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0. Summary

0.1. Rationale and results for the project

Air-drying and storage of digested sludge (aerobic, anaerobic or lagoon-based treatment) is the principal method of sludge management in the State of Victoria. Current Victorian State Guidelines prescribe digestion with a minimum storage period of dewatered biosolids for three years as one method for producing Treatment Grade T1 material. T1 graded products can be applied as fertilisers to land without restrictions related to microbiological safety. A number of key nutrients (nitrogen, phosphate and organic carbon) are, however, substantially lost from stockpiles during this storage period. This is a contributory factor restricting opportunities for recycling biosolids in Victoria.

A primary issue in the current regulation for application of biosolids to land is microbial safety. This has resulted in conservative regulation, due to the absence of applicable data regarding microbial safety for standard treatment processes. In addition, although air drying for three years is listed as a T1 process, there are no recommendations in the Guidelines for air-drying and storage for restricted grade products (Treatment Grades T2 and T3).

The project consisted of four parts; following the die-off of indicator pathogens during air-drying and stockpiling treatment processes, investigating the potential for regrowth of pathogens in biosolids, observing the loss of nitrogen nutrient compounds during storage and reviewing the literature on the effects of air-drying and storage on sludge properties, indicators, pathogens and nutrients.

0.1.1. Die-off of indicator micro-organisms during the sludge air drying and stockpiling processes

First we evaluated the die-off of indicator micro-organisms during the sludge air drying and stockpiling processes at two wastewater treatment plants (WWTPs) in the greater Melbourne area; Eastern Treatment Plant, Melbourne Water Corporation (ETP, MWC) and Mt Martha Plant, South East Water Limited (MM, SEWL). *E. coli* represented pathogenic enteric bacteria, coliphages represented enteric viruses, and *Clostridium perfringens* was used as an indicator for *Giardia* and *Cryptosporidium* parasites.

In drying-pans from both WWTPs;

- *E. coli* showed substantial die-off during pan drying, at both treatment plants.
- Levels of *E.coli* dropped from 1 x 10^6 cfu/g DS (T3 grade) on entry into the drying pan to <1000 cfu/g at 6 to 7 months (equivalent to T2 limit) and <100 cfu/g by 8 to 10 months (equivalent to T1 limit).
- There were minor differences between the two treatment plants in the length of time required to reach each treatment grade.
- Removal of coliphages was similar to that of *E. coli*.
- *Salmonella* spp. were not detected in a selected range of drying-pans.
- Levels of *C. perfringens* were about 10^7 cfu/gDS on entry to the drying pan and remained numbers static.

In stockpiles from both WWTPs;

- No *E. coli* or coliphages were detected (limit 20 cfu/g DS or pfu/g DS) in any stockpiles sampled (from 6 months to 3 years of age).
- *Salmonella* spp. were not detected in a selected range of stockpiles.
- Levels of *C. perfringens* remained relatively static during stockpile storage.

0.1.2. Potential for regrowth of E. coli and Salmonella in biosolids

Secondly, we examined the potential for regrowth of *E. coli* and *Salmonella* in biosolids from both drying-pans and one year-old stockpiles. No regrowth was observed, even with added *E. coli* M34 (isolated from a drying pan) or *Salmonella dublin* (isolated from a calf with diarrhoea).

0.1.3. Mineral nitrogen release during the air-drying and stockpiling process

Thirdly, we evaluated the mineralisable nitrogen content during the air-drying and stockpiling process to ascertain when the major loss of mineralisable nitrogen takes place. Our results showed that biosolids can significantly improve the nitrogenous content of aerobic soils, but provide less improvement in water-logged soils. Biosolids, from drying-pans and stockpiles of 1 and 2 years age, were added to soils at standard ratios. The addition of biosolids to aerobic crumbed soils resulted in increased levels of mineral nitrogen, both ammonia and nitrate, on Day 0. After 70 days incubation at 20 ^{o}C, all biosolids samples showed substantial conversion of ammonia to nitrate. In contrast, under saturated (anaerobic) conditions levels of mineral nitrogen were highest in drying-pan/soil mixtures. The stockpile/soil mixtures showed substantial loss of mineral nitrogen and the appearance of nitrite.

In addition, the content of phosphate and water holding capacity of biosolids decreased during drying and stockpiling.

0.2. Recommendations

The data from these three parts of the project have been used to propose suitable beneficial uses for Treatment Grades T1, T2 and T3 air-dried and stored biosolids.
Summarized in Figure 1, below, are the current scheme and key proposed recommendations for air-drying and storage of biosolids for microbial safety.

0.2.1. Treatment grades

Procedures for treatment plants with anaerobic digesters, such as MWC ETP and SEWL MM, to provide treatment grades of biosolids:

- For production of T1 grade biosolids, air-dry biosolids in pans for 8-10 months (from pan to land)
- For production of T2 grade biosolids, air-dry biosolids in pans for 6-7 months (from pan to land)
- For production of T3 grade biosolids, use biosolids directly from the anaerobic digesters (from pump to land)

The above recommendations are based on values of *E. coli* and coliphage removal. These recommendations, however, require a caveat, that confirmation is needed of the potential removal of both enteric viruses and parasites before the product is permitted for unrestricted use. This will require use of a laboratory simulation system, to properly test the effects of air-drying treatment processes on the removal of these pathogens. Such a laboratory-based approach, with PC2 level microbial safety conditions, is necessary to ensure safe work. This avoids the risks of spiking field processes with pathogens, which might lead to cases of diseases in plant workers or other people; a situation that must be avoided.

Furthermore it is recommended that analysis should be repeated over at least two further seasons, to determine whether the kinetics of removal of pathogen indicators across the air drying and storage processes that are reported in this report are reproducible.

It is suggested that hazard analysis and critical control points (HACCP) management be utilized to consistently provide the robust removal of pathogen indicators by each individual process, and to set goals for standard log decreases of the numbers of pathogen indicators.

The recommendations for production of T1, T2 and T3 treatment grades biosolids apply specifically to plants with the same process structure as the two plants investigated in this project. Notably, this includes in-line mesophilic anaerobic digestion as well as drying pans and storage areas.

Other types of treatment plants in Victoria, such as with the common lagoon-based treatment (e.g., without anaerobic digesters), require investigations to determine when T1, T2 and T3 grades of biosolids are produced. As each process should have characteristic values of pathogen removal, investigations should characterise the log decrease data for indicator pathogens in each process.

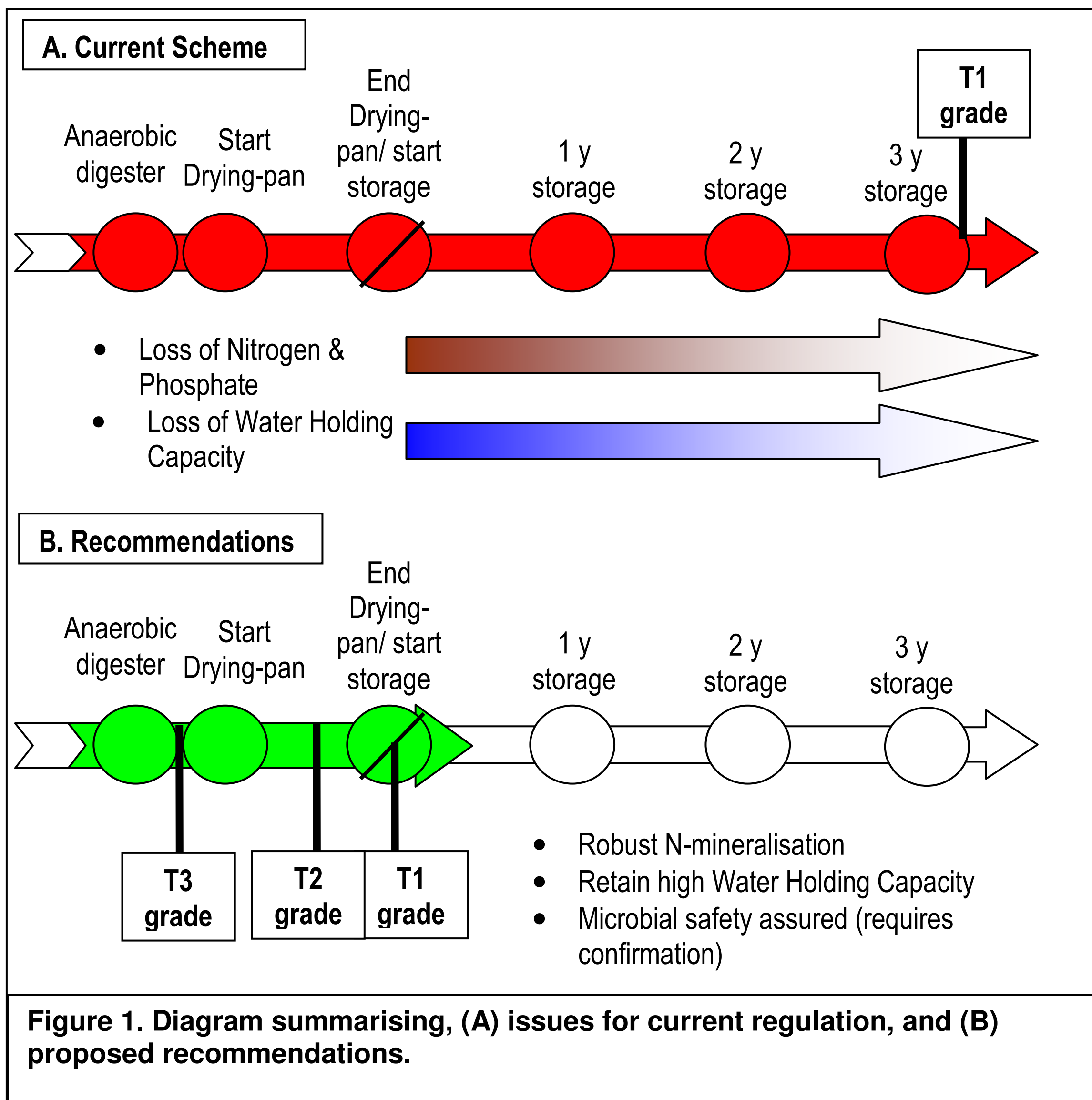

Figure 1. Diagram summarising, (A) issues for current regulation, and (B) proposed recommendations.

0.2.2. Avoiding regrowth

Wetting of dried biosolids (to 40-50% moisture content) does not directly lead to regrowth of pathogen indicators. Dried biosolids may be stored in the open before use, but under conditions that do not allow pools of water to accumulate over the biosolids, as the pools may allow growth of pathogens due to contamination by pathogens from animals in the environment (Zaleski et al., 2005). Furthermore, it is recommended to investigate what extent of water saturation is necessary for regrowth to potentially occur in dewatered biosolids.

0.2.3. Indicators

To provide improved knowledge for removal of pathogens compared to removal of pathogen indicators, laboratory trials simulating the digestion, drying and stockpiling stages, are required to further explore the relationship between representative pathogens and chosen indicators under controlled conditions.

Clostridium perfringens is not recommended as an indicator for the decaying presence of protozoan pathogens during sludge treatment by operational plants.

0.2.4. Which serotypes of *Salmonella*?

The Environmental Protection Agency (EPA) regulations (EPA, 2004) require direct analysis for the genus *Salmonella*, but do not describe which particular serotypes should be detected. In terms of microbial safety, it is proposed that only *Salmonella enterica* subsp. *enterica* be mandated for verification and monitoring of T1, T2 and T3 grades for sludge treatment, as other subspecies of *S. enterica*, along with *S. bongori*, are usually isolated from cold-blooded animals and the environment, and generally do not cause disease in humans.

0.2.5. Nutrient content

Conservatively, stockpiles should not be more than 1 year old to retain optimal nutrient content for land application. This is recommended due to the decline in nutrient content during storage, particularly of nitrogen components, and the increasing stability of the organic nitrogen fraction.

A significant degree of unexplained variability in nitrogen content between a number biosolids stockpiles was observed. This requires further investigation of nutrient contents in air-dried and stored biosolids. This includes examining the effect of variant operational treatments of biosolids on nutrient content.

Further investigations of nitrogen mineralisation by biosolids should include a range of Australian soils, to assess the potential range of rates for nitrogen mineralisation in different types of soil. In addition, it may be concluded that in applying biosolids to soil the characteristics of the microbial flora in the soil should be investigated to assess the potential for nitrogen mineralisation.

User guidelines for land application of biosolids should be developed to ensure optimum use of biosolids for fertilization of crops and other plants. These guidelines should be informed by nutritional information in applying biosolids to land, including about nitrogen mineralisation.

0.2.6. Who took part in the project?

The funding was awarded to the CRC for Water Quality &Treatment, with research performed at RMIT with consultation from Imperial College London (UK).

0.2.7. When was the project performed?

June 2007 to June 2008.

1. Introduction

Biosolids are the treated residual product resulting from urban wastewater treatment. In Australia, urban wastewater treatment has traditionally involved several stages, including grit removal, primary sedimentation, and biological treatment (activated sludge process). The sludge produced from these processes is stabilized by a range of treatments. In the State of Victoria amongst metropolitan WWTPs, primary mesophilic anaerobic digestion is often coupled with air-drying in pans followed by stockpiling. In contrast, across regional Victoria there are 172 smaller plants, which mainly use long-term lagoon-based treatment, followed by stockpiling. Due to the availability of large tracts of land, air-drying followed by stockpiling of digested sludge has been possible and is the principal method of sludge management in Victoria.

With the increasing community awareness of the need to recycle and avoid wastage of resources, biosolids are seen as a valuable resource. In addition, with the distribution of treatments plants across Victoria there are likely to be advantages in local usage of biosolids, both to reduce transport costs and contribute to local communities. While biosolids can provide useful nutrients for plant growth (Beshah et al., 2008; Singh and Agrawal, 2008), much of their nutrient value may be lost during stockpiling.

Currently, in Victoria, approximately 2 million t dry solids (DS) of sludge are either stored in lagoons or stockpiled; <5 % of annual sludge production (66,700 t DS) is used beneficially (DNRE, 2002), equivalent to little more than 3000 t DS y^{-1}. The main reason for this is that current Victorian State Guidelines prescribe digestion with a minimum storage period of three years as one method for producing Treatment Grade T1 material, that is material that can be applied to land without restrictions related to microbiological quality. Moreover, there are no recommendations in the Guidelines for air-drying and storage for restricted grade products (Treatment Grades T2 and T3). These restricted grades represent the main outlets for biosolids in all other countries where recycling to land is a well developed and accepted practice. The use of these products is coupled with land use restrictions to allow the natural attenuation of any residual pathogenic organisms that may be present in the sludge after treatment. Restrictions are based on the well established multi-barrier approach to protect human health from infectious enteric disease when sewage sludge is recycled to farmland (WHO, 1981; US EPA, 2003). Although EPA Victoria has provided some site specific approvals for biosolids air-dried for less than 3 years to be land applied as a T3 product, the lack of clear guidance is a significant barrier to the expansion of beneficial reuse programmes for biosolids in Victoria.

Without clear guidance and supporting information on the pathogen removal efficiency of air drying, it is difficult to demonstrate due diligence in the use of biosolids from air drying, have certainty in planning for regulatory approvals, and have robust systems to ensure reliable production of a specified product quality.

Overcoming these barriers and establishing confidence in using biosolids air dried for less than 3 years will have important benefits through increasing the agronomic value of the biosolids produced. This is because the nutrient value of biosolids diminishes during storage owing to the loss of mineral nitrogen (N) by volatilisation of the ammonia content and stabilisation of the organic nitrogen fraction, reduced solubility of phosphorus (P) and degradation of the organic matter content. Over time, this compromises the quality and value of the ‘treated’ material as a soil improver and fertiliser. While shortening the storage period has the benefit of increasing the agronomic value of the biosolids, due account must be taken of the implications for the microbiological quality of the sludge. Storage after mechanical or solar/air drying for periods of between 3 to 6 months to meet appropriate microbiological criteria for restricted agricultural application is commonly practised in other countries with climates ranging from temperate (e.g. UK) to arid (e.g. Egypt).

2. Description of Project

2.1 Objectives

The ultimate aim of this project is to provide revised recommendations for production of grade T1 and restricted grades T2 and T3 biosolids, using air-drying and storage. Such recommendations will be based on microbiological data and data on nutrient availability for plants during air-drying and stockpiling processes. This would increase the flexibility and opportunities for beneficially recycling biosolids that are 'fit-for-purpose' for land application including, for example, for the production of processed crops and in land restoration, which are potentially major outlets for sludge in Australia as in all other countries with well developed recycling programmes.

The aims of this project were to (i) provide preliminary data on the decline in numbers of bacterial and viral indicators of pathogenic microorganisms during sludge treatment, (ii) provide data on the ability of selected bacterial pathogens to grow in dried sludge that has been re-wetted, e.g. by rain (iii) provide data on the nutrient value for plant growth of sewage sludge throughout the drying and stockpiling process and (iv) produce a literature review on the effect of air-drying and sludge-storage on properties of biosolids.

The original research proposal was for this research to be carried out in two stages; the first stage is presented in this report. Stage 1 includes foundational work, valuable in its own right, but which will also underpin successful completion of Stage 2, which is proposed to follow the recommendations in section 5, Discussion and Recommendations. Stage 2 research will be undertaken should an application for future Smart Water Funding be successful.

As required for reporting on the final quarter of work, this report also includes:

- Final results and analysis of die-off of microbial indicators and recommendations regarding suitability of biosolids at key phases in air drying and storage for T2 and T3 uses.
- Data from the winter regrowth experiment and recommendations regarding conditions likely to lead to regrowth of *E. coli and Salmonella* in air dried and stored biosolids.
- Data on nutrient value of pan-dried and stockpiled biosolids and recommendations on the optimal stages in the treatment process and optimal storage conditions for preserving nutrient value.
- Literature review

2.2 Research Outline

The project evaluated the die-off of indicator micro-organisms during the sludge air drying and stockpiling process. It also evaluated the mineralisable nitrogen content during this process to ascertain when the major loss of mineralisable nitrogen takes place. The data from these two parts of the project have been used to make preliminary proposals for the beneficial uses for Treatment Grades T1, T2 and T3 air-dried and stored biosolids. The issue of possible regrowth of bacteria in treated sludge which is re-wetted by rain was also investigated to further assist in the safe use of air-dried and stored biosolids.

2.3 Methods/Project Overview

This section provides project outlines and, rationale and outcomes of each of the three subprojects that are the subject of this review.

2.3.1. Monitoring survival of indicators across two operational treatment processes

Aim: To establish a "snap-shot" of indicator survival across the air-drying and stockpiling processes of two full-scale operational plants.

Rationale:
Since the concentrations of pathogens and parasites in sewage sludge are low, indicators which are naturally present in larger numbers are generally used to estimate the levels of pathogens present in water or sewage sludge. F-specific coliphages have frequently been used as indicators of enteric viruses, *E. coli* is used as an indicator for the *Enterobacteriaceae* and *C. perfringens* is used as an indicator of protozoan parasites in water quality testing (UK Environment Agency, 2002). Survival of both of these indicators across the air-drying and stock-piling process could provide an indication of the efficacy of the process for the removal of bacterial and viral pathogens.

Methods: As the concentrations of pathogens and parasites in sewage sludge are small and generally not detectable, we monitored indicators of pathogens, which are naturally present in the sludge in larger numbers than pathogens. The indicators chosen were coliphages as indicators of enteric viruses, *E. coli* as an indicator for the *Enterobacteriaceae* and *C. perfringens* as an indicator of protozoan parasites (UK Environment Agency, 2002). Selected samples were also examined for *Salmonella* species by standard filtration methods, with some modifications.

Sludge was sampled at: (i) the time of entry to the drying pan, (ii) in different drying pans representing sludge at different stages of the drying process e.g., 1 month, 3 months, 12 months (iii) up to three depths within stock-piles representing storage between 6 months and 2 years of storage. Depths were 0.0-0.2 m at the surface of the pile, and depending on the size and height of the pile, at depths in the range e.g. of 0.4-0.6 m and 0.9-1.1 m.

Results:
E. coli showed substantial die-off during the drying-pan phase at both treatment plants. Levels dropped from 1 x 10^6 cfu/g DS (T3 grade) on entry into the drying pan to <1000 cfu/g at 6 to 7 months (equivalent to T2 limit) and <100 cfu/g by 8 to 10 months (equivalent to T1 limit) (Figures 2 and 3). Removal rates of coliphages were similar to the removal rates of *E. coli* (Figure 6). There were minor differences between the two treatment plants in the length of time required to reach each treatment grade (Figures 2 and 3). No *E. coli* or coliphages were detected (limit 20 cfu/g DS or pfu/g DS) in any stockpiles sampled (from 6 months to 3 years of age). *Salmonella* spp. was not detected in the selected range of drying-pans and stockpiles. Levels of *C. perfringens* were about 10^7 cfu/g DS on entry to the drying pan, and showed only minor changes throughout pan drying and stockpiling (Figures 9 and 10).

2.3.2. Controlled laboratory investigation – regrowth of *E. coli* and *Salmonella*

Aim: To establish whether *E. coli* and *Salmonella* regrow in stockpiles that have been wetted by rain.

Rationale:
While regrowth of bacteria such as *Salmonella* and *E. coli* has not been reported for properly digested sludge (EPA Vic, 2004) with a DS content above 25% (Bellemain and Bagnall, 2003), it is still perceived as being a concern.

Methods:
Samples of both stockpiles and a drying pan were taken from the same two full-scale operational plants. Samples from stock-piled sludges one year of age, were taken at multiple depths (down to 1.0 m), and composite samples, comprising 10 sub-samples, were taken from a drying pan prior to harvest. The water content of the biosolids was adjusted, under controlled laboratory conditions, to simulate representative rainfall and temperature conditions. The moist and saturated biosolids had moisture contents of 5-10%, and 40-50% respectively.

Two bacterial isolates, *E. coli* M34 (isolated from a drying pan at Mt Martha in this project) and *Salmonella* Dublin isolated from a calf with diarrhoea, were added to the biosolids. The regrowth of *E. coli* strain M34 and *Salmonella* Dublin in these saturated sludges and moist controls was monitored over a period of up to four weeks, by performing standard plate counts.

Results:
No regrowth occurred in either saturated biosolids (40-50% moisture) or moist biosolids (5-10% moisture) without added bacteria, over 4 weeks of incubation time. Moreover in moist biosolids with added bacteria, survival of both *E. coli* M34 and *Salmonella* Dublin decreased by 5 logs, while in saturated biosolids the strains mainly had stable presence or decreased survival.

2.3.3. Controlled laboratory investigation - mineralisable nitrogen release

Aim: To establish the change in phyto-available nitrogen content of biosolids during the air-drying and stockpiling process.

Rationale: Nitrogen is present in biosolids in a number of forms; ammonia, nitrate/nitrite and organic matter. While ammonia and nitrate applied to soil in sludge are readily phyto-available, these compounds may be leached or otherwise lost from biosolids during storage. Organic nitrogen only becomes phyto-available through mineralisation. Organic nitrogen stabilisation takes place during the air-drying and storage process and it is hypothesised that with time, the amount of mineralisable nitrogen in the sludge will decrease. It may be the case that the maximum agronomic advantage from the organic nitrogen contained in sludge is obtained by using the biosolids well before the end of the prescribed air-drying and storage treatment periods.

Many soils in Australia suffer from nutrient and organic matter deficiency with an annual application of fertiliser essential to maintain financially viable levels of crop and pasture production. The application of biosolids to agricultural land is considered of benefit as it supplies many of the essential nutrients for crop growth including phosphorus, nitrogen, potassium, sulphur and metals.

Methods:
A standard soil-incubation protocol was followed to determine the change in mineralisable nitrogen content of the biosolids during air-drying and storage. A reference soil was selected (a typical sandy loam, pH 6.5) for comparison with published work using this technique. Biosolids were thoroughly mixed with the soil at a rate equivalent to 10 t DS ha^{-1} to avoid inhibition of nitrification from excessive concentrations of ammonia-N in the sludge. Approximately 100 g quantities of the sludge-soil mixtures were transferred to partially sealed plastic containers and maintained in a temperature-controlled incubator in the dark at 20°C. Sufficient samples were prepared for three replicates of soil to be removed at intervals of 0, 5, 10, 20, 40 and 70 days. The concentrations of ammonium-nitrogen, nitrate-nitrogen and nitrite-nitrogen were determined by 2 M KCl extraction followed by automated colorimetric analysis of the filtered extract. In addition, bicarbonate extractable phosphorus content was determined using a colorimetric autoanalysis technique.

Samples were also be analysed for total nitrogen by standard Kjeldahl digestion and the organic matter content (volatile solids, VS) was measured by a standard loss-on-ignition method.

Results:
Our results suggested that biosolids from drying-pans could be used to effectively fertilise both aerobic moist and anaerobic saturated soils, though best results would be expected with aerobic soils. In contrast, it appears that stockpile biosolids should be applied only to aerobic soils, due to the rapid disappearance of mineral nitrogen under saturated conditions. Most biosolids samples from pans and stockpiles had high levels of extractable phosphate. As with mineral nitrogen, our results suggest that for preserving the phosphate content of biosolids, the storage period should be minimised.

2.3.4. Literature review

Aim: To obtain information that will enhance performance of the project and assist in results interpretation.

A literature review was carried out as part of an Environmental Engineering MSc project at Imperial College, London. The literature review covered the effect of air-drying and storage on sludge properties, pathogens, indicators and nutrients. It also included a review of guidelines on air-drying and storage treatment processes and biosolids use as given in Australian and international biosolids guidelines (Appendix C).

3. Project Details

3.1. Project 1: Monitoring Survival of Indicators Across Two Operational Treatment Processes

3.1.1. Aim

To establish a “snap-shot” of indicator survival across the air-drying and stockpiling processes of two full-scale wastewater treatment plants (WWTPs) in the greater Melbourne area; Eastern Treatment Plant, Melbourne Water Corporation (ETP, MWC) and Mt Martha Plant, South East Water Limited (MM, SEWL).

3.1.2. Rationale

The concentrations of pathogens and parasites in sewage sludge are small and generally not detectable. Hence it was decided to measure indicators of pathogen survival which are naturally present in the sludge in considerably larger numbers. F-specific coliphages have frequently been used as indicators of enteric viruses. These coliphages can be measured using a double layer agar method. *E. coli* is generally used as an indicator for the *Enterobacteriaceae* which include many enteric bacterial pathogens, particularly *Salmonella* spp. and *E. coli* pathotypes. The filtration method for measurement of *E. coli* in sludge is well developed and reasonably robust. Survival of both of these indicators across the air-drying and stockpiling process gives an indication of the efficacy of the process for the removal of bacterial and viral pathogens. Selected samples were also examined specifically for *Salmonella* species by filtration methods. Levels of *C. perfringens* were determined in the first set of samples from both treatment plants, since this organism is used as an indicator of protozoal parasites in water quality testing (UK Environment Agency, 2002).

3.1.3. Methods

<u>Sampling at operational sites</u>

Samples were collected at two operational sites (ETP, MWC and MM SEWL). Samples included material from primary sludge, the dissolved air flotation (DAF) stream, output from the anaerobic digester (MAD output), pans and stockpiles. Pan samples were colleted at the time of entry to the drying pan and from different drying pans representing sludge at different stages of the drying process e.g., 1 month, 3 months, 12 months. Stockpile samples usually consisted of three composite samples taken across three different locations at three different depths up to three depths within stock-piles representing storage between 6 months and 2 years. Depths were 0.0-0.2 m at the surface of the pile, and depending on the size and height of the pile, at depths in the range e.g. of 0.4-0.6 m and 0.9-1.1 m. Where frequent turning was practiced, which is common in the initial storage period to accelerate drying, only a single depth was available (e.g., in the range 0.0–0.2 m) as regular mixing ensures the homogenisation of the sludge.

<u>Microbiology methods</u>

After collection, samples were transported to the laboratory and stored at 4°C overnight before analysis. The numbers of *E. coli*, *Salmonella* spp. F-specific coliphage (MS-2) and *Clostridium perfringens* were estimated by modifications of standard methods used for water and/or sewage analysis (Appendix B).

Table 1. Samples taken from Mount Martha WWTP (South East Water Limited)

Type of sample	Sample No*	Sample Date	Location/date**	Depth (m)	Age† (months)	DS%
Primary sludge	M0a	13/11/2007	1^{O} sludge		Fresh	3.6
	M0a2	03/12/2007	1^{O} sludge		Fresh	4.7
DAF	M0b	13/11/2007	DAF stream		Fresh	4.1
	M0b2	03/12/2007	DAF stream		Fresh	3.7
MAD output	M1a	16/10/2007	MAD output P. 2		Fresh	1.5
	M1b	13/11/2007	MAD output P. 5		Fresh	1.7
	M1c	03/12/2007	MAD output P. 5		Fresh	2.3
Drying-pan	M2a	16/10/2007	P. 3		6	4.7
	M2b	16/10/2007	P. 3		6	4.6
	M2c	16/10/2007	P. 3		6	4.5
	M3a	13/11/2007	P. 3		7	5.3
	M3b	13/11/2007	P. 3		7	4.8
	M3c	13/11/2007	P. 3		7	5.2
	M4a	03/12/2007	P. 3		8	5.9
	M4b	03/12/2007	P. 3		8	7.0
	M4c	03/12/2007	P. 3		8	6.5
	M5a	22/04/2008	P. 3		12	31.9
	M5b	22/04/2008	P. 3		12	36.9
	M5c	22/04/2008	P. 3		12	33.8
Stockpile	M6	16/10/2007	S Feb 07	0-0.2	20	62.4
	M7	16/10/2007	S Feb 07	0.4-0.6	20	53.4
	M8	16/10/2007	S Feb 07	0.9-1.1	20	51.8
	M9	16/10/2007	S Feb 06	0-0.2	32	69.6
	M10	16/10/2007	S Feb 06	0.4-0.6	32	56.9
	M11	16/10/2007	S Feb 06	0.9-1.1	32	55.6
	M12	04/02/2008	S Feb 07	0-0.2	24	85.5
	M13	04/02/2008	S Feb 07	0.4-0.6	24	55.9

*a, b, and c are replicates
**DAF, dissolved air flotation
DS, dry solids
MAD, mesophilic anaerobic digester.
P drying pan
S stockpile
†Ages of pans and dates from the end of filling, ages of stockpiles date from end of filling of associated pan (add 12 months to piled age).

Table 2. Samples taken from Eastern Treatment Plant (Melbourne Water Corporation)

Type of sample	Sample No*	Sample Date	Location/date**	Depth (m)	Age† (months)	DS%
Primary sludge	E0	13/11/2007	1º sludge		Fresh	6.0
MAD output	E1a	13/11/2007	MAD output. 5		Fresh	1.8
	E1b	03/12/2007	MAD output. 5		Fresh	1.9
Drying-pan	E2a	13/11/2007	P SDP 33		8	12.6
	E2b	13/11/2007	P SDP 33		8	9.2
	E2c	13/11/2007	P SDP 33		8	10.1
	E3a	03/12/2007	P SDP 33		9	13.5
	E3b	03/12/2007	P SDP 33		9	12.6
	E3c	03/12/2007	P SDP 33		9	12.6
	E4	04/02/2008	P SDP 33		11	93.8
Stockpile	E5	04/02/2008	S Feb 07††	0-0.2	24	26.8
	E6	04/02/2008	S Feb 07††	0-0.2	24	31.8
	E7	04/02/2008	S Feb 07††	0-0.2	24	29.3
	E8	04/02/2008	S Feb 06	0-0.2	36	83.0
	E9	04/02/2008	S Feb 06	0.4-0.6	36	71.3
	E10	22/04/2008	S Apr-08	0-0.2	12	80.9
	E11	22/04/2008	S Apr-08	0.4-0.6	12	78.4
	E12	22/04/2008	S Apr-08	0.9-1.1	12	78.1

*a, b, and c are replicates
**DAF, dissolved air flotation
DS, dry solids
MAD, mesophilic anaerobic digester.
P drying pan
S stockpile
†Ages of pans and dates from the end of filling, ages of stockpiles date from end of filling of associated pan (add 12 months to piled age).
†† Samples E5, E6 AND E7 were collected from a stockpile that had been flattened recently by bulldozing and only surface samples were available

3.1.4. Results

Counts of *E. coli* in drying pans over the drying period

Drying pan SDP33 (ETP, MWC)

Drying pan SDP33 was filled in March 2007 and harvested in February 2008. Samples collected retrospectively from the digester output showed an average number of *E. coli* of 1 x 10^6 cfu/gdw. Samples collected from the drying pan in November 2007, December 2007 and February 2008 showed a steady decline in the numbers of *E. coli,* reaching undetectable levels by February 2008. A virtual trend line for removal of *E. coli* is presented (Figure 2). The T2 limit was reached by about 6 months digestion, while the T1 limit was be reached before 9 months digestion.

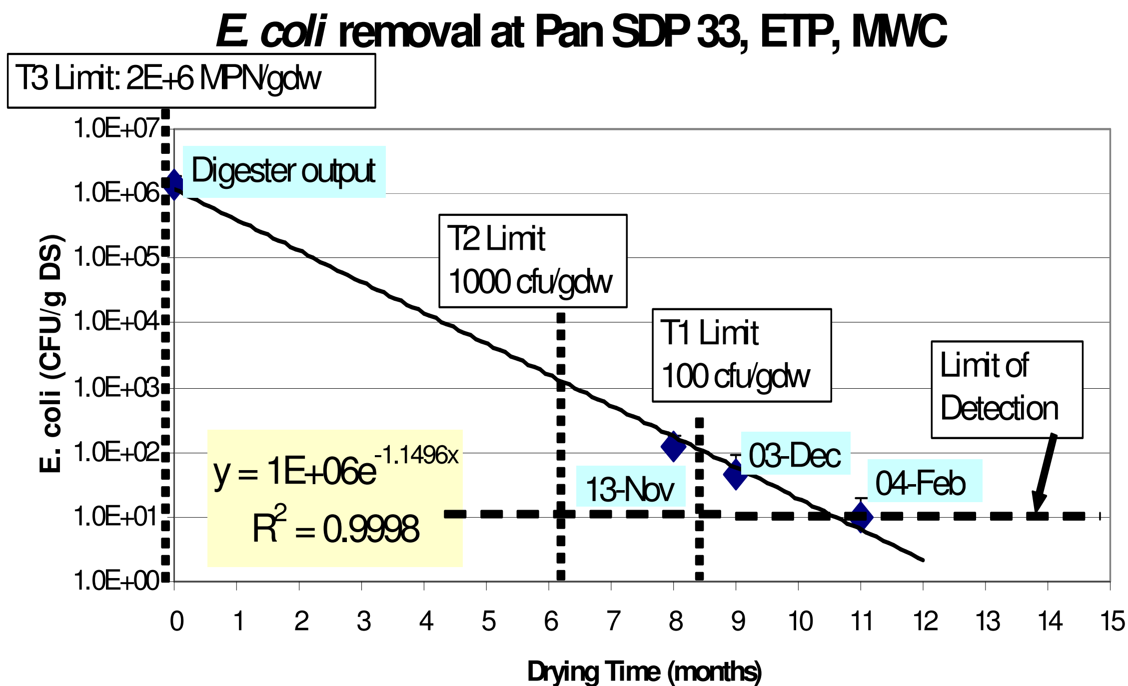

Figure 2. Levels of *E. coli* at Pan SDP 33, ETP MWC

♦ data points showing average of three values, with top error bars of 1 standard deviation (1 STD). The drying times for reduction of *E. coli* prevalence to the required values for treatment grades T1 and T2 are indicated by vertical dotted lines. The limit of detection (20 CFU/g DS) is indicated by a horizontal dashed line. The fitted equation for the trend line (black line) and correlation coefficient are shown in the yellow box.

Drying pan 3 (MM, SEWL): E. coli
Pan 3 was filled in April 2007 and harvested in April 2008. Retrospective samples for the output of the anaerobic digester showed average *E. coli* counts of 5 x 10^5 cfu/gdw. The numbers of *E. coli* showed a similar rapid decline to that observed for pan 33 (ETP, MWC), reaching the T2 limit in seven months, T1 limit just before 10 months and levels were below the limit of detection by 12 months (Figure 3).

The times for reaching T1 and T2 limits are slightly greater for pan 3, MM, SEWL than for pan SDP33 at ETP, MWC. The difference may be due to the extra regular stirring of drying pans that occurs at the ETP, MWC. Nevertheless, given the similarity of treatment processes at MM SEWL and the ETP MWC and the difference in scale of these two plants, it is interesting how close the periods for reaching T2 and T1 values are at these plants.

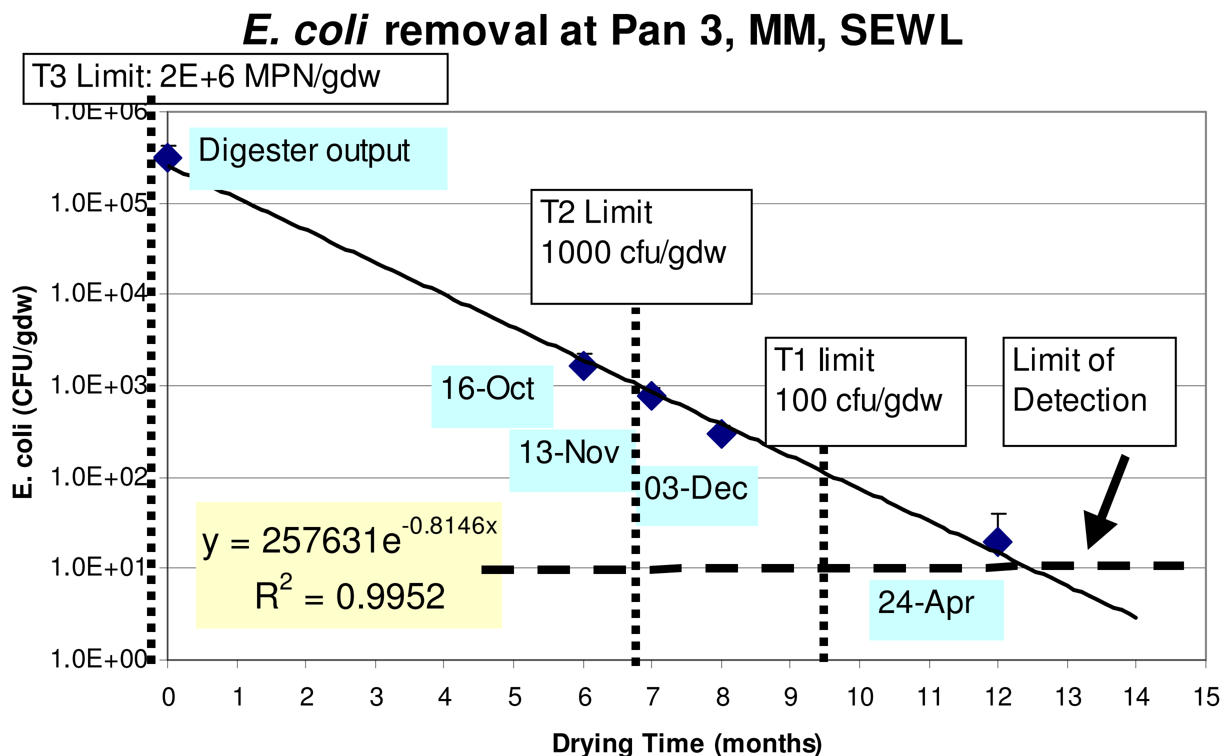

Figure 3. Removal of *E. coli* at Pan 3, MM SEWL
♦ data points showing average of three values, with top error bars of 1 standard deviation (1 STD). The drying times for reduction of *E. coli* to the required values for treatment grades T1 and T2 are indicated by dotted lines. The limit of detection, 20 CFU/g DS, is indicated by the horizontal dashed line. The fitted equation for the trend line (black line) and correlation coefficient are shown in the yellow box.

Summary of treatment grades reached at the different stages of the treatment process

A summary of treatment grades reached in the standard treatment processes at MM, SEWL is presented in Figures 4 and 5. Each treatment process had a characteristic level of pathogen indicator removal. The level of *E. coli* decreased by 2 logs after anaerobic digestion and by another 4 logs in the drying pan.

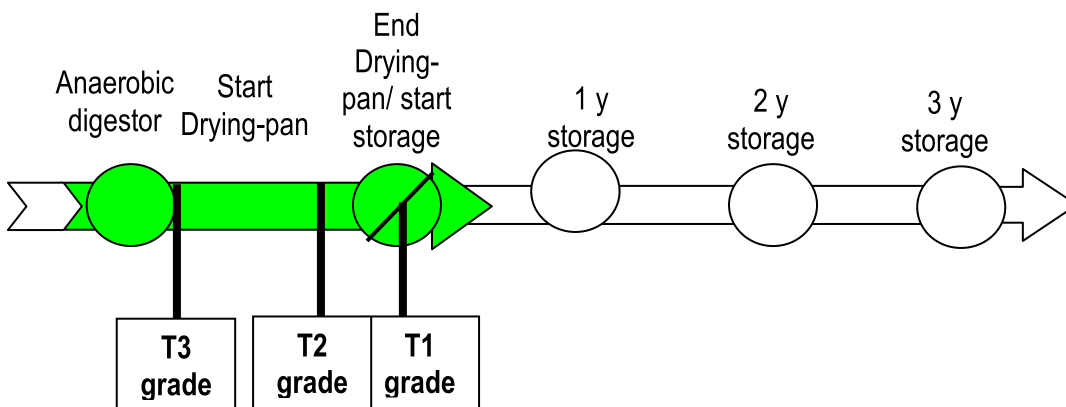

Figure 4. Summary of treatment grades reached in standard treatment processes.

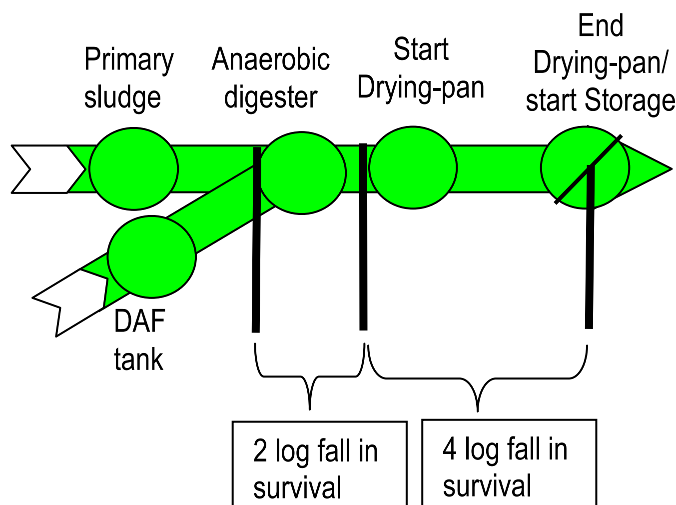

Figure 5. *E. coli* removal versus treatment process at Mt Martha WWTP.

Coliphage counts in drying pans over the drying period

Which coliphages were counted?

The coliphage assay method was designed to detect both F-specific as well as other *E. coli* coliphages. The F-specific coliphages reproduce only under conditions allowing growth of the specific *E. coli* host, as the F-pilus required for F-phage attachment may only be present on growing cells. In contrast, other *E. coli* coliphages may reproduce by the *E. coli* hosts during the stationary phase. Thus our assay, which uses an F-pilus-producing *E. coli* host, supports both F-specific and other *E. coli* coliphages and thus provides a conservative detection of *E. coli* bacteriophages (coliphages).

Drying pan SDP33 (Melbourne Water)

In pan SDP33 in ETP, F-specific and other *E .coli* coliphages were removed at a similar rate to *E. coli*, reaching the limit detection at 11 months of air-drying. The numbers of *E. coli* coliphages were reduced by about 3 logs between the output of the anaerobic digester (~1 x 10^4 pfu/g) and in the drying pan by approximately 9 months after filling was completed (Figure 6).

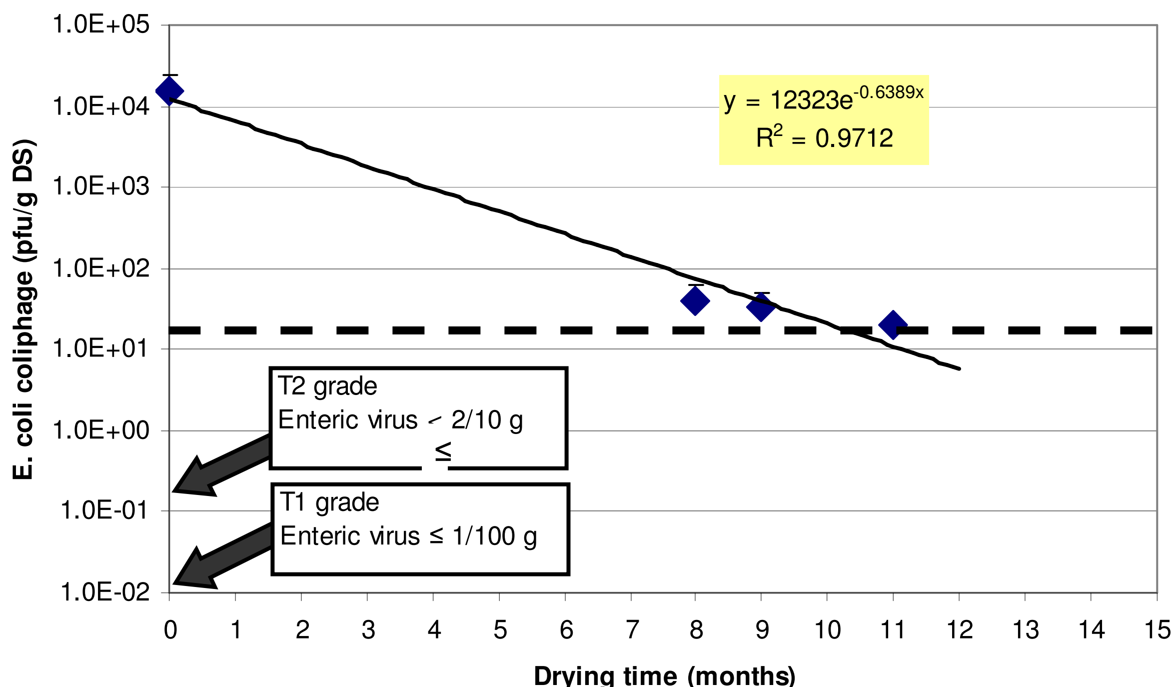

Figure 6. Summary of removal of coliphages in pan SDP 33 at ETP, MWC

♦ data points showing average of three values, with top error bars of 1 standard deviation (1 STD). The limit of detection, 20 pfu/g DS, is indicated by the horizontal dashed line. The values for treatment grades T1 and T2 of enteric viruses are indicated by arrows, which are well below the limit of detection. The fitted equation for the trend line (black line) and correlation coefficient are shown in the yellow box.

Drying pan 3 (MM SEWL)
Due to the low numbers of detectable coliphages in Pan 3, samples were taken further upstream, from the MAD output, DAF tank, and primary sludge. Three pan samples were collected (6, 7 and 8 months after filling was completed). Four stockpile samples were collected on different dates from stockpiles aged 20, 24 and 32 months. As expected, the highest coliphage numbers were observed in the primary sludge and material from the DAF tank. Interestingly, the level of coliphages decreased across both the digester (2 logs) and pan 3 (about 2 logs), while no coliphages were detected in stockpiles (Figure 7). The same data, relating to pan samples only, is presented in Figure 8.

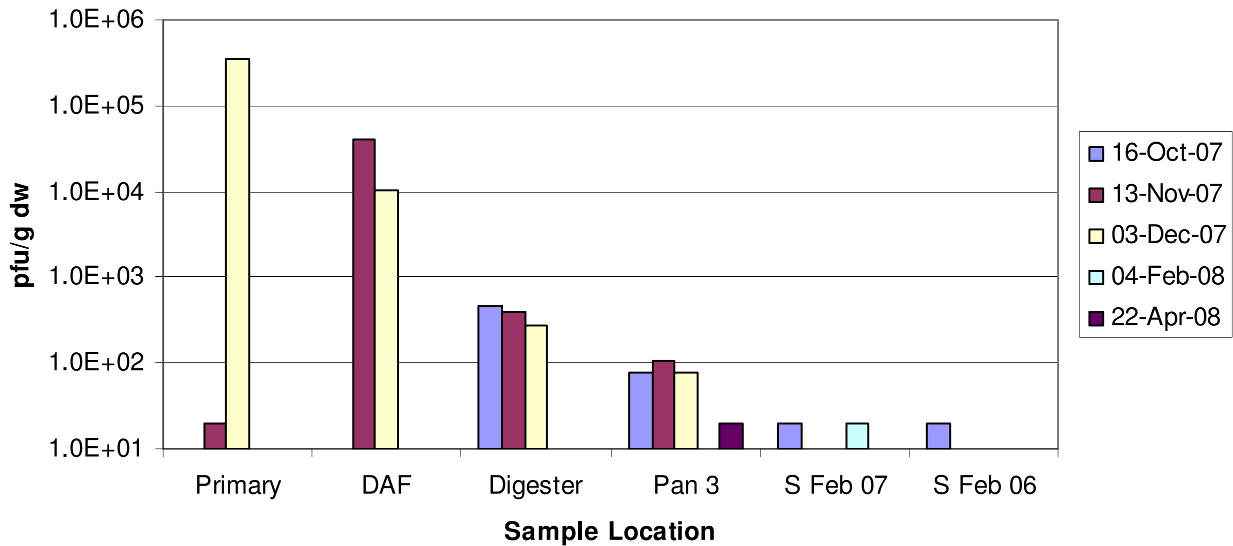

Figure 7. Levels of *E. coli* coliphages in pan 3 MM, SEWL
Values are shown for an average of three assays for each sample. The limit of detection, 20 pfu/g DS, is indicated by the horizontal dashed line.

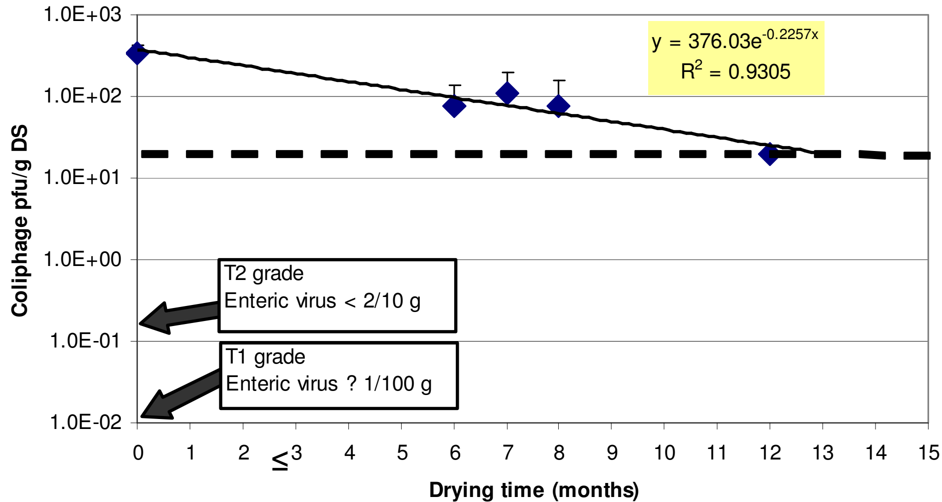

Figure 8. Summary of removal of coliphages in pan 3 at Mt Martha (SEWL
♦ data points showing the average of three values, with top error bars of 1 standard deviation (1 STD). The limit of detection, 20 pfu/g DS, is indicated by the horizontal dashed line. The values for treatment grades T1 and T2 of enteric viruses are indicated by arrows, which are well below the limit of detection. The fitted equation for the trend line (black line) and correlation coefficient are shown in the yellow box.

Discussion of coliphage results.
The coliphage data indicates the importance of each specific process in removing or retaining coliphages, from digester to pan to stockpile. These results support a potential HACCP management to consistently remove or retain loss of pathogen microbes by each individual process.

Stockpile samples (ETP MWC and MM SEWL): *E. coli* and coliphage

Samples from stockpiles at MM, SEWL, ranging in age from 12 to 36 months, failed to yield *E. coli* or coliphage, with one exception (Table 1). Small numbers of *E. coli* were detected in the surface level of stockpile Feb-07 at MM, SEWL at the sampling run of February 2008 (24 months of age). As previous sampling of the same stockpile had not shown any detectable presence of this indicator and as no *E. coli* were detected at a lower level (0.5 m), these results suggest that contamination of the stockpile had occurred recently, presumably due to faecal contamination by environmental animals or birds.

Samples were taken at the ETP, MWC from stockpiles aged 20, 24 and 32 months after the date of filling the associated pan (Tables 1 and 2). No *E. coli* or coliphage was detected in these samples (limit of detection 20 cfu/g DS or pfu/g dw.

Clostridium perfringens levels throughout the treatment process

Background on the use of C. perfringens as an indicator for the presence of protozoal cysts
Clostridium perfringens has been suggested as an indicator of the presence of protozoal cysts, since methods for the detection of live cysts are laborious, expensive and not entirely reliable. The most commonly used method for enumeration of live *Cryptosporidium* and *Giardia* (oo)cysts uses vital dye staining or *in vitro* excystation. Both methods are known to over estimate infectivity. The ability of *Cryptosporidium* cysts to initiate infection has been linked to finite carbohydrate energy reserves which are consumed in direct response to environmental temperature. As the environmental temperature increases, the reserves are depleted more rapidly and infectivity declines.

Treatment-related loss of infectivity of cryptococcal cysts
King et al (2005) looked at the loss of infectivity of *Cryptosporidium* cysts stored in water at a variety of temperatures likely to be found in water storage, treatment and distribution systems. For cysts stored at 30℃, a 4 log decline in infectivity took place after 360 hours (~15 days). At 40℃, the same 4 log decline in infectivity occurred after only 98 hours (~4 days). As anaerobic digesters are generally run at 35℃ with a nominal retention time of 15 days, a 4 log reduction in infectivity is likely to occur unless energy can be sourced from the surrounding medium. Currently the chance of this occurring is unknown. In order for *C. perfringens* to be a reliable indicator of the presence of live protozoal cysts, the decay kinetics would need to follow a similar pattern.

Estimation of the numbers of cryptococcal cysts entering drying pans
To track the loss of infectious cysts through the water treatment process, it can be assumed that raw sewage has a concentration of *Cryptosporidium* cysts with a mean concentration of 350 cysts/L. Note that this figure is one derived by using the vital dye staining method, hence many of these cysts may in fact be non infectious. Using the CIWEM 1996 calculation showing that 100L of raw sewage gives rise to around 1 L of sludge at 2.7% w/w, the density of *Cryptosporidium* cysts in the sludge sent for anaerobic digestion is 350 x 100 = 35,000 cysts/L. This assumption is ultra conservative in that no allowance is made for inactivation of cysts in the activated sludge process. As filter feeding and grazing protozoa will ingest and consume cysts, there is likely to be a reduction in infectious cysts. Assuming that a 4 log reduction in infectivity occurs in the anaerobic digester based on the temperature of 35℃ and a nominal retention time of 15 days, the final count in the digested sludge is likely to be 3.5 cysts/L of sludge.

Levels of C. perfringens in MAD output and Pan 33 (ETP MWC)
Samples were collected from the MAD output and from pan 33 on two occasions, 8 months and 9 months after filling pan 3 (Figure 9). The data showed only a half log reduction between the anaerobic digester and the drying pan after 3 weeks.

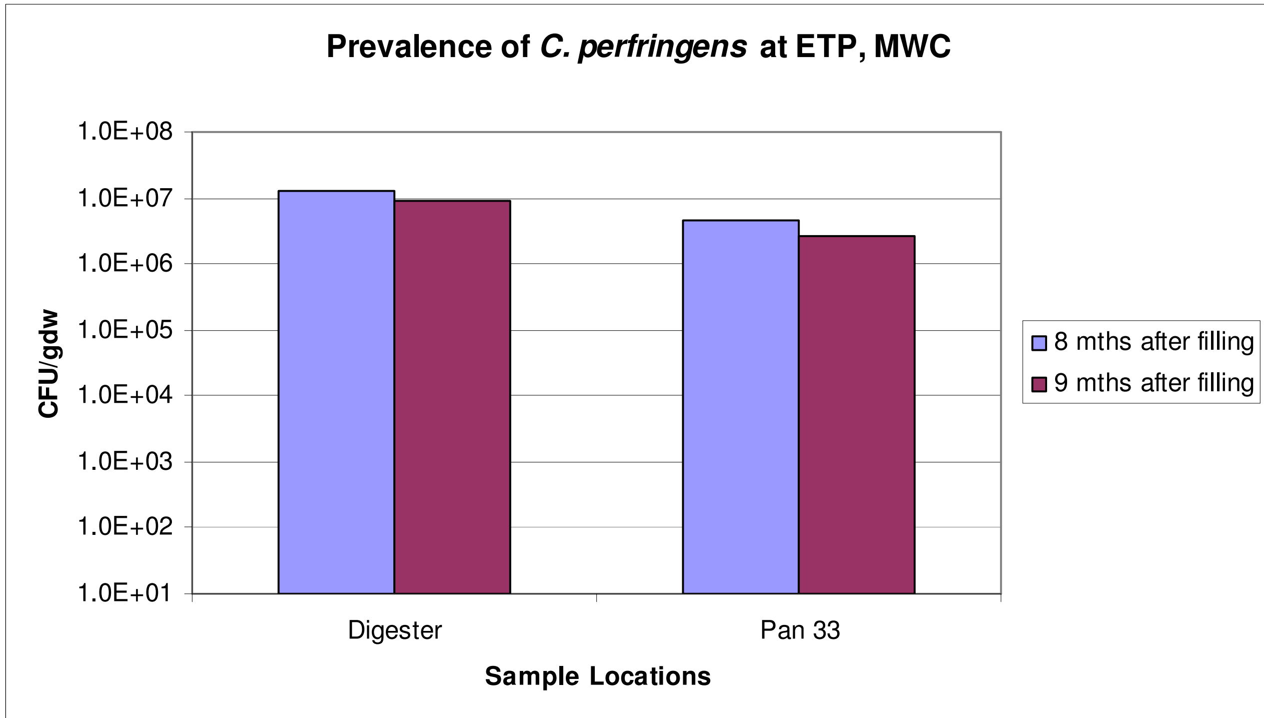

Figure 9. Prevalence of *C. perfringens* during pan air-drying at ETP MWC
Values are shown for an average of three assays for each sample. The limit of detection, 20 cfu/g DS, is indicated by the horizontal dashed line.

Levels of C. perfringens in primary sludge, DAF, MAD output, Pan 3 and stockpiles (MM, SEWL)
Samples collected from primary sludge, the DAF stream, output from the anaerobic digester, Pan 3 (6, 7, and 8 months after filling) and stockpiles (20 and 32 months after filling the associated pan). There was an increase in the numbers of *C. perfringens* between primary sludge and pan samples, followed by a decrease of ~one log in stockpile samples (Figure 10). Stockpile samples, however, still showed high numbers of *C. perfringens* (~5 x 10^6cfu/g).

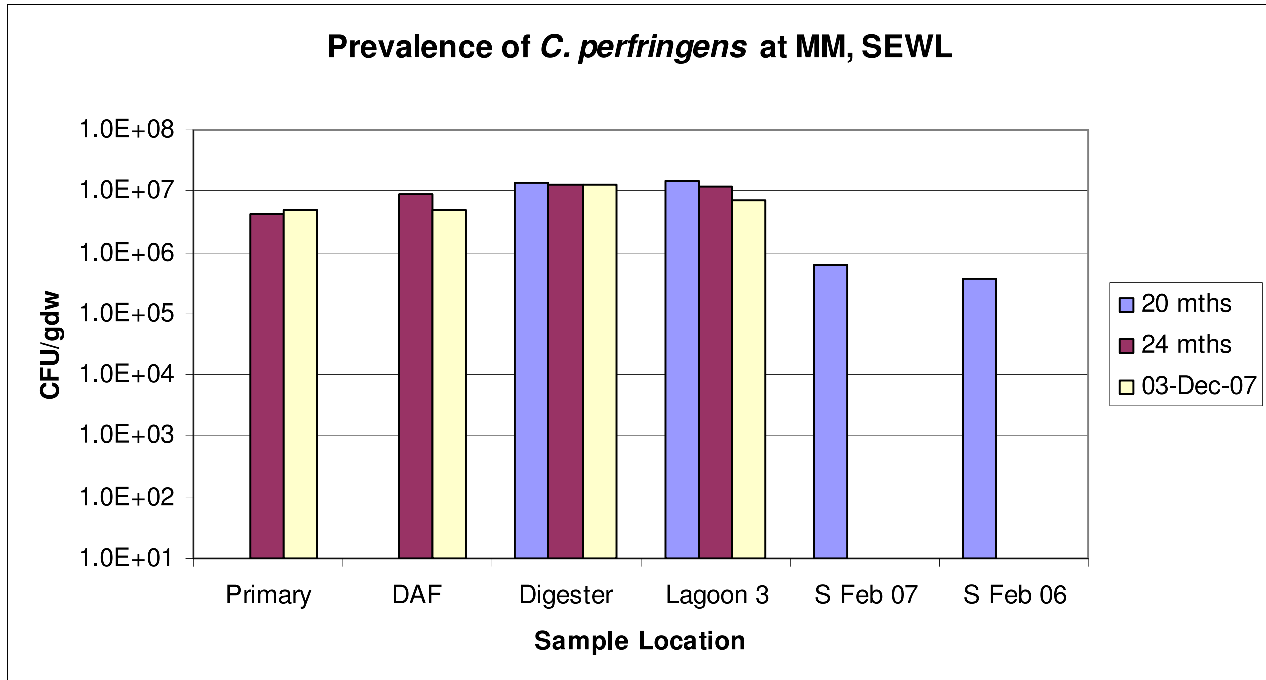

Figure 10. Prevalence of *C. perfringens* during the MAD process and pan air-drying at MM, SEWL. Values are shown for an average of three assays for each sample. The limit of detection, 20 cfu/g DS, is indicated by the horizontal dashed line.

Culture of output of anaerobic digester, drying pans and stockpiles for *Salmonella* spp

A selected set of samples from the output of anaerobic digesters, drying pans and stockpiles was examined for the presence of *Salmonella* spp. No *Salmonella* was detected in any sample (limit of detection 20 cfu/g DS).

The standard method used in this study for detecting *Salmonella* do not adequately detect the human pathogens, *S. enterica* subsp. *enterica* serovars Typhi and Paratyphi (The Blue Book 2002, referenced in Appendix B). The presence of these serovars can be determined by alternative procedures, but no standard methods are currently available to evaluate these serovars.

3.1.5. Discussion

A summary of the reduction in the numbers of *E. coli*, coliphages, *Salmonella* and *C. perfringens* is presented in Table 3.

E. coli showed substantial die-off during the drying-pan phase at both treatment plants. Levels dropped from 1 x 10^6 cfu/g DS (T3 grade) on entry into the drying pan to <1000 cfu/g at 6 to 7 months (equivalent to T2 limit) and <100 cfu/g by 8 to 10 months (equivalent to T1 limit) (Figures 2 and 3). The rate of removal of coliphages was similar to that of *E. coli* (Figure 6). There were minor differences between the two treatment plants in the length of time required to reach each treatment grade (Figures 2 and 3). No *E. coli* or coliphages were detected (limit 20 cfu/g DS or pfu/g DS) in any stockpiles sampled (from 6 months to 3 years of age). *Salmonella* spp. was not detected in the selected range of drying-pans and stockpiles. Levels of *C. perfringens* were about 10^7 cfu/g DS on entry to the drying pan, and showed only minor changes throughout pan drying and stockpiling.

Table 3. Summary of pathogen indicator levels in treatment processes at the two full-scale plants in Victoria

Indicator	Anaerobic Digester*	Drying Pan*	Stockpile*
E. coli	10^5-10^6	10^5-10^6 → < 20	<20
Coliphage	5x10^2-10^4	5x10^2-10^4 → < 20	<20
Salmonella	<20	<20	<20
C. perfringens	10^7	5x10^6-10^7	5x10^5

* Units cfu/g DS or pfu/g DS

3.2. Project 2. Controlled Laboratory Investigation – Regrowth of E. coli and Salmonella

3.2.1. Aim

To establish whether *E. coli* and *Salmonella* regrow or survive in stockpiles that have been wetted by rain, (a) without added bacteria (b) with *Escherichia coli* or *Salmonella* Dublin added separately.

3.2.2. Rationale

While regrowth of bacteria such as *Salmonella* and *E. coli* has not been reported for sludge properly digested (EPA Vic, 2004) and having a DS content above 25% (Bellemain and Bagnall, 2003), it is still perceived as being a concern by some. The data obtained from this experiment is expected to establish whether regrowth of *E. coli* and *Salmonella* is likely to occur under climatic conditions typical of Melbourne and whether sludge age has an impact on regrowth risks.

3.2.3. Methods

Samples of both stockpiles and a drying pan were taken from the same two full-scale operational plants as described in previous sections. Samples from two stockpiles 1 year of age, were taken at multiple depths (down to 1.0 m), and one composite sample, comprising 10 sub-samples, was taken from a drying pan prior to harvest. These biosolids were dried and wetted under controlled laboratory conditions to simulate representative rainfall and temperature conditions. The moist and saturated biosolids had a moisture content of 5-10%, and 40-50%, respectively. In comparison, the average moisture content of stockpile samples in this project was 37.5% (mean) or 37.6% (median), with moisture in samples ranging from 6.2% to 73.2% .

Two bacterial isolates, *E. coli* M34 (a potential robust survival strain isolated from a drying pan at Mt Martha in this project) and *Salmonella* Dublin (isolated from a calf with diarrhoea), were added to the biosolids at levels of 10^6 cfu/ g biosolids. The regrowth of *E. coli* strain M34 and *S.* Dublin in these saturated sludges and moist controls was monitored over a period of up to 4 weeks, by performing standard plate counts. Samples were taken within one to two hours of addition of the bacteria to the biosolids, after 2 weeks and after four weeks. The percent dry solids and water holding capacity of samples was measured by standard methods.

Details of the methods used are given in Appendix B.

3.2.4. Results

Bacterial survival in biosolids without added bacteria

No regrowth occurred in either saturated biosolids (40-50% moisture) or moist biosolids (5-10% moisture) without added bacteria, over 4 weeks of incubation time.

Bacterial survival in moist biosolids

The same strains of *E. coli* and *S.* Dublin were seeded into the same sets of moist biosolids. In contrast to saturated biosolids, there was a steep reduction (~ 3 to 4 logs) in the viable counts of both species within one to two hours of adding the inoculum. This was attributed to the inability of the bacteria to adapt to the change from an overnight liquid culture to the dry salty conditions of the biosolids. By four weeks of incubation, the viable counts of both *E. coli* and S. Dublin were below the limits of detection (<100 cfu/g) (Figures 11 and 12).

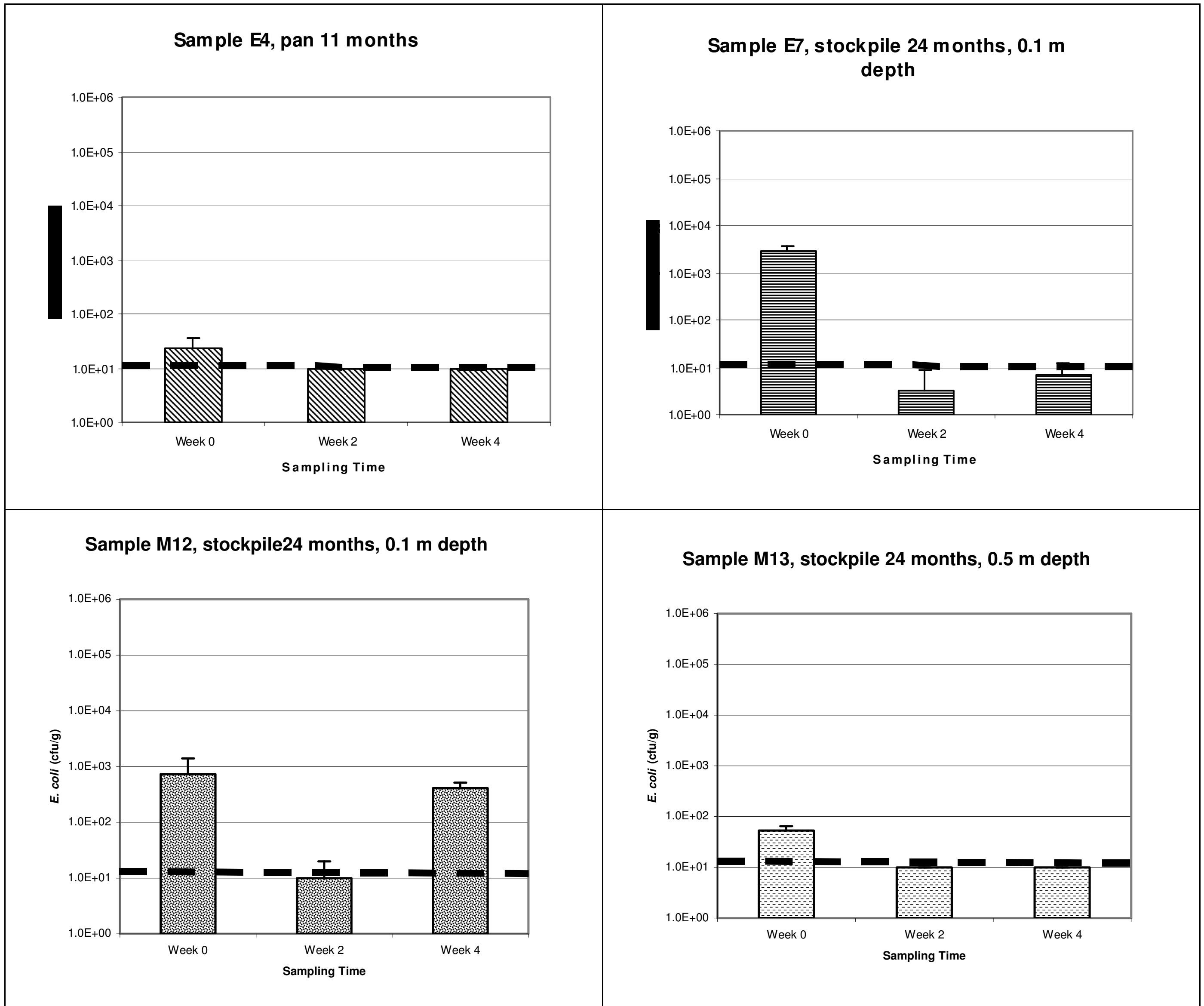

Figure 11. Survival of *E. coli* in moist biosolids.
An environmental strain of *E. coli* was added to a concentration of 1.0E+6 cfu/g to each biosolids sample.
Dashed line shows limit of detection (10 cfu/g).
Errors bars are 1 standard deviation (STD).

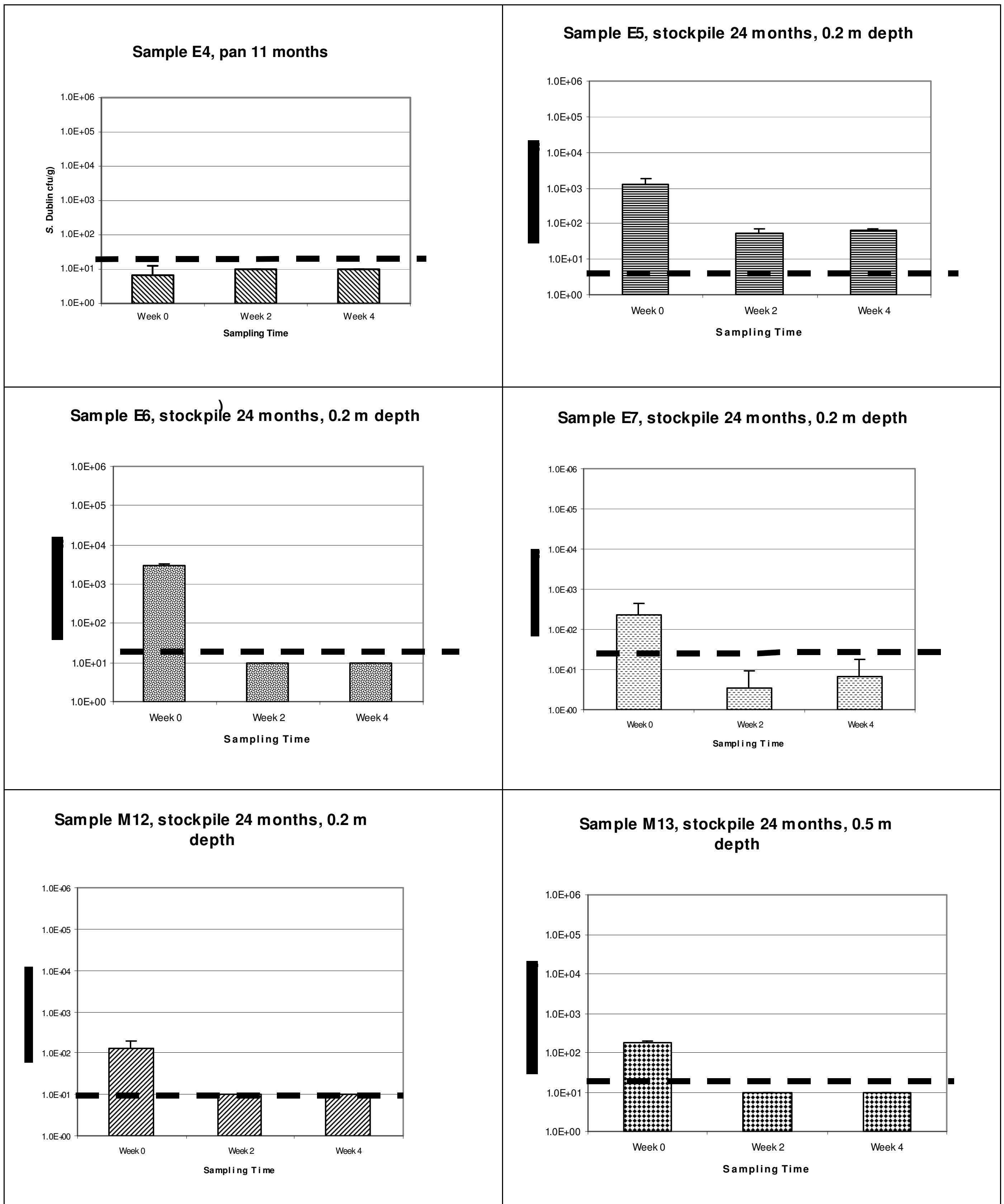

Figure 12. Survival of *Salmonella* Dublin in moist biosolids.
Salmonella Dublin, a clinical isolate from a calf, was added to a concentration of 1.0E+6 cfu/g to each biosolid. Dashed line shows limit of detection (10 cfu/g). Errors bars are 1 standard deviation (STD).

Bacterial survival in saturated biosolids seeded with *E. coli* M34

For all saturated biosolids, unchanged or decreasing numbers of added *E. coli M34* were observed. No regrowth was observed. The levels of *E. coli* M34 over the four-week incubation period for one pan sample (E4) and three stockpile samples (E7, M12 and M13) are presented in Figure 13. Within one to two hours of the addition of the bacterial load, when the first viable counts were performed, levels of *E. coli* dropped by half to one log presumably due to the inability to adapt to the changed conditions. While three of the four samples showed stable numbers of *E. coli* over time, one stockpile sample (M13, aged 2 years) showed a large reduction in bacterial numbers to <100 cfu/g by four weeks.

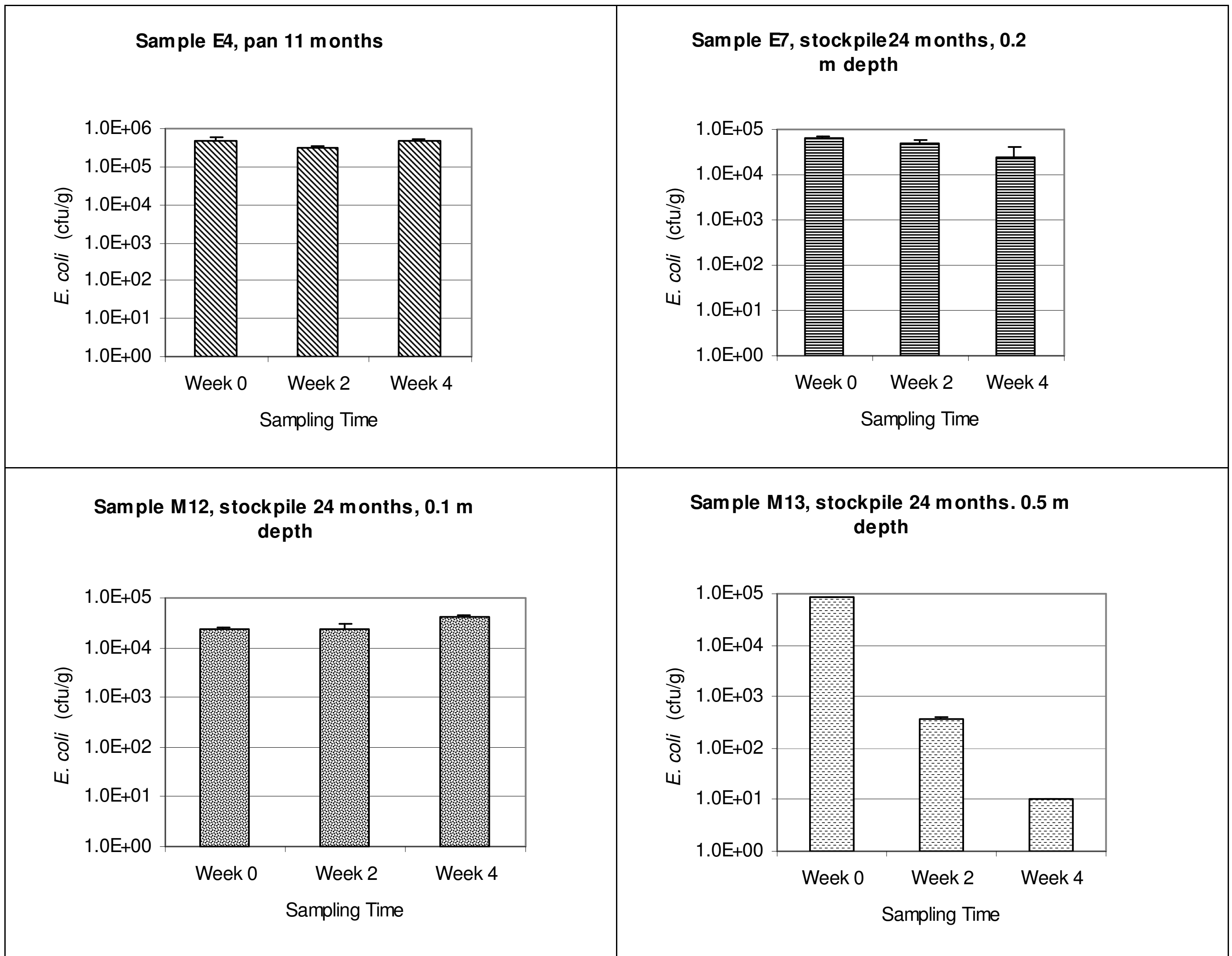

Figure 13. Survival of *E. coli* in saturated biosolids.
An environmental strain of *E. coli* was added to a concentration of 1.0E+6 cfu/g to each biosolids sample. Broken lines show limit of detection (10 cfu/g).
Errors bars are 1 standard deviation (STD).

Bacterial survival in saturated biosolids seeded with *Salmonella* Dublin

When *S.* Dublin was added to biosolids, numbers of that bacterium were unchanged or slightly reduced, and no significant regrowth was seen. Figure 14 shows the changes in the numbers of *S*. Dublin, added to one pan sample (E4 11 months) and five stockpile samples (all 24 months) over the four-week period of the experiment. One pan sample and one stockpile sample showed no changes or only minimal changes in the numbers of *S.* Dublin over time. The apparent increase in the number of *S.* Dublin in stockpile sample E7 is less than half a log over 4 weeks and within a 3 standard deviation value, so is unlikely to be significant. One stockpile sample showed a 2-log reduction and three other samples, including M1 (which showed a marked reduction in the numbers of *E. coli* over time) showed substantial loss of viable *S.* Dublin over time.

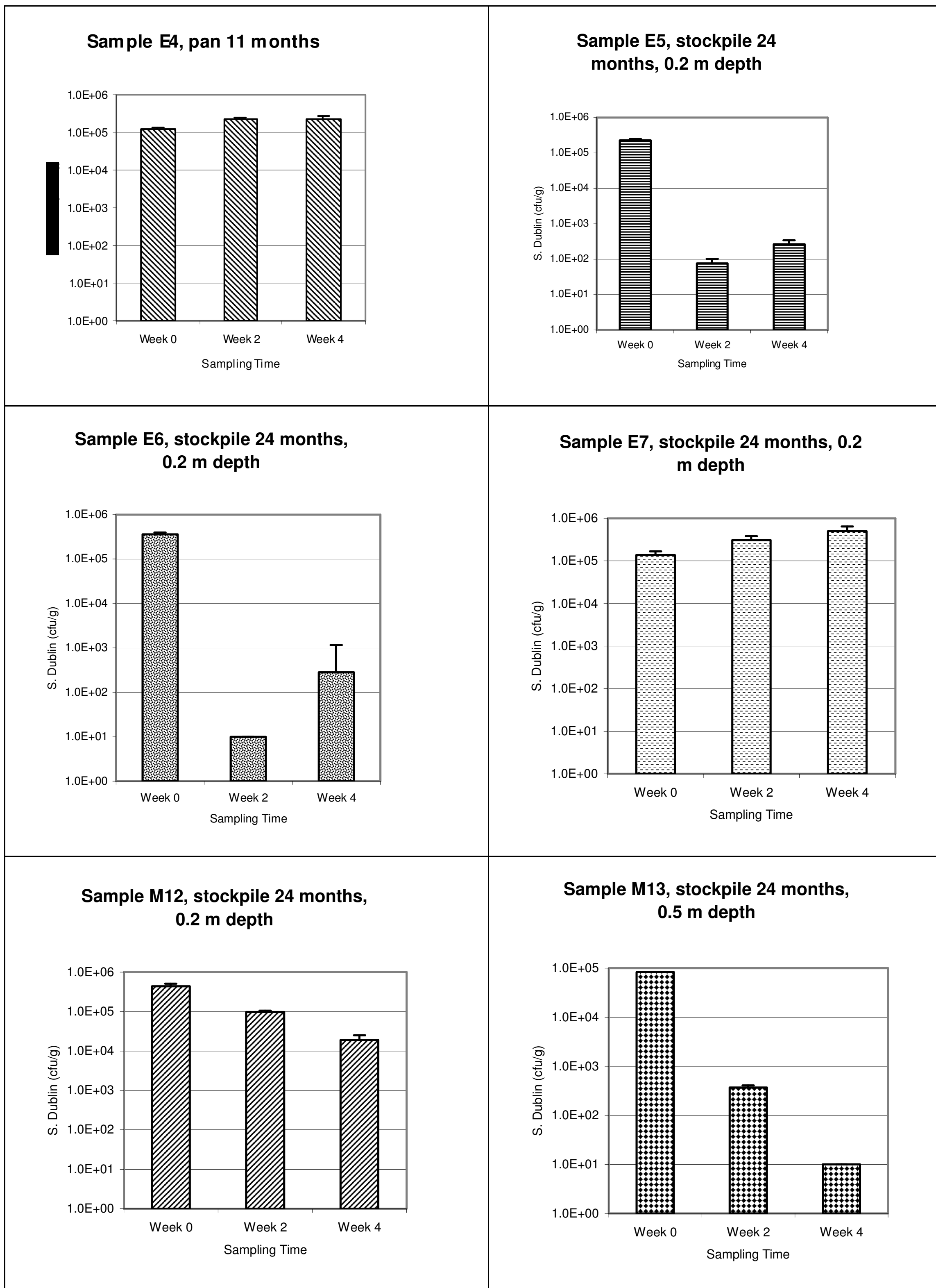

Figure 14. Survival of *Salmonella* Dublin in saturated biosolids.
S. Dublin was added to a concentration of 1.0E+6 cfu/g to each biosolids sample.
Broken lines show limit of detection (10 cfu/g). Errors bars are 1 standard deviation (STD).

3.2.5. Discussion

Neither *E. coli* nor *S.* Dublin were observed to grow in either saturated or moist biosolids. Therefore, it can be concluded that similar biosolids products can be stored outside, as any contamination of bacteria by animals and birds would not allow regrowth. Nevertheless, it has been reported that regrowth of *Salmonella* spp. can occur following contamination in large pools of water that overlay biosolids (Zaleski et al., 2005). Thus biosolids products could be stored outside, but pools of water should not be allowed to overlay the products.

In future experiments, bacterial cultures should be stressed prior to adding them to biosolids samples to avoid the large drop in numbers that was observed when fresh overnight cultures were added to moist biosolids. However, this drop in the initial inoculum, when above the limit of detection, was invariably followed by a further drop by week 2.

3.2.6. Water Holding Capacity

The moisture content of biosolids is defined as the actual water percentage of the total weight. In contrast, the water holding capacity (WHC) of biosolids is defined as the maximum water content that biosolids can carry while remaining in solid form. Biosolids tend to have significantly higher WHC values than soil, so that in land application biosolids may provide improved WHC of the soil, to support growth of crops and horticultural plants under drying weather and climatic conditions.

In this study, water holding capacity of biosolids averaged 83.0%, ranging from 29.0%, for a surface sample of a 2006 stockpile (E8, 36 months), to 257% for a pre-harvest pan sample (M5a, 8 months). This suggests that the water holding capacity of stockpiles may reduce during storage, especially on the surface layer.

In addition, it can be concluded that biosolids may potentially take up substantial amounts of water when wetted, which might possibly reduce the inhibition of regrowth.

Effect on soil properties

In comparison to these biosolids, soil samples exhibited a water holding capacity of about 30% (data not shown). In application to land these results indicate the importance of biosolids for providing holding water for plants and crops.

3.3. Project 3. Controlled Laboratory Investigation - Mineral nitrogen release

3.3.1. Aim

To establish (a) the change in phyto-available nitrogen (mineral nitrogen compounds, namely; ammonia, nitrate, nitrite) content of biosolids during the air-drying and stockpiling process, and (b) the potential release of mineral nitrogen compounds from the organic components of biosolids (nitrogen mineralisation), *i.e.* the agronomic effectiveness of the biosolids as a source of nitrogen above that supplied by the soil.

3.3.2. Rationale

Soil is a major source of nutrients needed by plants for growth. The three main nutrients are nitrogen, phosphorus and potassium. Australia, however, has a high proportion of nutrient-poor soils compared to other continents located at similar latitudes (Specht & Specht, 1999; Young & Young, 2001) (Table 4). Fragile soil structure and a susceptibility to water-saturation are other common features of Australian soils, while large areas are naturally affected by salt or acidity. These soil characteristics restrict particular agricultural activities, sometimes ruling out agricultural activity altogether (Australian Bureau of Statistics, 2008a).

Table 4 Chemical Properties of the soil used in this study‡

Analyte	Soil
DS (%)	96.9
pHw	6.5†
pH($CaCl_2$)	5.5†
EC (µS/cm)	67†
CEC (meq/kg)	6.8†
Volatile solids (%)	4
Total-C (%)	2.04†
Total Kjeldahl N (%)	0.175
C/N	12.24†
Available P mg/kg DS)	2
NH_4-N (mg/kg DS)	1.24
NO_3-N (mg/kg DS)	8.08
NO_2-N (mg/kg DS)	0.01

‡ Values from this study, † values from Beshah et al. (2008). DS, dry solids; pHw, pH by soil mixed with pure water method; pH($CaCl_2$), pH by soil mixed with $CaCl_2$ solution method; EC, electrical conductivity; CEC, cation exchange capacity; C, organic carbon; P, phosphate.

In Australia long-term weathering has depleted nutrients even at depth. The soils are generally infertile by world standards, with deficiencies in phosphorus and nitrogen. The soils in Australia are particularly poor in available nutrients, being composed largely of kaolin and quartz. Agricultural development of these soils has not been possible until comparatively recently because of extreme deficiencies of phosphorus, potassium and nitrogen, and widespread deficiencies of the minor elements copper, zinc, molybdenum, and manganese. In contrast, soils on floodplains are younger and more fertile. Very few are considered good quality soils for agriculture. To offset nutrient deficiencies, superphosphate and nitrogenous fertilisers are widely used, particularly on pasture and cereal crops. Indicative of the enduring importance of these fertilisers is shown by the continuous consumption of these resources through many decades (Figure 15). Prior to 1973-74 the general consumption ratio of elemental N:P:K had been 2:6:1, but by 1983 the ratio had changed to almost 3:3:1, and by 1998 to about 5:5:1. This variation has resulted from a combination of reduced consumption of phosphatic fertilisers with an increased

consumption of nitrogenous fertilisers (Australian Bureau of Statistics, 2008a; Australian Bureau of Agricultural and Resource Economics, 2008).

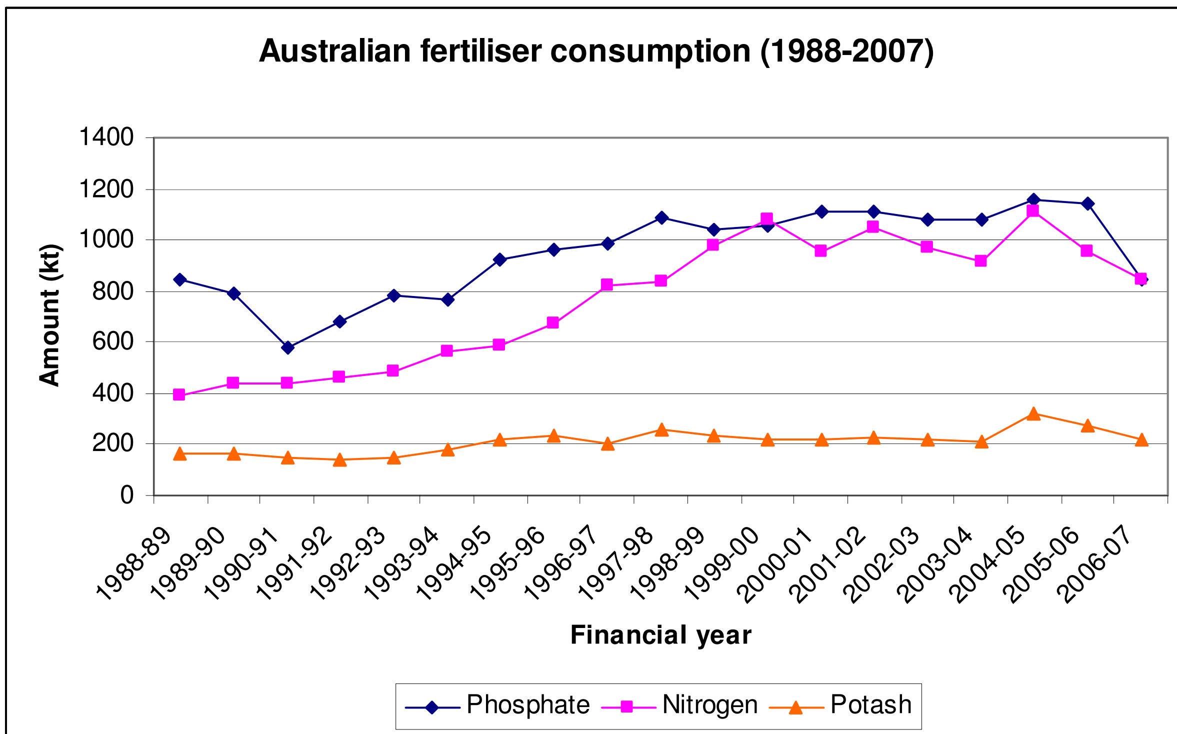

Figure 15. Australian fertiliser consumption (1988-2007). Source of data: Australian Bureau of Agricultural and Resource Economics (2008).

While chemical fertilisers have been required to support optimal growth of crops in Australia and around the world, there are finite limits of these resources, which have dramatically caused increases in the prices of these in recent times (Figure 16).

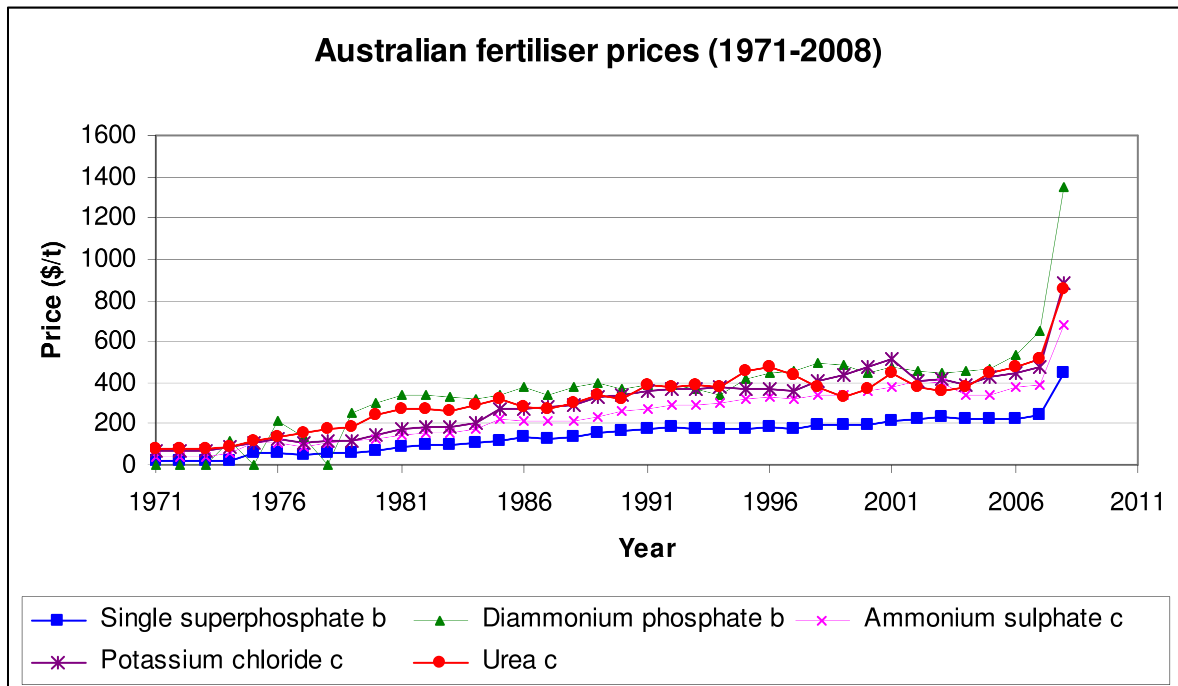

Figure 16. Australian fertiliser prices (1971-2008). Average price paid by Australian farmers at 30 June; b Bulk price. c Bagged price from 1970 to 2001, bulk price from 2002 onwards; 0 data not available. Source of data: Australian Bureau of Agricultural and Resource Economics (2008).

In contrast to the limiting presence and properties of chemical fertilisers, organic materials, including biosolids can provide renewable application and a higher range of beneficial properties. These include two specific properties, (a) measured release of mineral nitrogen compounds, to avoid contamination of waterways due to leaching at excessive concentrations, and (b) improved water holding capacity of soils, to aid plant growth under drying conditions. The rise in prices of chemical fertilisers may provide an increased opportunity for biosolids to be applied to land.

3.3.3. Properties of Biosolids

Before applying biosolids to land it is useful to consider the properties of these resources. By inspection of the soil used in this study, which has typical deficiencies of nutrients (Table 4) it can be concluded that the presence of four key properties in biosolids, namely; organic solids, total Kjeldahl nitrogen , mineral nitrogen compounds and phosphate would be highly valuable. The content of volatile (organic) solids and total Kjeldahl nitrogen (organic nitrogen) of biosolids used in this study are shown in Table 5. Table 6 shows the mineral nitrogen and phosphate components. These nutrient properties of biosolids were generally substantially higher than in the soil. It can be concluded that in principle biosolids from both air-drying pans and stockpiles can highly compliment the properties of soils to promote growth of crops.

Table 5. Volatile Solids (VS) and Total Nitrogen Content of Biosolids from Two Plants †

Sample code	Sample identification	Sampling date	Age† (months)	Consistency and depth (m)	%DS	Volatile Solids %DS	Total Nitrogen %DS
M1a	MAD Pump (P no. 2)	Oct-07	0	Liquid	1.5	76.9	18.8
M1b	MAD Pump (P no. 2)	Nov-07	0	Liquid	1.7	76.1	16.1
M4b	P No. 3	Dec-07	8	Liquid	7.0	70.5	5.8
M4c	P No. 3	Dec-07	8	Liquid	6.5	71.8	5.5
M5a	P No. 3	Apr-08	12	Solid	31.9	ND	2.7
M5b	P No. 3	Apr-08	12	Solid	36.9	41.5	2.8
M12	S Feb-07 0.1 m	Feb-08	24	0.1	85.5	26.3	1.1
M13	S Feb-07 0.5 m	Feb-08	24	0.5	55.9	29.5	1.8
E1a	MAD Valve No. 5	Nov-07	0	Liquid	1.8	70.3	4.4
E1b	MAD Valve No. 5	Dec-07	0	Liquid	1.9	70.7	11.3
E3b	P SDP 33	Dec-07	9	Liquid	12.6	43	3.0
E3c	P SDP 33	Dec-07	9	Liquid	12.6	40.5	3.4
E6	S Feb-07 BSP 38	Feb-08	24	0.1	31.8	ND	5.2
E7	S Feb-07 BSP 38	Feb-08	24	0.1	29.3	59.1	4.6
E8	S Feb-06 BSP 48	Feb-08	36	0.1	83.0	16.4	0.5
E9	S Feb-06 BSP 48	Feb-08	36	0.5	71.3	10.8	0.8
E10	S Apr-08	Apr-08	12	0.1	80.9	14.3	0.8
E11	S Apr-08	Apr-08	12	0.5	78.4	15.6	1.0
E12	S Apr-08	Apr-08	12	1.0	78.1	16.6	1.0
C^	Soil, Surbiton Park	Feb-07	NA	Soil	96.9	4	0.2

MAD, anaerobic digester output; P, drying pan; S, stockpile; VS, volatile solids (Loss on Ignition at 850°C).
† Ages of pans and date from the end of filling, ages of stockpiles date from end of filling of the associated pan (so added 12 months to the piled age). NA, not applicable; ND, not determined. Dry solids values were determined at the time of sample collection.

The sample lists from Tables 5 and 6 overlap, but do not exactly correspond, due to using all the remaining amounts of some samples in the analysis for Table 7.

Table 6. Levels of Mineral Nitrogen Compounds And Extractable Phosphate In Biosolids

Sample code	Identification*	Sampling date	Age‡ (months)	Consistency or depth (m)	NH_4-N mg/kg DS	NO_2-N mg/kg DS	NO_3-N mg/kg DS	Extractable phosphate Mean mg/kg DS	Extractable phosphate STD	Concentration of phosphate in soil/biosolids mixtures Ω Solids (Ratio to soil of 1:99)	Concentration of phosphate in soil/biosolids mixtures Ω Liquids (Ratio to soil of 1:9)
E3	P SDSP33	Dec-07	9	liquid	8600.2	1.3	0.6	5263	174	52.6	
E4	P SDSP33	Feb-08	11	solid	1455.9	0.5	16.4	622	50	6.22	
E5	S Feb-07 BSP38	Feb-08	24	0.1	9763.3	3.0	15.1	3580	680	35.8	
E6	S Feb-07 BSP38	Feb-08	24	0.1	8182.5	3.4	12.2	3059	401	30.6	
E7	S Feb-07 BSP38	Feb-08	24	0.1	8139.9	3.3	13.7	4467	221	44.7	
E8	S Feb-06 BSP48	Feb-08	36	0.1	65.2	0.1	715.3	492	14	4.9	
E9	S Feb-06 BSP48	Feb-08	36	0.5	557.4	2.5	62.1	140	1	1.4	
M4	P Pan 3	Dec-07	8	liquid	11093.4	0.0	5.4	6157	518		615.7
M6	S Feb-07	Oct-07	20	0.1	78.2	1.2	998.3	666	47	6.7	
M7	S Feb-07	Oct-07	20	0.5	968.8	9.7	648.1	676	19	6.8	
M8	S Feb-07	Oct-07	20	1.0	2522.9	2.3	64.7	860	12	8.6	
M9	S Feb-06	Oct-07	32	0.1	259.5	2.1	775.4	1094	55	10.9	
M10	S Feb-06	Oct-07	32	0.5	644.0	1.0	1045.3	1368	52	13.7	
M11	S Feb-06	Oct-07	32	1.0	1782.2	0.9	45.9	1726	119	17.3	
M12	S Feb-07	Feb-08	24	0.1	77.8	0.2	913.4	479	18	4.8	
M13	S Feb-07	Feb-08	24	0.5	1236.4	0.8	1399.2	445	12	4.5	
C6	Surbiton Park	Feb-08	NA	solid	1.2	0.0	8.1	2	0	NA	NA

* P, drying pan; S, stockpile.

‡ Ages of pans and date from the end of filling, ages of stockpiles date from end of filling of the associated pan (so add 12 months to the piled age). Ω Theoretical values if biosolids were added to soils at standard rates. STD, standard deviation for data of extractable phosphate.

Blue = samples used for soil/biosolids mixtures (refer to Table 8)

The three samples (E5, E6, E7) were from the same depth of same stockpile. This stockpile was flattened and it was not possible to collect deeper samples. The results are internally consistent, but appear to be too high. For further discussion, see the legend to Table 8 (p. 58).

Dry Solids (DS)

The percentage of DS in air-drying treatment of biosolids is important as (a) this significantly corresponds to the potential removal of pathogens, and (b) the cost of transporting biosolids for applications. The DS contents of the different sludge products (Table 5) reflected the extent of dewatering by air-drying and storage by stockpiling. The mean percent DS from the MAD output varied from 1.6% (Mt Martha) to ~1.8% (ETP). Pan samples had a mean DS content of 18% (range 7 to 37), while the DS content of stockpiles ranged from 29% to 86%.

Volatile solids (VS)

The presence of organic components in biosolids is important both (a) as the source for releasing mineral nitrogen compounds, and (b) to improve the water holding capacity of soils in land application. In practice the content of organic matter is represented by the test for content of VS. The presence of VS in general followed an expected pattern, with the highest concentrations found in drying-pan samples and the lowest values in the soil (Table 5). The VS content of the output from anaerobic digesters of both WWTPs was >70% of DS, which compares favourably with VS values of raw sludge in the UK (typically 70-75% DS). After pan digestion for 8 to 12 months, the VS values ranged from 41% to 72% of DS, which is also in the range reported in the UK (55-60%). The lower values in some pan samples from ETP may reflect the extended drying phase at the Victorian plants compared to UK processes. Although stockpile samples had considerably lower levels of VS then pan samples, all stockpiles, including one 36 months of age (ETP, BSP 48), still showed substantially higher levels of volatile solids than the soil sample.

Two stockpiles from ETP had very low levels of VS (10 to 17%). This could have been due to a sampling error due to the presence of clay material. It is suggested that in future samples should be taken from stockpiles associated with drying-pans that have cement bases rather than clay bases. This will be possible since ETP is currently replacing all clay bases with cement.

The decline in VS from the output of anaerobic digesters through pan drying to stockpiling is presented in Figure 17. In contrast to the variability of the MAD data for Kjeldahl nitrogen (see the next section), the VS data was less variable and fitted the trend line.

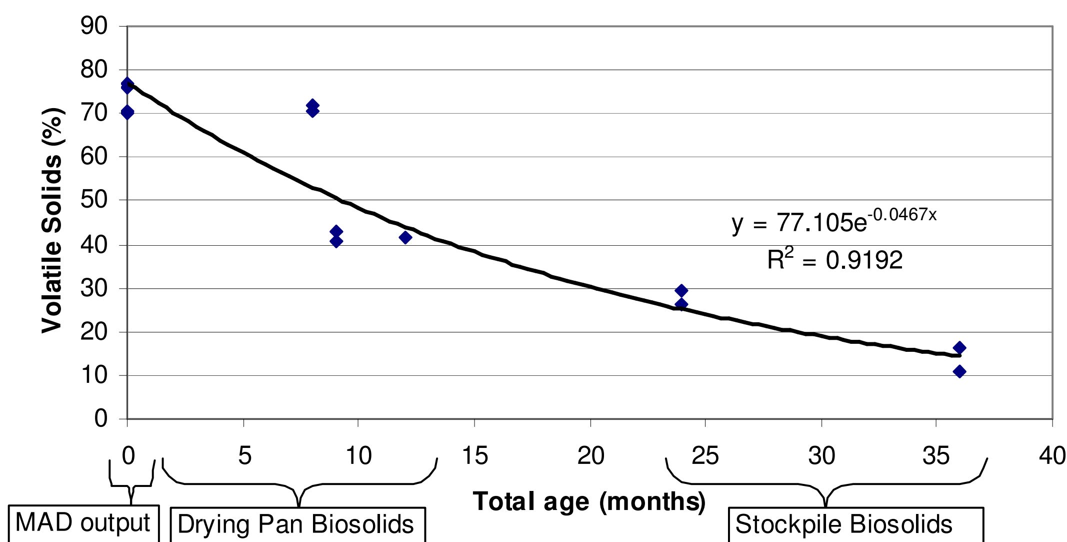

Figure 17. Reduction in content of volatile solids during air-drying and storage of biosolids

Kjeldahl nitrogen

The total Kjeldahl nitrogen content corresponds to the organic nitrogen fraction of the biosolids plus ammonia, thereby more directly representing the source for release of NO_3–N and NO_2-N compounds than volatile solids. In contrast, the total nitrogen content includes both the mineral nitrogen and organic nitrogen fraction values. The total mineral nitrogen (NH_4-N, NO_3–N and NO_2-N), and total Kjeldahl nitrogen (TKN) levels were substantially higher in biosolids than in the soil (Table 4).

Since nitrogen is a major component of volatile solids it is be expected that reduction of the content of nitrogen in biosolids may also occur over time. The levels of total Kjeldahl nitrogen in biosolids of different ages, from the output of anaerobic digesters, drying pans and stockpiles from both plants, was plotted against the ages of the biosolids (Figure 18). There is clear exponential reduction of total Kjeldahl nitrogen content as biosolids age, with drying pan samples containing substantially higher levels of total nitrogen than stockpiles. Three of the four MAD values of total Kjeldahl nitrogen were substantially higher than expected and two values were excluded from the calculations as outliers. Since other nitrogen values were consistent with expectations, the variability of the MAD samples was attributed to either errors in sampling, or possibly to high nitrogen inputs to the plants. In future samples should be also taken upstream of the MAD, as well as from the MAD output, to aid correct assessment of the data. Attention should also be paid to whether the output line has been cleared properly before sampling.

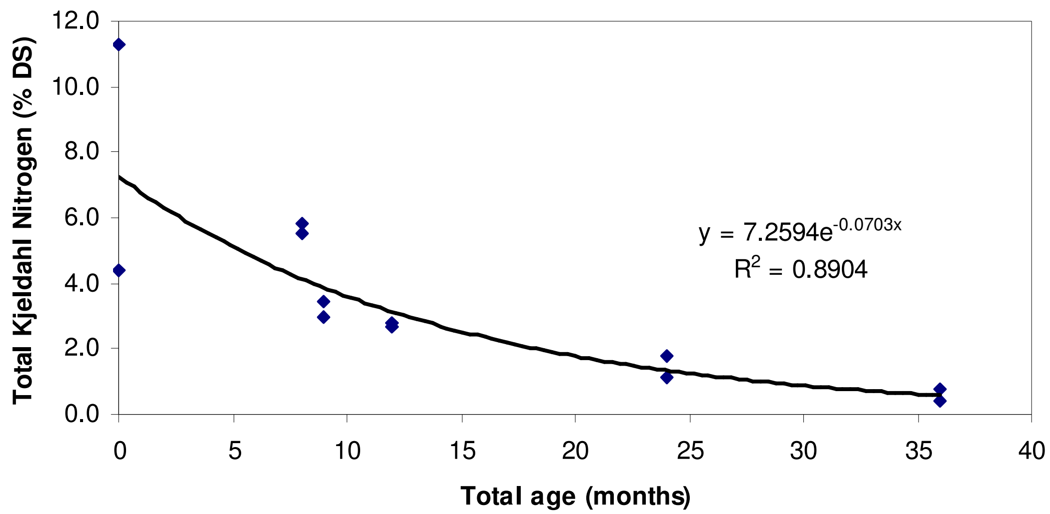

Figure 18. Reduction in total Kjeldahl nitrogen content during air-drying and storage of biosolids

Relation between total Kjeldahl nitrogen and volatile solids

While total Kjeldahl nitrogen provides a direct measure of the content of organic nitrogen in biosolids, in practice it is easier to analyse the VS variable, an indirect indicator of organic matter. Values of these variables were plotted, (Figure 19) exhibiting a close relationship with a R^2 value of 0.93.

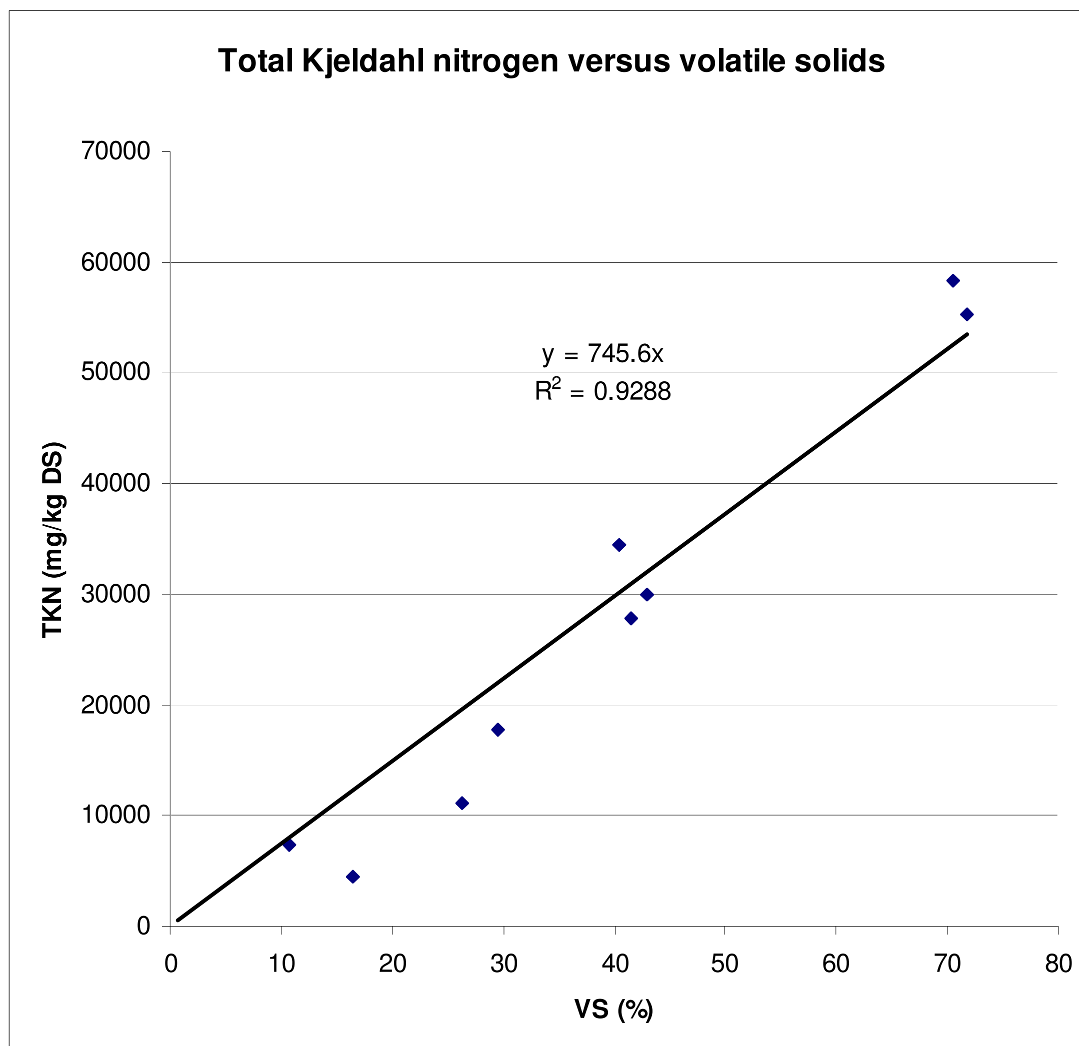

Figure 19. Relationship between levels of total Kjeldahl nitrogen and volatile solids in biosolids. The MAD output data was excluded from this plot due to its high variability, as discussed above.

Levels of ammonia

Most of the mineral nitrogen in biosolids was in the form of NH_4-N. Although there was much variation, samples from pans and younger stockpiles (<1 y) generally showed the highest levels of ammonia, compared to substantially lower levels in older stockpiles, e.g., 32 to 36 months old and there were lower concentrations in the surface of stockpiles compared to 0.5 or 1.0 m depths (Table 6).

Nitrate and nitrite

In general, the levels of NO_3 were higher in stockpile samples than pan sludge, which is wet and anaerobic. Some of the older stockpiles had a predominance of NO_3-N, mainly at the shallower depths, suggesting that NH_4-N was being transformed to NO_3-N by nitrification. The levels of NO_2 were low in pans and higher in some stockpiles, probably reflecting different environmental conditions (Table 6).

Nitrogen mineralisation

A unique property of biosolids, compared to chemical fertilisers, is the capability to release mineral nitrogen compounds from the organic content during incubation with soil. Nitrogen is present in biosolids in four major forms; organic matter and the

mineral nitrogen compounds ammonia, nitrate and nitrite. In order to provide nitrogen in a form that is available to plants, the organic matter must be mineralised to NH_3 and NO_3.This process has the potential to provide measured release of mineral nitrogen over time, to provide the mineral nitrogen requirements for growth of crops, while avoiding contamination of water ways due to leaching of mineral nitrogen compounds when excessively applied.

In summary, the initial mineral nitrogen content of biosolids provides an 'upfront' fertilisation value, while the liberation of mineral nitrogen compounds from the organic matter potentially provides a slow release fertilisation value.

Levels of extractable phosphate in biosolids

In soils, phosphorus occurs in the form of phosphate. Most soils of Australia have only 10–400 ppm total phosphorus, and some have as little as 1 ppm phosphorus and are unsuitable for agriculture without intensive fertilization. A survey of 77 samples of arid-zone soils of Australia yielded a mean of 240 ppm total phosphorus, compared to 643 total ppm in 38 soil samples from arid zones on other continents (Charley & Cowling, 1968; Stafford Smith & Morton, 1990). Forms of phosphorus readily available to plant roots range from 2.8 ppm on sand dunes with hummock grasses (*Triodia* spp.) to 18.8 ppm on river floodplains (Charley & Cowling, 1968). In contrast, the black earths of small areas of eastern Australia, the most fertile on the continent, have up to 5,000 ppm available P (Williams & Raupach, 1983).

In this study most biosolids samples from pans and stockpiles had high levels of extractable phosphate. Early pan samples (E3, M4, aged 7 and 8 months respectively) contained the highest levels of phosphate, while a later pan sample (E4, aged 11 months) and stockpile samples exhibited lower levels. This suggests that some extractable phosphate was lost when removing water from pans to aid air-drying. Stockpile ETP 2006 (aged 32 months) was an exception. The low levels of phosphate detected in this stockpile may have been caused by leaching by rain and or transformation to less soluble forms, related to the age of the stockpile. Nevertheless young stockpiles, such as E5-E7 (aged 24 months), still contained substantial levels of phosphate (Table 6). The levels of extractable phosphate were very low in the soil used in this study (Table 4). Thus most biosolids, except for one sample from stockpile, if applied to soil would add phosphate to the soil,

Stockpile Feb-07 (MM SEWL) was sampled twice, 4 months part, showing a reduction of 31% of the level of extractable phosphate over that time (Table 6).

Taken together, these results suggest that phosphate may have been leached from stockpiles during storage, presumably due to rainfall (Batziaka et al, 2008) and or the phosphate minerals were transformed to less soluble forms (Smith et al., 2002). As with nitrate, in can be concluded that for preserving the phosphate content of biosolids, the storage period should be minimised.

3.3.4. Methods

Sampling at Operational Sites

Sampling was undertaken at the same two full-scale WWTP plants using the same methods as described under "Monitoring survival of indicators across two operational treatment processes". The ages of all samples were calculated from the end of filling of the associated drying pan. All biosolids samples were frozen prior to use to prevent loss of nitrogen.

Samples for determining properties of biosolids of different ages

The samples used for this study are listed in Tables 5 and 6. In order to obtain values for material entering the lagoons, samples were obtained from the output from the anaerobic digesters at the two WWTPs as well as pans and stockpiles of different ages. Samples were sent to the ALS Laboratory Group, Melbourne for analysis of VS, nitrate, nitrite, ammonium and phosphate. Timing and availability of samples did not allow the same set of samples to be used for all types of analysis.

Samples used for studying biosolids-soil mixtures

A subset of samples was used for preparing the biosolids-soil mixtures (Table 7). The samples were selected to represent pan samples at the different ages (8 to 11 months) and stockpile samples at depths of 0.5 and 1 meters and aged from 20 to 36 months. Different sets of samples were used in experiments using moist soil and saturated soil due to the sample availability.

Table 7. Samples used for biosolids-soil mixtures

(a) Biosolids added to moist soil

Pan (P) or Stockpile (S)	Depth (metres) (stockpile)	Identification of pan or stockpile by end of filling associated pan and number (where available)	Code	Age (months)
P		P SDP 33	E4	11
S	0.1	Feb-07 BSP 38	E5	24
S	0.1	Feb-07 BSP 3	E6	24
S	0.1	SFeb-07 BSP 38	E7	24
S	0.1	SFeb-06 BSP 48	E8	36
S	0.5	Feb-06 BSP 48	E9	36
S	0.1	Feb-07	M12	24
S	0.5	Feb-07	M13	24
Controls				
Soil only			C6	
Soil+NH_4-Cl 1%			C7	
Soil+$NaNO_3$ 1%			C8	

(b) Biosolids added to saturated soil

Pan (P) or Stockpile (S)	Depth (meters) (stockpile)	Identification of pan or stockpile by end of filling associated pan and number (where available)	Code	Age (months)
P		SDP 33	E3	9
P		P 3	M4	8
S	0.1	Feb-07	M6	20
S	0.5	Feb-07	M7	20
S	1.0	Feb-07	M8	20
S	0.1	Feb-06	M9	32
S	0.5	Feb-06	M10	32
S	1.0	Feb-06	M11	32
Controls				
Soil only			C1	
Soil+NH_4-Cl 1%			C2	
Soil+$NaNO_3$ 1%			C3	

Selection of Soil.

A typical sandy loam (pH 6.5) was selected for comparison with published work and as a representative of many land areas in Australia. The soil had not been subject to recent fertilization, had poor nutritional properties and was classified as a tenosol soil (Table 4).

Tenosol has a weakly developed soil profile which is typically very sandy and without obvious horizons. Generally, tenosol soils have a very low agricultural potential with very low chemical fertility, poor structure and low water-holding capacity. The low cation exchange capacity (CEC) is typical of sandy soils and indicates a low ability to bind ions such as ammonia.

Preparation and Incubation of Soil-Biosolids Mixtures

A routine biosolids-soil incubation procedure was followed (Smith et al. 1998). Two soil conditions were chosen to examine the effect of different soil conditions on nitrogen mineralisation; moist aerobic crumbed soil conditions (adjusted to 86-89% DS) and saturated anaerobic soil conditions (adjusted to 76-80% DS). Biosolids were added to soil at standard rates for liquids (defined as 6-12% DS) or solids (defined as 27-94% DS), i.e. they were added to fresh soil at a wet-weight ratio of 1:9 for liquid samples, and 1:99 for dry biosolids samples. These ratios are equivalent to normal rates applied by the Thames Water's 'TERRA ECO-SYSTEMS' recycling to land operation, for 100 m^3 ha^{-1} for liquids (Smith et al., 1998).

If samples were dry, 10 g of biosolids were added to 990 g of soil. The mixture was made up to the required DS content (86-89% DS for moist aerobic crumbed soil or 76-80% DS for saturated anaerobic soil). In practice, it was more effective to add the moisture to the biosolids prior to addition to the soil for efficient mixing. If samples were liquid, 100 g of biosolids were added to 900 g of soil. Extra water was added if necessary to reach values of 86-89% DS for moist aerobic crumbed soil or 76-80% DS for saturated anaerobic soil. For data analysis the wet weight dilutions were converted to dry weight dilutions, using values for the percentage dry solids for the soil and for each biosolids sample. A number of measures were taken to ensure the uniform mixing of biosolids and soil samples to minimise variation between the experimental replicates. Stockpiled biosolids samples are difficult to mix uniformly with soil and were ground to ≤5 mm prior to soil incorporation. The amended samples of soil were thoroughly mixed together using a hand-held electric mixer.

Incubation of Soil-Biosolids Samples

A standard soil-incubation protocol was followed to determine the change in mineralisable nitrogen content of the biosolids during air-drying and storage. Work by Smith et al. (1998 a-c), Smith et al. (2004) and Bellett-Travers et al. (2003) has confirmed that data on mineralisable nitrogen generated by this test is comparable with field measurements of crop response to biosolids nitrogen. Approximately 100 g quantities of the sludge-soil mixtures were transferred to partially sealed (to aid gas exchange and limit dehydration) plastic containers and maintained in a temperature-controlled incubator in the dark at 20°C. Sufficient samples were prepared for three replicates of soil to be removed at intervals of after 0, 5, 10, 20, 40 and 70 days of incubation. The experimental treatments were replicated three times for each time of removal from the incubator. Soil moisture status was monitored during the incubation period but water loss was minimal and required no adjustment. Samples were removed from the incubator and immediately prepared for chemical analysis or were preserved by freezing at -20°C. This is a standard soil storage technique in nitrogen transformation studies (Smith et al. 1998 a-c).

Controls

Controls included soil without additions to determine whether the soil alone could mineralise nitrogen, based on its existing component of organic nitrogen compounds. The results from this control were subtracted from results for soils amended with biosolids to determine the improvement in mineralisation provided by the biosolids. Nitrate and ammonia were separately added at 1% to different samples of soil, for observing denitrification and nitrification, respectively. Under normal conditions ammonia is converted to nitrate, given that bacteria providing nitrification activity are present and active.

Analysis of Soil-Biosolids Samples

Biosolids-soil mixtures were analysed for total nitrogen by the standard Kjeldahl method, and the organic matter content (VS) was measured by a standard loss-on-ignition method (at 850^oC). After extraction with 2M KCl and filtering, filtrates were sent to the Environmental Analysis Laboratory, CSIRO Land and Water, Urrbrae, South Australia for chemical analysis. Ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2), concentrations were determined by automated colorimetric analysis (Eaton et al, 2005).

The levels of the different nitrogen species were determined in biosolids-soil mixtures at intervals over the 70 day incubation period. The levels of phosphate was not determined during incubation of biosolids-soil mixtures, as it is known that phosphate levels are generally stable over time, in the absence of uptake by plants, or leaching by groundwater.

Calculations were performed to measure the agronomically effective nitrogen release from biosolids, based on the difference from the unamended control. The standardised mineral nitrogen released from the biosolids by day 70 was calculated as the amount of nitrogen released by day 70 as a percentage of the organic nitrogen component originally present in the biosolids, irrespective of the soil nitrogen content. The standardised total mineral nitrogen added by the biosolids was calculated as the mineral nitrogen initially present in the biosolids plus the released nitrogen as a percentage of the total nitrogen present in the added biosolids.

3.3.5. Results

Release of mineral nitrogen in biosolids-soil mixtures: moist soil conditions

Total mineral nitrogen present in biosolids-soil mixtures

A plot showing the levels of total mineral nitrogen present in the biosolids over time is presented in Figure 20a. Under moist soil conditions all biosolids provided increased total mineral nitrogen over time (nitrite, nitrate plus ammonia) compared to soil alone. The lowest levels of mineral nitrogen were provided by the two oldest stockpiles (E8 and E9)

Rates of nitrogen mineralisation (change in mineral nitrogen content per day) were generally positive over the incubation time, with peak rates occurring at 5-10 days (Fig. 20b).

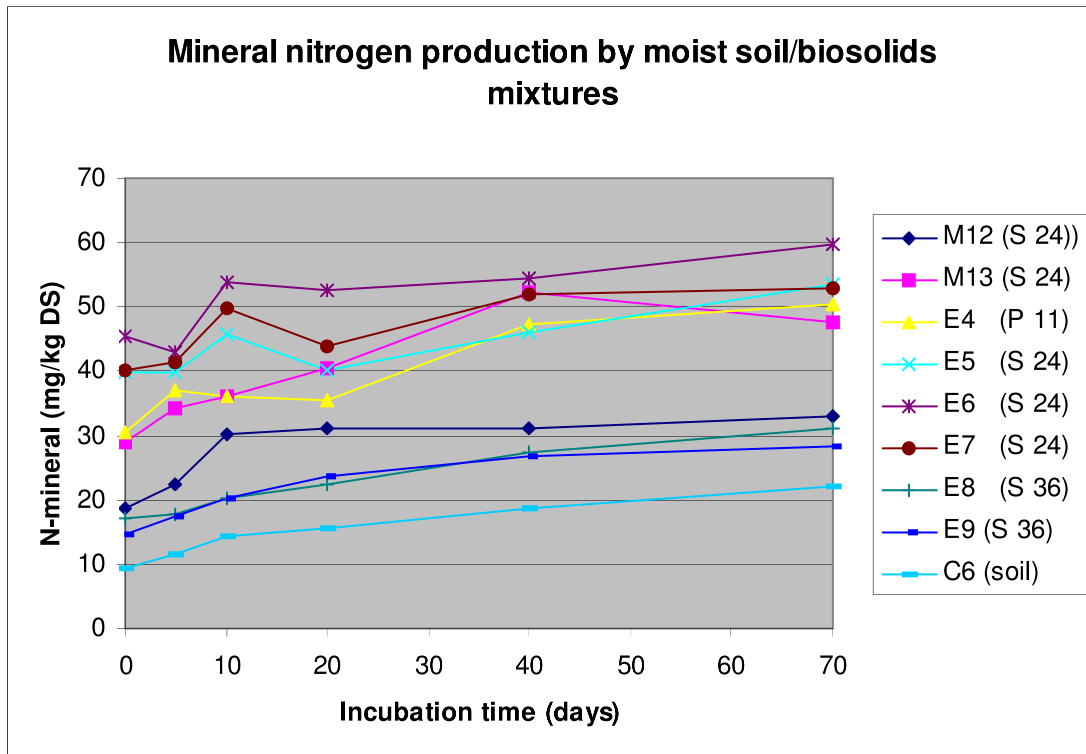

Figure 20a. Increase in total mineral nitrogen content in moist soil amended with biosolids due to mineralisation of organic nitrogen during incubation over 70 days. P pan, S stockpile. Figures are the ages since filling associated pan. Concentrations refer to the concentrations in the soil-biosolids mixtures.

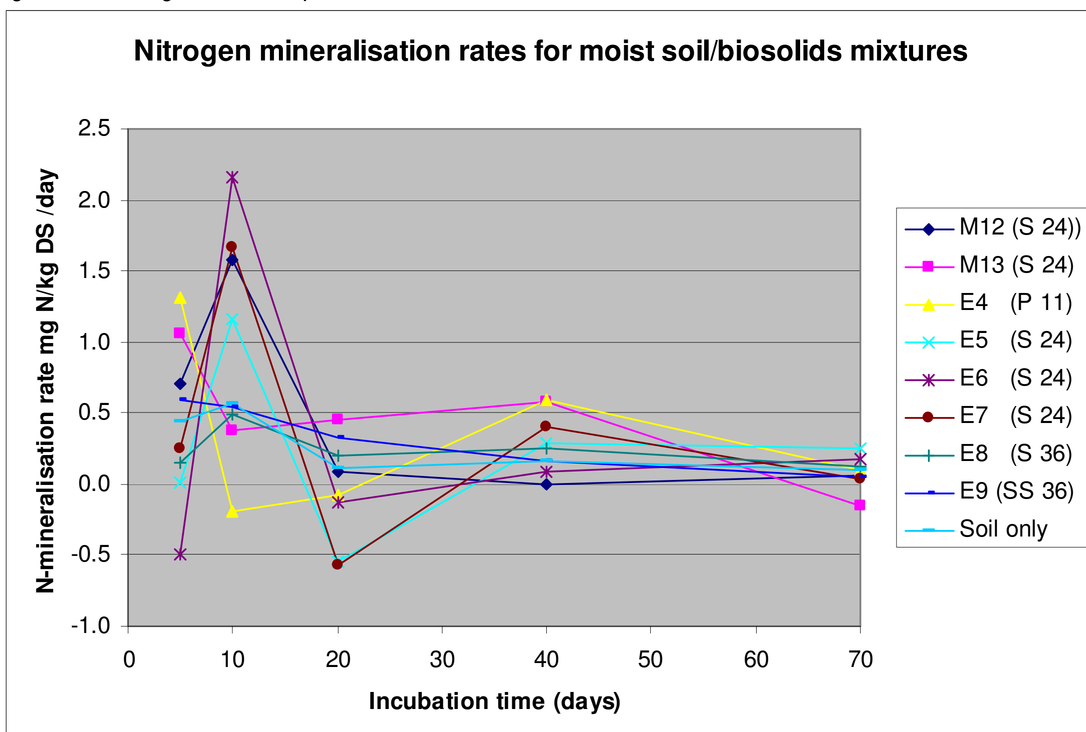

Figure 20b. Nitrogen mineralisation rates over 70 days. P pan, S stockpile. Figures are the ages since filling associated pan. Concentrations refer to the concentrations in the soil-biosolids mixtures

Levels of nitrate and nitrite from ammonia in biosolids-soil mixtures

The concentrations of ammonium, nitrate and nitrite on day 0 and day 70 are presented in Figures 21and 22 and Table 8. On day 0, all biosolids, both from pans and stockpiles of one and two years age, contributed inorganic nitrogen in the form of both ammonia and nitrate (Figure 21). The presence of NO_3-N in the pan samples suggests the dried biosolids have undergone some aerobic conversion from NH_4 to NO_3. After 70 days incubation at 20 ^oC, ammonia was substantially converted to nitrate, for all biosolids samples. No nitrite was detected in pan samples or soil samples during the 70 day incubation period (Figure 22). These results indicate that nitrification activity and absence of denitrification. Less total mineral nitrogen was added by the older stockpile material (E8 and E9) compared to more recently stockpiled biosolids (E5-E7) (Figure 22, Table 8 [total mineral nitrogen added by biosolids]).

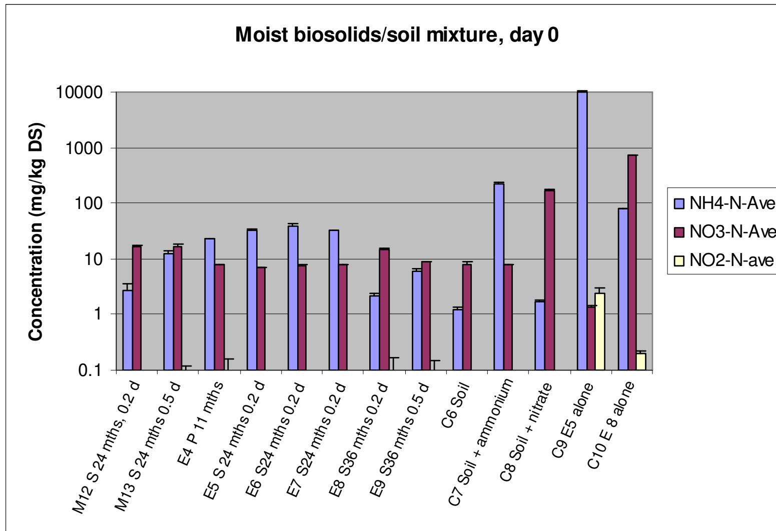

Figure 21. Levels of ammonia and nitrate and nitrite in initial samples of moist soil/biosolids mixtures. Moist biosolids-soil mixtures. Concentrations refer to the concentrations in the soil-biosolids mixtures

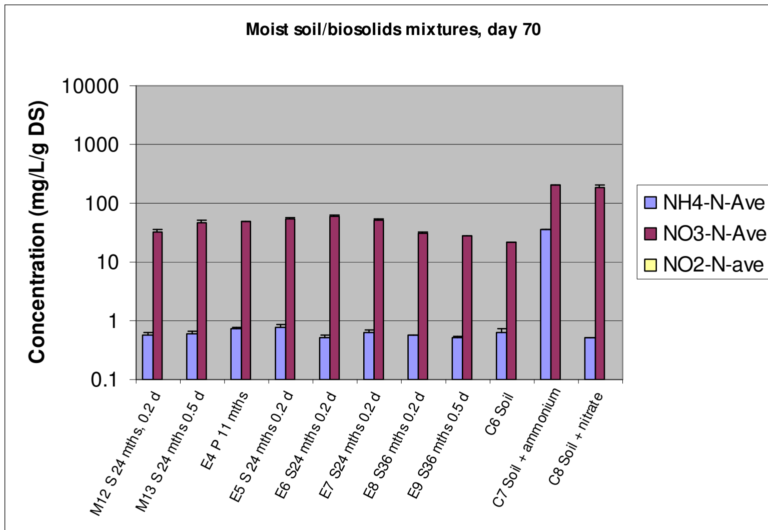

Figure 22. Levels of ammonia and nitrate in samples of moist soil/biosolids mixtures at day 70 of incubation. Moist biosolids-soil mixtures. Concentrations refer to the concentrations in the soil-biosolids mixtures

	Code	Age (months)
S Feb-07 0.1 m	M12	24
S Feb-07 0.5 m	M13	24
P SDP 33	E4	11
S Feb-07 BSP 38 0.1 m	E5	24
S Feb-07 BSP 38 0.1 m	E6	24
S Feb-07 BSP 38 0.1 m	E7	24
S Feb-06 BSP 48 0.1 m	E8	36
S Feb-06 BSP 48 0.5 m	E9	36
Soil	C6	
Soil+NH4-Cl 1%	C7	
Soil+NaNitrate 1%	C8	
E5 alone	C9	
E8 alone	C10	

Sample identification: S, stockpile; P, drying-pan.
mths months
d depth of sample
Location; E, ETP; M, Mt Martha WWTP.
Note E5, E6 and E7 are effectively replicates.
C, controls; C6, soil alone.
NH_4-N, ammonia-N; NO_3-N; nitrate-N; NO_2-N, nitrite-N
Error bars show 1 standard deviation (STD) value.
Note log scale on Y axis

To further explore the conversion of ammonia to nitrate and nitrite, plots were prepared showing the levels of ammonia, nitrate and nitrite in pan samples and soil (Figures 23 and 24).

Pan sample E4 provided increased total mineral nitrogen (N-Sum) over time. No nitrite accumulation was detected, indicating effective transformation of ammonia to nitrate (Figure 23). The control soil also provided an increase in total inorganic nitrogen (N-Sum) over time, but at a lower level compared to soils amended with biosolids (Figure 24). Data from one stockpile (M13 24 months) showed similar rates of conversion as for the pan sample, E4 (data not shown).

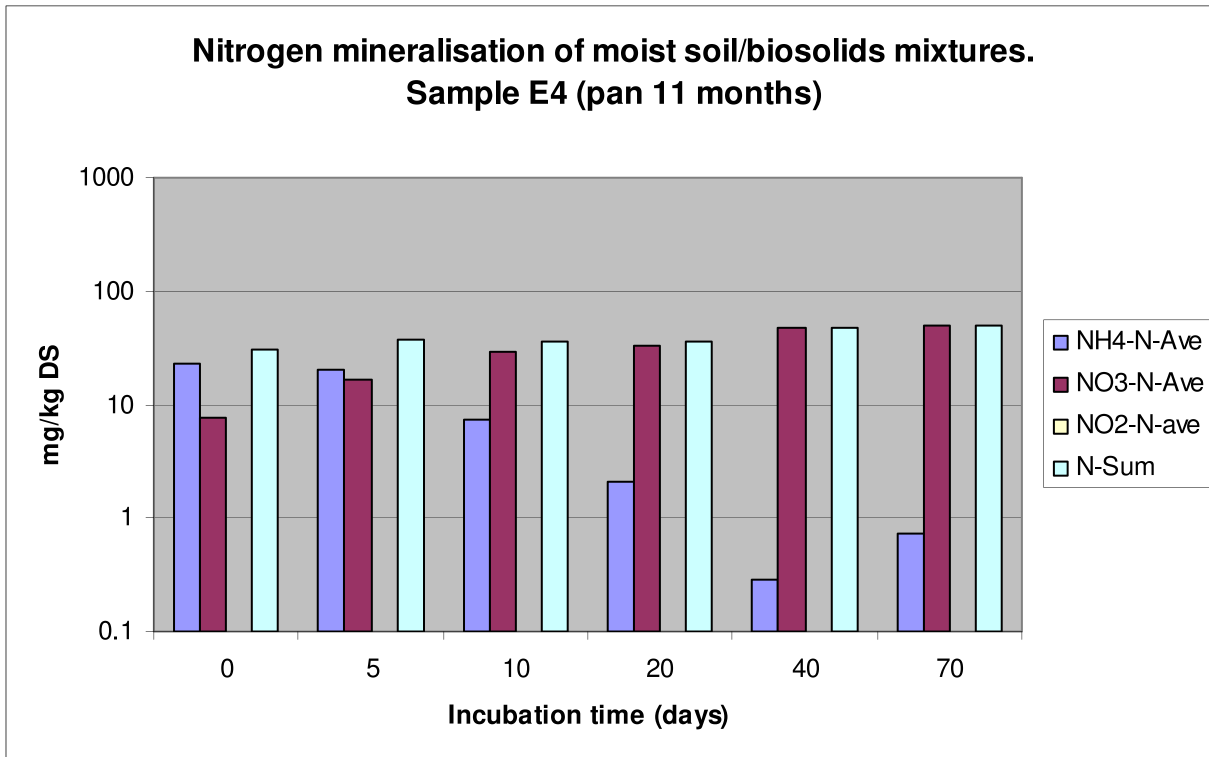

Figure 23. Conversion of ammonia to nitrate by pan biosolids.
Bars show the average of three values. Concentrations refer to the concentrations in the soil-biosolids mixtures.

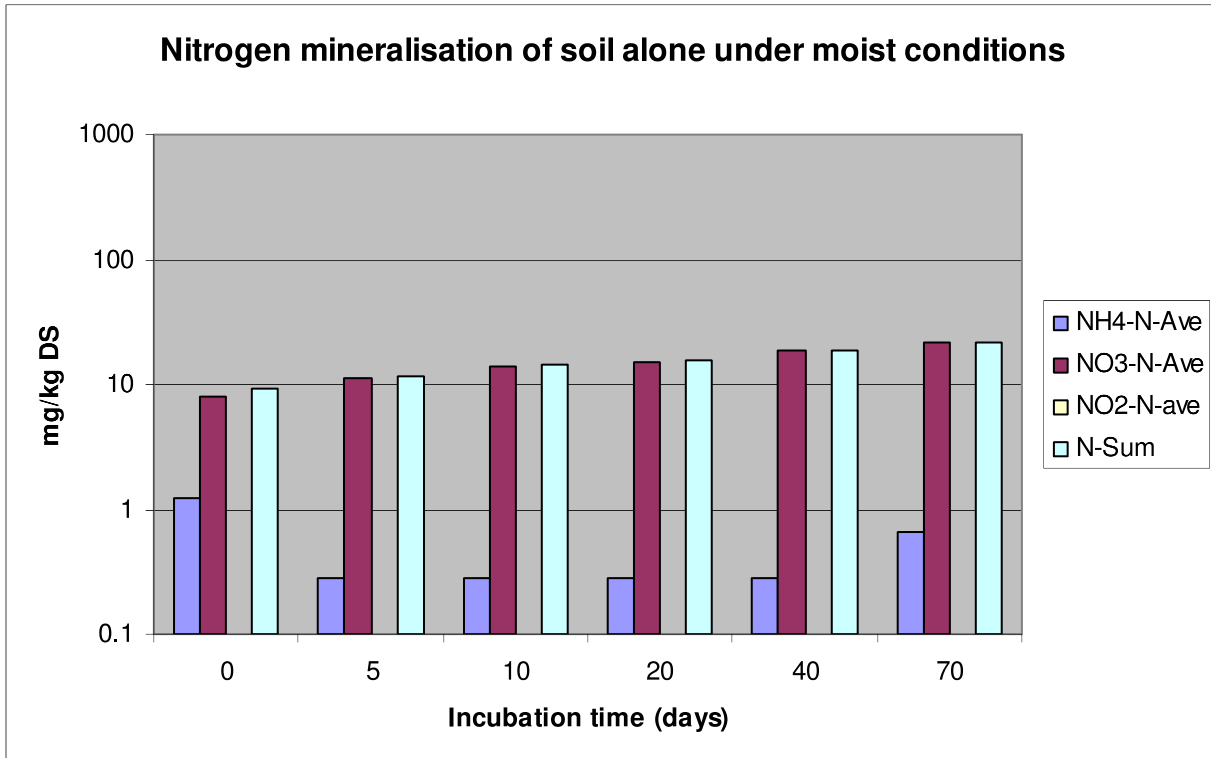

Figure 24. Conversion of ammonia to nitrate in soil alone.
Bars show the average of three values. Concentrations refer to the those of soil-biosolids mixtures

Production of mineral nitrogen from organic components in moist soil amended with biosolids

The soil used in this study had a poor nutrient quality, which contained only 9.33 mg/kg DS mineral nitrogen (Tables 4 and 8, column 3]). The mineral nitrogen content provided 'up-front' by biosolids at day 0 (Table 8, column 4) was calculated by subtracting the amount of mineral nitrogen present in the soil on day 0 (Table 8 column 3 soil only) from the amount in soil and biosolids (Table 8 column 3 biosolids). At the applied rate of 1 in 100, w/w, all biosolids provided appreciably additional mineral nitrogen in the soil, 0.5- to 4-fold, compared to the background soil concentration.

By day 70, all mixtures contained higher concentrations of total mineral nitrogen than soil alone (Table 8, column 5). This amount includes the mineral nitrogen provided by biosolids at day 0 as well as the amount produced by mineralisation of the organic component over the incubation time of 70 days.

To compare mineral nitrogen released from the organic components of different materials directly it is necessary to calculate this relative to the amount of nitrogen added to the soil, since soils differ in their organic and mineral nitrogen content. The amounts of mineral nitrogen released from the biosolids, due to mineralisation of the organic component, are shown in Table 8, column 7. This was calculated by subtracting the amount of mineral nitrogen released from the soil only (Table 8 column 6, soil only, 12.63) from the amount released from the soil and biosolids (Table 8, column 6, biosolids). The mineral nitrogen released from biosolids varied between 0.96 and 7.11 mg/kg DS, with the highest amounts released from the pan sample and one stockpile sample, aged 24 months, taken at a depth of 0.5 m.

The values for the standard mineral nitrogen released from the biosolids were also calculated, to estimate the percentage of the organic nitrogen initially present in the biosolids that was mineralised (Table 8, column 8). This was calculated by dividing the net total nitrogen released from the biosolids (Table 8 column 7) by the organic-N content provided by the biosolids. The standardised mineral nitrogen released by biosolids ranged from 0.3%, to 6.9%, with an average of 2.5%. In contrast, Smith and Durham (2002) reported higher rates, 4% to 57%, for thermally-dried biosolids added to two-types of agricultural soils, in the UK. Also, given the fall in total Kjeldahl nitrogen content with age of biosolids (Figure 18) it may be expected that the mineral-N released values would also fall with age. There was no clear relationship, however, between the age of biosolids and the mineral nitrogen released from biosolids. These differences may reflect variable activation and growth of indigenous microbes required to mineralise organic nitrogen fractions in the biosolids. Also, the stability of the organic-N fractions, due to extent of pan-drying and storage, may have contributed to the low levels of mineral-N release.

Finally, the total mineral nitrogen (initial plus released values) provided by the biosolids was calculated (Table 8 column 9, and standardised (Table 8 column 10). The total mineral N added by biosolids accounted for 9.8 to 24.1% of the total nitrogen added by biosolids to the soil. Comparing the standardised mineral nitrogen release (column 8) and standardised total mineral-N (column 10) we see the mineral nitrogen released contribution by the biosolids over 70 days was about an order of magnitude less than the 'up-front' contribution.

Table 8. Production of mineral nitrogen in moist conditioned soil amended with biosolids

1a	1b	1c	2a	2b	2c	3	4	5	6	7	8	9	10
Sample						Mineral Nitrogen					Standardised mineral N released from biosolids by Day 70†	Total mineral N added by biosolids by Day 70‡	Standardised total mineral N added by biosolids by Day 70Ω
						Soil + biosolids at Day 0	Provided by biosolids at Day 0	Soil + biosolids at Day 70	Released by soil + biosolids by Day 70	Released from biosolids by Day 70			
Code	Age (mths)	P/S	Dilution (at dry weight) Ψ	TN* mg/kg DS	DS %	mg/kg DS	mg/kg DS	mg/kg DS	mg/kg DS	mg/kg DS	% DS	mg/kg DS	% TN
E4	11	P	0.00969	29900	93.8	30.54	21.21	50.28	19.74	7.11	2.65	28.32	9.8
E5	24	S	0.00279	48600	26.8	39.87	30.54	53.58	13.71	1.08	1.03	31.62	23.4
E6	24	S	0.00331	51700	31.8	45.53	36.20	59.66	14.13	1.50	1.11	37.70	22.0
E7	24	S	0.00305	45500	29.3	40.08	30.75	53.03	12.95	0.32	0.30	31.07	22.4
E8	36	S	0.00857	4490	83.0	17.06	7.73	31.04	13.98	1.35	4.40	9.08	23.6
E9	36	S	0.00738	8140	71.3	14.60	5.27	28.18	13.59	0.96	1.75	6.22	10.4
M12	24	S	0.00883	11400	85.5	18.80	9.47	32.97	14.17	1.54	1.69	11.01	10.9
M13	24	S	0.00580	18300	55.9	28.85	19.52	47.48	18.63	6.00	6.93	25.53	24.1
Soil						9.33	NA	21.96	12.63	NA	NA	NA	NA
Soil+NH_4-Cl 1%						229.35	NA	234.98	5.63	NA	NA	NA	NA
Soil+$NaNO_3$ 1%						173.14	NA	186.90	13.76	NA	NA	NA	NA

P, pan, S, stockpile; TN, total nitrogen in unamended biosolids sample (TKN + nitrite and nitrate); Mineral N content includes ammonia, nitrate and nitrite;
Ψ, The wet weight dilutions of biosolids in soil were 1 in 100, then for data analysis the wet weight dilutions were converted to dry weight dilutions, using DS values of biosolids and soil (96.9%, Table 4). For example for E4 the dry weight dilution = 1 * 0.938 /(99 * 0.969 +1 * 0.938) =0.0097.
* Data of sample E3 (from the same pan) used for this analysis as data for sample E4 not available, Data for E5 as average values of E6 and E7 as data for E5 not available;
†, this represents the % of the organic N in the biosolids that was released by day 70, (day 70 - day 0) mineral N / Norg [Norg = TN addition (TN * Dilution at dry weight) – mineral N added by biosolids at Day 0];
‡, includes the organic and the mineral component, mineral N provided by biosolids at day 0 plus released mineral N from biosolids by day 70;

Ω, this represents the % of the mineral N due to mineralisation of the organic matter in the biosolids, total mineral N added to soils by biosolids by day 70 / (TN * Dilution at dry weight)*100. Results are averages for triplicate analysis.

Sample calculation:

Sample E4.

Column 3 Measured amount of total mineral-N in the biosolids/soil mixture on day 1 = 30.54

Column 4 Amount provided by the biosolids alone = 30.54-9.33 (contribution of the soil) = 21.21

Column 5 Measured amount of total mineral-N in the biosolids/soil mixture on day 70 50.28

Column 6 Amount released from the biosolids/soil mixture by day 70 = 50.28-30.54 (contribution of the soil) = 19.74

Column 7 Amount provided by the biosolids alone by day 70 = 19.74-12.96 (contribution of the soil) = 7.11.

Column 8 % of the **organic**-N (=TN – mineral N provided by biosolids on day 0) in the biosolids that was released by day 70 =7.11/[(29900*0.00969) – 21.21)]*100 = 2.65 (0.00969 is the dry weight dilution factor).

Column 9 Total mineral nitrogen added by biosolids by day 70 (initial N and released N) = 21.21+7.11

Column 10 The total mineral-N provided by the biosolids, that was released by day 70 or was initially provided, as % of total N in the biosolids = (28.32/(29900*0.00969)*100 = 9.8

Notes:

E5, E6 and E7 are replicates. The TN values for stockpile samples E5, E6 and E7 appear to be too high, given their age, though are consistent with the mineral-N contents. See also Table 6. Dry weights were determined specifically at this experiment.

Release of mineral nitrogen in biosolids-soil mixtures: saturated soil conditions

Total mineral nitrogen over the incubation period of 70 days

The levels of total mineral nitrogen generally decreased over the incubation period. The only exception were two pan samples (M4, aged 8 months and E3 aged 9 months), that retained their mineral nitrogen content (Figure 25).

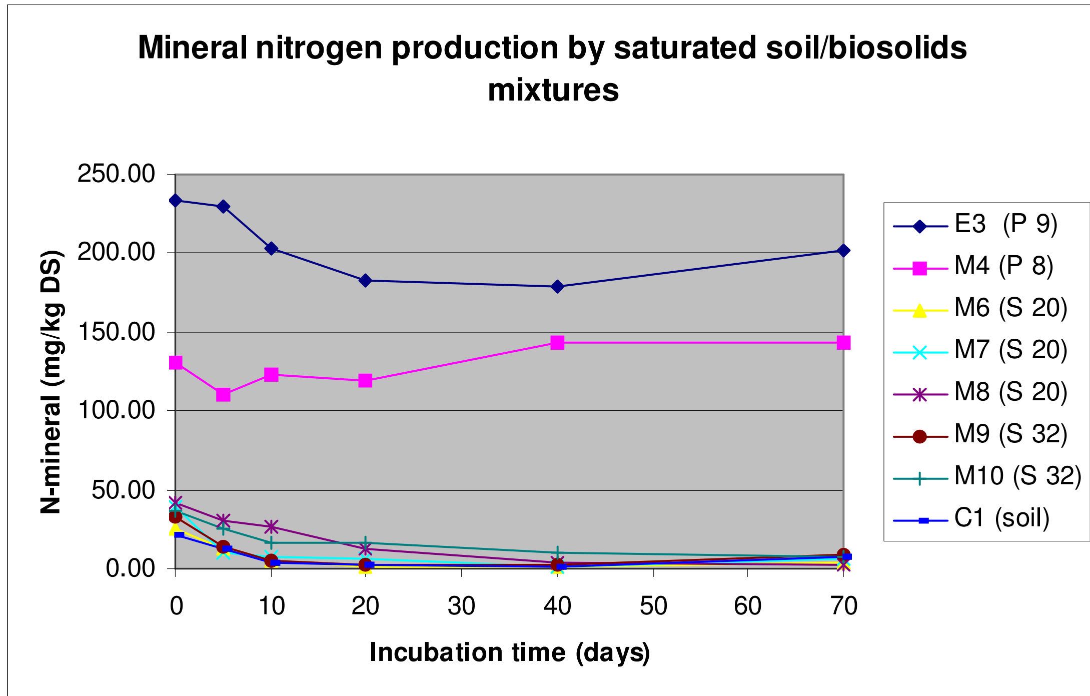

Figure 25. Nitrogen mineralisation under saturated conditions

P pan, S stockpile. Figures are the ages since filling associated pan. Concentrations refer to the concentrations in the soil-biosolids mixtures

The mineralisation rates are shown in Figure 26. Under these conditions only one sample provided positive rates of nitrogen mineralisation (pan sample E3, aged 8 months).

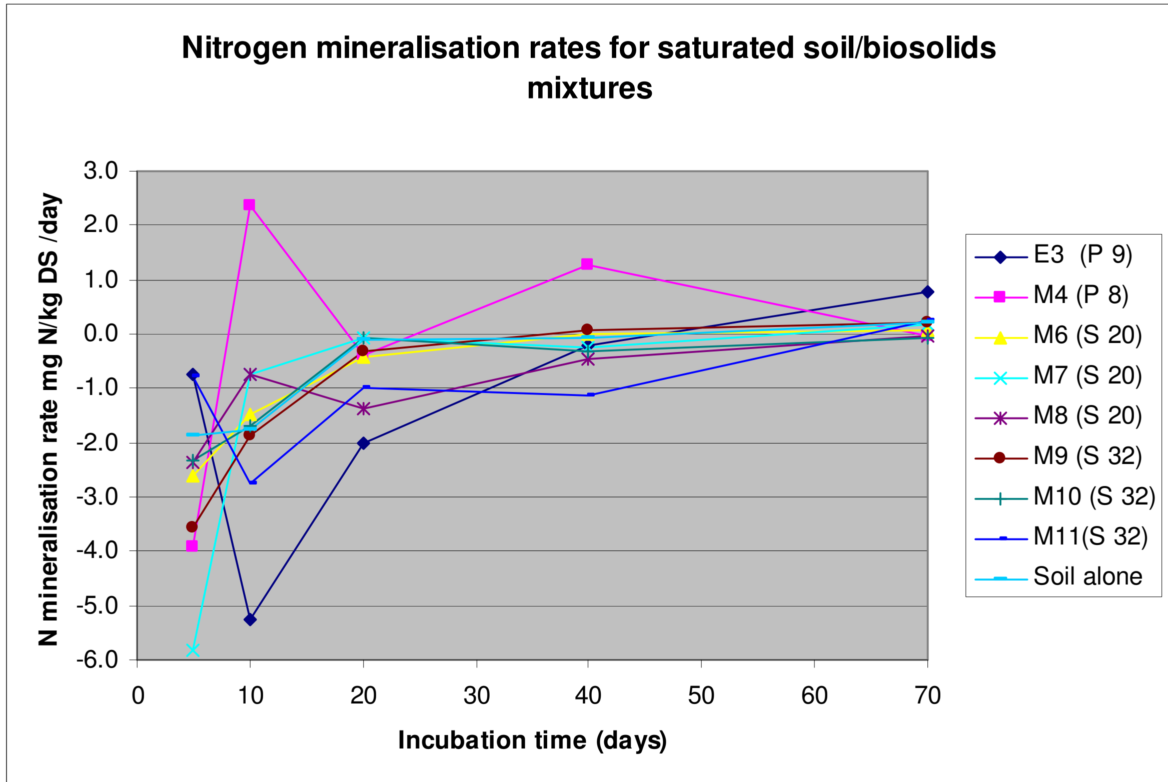

Figure 26. Rates of Nitrogen mineralisation under saturated conditions.
P pan, S stockpile. Figures are the ages since filling associated pan. Concentrations refer to the concentrations in the soil-biosolids mixtures

Mineral nitrogen species

Day 0

All biosolids, both from pans and stockpiles of 1 and 2 years age added mineral nitrogen, on Day 0. The two liquid pan samples (E3 and M4) added substantially more ammonia compared to stockpiles (M6 to M11) (Figure 27). The concentration of ammonia increased with stockpile depth, but nitrate concentrations were not affected by depth. Mineral nitrogen profiles were similar for the two stockpile ages.

Day 70

Under saturated conditions, controls showed a loss of NO_3^- and production of NO_2^-, indicationg that denitrification was occurring under these conditions. After incubation for 70 days at 20 ^oC, pan samples showed conversion of ammonia to nitrate, however, stockpile biosolids substantial loss of both ammonia and nitrate occurred, associated with the appearance of nitrite for some samples (Figure 28). This indicated the occurrence of simultaneous nitrification and denitrification in the stockpile amended soil, due to the presence of anaerobic microsites. In the case of soil alone (C1) substantial loss of nitrate occurred. This may have occurred due to decreased conversion of nitrite to nitrate during nitrification or conversion of nitrite to ammonia in dissimilatory reduction, due to anaerobic conditions (Figure 28).

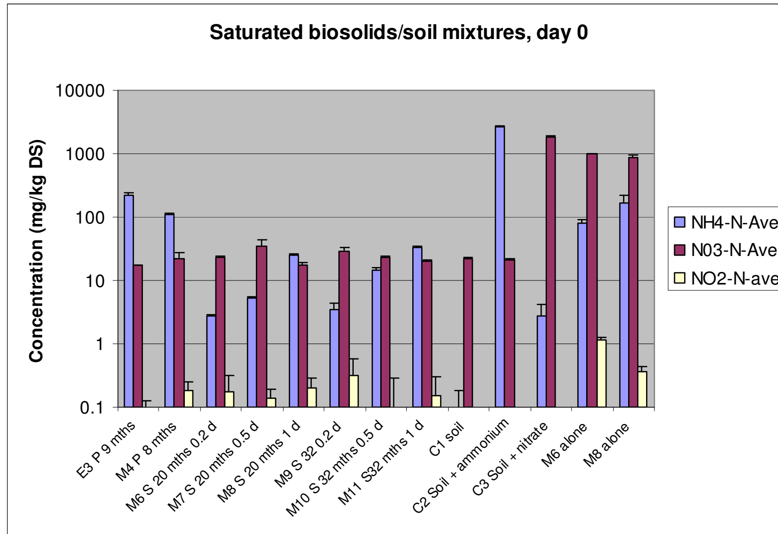

Figure 27

Levels of ammonia, nitrate and nitrite in initial samples of saturated soil.

Concentrations refer to the concentrations in the soil-biosolids mixtures

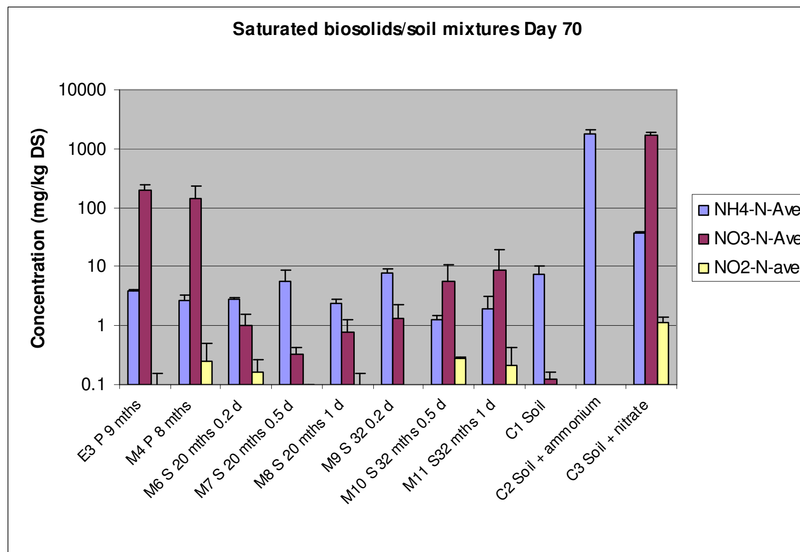

Figure 28.

Levels of ammonia and nitrate in samples of wetted soil at day 70 of incubation.

Concentrations refer to the concentrations in the soil-biosolids mixtures

		Age (months)	
P SDP 33	E3	9	Sample identification:
P3	M4	8	S, stockpile;
S Feb-07 0.1 m	M6	20	P, drying-pan.
S Feb-07 0.5 m	M7	20	mths months
S Feb-07 1.0 m	M8	20	d depth of sample
S Feb-06 0.1 m	M9	32	Location;
S Feb-06 0.5 m	M10	32	E, ETP MWC;
S Feb-06 1.0 m	M11	32	M, MM SEWL
Soil only	C1		C, controls
Soil+NH4-Cl 1%	C2		Error bars show 1 STD value.
Soil+NaNitrate 1%	C3		Note log scale on Y axis
M6 alone	C4		
M9 alone	C5		

Levels of ammonium, nitrate and nitrite over the incubation period of 70 days

To provide a more detailed picture of the profile of the different species of mineral nitrogen over time, a representative pan sample (E3) was analysed for ammonium, nitrate and nitrite over the 70 day incubation period (Figure 29). The same data was generated for soil alone (Figure 30).

Under these conditions pan sample E3 showed a stable level of total mineral nitrogen (N-sum), along with substantial conversion of ammonia (NH_4-N) to nitrate (NO_3-N). Nitrite (NO_2-N) was detected at days 10 and 40 in the sample, indicating action of dissimilatory reduction in the anaerobic conditions or poor nitrification: Most other samples (both pan and stockpile) showed the production of nitrite and at a range of times over the incubation period (data not shown). Nitrite may be occasionally detected when measuring nitrification processes in the soil as an intermediate compound in the biological oxidation of mineral nitrogen.

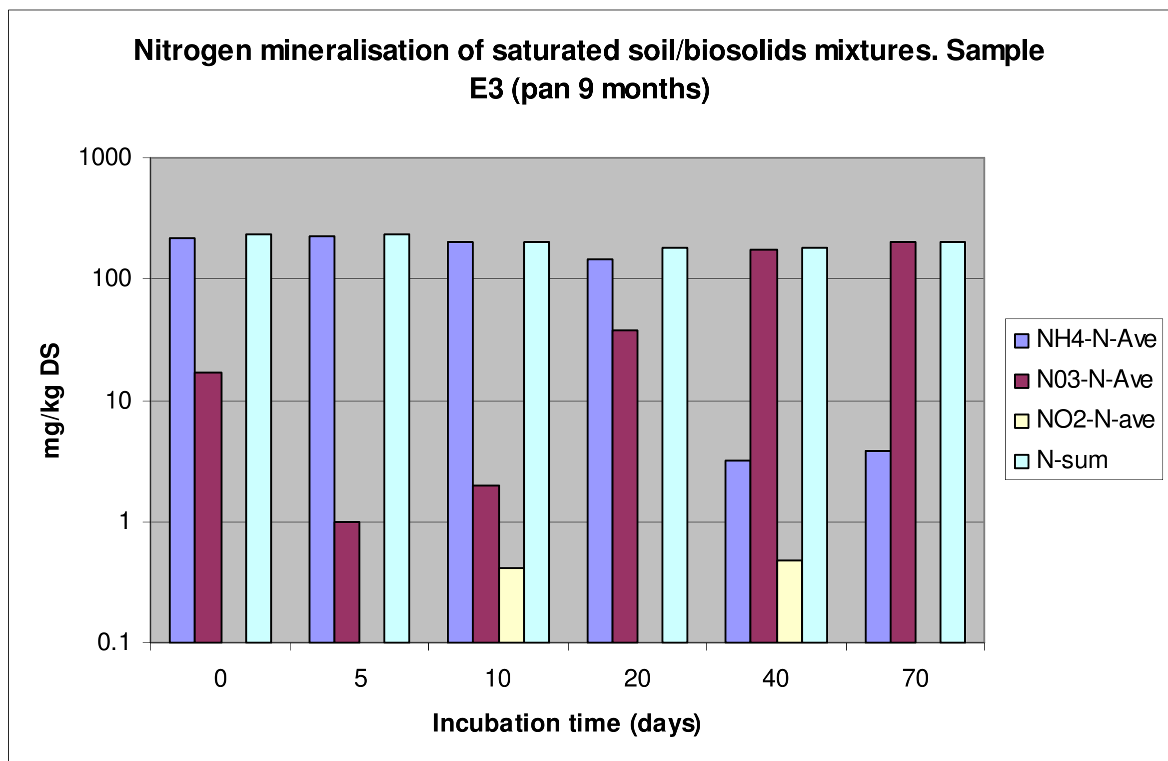

Figure 29. Conversion of ammonia to nitrate by pan biosolids in saturated soil.
Bars show the average of three values
Concentrations refer to the concentrations in the soil-biosolids mixtures

Under saturated conditions, the soil control (C1) showed reduction of total inorganic nitrogen (N-sum). Nitrate was lost from the soil presumably due to denitrification, whereas NH_4-N accumulated from the mineralisation of residual soil organic matter (Figure 30). The apparent differences in NO_3-N accumulation in soil amended with biosolids from the drying pans and the unamended control may be because the biosolids improved the soil structure and aeration reducing the risk of denitrification. Denitrification losses were relatively marginal from the experiment overall as no NO_3-N was lost from the C3 control.

It can be concluded that biosolids from drying-pans can be used to effectively fertilise both moist (aerobic) and saturated (anaerobic) soils, though best results would be expected with aerobic soil. In contrast, it appears that stockpile biosolids should be applied only to aerobic soils, due to the rapid disappearance of nitrate under the saturated condition.

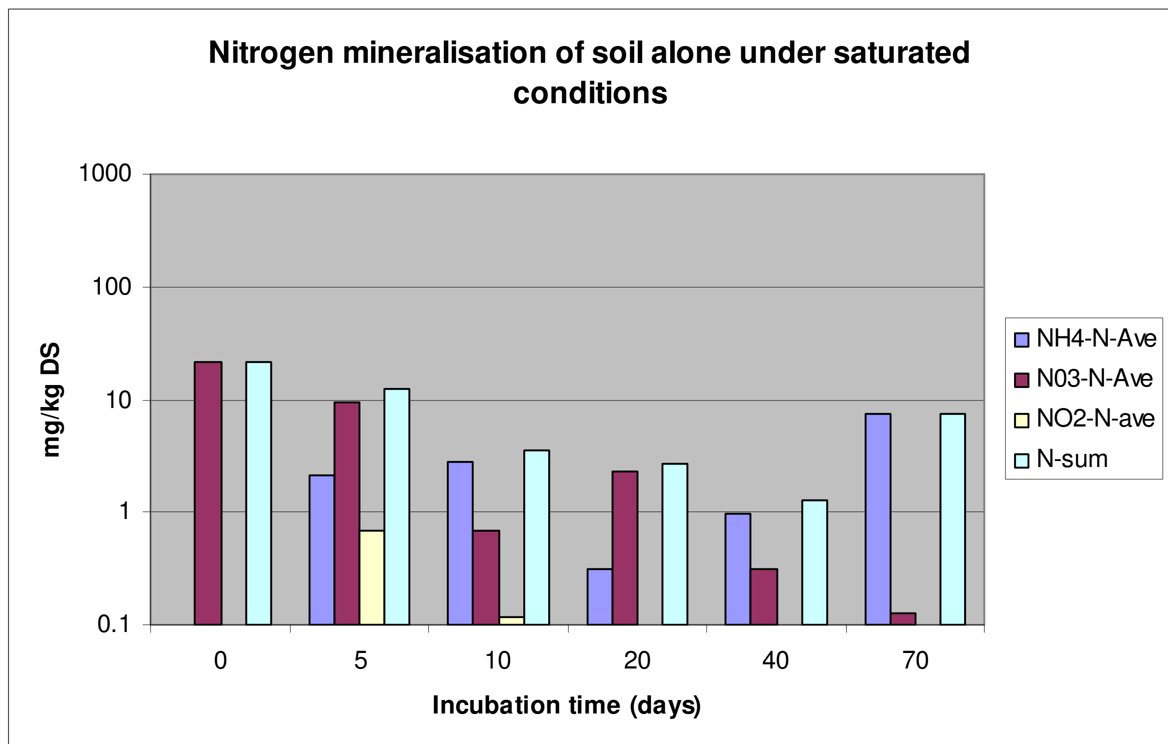

Figure 30. Group Conversion of ammonia to nitrate in saturated soil alone.
Bars show the average of three values
Concentrations refer to the concentrations in the soil-biosolids mixtures

3.3.6. Discussion

Nitrogen release from biosolids added to soil

The mineral nitrogen content in pan samples was generally similar to mechanically dewatered digested biosolids, which can contain 7000-9000 mg/kg NH_4-N DS (Smith and Durham 2002). In contrast, stockpiles tended to contain significantly less mineral nitrogen, except for three surface samples from 24-month stockpiles (Tables 7 and 8). In terms of the mineral nitrogen content, up to 90 % may be lost during the pan drying stage by NH_3 volatilisation. Therefore, to maximise the nitrogen value, biosolids should be considered to be provided for land application after first reaching the required microbial safety treatment grade.

A key aspect of the work was to examine the effects of sludge age on mineral nitrogen release. In this study standardised mineral nitrogen releases ranged from 0.24% to 3.47%, while Smith and Durham (2002) reported higher rates, 4% to 57%, over a comparable period for thermally-dried biosolids added to two-types of agricultural soils, in the UK. These differences may reflect variable activation and growth of indigenous microbes required to mineralise organic nitrogen fractions in the biosolids.

In addition, although TKN contents decreased with storage age (Table 5), similar mineral nitrogen production was observed across the range of storage ages (Table 8). Also, there was little overall difference in the amount of mineral nitrogen produced in relation to stockpile depth although the balance of NH_4-N and NO_3-N varied (data not shown). These results indicated that the contents of organic nitrogen were not limiting for nitrogen-mineralisation.

There were some interesting dynamics taking place and evidence of denitrification in some of the saturated soil treatments but not others. Loss of NO_3^- was observed in all saturated controls, as well as the stockpile amended soils, however, the pan samples showed an accumulation of NO_3 (Figures 27 and 28).

The higher organic matter status of pan material could improve the physical properties of soil, increasing aeration and reducing the risk of denitrification. The stockpiled sludge may not have these ameliorating properties and was very soil like in texture and contained a significant amount of clay material, which would also promote denitrification. It is uncertain why the NO_3-N control did not show a denitrification response, perhaps the natural salt in the soil (1000 mg N/kg is a large extra content of nitrate salt) perturbed the fragile microbial community and reduced/prevented microbial activity including denitrification.

Soil amended by sewage sludge mixed with compost provided significantly higher nitrogen mineralisation rates than soil amended with chemical fertiliser, at two Japanese farms (Zaman et al. 2002). This was apparently due to more active microbial flora present in the sludge combined with compost, compared to the soil alone. In addition, Luxhøi et al. (2008) observed that the indigenous microbial flora in soil was the prime determiner of nitrogen mineralisation activity in soil. Therefore, the low nitrogen mineralisation activity observed in the soil used in the present study may have been due to the low activity of the indigenous microbial flora. It may be concluded that in applying biosolids to soil the characteristics of the microbial flora in the soil should be investigated to assess the potential for nitrogen mineralisation.

The properties of biosolids treated by different methods can also affect the potential nitrogen mineralisation rates in amended soils (Smith and Durham 2002). The rate and extent of mineralisation of organically-bound nitrogen depends upon the stability of the sludge organic nitrogen fraction applied to soil. The concentration of volatile solids is often

taken as a chemical indicator of the extent of sludge stabilisation and usually declines with increasing stabilisation of sludge organic matter. The degradation of the organic nitrogen fraction applied to soil in biosolids can follow two potentially different mechanisms depending upon the stability of the organic component. For example, organic nitrogen in highly-stabilised sludge products resists further mineralisation in soil, and biosolids treated to this extent may have low content of mineralisable nitrogen. Alternatively, however, less efficiently stabilised organic matter has an elevated C:N ratio and may potentially immobilise nitrogen temporarily during the microbial turnover of organic carbon added to soil in sludge, and potentially limit the net release of mineral nitrogen. In this study positive nitrogen mineralisation was observed in moist soil amended with biosolids, with a range of volatile solids contents (10.9% to 59.1%). However, no statistically significant correlation was observed between nitrogen mineralisation and the content of volatile solids, indicating this was not a limiting factor in nitrogen mineralisation here. In contrast, nitrogen mobilisation, as indicated by declining mineral nitrogen contents in soil (Figure 30), generally occurred under the saturated conditions, with the exception of soils amended with drying-pan biosolids. This indicates the importance of environmental conditions on assessing the potential fate of mineral nitrogen from land applied biosolids.

Overuse of nitrogen fertilisers may lead to leaching of nitrate into waterways or production of nitrous oxide, a greenhouse gas. At the Australian Centre for International Agriculture Reseach (ACIAR) work has begun to quantify water and fertiliser inputs to determine appropriate levels to maintain current yields (ACIAR project LWR/2003/039). Already modelling of these inputs, using a water and nitrogen management model developed through past ACIAR research, has determined that fertiliser application rates can be significantly reduced. This will help increase available income as less is spent on fertiliser, as well as reducing excess nitrogen that either leaches into soils as nitrate or oxidises to form nitrous oxide (Australian Bureau of Statistics, 2008b).

Nitrogen Limited Biosolids Application Rate

How do these results relate to the definition of the Nitrogen Limited Biosolids Application Rate (NLBAR)? Biosolids are usually applied to land at the rate of one NLBAR, the rate which provides the amount of nitrogen required to produce optimal growth of a particular type of crop. How is this available amount of nitrogen calculated? Because part of the nitrogen in biosolids is in organic form, it is not readily available for plant use immediately after application. The available nitrogen content of the biosolids after application includes the mineral nitrogen (nitrate, nitrite, ammonia) and the mineralisable fraction of the organic content of the biosolids. The mineralisable fraction is defined as the amount of the organic content mineralised in the year following application. As this amount is normally not known in advance the available nitrogen is usually estimated. There are a number of ways of estimating the amount of nitrogen available from biosolids, which include the following method for evaluating liquid digested sludge (Hall and Williams, 1984; Davies, 2002):

(1) Available Nitrogen (Year 1) = ammonia nitrogen + 0.15 (Total Kjeldahl nitrogen – Ammonia N)

For agricultural applications, the NLBAR, in dry solid tonnes per hectare, is based on the crop requirements and the available nitrogen content of the biosolids in the soil. The higher the available nitrogen in the soil, the lower the NLBAR.

(2) NLBAR (t/ha) = Crop Requirement (kg/ha) / Available Nitrogen (kg/t)

In examining the formula for estimating available nitrogen (1) we see that it assumes a single mineralisable fraction, when the actual rate of mineralisation may vary between different soil and climatic conditions. Nevertheless, for conventionally treated, mechanically dewatered anaerobically digested cake this formula provides a reasonable estimate and typically gives a value of about 30 % N availability relative to the total N

content (i.e., from the NH4-N + mineralisable-N) . Our results suggest that, for air-dried sludge that has been stored by lagooning and then in stockpiled after air drying, the 15 % mineralisable N value may be an overestimate. This could be expected from the increased stabilisation of the organic N fraction during lagooning and stockpile storage (as indicated by the increased VS destruction). Readily mineralisable N in sludge is released relatively quickly in the field (it is not slow release as is often mistakenly suggested). The incubation experiment was designed to provide optimum conditions to maximise the mineralisation rate so, in general, the majority of the available organic N would be mineralised within the 70 day incubation period. 70 days was selected for the duration of the incubation based on our experience of sludge incubation experiments. These results should be regarded as provisional, however, as the number of sludge type/soil type conditions tested were restricted. Further work is necessary to confirm this behaviour, but the results emphasise that the generic N-mineralisation rate values quoted in the Victorian Guidelines may need adapting to account for particular process conditions and currently they do not consider the effects of long-term storage on N availability.

The balance between nitrification and denitrification

Organic matter contains the ratio C:N:S:P as roughly 100:10:1:1 (Probert, 1988). Most of the nitrogen in organic matter is present as amino acids unavailable to plants and does not leach out. Levels of nitrogen in soil are generally in the order of > 1000 mg/kg for sandy soils (1750 mg/kg in this report, Table D2), > 2000 mg/kg for loams and > 2500 mg/kg for clays (Walker and Reuter, 1996). Since the heterotrophic organisms use organic carbon as energy they consume the organic matter and form fresh organic matter, competing with plants for the available nitrogen. Autotrophs obtain energy from oxidation of inorganic salts and their carbon from CO_2 in the atmosphere.

Monnett *et al*., (1995) reported that maintaining reclaimed water in the upper microbiologically active part of the soil profile was important to denitrification. They also stated that denitrification is enhanced in anaerobic conditions. A supply of NO_3- or NO_2 - in soil is a prerequisite for denitrification.

High NO_3- increases the rate of denitrification and exerts a strong influence on the ratio of nitrous oxide to elemental nitrogen in the gases released from soil by denitrification. This finding is important as it contradicts the processes by which nitrogen is accounted for in nutrient balances. The direction that nitrogen takes in mineralisation or denitrification is dependent upon the carbon:nitrogen ratio (C:N). If the decomposing organic matter has a high C:N ratio (above 30:1) the microbes will utilise the NH_4 + and NO_3 - present in the soil to continue decomposition and mineral nitrogen will not be available to the plants. When C:N is low (below 20:1), there will normally be a release in mineral nitrogen in the soil that is available for plants and microbes.

Large populations of denitrifying organisms are present in arable soil and most numerous in the vicinity of plant roots. Carbonaceous exudates from actively functioning roots are believed to support the denitrifying bacteria in the rhizosphere. The potential for denitrification is immense in most field soils but conditions must arise which cause these organisms to shift from aerobic respiration to a denitrifying metabolism, involving use of NO_3 - as an electron acceptor in the absence of oxygen. Waterlogging is sufficient to cause this change of state. Denitrification can operate in seemingly well-aerated soil, presumably in anaerobic micro-sites where biological oxygen demand exceeds supply. The increased application of water alone is likely to increase vegetation mass, increase soil microbial activity and lead to a faster denitrification.

Therefore, application of biosolids to land should require aerobic conditions of soils, to allow the use of both pan and stockpile biosolids. Nevertheless pan biosolids appear to

contain compounds and or organisms that allow nitrate production in both moist soil and saturated soils.

4. Issues Arising from this project

The level of work required for this project was slightly underestimated, in part due to the requirement for confirmation of microbial indicators. So we are grateful to Vennessa Fleming for putting in significantly more hours than her part-time contract required. Furthermore, the regrowth experiment needed particularly extra work as well as resources, so we are grateful to Daniela Petrovska, an undergraduate work-experience student, and Sneha Pai, a Masters student, for supporting this experiment.

Moreover, it was noticed on site visits to ETP MWC that there were differences in the way stockpile material was treated at different years, such as being graded out to dry (Feb-07) or being simply stockpiled (Feb-06, Apr-08) and grazed by cows (Feb-06). It appears possible that different ways that biosolids are treated may lead to different contents of nutrient components for land application. The affects of different treatment operations of biosolids on nutrient contents, therefore, should be investigated.

5. Discussion and Recommendations

5.1. Treatment grades

The decrease in numbers of pathogen indicators during the standard air-drying processes at the two operational plants indicated that the T1 grade of biosolids could be produced much earlier than allowed by the current regulations, and that conditions for T2 and T3 grades could also be specified for restricted use for applications on agricultural land. The absence of pathogenic indicators in stockpiles at the two plants has also been seen in stockpiles at Bolivar WWTP, South Australia (Desmier et al., 2004).
Procedures for treatment plants with anaerobic digesters, such as MWC's ETP and SEWL's Mt Martha, to provide treatment grades of biosolids:

- For production of T1 grade biosolids, air-dry biosolids in pans for 8-10 months (from pan to land)
- For production of T2 grade biosolids, air-dry biosolids in pans for 6-7 months (from pan to land)
- For production of T3 grade biosolids, use biosolids directly from the anaerobic digesters (from pump to land)

The above recommendations are based on values of *E. coli* and coliphage removal. These recommendations, however, require a caveat, that confirmation is needed of the potential removal of both enteric viruses and parasites (such as *Giardia* and *Taenia saginata*) before the product is permitted for unrestricted use. This will require use of a laboratory simulation system, to properly test the effects of air-drying treatment processes on the removal of these pathogens. Such a laboratory-based approach, with PC2 level microbial safety conditions, is necessary to ensure safe work. This avoids the risks of spiking field processes with pathogens, which might lead to cases of diseases in plant workers or other people, a situation that must be avoided.

Furthermore it is recommended that analysis of microbial removal in the two metropolitan plants should be repeated over at least two further seasons, to determine whether the kinetics of removal of pathogen indicators across the air drying and storage processes that are reported here are reproducible.

The recommendations for production of T1, T2 and T3 treatment grades biosolids apply specifically to plants with the same process structure as the two plants investigated in this project. Notably, this includes in-line mesophilic anaerobic digestion as well as drying pans and storage areas.

Other types of treatment plants across regional Victoria, e.g., with lagoon-based treatment, require investigations to determine when T1, T2 and T3 grades of biosolids are produced. As each process should have characteristic values of pathogen removal, investigations should characterise the log decrease data for indicator pathogens in each process. Moreover different types of plants may suit the different climatic conditions across Victoria.

It is suggested that data be produced to support the development of HACCP management systems, to consistently provide the robust removal of pathogen indicators by each individual process, and to set goals for standard log decreases of the numbers of pathogen indicators. Therefore, future studies should examine calibration of pathogen indicator removal data against standard physical and or chemical data, especially those collected in-line, to aid HACCP management.

5.2. Avoiding regrowth

Wetting of dried biosolids (to 40-50% moisture content) does not directly lead to regrowth of pathogen indicators. Dried biosolids may be stored in the open before use, but under conditions that do not allow pools of water to accumulate over the biosolids, as the pools may allow growth of pathogens due to contamination by pathogens from animals in the environment (Zaleski et al., 2005). Furthermore, it is recommended to investigate what extent of water saturation is necessary for regrowth to potentially occur in dewatered biosolids.

5.3. Indicators

To provide improved knowledge for removal of pathogens compared to removal of pathogen indicators, laboratory trials simulating the digestion, drying and stockpiling stages, are required to further explore the relationship between representative pathogens and chosen indicators under controlled conditions. Optimally, an indicator should be found for each group of pathogens (bacteria, viruses, helminths and protozoa). An indicator may be process-specific, that is useful only for a certain treatment process. Such a restriction may allow more flexible choices of indicators among current limited practical options.

The survival of *Clostridium perfringens* during treatment processes does not follow the potential removal of protozoan parasites, especially during anaerobic digestion Moreover, since *C. perfringens* is commonly found in the environment it is likely that its presence in agricultural soil amended with biosolids will not provide an effective indication of the potential risk to health and decay of enteric protozoa. *Clostridium perfringens* is not recommended as an indicator for the decaying presence of protozoan pathogens during sludge treatment by operational plants.

5.5. Which species of Salmonella?

5.5.1. *Salmonella* species that cause disease in humans

The EPA regulations (EPA, 2004) require direct analysis for the genus *Salmonella*, but do not describe which particular strains or species should be detected. The genus *Salmonella* is currently described as composed of 2 species, *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* has been subdivided into six subspecies. Of these only *S. enterica* subsp. *enterica* is commonly isolated from humans and warm-blooded animals. In contrast, the other five subspecies, II, IIIa, IIb, IV, and VI and *S. bongori* are usually isolated from cold-blooded animals and the environment. These five subspecies are considered rare human pathogens, which make up about 1 to 2% of *Salmonella* isolates reported to the U.S. National *Salmonella* surveillance system (Centers for Disease Control and Prevention, 2004). For identification, subspecies I strains exhibit O serotypes A, B, C_1, C_2, D_1 and E_1 (O:2 to O:10), while subspecies II, IIIa, IIIb, IV and VI are primarily found in O groups O:11 to O:67 (Murray et al., 2007).

In terms of microbial safety, it is proposed that only *Salmonella enterica* subsp. *enterica* strains, including *S. typhi* and *S. paratyphi*, be mandated for verification and monitoring of T1, T2 and T3 grades for sludge treatment, as other subspecies of *S. enterica*, along with *S. bongori*, are usually isolated from cold-blooded animals and the environment, and generally do not cause disease in humans.

5.5.2. Detection of *S*. Typhi and *S*. Paratyphi

Limits are prescribed in the EPA 503 regulation (USA EPA, 1994) for total numbers of *Salmonella* species in sludge as an end-product standard therefore specifying species may be unnecessary. However, current standard methods for detecting the presence of

Salmonella (UK Environmental Agency (2002) do not adequately detect the pathogenic *S. enterica* subsp. *enterica* serovars Typhi and Paratyphi. These serovars belong to the *S. enterica* subsp. *enterica* group of serovars that are commonly isolated from humans and warm-blooded animals. The presence of these serovars can be determined by alternative procedures, but no standard methods are currently available to evaluate these serovars.

To fully evaluate the presence of *Salmonella enterica* subsp. *enterica* strains will require development of a standard method to quantitatively detect the two serovars, *S. typhi* and *S. paratyphi*, as none currently exists (see Appendix B). This is necessary for detection as the levels of these serovars in sewage sludge in Victoria are likely to be much lower than for other salmonella serotypes.

We suggest that any regulatory requirement for monitoring a specific pathogen or indicator make reference to an existing corresponding standard method. Without relevant standard methods it may be difficult to properly either develop or comply with such regulatory requirements.

5.6. Nutrient content

Our data on loss of nitrogen and phosphate nutrients during storage supports previous conclusions that storage reduces both nitrogen and phosphorus availability of biosolids. Conservatively, stockpiles should not be more than 1 year old to retain optimal nutrient content for land application.

A significant degree of unexplained variability in nitrogen content between a number biosolids stockpiles was observed. This requires further investigation of nutrient contents in air-dried and stored biosolids. This includes examining the effect of variant operational treatments of biosolids (such as flattening of stockpiles by bulldozing) on nutrient content.

The low nitrogen mineralisation activity observed in the soil used in this study may have been caused by the low activity of the indigenous microbial flora in this type of soil. Further investigations of nitrogen mineralisation by biosolids should include a range of Australian soils, to assess the potential range of rates for nitrogen mineralisation in different types of soil. In addition, it may be concluded that in applying biosolids to soil the characteristics of the microbial flora in the soil should be investigated to assess the potential for nitrogen mineralisation.

These kinds of nutritional information for land application of biosolids would support user guidelines for optimum use of biosolids for fertilization of crops and other plants. These guidelines would help ensure effective land application, thereby maximising both user satisfaction and competition against other sources of fertilizers.

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8. Appendices

Appendix A: The nitrogen cycle

Appendix B: Detailed methods

Appendix C: Literature review