65                        | <0.3               | 0.48 | 397 (354)                | 700  | nd                      | nd   |
| LSD <sub>0.05</sub>       | ns                 | ns   | ns                       | ns   | -                       | -    |
| Compost(70t/ha)           | <0.3               | <0.3 | 16.66                    | <10  | nd                      | nd   |
| Dewatered biosolids(06)   | 2.3                | -    | 4300                     | -    | <3                      | <3   |
| Dewatered biosolids(07)   | -                  | 43   | -                        | 590  | <3                      | <3   |
| Composted biosolids (07)  | <0.3               | <0.3 | 70                       | -    | nd                      | nd   |
| Infective dose            | 10 <sup>4</sup> /g |      | 10 <sup>6</sup> cfu/g    |      | 10 <sup>2</sup> /25g    |      |
| EPA, Victoria limit       | <100/g sample      |      | -                        |      | <1/5g sample            |      |

Figures in parenthesis indicate standard deviations of triplicate measurements; cfu- refers to colony forming units

## ***Economic valuation***

The two estimates for the value of dewatered biosolids were determined, the first based on the seed yield, the other based on the nutrient value of the biosolids.

By determining the rate of change in the seed yield of canola per unit of biosolids applied, it was calculated that the optimum rate of application was 54 t/ha dewatered biosolids, which would provide gives 4.5 t/ha of seed. Using the farm gate price of canola seed around Melton of A\$ 470/tonne of canola seed, the value of one tonne of dewatered biosolids was calculated to be A\$39. The value of composted biosolids calculated in this way was A\$13.5.

To calculate the value based on nutrient content, the market price of each nutrient was obtained and multiplied by the concentration of the nutrients in the biosolids. Tables 18 and 19 provide these data. Using this method, dewatered biosolids were valued at A\$20.54 per tonne while composted biosolids were valued at A\$13.25.

Table 18 Average quantity of plant available nutrients (NPK) contained in dewatered biosolids (kg/t) and the price/kg and the nutrient values of dewatered biosolids price/tonne of biosolids (in Aus\$).

| Plant available Nutrients          | Market price/kg of nutrients ( Aus\$) | Quantity of nutrient/tonne of biosolids (kg/t) | Value of each nutrients in Aus \$ ( price/t of dewatered biosolids) |
|------------------------------------|---------------------------------------|--|---|
| N                                  | 1.70                                  | 10.2   | 17.34   |
| P                                  | 3.37                                  | 0.69   | 2.32  |
| K                                  | 1.20                                  | 0.73   | 0.88  |
| Price/tonne of dewatered biosolids | -                                     | -  | 20.54   |

Table 19 Average quantity of plant available nutrients (NPK) contained in composted biosolids (kg/t) and the price/kg and the nutrient values of composted biosolids price/tonne of biosolids (in Aus\$).

| Plant available Nutrients                       | Market price/kg of nutrients (Aus\$) | Quantity of nutrient/tonne of biosolids (kg/t) | Value of each nutrients in Aus \$( price/t of composted biosolids) |
|---|--------------------------------------|--|--|
| N   | 1.70                                 | 3.08   | 5.24   |
| P   | 3.37                                 | 0.76   | 2.56   |
| K   | 1.20                                 | 4.54   | 5.45   |
| Total Price of NPK/tonne of composted biosolids | -                                    | -  | 13.25  |

The energy value of total canola oil produced per hectare of land using the optimum (54 t/ha) dewatered biosolids application was estimated to be 71,604 MJ/ha.

### Summary of Key Findings

In general, results of this study have shown that the biosolids produced at WWRWP can be used as a source of plant nutrients without significantly contaminating the soil with heavy metals and pathogens, in the short term. Longer periods at higher rates could create problems with nitrogen leaching and contamination with Cu and Zn. Strategies would need to be put in place to periodically decontaminate the soil, perhaps by using phytoremediation with a crop which could be safely removed from the site for none edible purposes.

### In particular the study found that:

- incorporation of biosolids into the soil significantly increased the seed and plant biomass yields of both canola and oats, and the increase in yield and yield components of both crops surpassed that of conventionally fertilized control plots.

- the response of canola to the application of biosolids in terms of yield and yield components was greater than oats crops for dewatered and composted biosolids in both years of the experiments.
- biosolids applications significantly increased the total and extractable nitrogen and phosphorus levels in soil at all application rates.
- the residuals of Olsen-P and total-N were significantly larger for the canola-oats cropping sequence than for the oats-canola crop sequence for both biosolids, demonstrating the importance of rotation as a means of controlling nutrient accumulations.
- after two years of repeated land applications of dewatered biosolids, the level of pathogens surviving in biosolids amended soils was very low and hence the biosolids comply with the bacterial requirements so far tested for unrestricted agricultural use.
- application of dewatered and composted biosolids significantly increased the total and DTPA extractable Cu, and Zn in the amended soil, but did not exceed the maximum EPA permitted ceiling limits for soils receiving biosolids used for plant production.
- the results of economic valuation clearly demonstrated that dewatered biosolids had greater value than either composted biosolids or conventional fertilizers. The evidence for this was in the significantly higher concentrations of total nitrogen levels in dewatered biosolids, which was reflected in the associated highest canola seed oil yield.
- dewatered biosolids generated from WWRWP can be incorporated into an energy cropping system using the optimal dewatered biosolids application rate (54 t/ha) for canola which would generate oil seed with an equivalent 59,670 MJ/ha energy.
- a crop rotation energy cropping system could be established at WWRWP as a sustainable biosolids management system.
- In the event of the biosolids being made available to the local agricultural community, the benefits of using these biosolids in a controlled manner include increased crop yields and a reduction in fertiliser costs.

## References

- BELL, M., G. BARRY, G. PU, Key Findings and Recommendations from the National Biosolids Research Program Queensland component, Draft Position Paper on Recommendations from the National Biosolids Research Program (NBRP) December 2007,  
<http://www.clw.csiro.au/research/biogeochemistry/organics/biosolids/documents/NBRPFinalSeminar2a-QldOverview.pdf> accessed 3/3/2010
- BENTON JONES, J. (2001) Laboratory Guide for Conducting Soil Tests and Plant Analysis.
- BESHAH, F. Incorporation of biosolids into an energy cropping system, PhD dissertation, RMIT University, submitted January, 2010
- BIDWELL, A. M. & DOWDY, R. H. (1987) Cadmium and zinc availability to corn following termination of sewage sludge applications. *Journal of Environmental Quality*, 16, 438-42.
- EPA VIC. (2004) Guidelines for Environmental Management. Biosolids land application. Publication 943. Melbourne, VIC, Australia.
- GALE, A. J. (2007) The Australian Biosolids Partnership and Public Perceptions. *Water Practice and Technology*.
- HOODA, P. S. & ALLOWAY, B. J. (1994) Changes in operational fractions of trace metals in two soils during two-years of reaction time following sewage sludge treatment. *International Journal of Environmental Analytical Chemistry*, 57, 289-311.
- KALRA, Y. P. & EDITOR (1998) Handbook of Reference Methods for Plant Analysis.
- KEENEY, D. R. & NELSON, D. W. (1982) Nitrogen in organic forms. In : A.L. Page, R.H. Miller, and D.R. Keeney, Eds. *Methods of Soil Analysis. Part 2. Agronomy No. 9.* American Society of Agronomy, Madison, WI
- WINBRP (2007) Draft position paper, Recommendations of the Australian National Biosolids Research Program on Biosolids Guidelines. *Biosolids Specialty III Conference*.
- NORTON, R. M. (1993) The effects of applied nitrogen on the growth, yield, water use and quality of rapeseed (*Brassica napus*) in the Wimmera. LaTrobe University, Bundoora.
- POLGASE, P. J. & ROBINSON, M. B. (1996) Sustainable Use of Biosolids in Plantation Forests, 1st Int. Conf. - Contaminants and Soil Environ., Adelaide.
- PRITCHARD, F. M., EAGLES, H. A., NORTON, R. M., SALISBURY, P. A. & NICOLAS, M. (2000) Environmental effects on seed composition of Victorian canola. *Australian Journal of Experimental Agriculture*, 40, 679-685.
- RAYMENT, G. E. & HIGGINSON, F. R. (1992) Australian Laboratory Handbook of Soil and Water Chemical Methods, Australian Soil and Land Survey Handbook, Inkarta Press, Australia
- SEARLE, P. L. (1984) The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. *Analyst (Cambridge, United Kingdom)*, 109, 549-568.

SOMMERS, L. E., PAGE, A. L., LOGAN, T. J. & RYAN, J. A. (1991) Optimum use of sewage sludge on agricultural land. Western Regional Res. Publ.W-124, Colorado Agric.Exp. Stn, Fort Collins

WARNE, M., MCLAUGHLIN, M., DIANE, H., BELL, M., BROOS, K., WHATMUFF, M., BARRY, G., NASH, D., PRITCHARD, D., STEVENS, D., PU, G. & BUTLER, C. (2007) Draft position paper, Recommendations of the Australian National Biosolids Research Program on Biosolids Guidelines. Biosolids Specialty III Conference.

WELCH W. R. (1995) The Oat Crop: Production and Utilization, London, Published by Chapman & Hall.

WESTERN WATER (2008) Environment report.

## **Appendix: Methods**

### **Measurement of soil moisture, pH and electrical conductivity**

The moisture content of soil and biosolids was determined as per method 2A1 (Rayment and Higginson, 1992)

The  $\text{pH}_w$  and  $\text{pH}_{ca}$  of soil and biosolids were measured as per Method 4A1 and 4B2 (Rayment and Higginson, 1992).

Electrical conductivity of soil and biosolids were measured as per method 3A1 (Rayment and Higginson, 1992)

### **Determination of exchangeable bases ( $\text{K}^+$ , $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ and $\text{Na}^+$ ) and cation exchange capacity**

The major cations in soils and biosolids were determined using 1 M  $\text{NH}_4\text{Cl}$  as an extracting solution according to Method 15A1 (Rayment and Higginson, 1992). Analysis was conducted using an Agilent Technologies Model 4500 series 300 ICP-MS with HP ChemStation software. The cation exchange capacity (CEC) of soil/biosolids samples was calculated as the ratio of milli-equivalent of total exchangeable bases (Na, Mg, Ca, K) to percent base saturation as per method 15B1 (Rayment and Higginson, 1992).

### **Determination of the bioavailable forms of heavy metals in soil and biosolids**

DTPA extractable metals were determined by the extraction of air dried soil for 2 hours using a solution which was 0.005 M in DTPA, 0.01 M in  $\text{CaCl}_2$  and 0.1 M with respect to triethanolamine (TEA). Analytical grade multi element mixed ICP-MS stock solution

containing Mn, Al, Fe, Cu, Cr, Cd, Co, Mo, Ni, Pb and Zn at a concentration of  $100 \text{ mgL}^{-1}$  was purchased from Graham B. Jackson, a certified reference material distributor based in Melbourne. An intermediate stock solution was prepared from the mixed stock standards at a concentration of  $1 \text{ mgL}^{-1}$  for each micronutrient. Calibration standards were prepared from the intermediate multi-stock solutions. Then 0.2-1 mL aliquots of soil and biosolid extracts were pipetted into 50 mL volumetric flasks and 0.5 mL (5000 ppb) Praseodymium internal standard was added to standards, samples and blanks to check for any matrix effect or instrument drift. The calibration standards, samples and blanks were diluted with 2 % nitric acid solution and analysed using ICP-MS.

### **$\text{HNO}_3/\text{H}_2\text{O}_2$ extractable heavy metals in soil, biosolids and plant matter**

The metal concentrations in soil, biosolids and plant matter were determined according to the procedures described by Benton Jones (2001) and analysed using the Agilent Technologies Model 4500 series 300 ICP-MS described above.

To validate the analytical data, standard reference materials CRM 031-040 (sewage sludge) and SRM1573a (Tomato leaves) for soil /biosolids and plant tissue respectively, were analysed along with the samples, and the percentage recovery of each analyte was calculated.

To check the reproducibility of the analytical data, five replicates of soil and biosolids samples were analysed and the coefficient of variation was determined.

### **Determination of total metals in soil and biosolids using X-Ray Fluorescence Spectrometry**

Total metals in soil/ biosolids and biosolids amended soil samples were analysed using a Bruker S4 pioneer Wave Length Dispersion- X-Ray Fluorescence Spectrometry, equipped with LiF, LiF (200), Ge, PET, OVO -55 crystals with a detection limit ranging between  $10\text{-}100 \mu\text{gg}^{-1}$  for soil (Schlotz and Uhlig, 2002).



X-ray fluorescence analysis was carried out by weighing 8 g of soil/biosolids and biosolids amended soil samples and 2 multimixes XRF pelleting tablets each weighing 1 g were added in each of the samples and ground using a Zirconium made Lab Technics Ring Mill. The fine particulate samples were transferred into an aluminum cup and packed using an Enerpac hydraulic pressure packer. The pressed pellets were analysed in triplicate.

For XRF analysis of total metals in soil/biosolids and biosolids amended soils samples, external calibration curves for each of the metals ((Cu, Cd, Co, Cr, Mo, Mn, Fe, Pb, Ni, Zn,) and major cations (Na, K, Ca, Mg, Al) were established by analysing eight soil standard reference materials (NCS DC 73319, NCS DC 73320, NCS DC 73321, NCS DC 73322, NCS DC 73323, NCS DC 73324, NCS DC 73325, NCS DC 73326). To validate the established calibration curves, Till1 and Till3 soil standard reference materials were treated as samples and analysed based on the already established calibration curves and the percent recovery was calculated.

### **Total nitrogen and carbon in soil and total nitrogen in plant matter**

In collaboration with Department of Primary Industry (DPI Werribee, State Chemistry Laboratory), 0.5 g of soil, biosolids and ground plant matter were weighed and analysed in triplicate to determine the percent total nitrogen in soil, biosolids and in plant matter. The Leco FP 2000 Carbon and Nitrogen Autoanalyzer instrument was calibrated using EDTA, Australian Soil and Plant Analysis council (ASPAC) standard reference materials (STD 75, STD 55 and compost5 for soil and biosolids) and ASPAC standard reference materials STD 143 tea leaves and STD 63 eucalyptus leaves were also analysed for total nitrogen concentrations to validate the accuracy of analytical results.

### **NO<sub>3</sub>-N in soil, biosolids and in plant matter**

Nitrate-N was determined by flow injection analysis. In this method nitrates are reduced to nitrite by a copper cadmium reduction coil. The nitrite ions react with sulfanilamide under

acidic conditions to form a diazo compound. This couples with N-1-naphthyl ethylenediamine dihydrochloride to form a reddish purple azo dye (Technicon Instrument Corporation, 1971).

Nitrate-N in soil and biosolids were extracted using 2 M KCl extracting solution (Keeny and Nelson, 1982).

For the analysis of nitrate in plant tissue, 0.2 g of ground plant was weighed and 50 mL of 2 % acetic acid was added and shaken for 30 minutes, filtered using whatman No 42 filter paper (11 cm) and stored in a cool (4 °C) place ( Karla, 1998).

Ammonium in soil and biosolids was determined by segmented flow analysis utilizing the Berthelot reaction (Searle, 1984).

### **Determination of Olsen-extractable P in soil and biosolids**

The empirical method of Olsen et al., (1954) has wide international acceptance as an indicator of soil P fertility. Olsen's method is based on extraction of air-dried soil with 0.5 M NaHCO<sub>3</sub> and adjusted to pH 8.5 with NaOH. Soil extraction is for 30 minutes at a soil/solution ratio of 1:20 as per Method 9C1 (Rayment and Higginson 1992).

### **Acetic acid extractable phosphorus in plant matter**

For the determination of phosphorus in plant matter, 0.2 g of ground plant samples were weighed in 125 mL plastic bottle in triplicate and 50 mL of 2 % acetic acid was added, shaken for 30 minute and filtered using a Whatman number 42 filter paper (Karla, 1998). The phosphorus concentration in plant extracts was determined using the stannous chloride reduction technique using flow injection analysis.

### **Seed analysis**

Samples of canola seed were sent to Agrifood Technology at Werribee, Victoria and seed oil content was extracted using solvent extraction and the concentration of oil in canola seed was determined by gas chromatography.

| Cost Item  | SWF Contribution | RMIT Contribution | Total    |
|--|------------------|-------------------|----------|
|  | \$15,000         | \$6,500           | \$21,500 |
| Salary of research assistant to put together this report | \$ 15,000        |                   |          |
| Payment of supervising staff                             |                  | \$ 6,500          |          |