Faculty of Health, Engineering and Sciences

Optimising nutrient extraction from chicken manure and compost

For the award of

DOCTOR OF PHILOSOPHY

A dissertation submitted by

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To my Mother and Father,

To my Sisters Salma and Ahlam,

To my Brothers Khaled, Adel, Mahmoud and Neserdeen,

To my Wife Marwa,

To my Children Safa and Daina,

To each who taught me goodness and humanity,

To each who instilled in me the meaning of hope and humanitarian highness,

To each who helped me,

I dedicate this work.

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Abstract

The global production of chicken manure is estimated to be 457 million tonnes per year. Chicken manure contains most nutrients that could be applied as a fertiliser to plants. Chicken manure has a high salinity level, high nitrogen and phosphorus content, low C/N ratio and contains pathogens. Hence, the disposal of chicken manure requires appropriate management to avoid environmental contamination. The main disposal practices are direct application to agricultural lands or composting. However, both of these methods require a high standard of management. This research investigates strategies for optimising nutrient extraction by leaching chicken waste. If the leachate can be extracted and treated effectively, then it may be applied to crops using an irrigation system where the rate and time of application are more readily controlled.

There are a range of methods used to extract soluble nutrients from animal manure at different scales. The main nutrient extraction methods produce either a manure leachate or manure tea. However, the quality (both nutrient and pathogen levels) of the leachate or tea is a function of the waste material properties and the extraction method used. Hence, the aim of this research was to identify strategies which optimised the extraction of the soluble nutrients from chicken manure or compost.

Fresh chicken manure and compost (ranging in age from 1 to 18 weeks) was packed into small columns which were subsequently leached. The leachate extracted from fresh manure and week 1 compost was found to contain higher concentrations of soluble nutrients than leachate derived from the week 6 and 18 composts. However, the leachate from the fresh manure also contained high levels of sodium and the leachate from both fresh manure and the young composts contained pathogens. On the other hand, leachate extracted from the older composts (≥4 weeks old) was free of pathogens.

Further column studies were conducted to (a) evaluate the effect of column length and packing density on the nutrient extraction, (b) identify strategies for eliminating pathogens in the leachate and (c) evaluate the effect of increasing the hydraulic pressure in the extraction process. In general, the concentration of soluble nutrients in the leachate was found to increase with both column length and packing density. To evaluate the sterilisation options, a range of treatments (hot water, acetic acid and hydrogen peroxide) were applied during leaching and different treatments (acetic acid, hydrogen peroxide, heating and ozone) were applied to the extracted leachate. The most effective treatments were found to involve leaching with hot water (although the concentration of soluble nutrients was greatly reduced) or applying ozone to the extracted leachate (which did not affect the nutrient levels). While the application of a low hydraulic pressure during leaching reduced the time required to extract nutrients the concentration of soluble nutrients per unit volume of leachate was reduced due to a reduced contact time.

This work has identified the effect of the waste material properties and extraction methodology on nutrients in the leachate. However, further work is required to (a) better understand the optimal column length to packing density ratio and the extraction pressure appropriate for specific waste materials, (b) evaluate strategies to mitigate the potential effect of high sodium levels found in the leachate, (c) evaluate the efficacy of nutrient extraction improvements, including the use of a recirculating leaching system, and (d) refine the nutrient extraction system design for commercial scale use.

Certification of Dissertation

I certify that the ideas, experimental work, analyses, results, and conclusions reported in this dissertation are my own work, except where otherwise specified acknowledged. I further certify that this work is original and has not been previously submitted for any another award, except where otherwise acknowledged.

Endorsement:

Abdalhakim Mohamed A. Ksheem **Date**

Professor Steven Raine, Principal Supervisor **Date**

Dr John McLean Bennett, Co- Supervisor **Date**

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Table of Contents

List of Figures

List of Tables

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometer
ANOVA	Analysis of variance
C	Carbon
C/N	Carbon to nitrogen ratio
Ca	Calcium
^oC	Degree Celsius
CO_2	Carbon dioxide
Cl	Chloride
CROSS	Cation Ratio of Soil Stability
DM	Dry mass
dS m^{-1}	Decisiemens per meter
EC	Electrical Conductivity
FAO	Food and Agriculture Organisation
K	Potassium
KCl	Potassium Chloride
Ksat	Saturated Hydraulic conductivity
LSD	Least Significant Difference
Mg	Magnesium
N	Nitrogen
Na	Sodium
NO_3	Nitrate
NO_2	Nitrite
P	Phosphorus
SOM	Soil Organic Matter
USDA	United States Department of Agriculture

CHAPTER 1: INTRODUCTION

1.1 Background

Various pressures on farming systems (e.g. fertiliser costs and greenhouse gases) have caused an increasing interest in the agronomic utilisation of animal manure, particularly regarding the use of chicken manure as an alternative source of nutrients. Many researchers (Kelleher et al. 2002, Sharpley, McDowell, and Kleinman 2004, Guo and Song 2009) point out that chicken manure contains a high concentration of nutrients. Moreover, Haynes and Naidu (1998) and Shafqat and Pierzynski (2010) also reported that chicken manure can potentially be used as a soil amendment and, where it has been applied to agricultural land, that it has led to improved soil physical and chemical properties. Table 1-1 summarises the typical physical and chemical properties of chicken manure.

Table 1-1 Typical physical and chemical properties of chicken manure[*]

Parameter	*Unit*	*Value*			
		(Guo and Song 2009)	(Moore et al. 1995)	(Guerra-Rodríguez, Diaz-Raviña, and Vázquez 2001)	(Guo, Labreveux, and Song 2009)
Moisture content	% (w w^{-1})	73.8	65.7	48.7	75
Organic matter	(g kg^{-1})	760	na[**]	853.8	791
Total organic C	(g kg^{-1})	349	289	387.7	377
Total N	(g kg^{-1})	37.3	46	35.6	40.4
Soluble NH_4	(g kg^{-1})	16.23	14	na	5.2
Soluble NO_3	(g kg^{-1})	0.014	0.4	na	0.002
Soluble NO_2	(g kg^{-1})	n.d[***]	n.d	na	n.d
Total P	(g kg^{-1})	13.5	21	15.5	15.1
Total K	(g kg^{-1})	35.7	21	37.9	38.5
Total Ca	(g kg^{-1})	22.9	39	na	25.5
Total Mg	(g kg^{-1})	6.5	5	na	7.2
Total Na	(g kg^{-1})	3.6	4.2	na	7.3
pH	-	7	na	8.8	7.1
EC	dS m^{-1}	23.6	na	na	25.1

[*] *based on dry mass.*

[**] *na = data is not available.*

[**] *n.d = not detectable.*

Demand for low cholesterol and inexpensive food has increased (Moore et al. 1995), which has also increased the poultry demand. Alexandratos and Bruinsma (2012) pointed out that the number of poultry (layers and broilers) in the world increased by 4.7% between 1997 and 2007 and predicted a further increase of 1.8% by 2050. In southern Queensland, Australia, egg production has increased to the point where this region supplies around 90-93% of eggs consumed in the state (Wilson 2010). As a result of such increases, concerns about the management of the manure and safe disposal of chicken by-products has grown (Moore et al. 1998, Warman and Cooper 2000). The most common disposal method for chicken manure is land application as a source of nutrients. Chicken manure contains high concentrations of nitrogen, phosphorus and potassium (Kelleher et al. 2002, Webber et al. 2011, Guo and Song 2009), which can improve the soil. However, over-application of chicken manure to agricultural land has caused some environmental issues. Repeated applications of chicken manure over a long period of time may lead to increased concentrations of soil nutrients and loss of these nutrients either by leaching or in runoff that may contaminate ground and surface water (Kingery et al. 1993). Field, Reneau, and Kroontje (1985) and Sharpley, McDowell, and Kleinman (2004) reported that over-application of chicken manure resulted in the loss of phosphorus in runoff from agricultural land, which contaminated surface water. Other problems associated with the application of chicken manure to agricultural land include (Moore et al. 1998): 1) increased leaching of nitrate into groundwater, and 2) release of pathogenic microorganisms, especially where improper management of the soil systems and compost has occurred (Vervoort and Keeler 1999b, Bitzer and Sims 1988, Moore et al. 1995). Environmental and pathogenic risks could be reduced through composting done correctly under good management (Eghball and Power 1999, Vervoort et al. 1998, Van Horn et al. 1995, Polprasert 2007). However, the composting process may result in the loss of nutrients such as Nitrogen through volatilisation of ammonia (NH_3^+), emission in forms of nitrogen oxide (N_2O) or by nitrate leaching (NO_3) (Martins and Dewes 1992, Mahimairaja et al. 1994, Eghball et al. 1997a, Jiang et al. 2011). During composting some of the inorganic nutrients are also converted to organic forms (Polprasert 2007, Van Horn et al. 1995). The rate of availability of these nutrients depends on breakdown of this organic matter (Dalzell 1987), which will be affected by the compost properties, methods and time of compost application, and environmental conditions (Rasnake, Thom, and Sikora 2004). However, the conversion of nutrient from an inorganic to an organic form has advantages and disadvantages. In organic form, nutrients are effectively in a “slow – release” form are less likely to leach from soil and require application at lower frequency. A disadvantage would be less immediately availability for plant growth.

The application to agricultural land of soluble nutrients extracted from manure or compost piles could improve the timing and management of nutrient application throughout the season and increase the efficiency of nutrient recycling. Some farmers have started to use water extraction from manure or compost as liquid organic fertiliser (Gross et al. 2008). This technique has received attention in arid and semiarid regions of the world because fresh water resources in these regions are scarce (Hamoda and Al-Awadi 1996). Furthermore, applying animal wastes through an irrigation system helps to encourage crop health and decreases the need for pesticide suppression (ROU 2007). Scheuerell and Mahaffee (2006) reported that the application of manure tea (where manure has been soaked in water and solids are filtered out in manure similar to making tea) to plants or soil helps to suppress disease-causing organisms through competition with beneficial organisms that improve the soil and foliar conditions.

There are a number of techniques used to extract nutrients in liquid form from manure or compost. These methods range from those suitable for domestic use to those suitable for commercial use. The most common methods are manure or compost extracts and tea (Litterick et al. 2004). Scheuerell and Mahaffee (2002) suggest manure/compost extract is produced by mixing the material with water, which is then filtered without being fermented. However, manure/compost tea is prepared by mixing the material with water, for a defined period to allow fermentation, and then filtered. Diver (2002) classified manure/compost tea, depending on the system of preparation, into two major types: 1) anaerobic "passive" and 2) aerobic "bucket-bubbler" (involving the use of an air pump). Diver (2002) and Ingham (2005b) defined another organic extract, compost leachate, which is "a dark-coloured solution that leaches out of the bottom of the compost pile". At the early stage of composting, compost leachate may contain a high concentration of soluble nutrients and pathogens; as a result it is not suitable for use by foliar application (Diver 2002), unless diluted.

Manure or compost tea and compost extract have fewer available nutrients than manure/compost leachate (Merrill and McKeon 1998b), but have been found to suppress plant diseases in terms of reduce and control soil borne disease (Scheuerell and Mahaffee 2002). Although manure extracts provide a liquid that is suitable for application to soil through some irrigation systems (ROU 2007), they may contain compounds, produced during the process, such as butyric acid ($CH_3CH_2CH_2$-COOH), nitrogen in ammonium form (NH_4^+) and hydrogen sulphide gas (H_2S) that can harm the roots of the plants (Ingham 2005b).

However, extracting the soluble nutrients from the manure or compost and then applying these through an irrigation system with improved regulation of concentration, application rate and timing of nutrient additions should reduce the effect of excess nutrients on the crop and environment. Hence, the aim of this research is to identify strategies which optimise the extraction of the soluble nutrients from chicken manure or compost.

1.2 Structure of thesis

This thesis contains eight chapters. Chapter 2 provides a general review of organic material in soil and its effect on soil properties. This chapter also briefly reviews the methods used to apply organic material to soil and methods for extraction of soluble nutrients from manure and compost.

Chapter 3 describes the general methodology used in this study to characterise manure and compost material and leachate extraction which were obtained throughout this study.

Chapter 4 reports on a laboratory study conducted to investigate the effect of compost maturity on the nutrients extracted by leaching the compost. This chapter also studied the effect of composting period on the rate of *E coli* and *Salmonella spp* in both the composted material and leachate.

Chapter 5 aimed to develop a method to optimise the extraction of nutrients from a manure or compost column by leaching. This chapter studies the effect of both column length and bulk density of the material packed into the column on the concentration of soluble nutrients in the leachate.

Chapter 6 reports on a study conducted to evaluate the effect of using different solutions to extract soluble nutrients from chicken manure. This chapter also investigated the effect of these solutions on pathogens in leachate. Furthermore, this laboratory work also evaluates the effect of leachate on the elimination of pathogens.

Chapter 7 presents the results for the use of a pressurised solution extraction system, evaluating the effects of pressure on nutrient extraction and discussing the performance of a functional, laboratory scale conceptual extraction system based on the results obtained from chapters 4, 5 and 6.

Chapter 8 presents a general discussion of this work along with the conclusion and future research.

Figure (1-1) shows the outlines of thesis and the connection between the chapters.

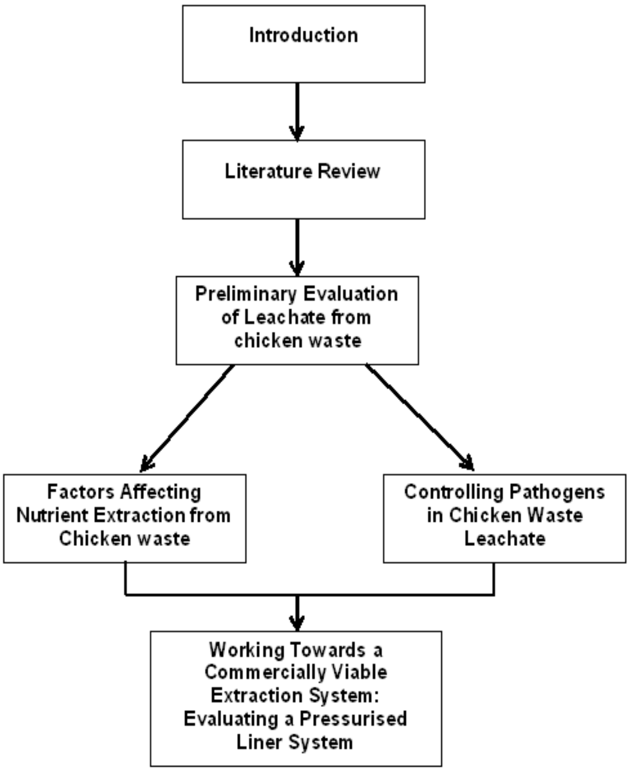

Figure 1-1 Thesis outline and structure

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This literature review provides a background to the application of poultry waste to agricultural land, and the main effects on soil properties associated with the application of manure or compost. Section 2.2 introduces chicken waste and the differences between chicken manure and chicken compost whereas in this dissertation the term “chicken manure” refers to chicken waste that has not been composted while the term “chicken compost” is chicken manure that has been composted. Section 2.3 discusses the common methods of chicken waste disposal including land application and composting. A brief overview of the techniques used to extract soluble nutrients from waste material is presented in section 2.4, along with the character of these extractable soluble nutrients. Nutrient solubility and movement will be discussed in section 2.5. Section 2.6 discusses methods of applying chicken waste as a solid or liquid organic extract or leachate, while section 2.7 focuses on the soil property impacts of applying these liquid extracts to agricultural land.

2.2 Chicken manure composition

In general, ‘chicken manure’ refers to a mixture of excreta while ‘chicken litter’ refers to a mixture of manure, bedding material, waste food, broken eggs and feathers (Kelleher et al. 2002, Tiquia and Tam 2002). The bedding materials consist of wood shavings or by products of agricultural production such as peanut, wheat or rice hull (Williams, Barker, and Sims 1999, Kelleher et al. 2002). Moore et al. (1995) suggested that dead birds are another waste associated with chicken manure.

Both chicken manure and litter contain high concentrations of plant nutrients, such as nitrogen, phosphorus and potassium, as well as micronutrients, which could be used as an organic fertiliser (Kelleher et al. 2002, Moore et al. 1995, Wilkinson 1979, Guo and Song 2009, Guo, Labreveux, and Song 2009, Sims and Wolf 1994). Table 2-1 summarises quantities of excreta and nutrient outputs at the end of housing period.

*Table 2-1 Estimated quantities of excreta and nutrient outputs at the end of housing period based dry matter**

Type of chicken	**Age**	**undiluted excreta (ton)**	**Nitrogen (N)[a] (Kg)**	**Phosphorus [a] (kg)**	**Potassium [b] (kg)**
1000[#] Laying hens (caged)	17 week and over	41	400	152.7	323.7
1000[#] Broilers		19[c]	330	96	282

* Adapted from (DEFRA 2010).

[a] nitrogen and phosphorus outputs based on nutrient balance estimates.

[b] potash outputs based on estimated undiluted excreta volumes and typical potash content of manures (@10% dry matter content for slurries).

[c] excretal output includes litter, where appropriate.

[#] 1000 refers to amount of nutrients in 1000 laying or broilers manure.

The concentration of nutrients in poultry manure differs according to poultry species, with higher concentrations of nitrogen and phosphorus in broiler birds than in meat and layer chickens manures (Williams 2010). The nitrogen in chicken waste exists in different forms, with the organic form in fresh manure about 60 – 80% urea (or uric acid) and protein (Kelleher et al. 2002, Mahimairaja et al. 1994). The large amount of the organic form of nitrogen can be converted to an (ammonia – ammonium) inorganic form where the total nitrogen is the sum of both organic and inorganic forms. Depending on environmental conditions, the ammonium form can be converted to nitrate by microorganisms in a process called nitrification (Kelleher et al. 2002).

Chicken manure is unlike other animal manures as it contains comparable concentrations of phosphorus to nitrogen. Moore et al. (1995) indicated that the range of nitrogen in chicken manure is 18 – 72 g kg^{-1} while the range of phosphorus in chicken manure is 14 – 34 g kg^{-1}. As plants generally require twice as much nitrogen as phosphorus, this may lead to the over application of phosphorus if waste is applied to amount of nitrogen required. As a result of the low mobility of phosphorus in the soil, applied phosphorus commonly remains near the soil surface. This increases the chance of phosphorus loss in runoff and resultant contamination of surface water. Table 2-2 shows the concentrations of macronutrients (N – P – K) generally required by most plants through the growth period. However, potassium (K) presents in chicken manure as a free ion (cation), while both calcium (Ca) and magnesium (Mg) present as salts such as calcium and magnesium carbonates ($CaCO_3$ or $MgCO_3$). NRC (1994) found that $CaCO_3$, $MgCO_3$ and $KHCO_3$ are required in a chicken feeding program to balance the osmotic pressure and pH throughout their bodies.

Table 2-2 Average concentrations of mineral nutrients in plant shoot dry matter that are sufficient for adequate growth[*]

Element	Abbreviation	(g 100 g^{-1} dry weight)
Nitrogen	N	1.40
Phosphorus	P	0.19
Potassium	K	0.98
Calcium	Ca	0.50
Magnesium	Mg	0.19

[*] Adapted from (Marschner 1995)

2.3 Chicken waste disposal

Chicken waste is typically applied it agricultural land either directly as manure or compost (Kelleher et al. 2002). This section outlines these options and their advantages and disadvantages.

2.3.1 Application of chicken manure to agricultural lands

Spreading chicken manure or litter on agricultural land is a traditional practice (Kelleher et al. 2002, Nicholson et al. 1999, Haynes and Naidu 1998) provides valuable nutrients for plant (Warren et al. 2008). The manure or litter can be applied to agricultural land to improve soil fertility (Nicholson et al. 1999) and soil chemical, biological and physical properties (Kingery et al. 1994). Hawke and Summers (2006) suggested that both soil chemical (e.g. cation exchangeable capacity, soil pH and EC and soil fertility) and physical properties (e.g. water holding capacity and aggregate stability) are affected by changes in soil biological properties, as a result of applying organic matter. The important biological property that is most usually affected is microbial biomass carbon (Wander et al. 1994). Haynes and Naidu (1998) found that the continued application of animal manure over a long time (over 140 years) increased the organic carbon in the soil by an exponential rate and that it impacted soil structure, and promoted the formation of water-stable aggregates. However, there are some negative effects that discourage the use of chicken manure as a source of nutrients in cropping systems compared with other sources of nutrients which include the potential for water contamination, spread of pathogens, the release of greenhouse gases.

While the production of chicken manure or litter is roughly constant during the year, chicken manure is usually applied in the spring (López-Mosquera et al. 2008) or prior to planting. Therefore, storage or continual application of chicken manure are often considered as the only solutions to the accumulation of chicken manure. Issues that are associated with storage or over-application of chicken manure, particularly where manure or litter is dumped as waste, include nitrogen emission, odour as well as chemical and biological contamination of ground and surface water (Nicholson et al. 1999). Sims and Wolf (1994) and Sharpley, McDowell, and Kleinman (2004) reported that the over application of chicken manure led to the contamination of groundwater by nitrate leaching, and surface water by phosphorus contained in runoff. Additionally, the cost of handling and spreading manure (per plant nutrient applied) is high compared with chemical fertilisers because of the high moisture and carbon content of chicken manure (Wilkinson 1979).

2.3.2 Composting chicken manure

Cooperband (2000) defined composting as "*the transformations of raw organic materials into biologically stable, humic substances suitable for a variety of soil and plant uses*". The product that results from composting is "*compost*" which is a rich source of organic matter, dark and easily crumbled, with an earthy aroma (not an aroma of decaying organic material). Kelleher et al. (2002) stated that compost is ideally characterised as being odourless with a fine texture, low moisture content, and is pathogen free. Compost can be applied to soil as a relatively dry source of nutrients compared with fresh manure (Eghball et al. 1997a).

The process of composting has been suggested (Moon 1997) as a suitable method of decreasing pathogens, as compared to raw organic matter. Pathogeneses and plant seeds are destroyed when compost windrow temperatures reach above 60 – 70^{o} C for a minimum of 4 days (Larney et al. 2003, Tiquia, Tam, and Hodgkiss 1996, Cooperband 2000). Composting is a biological thermophilic process, which requires oxygen to stabilise the organic by-product, and optimal moisture content for the development of microorganisms (Haug 1993, Zucconi and De Bertoldi 1987). This process may take between 4 to 6 weeks to reach the stabilised stage (Kelleher et al. 2002), so moisture content and oxygen availability need to be maintained over this period. If oxygen availability is not maintained then, anaerobic processes occur within the compost degrading the compost quality. Fungi grow at low nitrogen (Tuomela et al. 2000), do not grow well above pH 7.5 and are generally of fungi mesophlic growing between 25 – 30°C (Dix and Webster 1995). Therefore, fungi are not normally associated with the1` breakdown of chicken manure.

2.3.2.1 Anaerobic composting

The decomposition in anaerobic composting occurs without oxygen or with a limited oxygen supply. Anaerobic processes occur if low compost piles are not turned frequently, resulting in anaerobic conditions within the pile diminishing organic breakdown. Within anaerobic systems, compounds including methane, organic acids, hydrogen sulphide and other substances are produced by anaerobic micro-organisms (Misra, Roy, and Hiraoka 2003). These compounds accumulate and are not metabolised further. Many of these compounds have strong odours and some are phytotoxic. Additionally, as anaerobic composting is a low-temperature process, weed seeds and pathogens are often detected in the final product (Misra, Roy, and Hiraoka 2003, Monnet 2003). Hence, anaerobic composting does not meet the ideal characterisation of good quality compost as detailed by Keheller *et al*. (2002) and is not a suitable method for producing commercial land application compost products.

2.3.2.2 Aerobic composting

The main difference between aerobic and anaerobic composting is that the compost pile is provided with oxygen to allow aerobic reactions to occur. Provision of oxygen is usually by one of the following methods: 1) turning the compost pile by using a bucket loader or windrow turning machine, 2) in passive piles where the oxygen is provided through pipes, which serve as air ducts for passive air movement, and 3) in static piles oxygen is applied using blowers to force air into the pile via pipes installed as per passive piles (Rynk et al. 1992). In aerobic composting, carbon dioxide (CO_2), ammonia, water, heat and humus are produced by aerobic microorganisms. The intermediate compounds such as organic acids, which are produced by aerobic micro-organisms under aerobic composting, have been shown to continue to decompose through microorganism activity (Misra, Roy, and Hiraoka 2003). Most importantly, the resulting compost is a stable organic end product, as compared to compost produced in anaerobic systems (Misra, Roy, and Hiraoka 2003). The aerobic composting process is the preferred method to break down organic waste and produce commercial products for land application (Kelleher et al. 2002). Aerobic compositing takes 4 – 6 weeks to reach a final stabilised product that is odourless and pathogen free.

Applying compost to agricultural land instead of fresh manure has some advantages (e.g. free of pathogens and weed seeds), but may also have some drawbacks. Sikora (1998) and Cooperband (2000) listed the disadvantages of compost application which include:

1. The equipment cost and area of composting preparation.
2. The need for good management to increase the efficiency of composting.
3. The composting processes may contribute to contamination including emission of gases or volatile nitrogen into the air and leaching of elements into ground and surface water.
4. The low nitrogen concentration of compost leads to high application rates which may result in environment contamination particularly by nitrate and phosphorus and,
5. Slow release of nutrients to soil.

Furthermore, nutrients may be lost during the composting process resulting in a decreased nutrient content in the final product. Kithome, Paul, and Bomke (1999) found that the loss of nitrogen during composting chicken layer manure was between 47% and 62% of initial total nitrogen. Similarly, Ogunwande et al. (2008) found that the percentage of nitrogen lost by volatilisation ranged between 70.73% and 88.17% during composting. Martins and Dewes (1992) reported that most of the nitrogen (77.4%) lost from chicken manure during aerobic composting was in the form of emission gases (N_2O), with a lower quantity of nitrogen leached in the form of nitrate. They attributed the increased loss of nitrogen in the form of N_2O to increased total nitrogen in chicken manure and increases in the nitrification process. Losses are affected by temperature and pH with between 43% and 91% of nitrogen losses occurring in first 28 days (Ogunwande et al. 2008). Moreover, they found that the carbon to nitrogen ratio (C: N) significantly affected the loss of nitrogen with high loss occurrence associated with a low C: N ratio.

Fukumoto et al. (2003) reported that significant emission of N_2O occurred after turning where the surface of the pile moved to the centre the pile and the nitrate is denitrified, producing N_2O as a by-product. Hao et al. (2001) found when the aeration condition is brought about by a turning machine, emission of nitrogen (N_2O) was lower than with the passive aeration of a static pile. Shen et al. (2011) found that an increase in the intermittent rate, which is an aeration for 30 min and stopping for 30 min over first seven days and then continuous for the rest of the composting time, from 0.01 to 0.1 $m^3\ min^{-1}\ m^{-3}$ decreased the N_2O emission from 0.035% to 0.025% of total nitrogen loss. Thus, pile aeration may be used to control the emission of nitrogen gases.

2.4 Application methods of chicken manure

Chicken manure and litter can be land applied by various means. Both manure and litter are solid materials but may be found as slurry. Moore et al. (1995) defined a slurry as containing between 40 and 150 g kg^{-1} of manure. The methods of land application vary depending on the phase of the material.

2.4.1 Application of solid manure materials

Solid chicken manure or litter can be applied to agricultural land by different methods. Common methods are to broadcast it onto the surface and till it into the soil or to apply it in a narrow band on or beneath the soil surface (Johnson and Koenig 2011, Moore et al. 1995). Both broadcast and banding are usually done by spreader or large-bodied trucks where the use of spreading equipment relies on the handling and storing methods (Moore et al. 1995). Laguë, Landry, and Roberge (2005) suggested the benefits of applying animal manure solid to soil include: optimising the potential nutrients from manure; protecting surface and ground water from contamination; and minimising odour and gas emission to the atmosphere. On the other hand, many researchers (Shreve et al. 1995, Moore, Daniel, and Edwards 2000, Warren et al. 2008) have indicated that the application of chicken manure or litter to soil without adding amendments to reduce the loss of nutrients is one of disadvantages of this method.

Soupir, Mostaghimi, and Yagow (2006) demonstrated that the main issue associated with the application of animal manure is ground and surface water contamination by nutrients. Similarly Smith et al. (2007) identified both nitrogen and phosphorus derived from agricultural lands as contributors to surface water contamination. To reduce the contamination risk of chicken manure application, Sharpley, Kleinman, and Weld (2004) suggested using both chemical amendments, and physical treatment. Adding commercial amendments such as alum ($Al_2 (SO_4)_3.14\ H_2O$) or ferrous sulphate ($FeSO_4.7\ H_2O$) have been found to reduce the loss of nutrients as these chemicals reduce the solubility of nutrients by precipitation and adsorption (Moore and Miller 1994). Shreve et al. (1995) found that adding alum to broiler litter reduced the concentrations of phosphorus in runoff by 85%. Kithome, Paul, and Bomke (1999) found that applying alum reduced NH_3 loss by 45%. Physical treatment includes either incorporation or subsurface application litter or manure into soil. Subsurface banded application of chicken litter has been found to reduce the concentrations of nitrogen and phosphorus in runoff by 93% and 89% respectively (Pote et al. 2003, Sistani et al. 2010). Similarly, Giddens and Rao (1975) found the incorporation of chicken litter at a soil depth of 10 cm reduced the loss of nitrogen by volatilisation by 55% compared with a broadcast application. However, using these treatments may increase the cost of application, which then increases the cost crop of production. An alternative in irrigated cropping systems is to apply the nutrient from the manure or waste through the irrigation systems (Bond 1998). This may be an appropriate method of increasing the efficiency of nutrient applied and minimising the losses associated with their application to agricultural land.

2.4.2 Application of waste in a slurry or liquid form

Although less common, animal manure can be applied to agricultural lands as a liquid extract, slurry or leachate. ROU (2007) indicated that liquid compost extracts could be applied to soil either via irrigation systems or directly to plants by foliar applications. For example, wash-down waste from dairy milking sheds and yards are commonly irrigated onto pasture using sprinkler systems (Qld Government, 2010). However, Jarrett and Graves (2008) suggested that before applying liquid waste or compost to the soil there are some considerations that must be made:

1. Only the required amount of liquid manure or compost, as defined by soil/plant nutrient requirement, should be applied;
2. The liquid manure or compost should be applied at a rate that ensures no runoff post application; and,
3. It is necessary to use a suitable irrigation design and operating system for the particular leachate source, soil type, topography and conditions of the field.

Using surface irrigation systems to apply effluents or extraction solution derived from animal manure raises the risk of contamination from pathogens. Furthermore, surface irrigation may result in a non-uniform water distribution area in field leading to a non-uniform distribution of nutrients. Also, in arid countries, such as Libya, water is scarce, the evaporation rate is high. In these cases it is important to maximise water efficiency and efficient use of nutrients by the plant. Thus, sprinkler and drip irrigation systems are preferred methods for the application of fertiliser and organic solutions. Thompson, Huan-cheng, and Li (2009) showed that the advantages of subsurface drip irrigation (SDI) increased water use efficiency and reduced nitrate leaching. Lamm (2002) added other advantages including: 1) the reduction or elimination of runoff which, in turn, reduces nutrient loss (reduced water contamination by phosphorus and nitrate), and 2) improved nutrient management which means increased nutrient use efficiency.

2.5 Extraction of soluble nutrients from waste material

This section will focus on the preparation of manure and compost liquid extraction, which can be applied through sprinkler and drip irrigation systems. As discussed in section 2.4, the application of fresh chicken manure/litter directly to agricultural land has some disadvantages, which may be addressed in some cases by applying composted chicken manure. However, the release of nutrients from composts can often be slow, as it is reliant on the breakdown of organic matter (Syers and Craswell 2004). Furthermore, as composted material is most feasibly applied at the beginning of a cropping season, the risk of nutrient loss over time through volatilisation, fixation and runoff is increased. Applying nutrient from chicken manure or compost through an irrigation system may overcome these disadvantages, as the application rate may be better controlled; nutrients are immediately available and nutrients can be applied in-crop. There are a number of methods used to extract nutrients from manure or compost liquid. These methods range in scale from those suitable on a domestic level to those suitable on a commercial level. The aim in any method is to efficiently extract the majority of nutrients from the composted or fresh material, preferably using simple equipment to maximise the cost-benefit ratio. Factors affecting the extraction of soluble nutrients from fresh or composted material are: the initial material quality, amount of material to water ratio, aeration, fermentation nutrients, brewing time and filtration materials (Ingham 2005a, Scheuerell and Mahaffee 2002). The following section discusses the common nutrient extraction systems.

2.5.1 Anaerobic “passive” system

The passive method involves making compost liquid by inundating the manure or compost with water and leaving it to soak (Merrill and McKeon 1998a). Diver (2002) prepared passive organic tea by immersing a burlap bag filled with compost into a bucket or tank and stirring occasionally. Usually the brew time is long, taking between 7 to 10 days. This method has been used for hundreds of years in Europe (ROU 2007), and the resulting liquid is more a watery extract than a brewed and aerated tea. In this method aerobic microorganisms will consume oxygen from the water and after few days will create an anaerobic system. Although these techniques produce a liquid that may be suitable for land application through some irrigation systems, it contains many compounds produced during the anaerobic process, such as butyric acid ($CH_3CH_2CH_2$-COOH), nitrogen in ammonium form (NH_4^+) and hydrogen sulphide gas (H_2S). These may harm the roots of the plants. The two most common passive techniques are soaking in water and soaking-in-bag.

2.5.1.1 Soaking in water

This is the easiest way to prepare compost liquid where organic waste, normally chopped plant material or animal manure, is added to water at an approximate 1:25 ratio (waste : water) by volume (ROU 2007); it is then left for a short period of time to ferment. The period of fermentation depends on how long it takes the soluble nutrients to leach out and the amount of oxygen consumed by aerobic microbes (Merrill and McKeon 1998a). However, this technique has been reported as unsuitable for use in irrigation systems because it contains a large amount of solid particles that may clog emitters or droppers (ROU 2006). The concentrations of large particulates are too high for filtration to be economically feasible.

2.5.1.2 Soaking in bag

This technique produces a product that is cleaner than the prior method. The manure or compost is put into a bag made from a permeable material, usually hessian or burlap. The bag effectively acts as a crude filter: the tighter the weave, the cleaner the solution. A bag containing the manure or compost is soaked in water for one or two days to obtain a nutrient solution (Merrill and McKeon 1998a).

2.5.2 Aerobic “Bucket-bubble method” system

The aerobic system equipment setup and scale of production is similar to the passive system, except (a) an aquarium-size pump and air bubbler are used and (b) microbial food and catalyst mediums are added to the solution as a food source such as simple sugars, cane syrup or sugar beet for bacteria and cellulose, humic acids or other cellulose containing material for fungi for 2 – 3 days (Ingham 2005a). The purpose of the pump is to provide the microbes with oxygen and thus maintain aerobic conditions. The addition of oxygen to the organic liquid improves the quality of extracts by reducing harmful by-products such as butyric acid, nitrogen in ammonium form (NH_4^+) and hydrogen sulphide gas (H_2S). Merrill and Mckeon (1998a) found that this extract produced low nutrient concentrations and low concentration of organic acids considered harmful to plants.

2.5.3 Manure/Compost leachate

Compost windrow leachate is the solution that leaches from the bottom of a compost pile. This leachate is generally rich in soluble nutrients, but in the early stages of composting (raw manure) it may also contain pathogens and require further bioremediation to be suitable for foliar application or spreading (Diver 2002). Furthermore, compost leachate in the early stages is not suitable as a foliar spray due to likely increasing the salinity (ROU 2007). As this method produces the highest concentration of soluble nutrients, this study will focus on how to prepare manure or compost leachate to improve its solubility or application through irrigation systems.

2.6 Effects of applying chicken manure on soil

The application of chicken manure in solid or liquid phases may have effects on soil chemical, physical and biological properties. The use of manure or compost water extraction holds the potential for environmental impediments dependent on the characteristics of the extracts, climate of the site, and the characteristics of the immediate environment (Myers et al. 1998). Burns, King, and Westerman (1990) reported on a range of studies conducted from 1974 to 1987 evaluating manures applied in solid, slurry or liquid forms as an organic source of crop nutrients. In general, crop production was found to improve from application of manure to agricultural land. Hence, the need for the chemical fertilisers may be reduced by applying effluent nutrients (Bolan, Horne, and Currie 2004). However, the three major risks to the sustainable use of effluent in irrigated soils are: 1) excessive nitrate leaching to ground water, 2) increasing soil and groundwater salinity, and 3) exacerbating or introducing soil sodicity (Bond 1998). These three major risks rely on the nature of the effluent, application rate, land use and the location. Furthermore, there are biological issues that may arise, such as deterioration of soil carbon, introduction of pathogens, and decreasing populations of desirable microbes. This section will discuss the chemical, physical and biological effects arising from the application of solution extracted from manure or compost.

2.6.1 Physical soil properties

The changes in the soil physical properties resulting from the application of animal manure have been reported in many studies. The main effect of applying animal manures on the physical properties of soil are related to the accumulation of organic carbon in soil (Lee et al. 2009). Schjonning, Christensen, and Carstensen (1994) observed that increased soil organic carbon caused an increase in stability of aggregates, porosity, bulk density, hydraulic conductivity, and water holding capacity.

Water extracted from manure often contains relatively high salinity and sodicity (Bond 1998), which may affect soil structural integrity. Falkiner and Smith (1997) found that soil sodicity increased when the soil was irrigated with secondary treated sewerage effluent. Bond (1998) indicated that the concentration of the soil solution is the main factor that affects soil ESP, and the breakdown of soil structure as a result of the solution application. Edmeades (2003) reported that long term application of chicken manure to soil reduced saturated hydraulic conductivity and Balks, Bond, and Smith (1998) found that irrigation of soils with manure water extract led to decreased infiltration

Increasing organic carbon in the soil generally improves soil physical properties such as aggregation, bulk density and hydraulic conductivity (Kladivko and Nelson 1979), whereby the bulk density decreases and soil moisture content, field capacity and water holding capacity increase while aggregate size improves. Weil and Kroontje (1979) and Khaleel, Reddy, and Overcash (1981) reported that an increase in chicken manure application to soil resulted in an increase in soil water holding capacity.

2.6.2 Chemical soil properties

A particular concern associated with effluent irrigation is that excessive concentrations of NO_3 and soluble salts may build up in soil over time (Aldrich et al. 1997). This could result in pollution of ground and drinking water, as observed in the study by Linsley, Crow, and Warren (1976). Burns, King, and Westerman (1990) found that increasing application rates of swine effluent through a sprinkler irrigation system resulted in a rise of residual nutrients. This residual nutrient was then prone to leaching and pollution of ground water. While pollution of subsurface and groundwater by nitrate is an environmental concern, when the nutrient is derived from animal manure it can also be a concern for human health (Sharpley et al. 1990).

Nitrate use and leaching depends on the time of application and the type of irrigation system that is used, as well as the soil type. Miller et al. (2001) found that various application rates of cattle effluent had no significant effect on nitrogen uptake. However, they observed that soil texture had significantly influenced nitrogen uptake. Soil nitrogen uptake on clay loam was more than two times that on a fine sandy loam, while phosphorus uptake was three times greater in clay loam than fine sandy loam.

The application of waste water with a relatively high nitrate concentration has been demonstrated to cause a decrease in soil pH, although this decrease may be short-lived (Mohammad and Mazahreh 2003). King, Burns, and Westerman (1990) also found that increasing application rate of swine effluent through a sprinkler irrigation system resulted in a decrease in soil pH within 0 –15 cm of the surface. They explained this reduction in soil pH as a result of the nitrification of ammonium. However, Aldrich et al. (1997) found that the soil pH increased in the 0 – 15 cm depth, as a result of the difference in effluent pH (8.1) and initial soil pH (6.0). Vazquez-Montiel, Horan, and Mara (1996) explained that the increase in soil pH following long-term wastewater application is related to the wastewater chemistry and, in particular, the high content of basic cations such as Na, Mg, and Ca.

The total dissolved solid (TDS) of sewage effluent is often high, between 200 – 300 mg L^{-1}, and has the potential to be much higher where effluent is sourced from intensive rural industries and industrial processing (Feigin, Ravina, and Shalhevet 1991), such as feedlots and abattoirs, respectively. Consequently, Bond (1998) observed that the irrigation of soil using industrial processing waste led to an increase in the soil salt load. Aldrich et al. (1997) found that the soil electrical conductivity (EC) increased to a depth of 30 cm as a result of the difference between background soil EC (0.65 dS m^{-1}) and the EC of poultry effluent (1.458 dS m^{-1}). The increase was approximately 1.0 dS m^{-1} per year within the 15 – 30 cm depth. This was highlighted by Mohammad and Mazahreh (2003) who also observed salt accumulation in the soil as a result of salts contained in the wastewater. However, they also observed that the soil EC increased when irrigated with potable water, although to a lesser extent than where wastewater was used. Additionally, Aldrich et al. (1997) suggested that insufficient leaching would lead to increase in soil EC where applying cattle effluent Falkiner and Smith (1997) observed that the EC of soil irrigated with secondary treated effluent increased in the topsoil (0 – 5 cm) within the first season and then declined in the second season. They attributed the cause of this decrease in EC to rainfall of 199 mm that occurred between the end of irrigation season and the time of soil sampling. Hence, appropriate management of an irrigation system using effluent solution as a nutrient source may not result in long-term degradation of the soil condition.

2.6.3 Biological soil properties

Barkle et al. (2000) reported that irrigating soil with dairy effluent for two years resulted in an increase of organic carbon concentration in the soil within the 0 – 20 cm depth. Also, they found that microbial biomass in soil was augmented immediately after ceasing irrigation with the effluent. However, Falkiner and Smith (1997) and Sparling, Schipper, and Russell (2001) indicated that irrigating soil with effluent might result in a decline of organic carbon or no significant effect on soil carbon. They suggested the reduction in organic C was due to leaching of organic C from 0 – 10 cm depth of soil. This is consistent with Degens et al. (2000) who found applying high pH and SAR effluent to soil resulted in dissolution of organic C and other nutrients from topsoil, and accumulation at 10 – 50 cm. Additionally, heterotrophic soil microorganisms and their activities were observed to increase with the long term application of sewage and pig slurry (Stadelmann and Furrer 2004), although high application of sewage sludge decreased other microorganism such as autotrophic microorganisms (Algae) (Cameron, Di, and McLaren 1997). Interestingly, Sparling, Schipper, and Russell (2001) found that the long term irrigation with dairy effluent affected microbial biomass C, more than doubling it in the soil. However, this is a measure of total microbial biomass, and does not discern between desirable microbes and undesirable microbes.

Yeates (1995) found that 7 years of spray irrigation of sewage effluent throughout a 17-year-old Pinus Radiata plantation on dune sands caused a rise in earthworm and nematode populations and a reduction in the populations of some groups of litter arthropods such as spiders and aphids. They also observed that effluent irrigation appeared to raise the rate of litter breakdown due to increased moisture associated with irrigation leaching to a higher microbial population and nutrients concentration.

2.7 Chicken manure risk for human health

Chickens affected by zoonotic disease/bacteria excrete pathogens with their manure. Thus, applying fresh chicken manure without treatment may contaminate the environment. Fenlon et al. (2000) reported that pathogens could survive in soil for up to 29 days after manure application but after 29 days the number of pathogens declined pathogen transmission from land to human due to the application to agricultural lands of untreated animal manure has been demonstrated (Charles and Smith 2005). Thus, this section discusses the issue associated with pathogen transmission from land to humans, or directly to humans while handling material and during the extraction process. It will also consider the fate of pathogens in the soil and after application.

Pathogen transmission from land to humans can occur through the application of untreated animal manure and then consumption of fresh raw products that are eaten uncooked, such as vegetables (Warriner et al. 2009). Many researchers have found links between outbreaks of foodborne illness, which are associated with the consumption of fresh products contaminated with pathogens, such as *Escherichia coli* and *Salmonella spp* (Buck, Walcott, and Beuchat 2003, Warriner et al. 2009). Harris et al. (2003) indicated that minimal processing of fresh products which are often eaten raw, would present serious risk to human health as a result of pathogen contamination. Olaimat and Holley (2012) suggested microbial contamination may occur in the production, harvest, processing, storage, transportation and handling of produce. However, there may only be a weak opportunity for the transmission of pathogens from soil to plants because there are many factors that could affect the survival of pathogens.

Mubiru, Coyne, and Grove (2000) reported that soil moisture content is an important factor affecting the survival of enteric bacteria such as *E coli* or *Salmonella spp*. Jamieson et al. (2002) found that pathogens derived from animal manure in soil declined in line with the decrease of soil-water availability. Fenlon et al. (2000) added other factors that affected the survival of pathogens in soil including: soil pH, presence of a rhizosphere and oxidation-reduction potential. Furthermore, under field conditions, solar radiation may also affect the survival of pathogens in the soil (Jiang, Morgan, and Doyle 2002).

Composting is one treatment that may eliminate pathogens in manure. Storage of manure or litter in a heap before its application to agricultural land has been found to decrease the concentration of pathogens (Nicholson, Groves, and Chambers 2005). The reduction of pathogens in the heap relies on the management of the storage conditions including temperature of heap, pH, aeration and dry matter content (Kearney, Larkin, and Levett 1993, Kudva, Blanch, and Hovde 1998, Himathongkham and Riemann 1999, Nicholson, Groves, and Chambers 2005).

2.8 Summary and hypothesis

Applying composted material to agricultural land is generally preferable to applying raw manure in terms of pathogens, odour and nutrient availability. However, during the composting process some highly soluble nutrients, such as nitrogen or phosphorus, may be lost by leaching or in runoff. Furthermore, nutrient availability within the soil is contingent on organic matter breakdown, which may be slow. In contrast, extracted solutions have the distinct advantage of being able to be applied in-crop, rather than in bulk at the beginning of a season. Hence, extraction of soluble nutrients may be a cost effective and a more efficient means by which to apply nutrients from organic matter. However, the literature pertaining to extracting soluble nutrients from manure and compost is inconclusive in terms of the best method and does not necessarily lend itself to commercial scale use of soluble nutrient extracts. Drawbacks have been identified in both anaerobic and aerobic systems used to extract soluble nutrients from animal waste. For example, anaerobic systems likes “manure tea” either soak the manure in water or soak it in a bag. These systems produce extract with low nutrient concentration and harmful compounds such as hydrogen sulphide gas or butyric acid that may harm plant roots. They also contain large solid particles that may clog irrigation emitters. Therefore this research will evaluate the leachate method because the concentrations of soluble nutrients are expected to be greater concentration and harmful compounds are expected to be lower. The hypothesis of this research is that: it is possible to develop an improved method to extract soluble nutrient from chicken waste for use as an organic fertiliser.

2.9 Research significance and specific objectives

Manure or compost leachate has been defined as an organic liquid fertiliser. However, manure or compost leachate contains a high concentration of soluble nutrients; there is little information available about strategies and techniques to optimise the collection and use of manure or compost leachate. Similarly, there have been few studies conducted using manure or compost leachate as a liquid source of soluble plant nutrients, and there is a lack of information about the effects of leaching soluble nutrients from both raw manure and mature compost on a commercial scale. Thus, this review raises three main research questions that need to be addressed before the implementation of these strategies, and to develop an extraction method for soluble nutrients from chicken manure and manure compost on a commercial scale:

1. How does the composting process of raw chicken manure affect the quality of leachate in chemical and biological terms?
2. How does the design of the leaching column and water applied affect the efficiency of nutrient extraction?
3. Can the pathogenicity of leachate be controlled or treated by using routine methods?

To address each of these questions, the specific research objectives are to:

1. Evaluate the compost leachate derived from composts of different age (Chapter 4).
2. Evaluate methods to extract soluble nutrients from different materials (raw manure and mature compost) (Chapter 5).
3. Evaluate methods to eliminate the pathogens in leachate extracted from chicken fresh manure (Chapter 6).

4. Develop a prototype chicken fresh manure nutrients extraction system and evaluate its perfotmance (Chapter 7).

CHAPTER 3: GENERAL METHODOLOGY

3.1 Introduction

A series of laboratory experiments were conducted in order to evaluate the potential for the extraction of soluble nutrients from chicken manure and compost. The first experiment (chapter 4) evaluated the leachate derived from chicken compost of different ages and the second trial (chapter 5) investigated methods to optimise the soluble nutrients extracted from fresh and mature compost. The third experiment (chapter 6) investigated the effect of using different methods to sterilise manure leachate either before or after leaching, on pathogeneses (*E. coli and Salmonella.sp)*. This chapter reports the common methods of analysis and methods used throughout the study.

3.2 Collection of manure and compost samples

The fresh chicken manure was collected from Enviroganics Pty Company in Pittsworth, 55 km west of Toowoomba, Queensland. Engineers obtained the fresh chicken manure when the layer chicken sheds were cleaned out at McLean Farm, Pittsworth where the manure was removed from the sheds weekly and transported to the field. The fresh manure samples were taken prior to treatment, so the fresh manure contained some broken eggs e.g. (membrane and eggshell) as well as pellets (waste food that had fallen out of the feeding apparatus) and feathers. Subsequently, the compost samples were taken at different stages of decomposition.

The first stage of composting involved a mix of 20% sawdust “fine wood shavings” to 80% fresh manure (First week). Other compost samples were collected after 4 weeks; 6 weeks and 18 weeks at (mature compost). All piles were subject to rainfall. In each case, the samples were collected after the pile turning process was completed. The temperature inside each pile was measured by using a 1.2 m length thermometric probe and reports the reading daily. The pile was turned when temperature inside the pile reached or exceeded 70^{o}C. Fig 3-1 shows the average pile temperature during aerobic composting process in classic phases (mesospheric and thermophilic phases). Initially, the temperature in the pile was about 28^{o}C (mesospheric) and after the first week it increased to about 55^{o}C. The thermophilic phase started on day 21 and continued to day 90. Subsequently, the temperature in the compost pile gradually declined with mature compost indicated by temperature after 110 days.

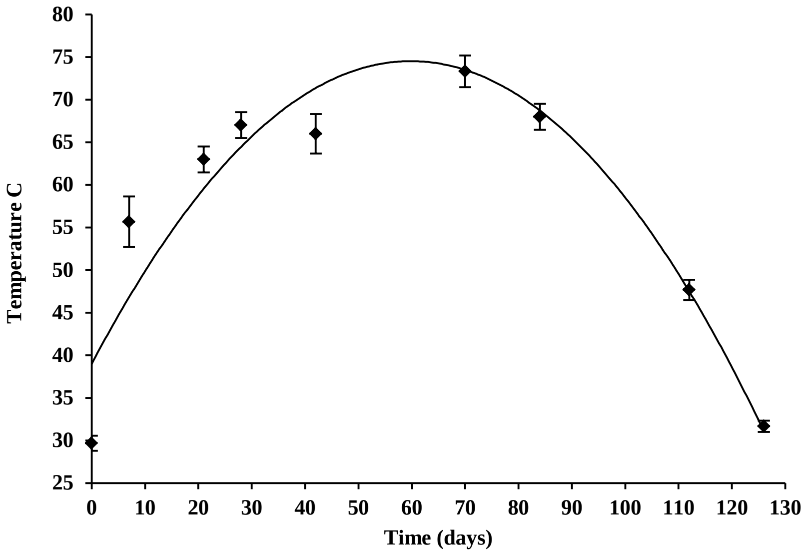

Figure 3-1 Evolution of temperature inside compost pile during composting process. Bars on plotted are standard error of three replicate

The compost pile dimensions were 100 m in length, 2 m in width and 1.5 m in height. The pile was nominally divided into five sections with each section being 20 m in length. Ten subsamples (~1.0 L each) were taken from each section and from both sides of pile (20 subsample per section) and mixed together to make one composite sample (20 L total volume for each section). Approximately 5 L was subsampled from the 20 L for experimental use. The five section samples were bulked in an esky cooler to produce a final sample size for each compost age of approximately 20 L which was transported to the laboratory for storage at < 4^{o}C. The samples were thoroughly homogenised by mixing in a rotary drum and three subsamples (0.5 L) were taken to measure physical and chemical properties. This process was undertaken for each set of experimental columns used in this dissertation.

3.3 Physical, chemical and biological properties of chicken manure and compost

The important physical properties of chicken manure and compost measured in all experiments were moisture content (%w w^{-1}), bulk and particle density (kg m^{-3}), porosity (%v v^{-1}), pore volume (m^3 m^{-3}) and %ash (%w w^{-1}). The chemical properties measured were electrical conductivity EC (dS m^{-1}), pH, total nutrients (mg g^{-1}), soluble nutrients (mg g^{-1}), exchangeable cations (*cmol k*g^{-1}) and cation exchange capacity CEC (*meq* 100^{-1}). The biological properties measured were organic carbon and pathogeneses (*Escherichia coli O157:H7 and Salmonella enterica*).

3.3.1 Moisture content

Determination of moisture content in chicken manure or compost was based on removing the water by oven-drying until constant mass. The moisture content (% w w^{-1}) was calculated from the sample weight before and after drying based on dry and wet weight (Peters et al. 2003, Agnew and Leonard 2003).

A 20 to 40 g (wet weight) of sample (manure – compost) was weighed and put in a clean aluminium container. The sample was placed in the oven at 105°C for 24 hr. After 24 hours, the sample was removed from the drying oven and put in the desiccator to prevent it absorbing humidity from the air. The dry weight was recorded and the moisture content was calculated by using the equation (3-1 and 3-2). This process was replicated three times for each sample. The results were reported based on dry weight.

$$\theta_w\% = \left(\frac{w_w - w_d}{w_w}\right) \times 100 \qquad \textit{Equation 3-1}$$

$$\theta_w\% = \left(\frac{w_w - w_d}{w_d}\right) \times 100 \qquad \textit{Equation 3-2}$$

where: w_w= wet weigh (g), w_d= dry weigh (g)

3.3.2 Bulk density and particle density

The bulk density of manure or compost is the mass of dry material per unit volume (kg m^{-3}). The particle density is the mass of solid material divided by the volume of the solid material (Agnew and Leonard 2003). Bulk density was determined using ASTM standards (Glancey and Hoffman 1996). By using a known beaker mass and volume, the beaker was filled with dry material and reweigh to measure the material mass. The bulk density was calculated using the following equation:

$$BD = \frac{M_{dry}}{V} \qquad \textit{Equation 3-3}$$

where: BD is the bulk density of material (kg m^{-3}), M_{dry} is the mass of dry material (kg), and V is the volume occupied by material (m^3).

Particle density was estimated based on the assumption that organic matter and inorganic solids had specific gravities of 1.55 and 2.65 g cm^{-3} (Haug 1993, Agnew and Leonard 2003). The ash percentage was determined in a muffle furnace based on three replicates of 2.5 g dried at 60° C for 48 hr (Thompson et al. 2001). The percentage of Ash and organic matter (%OM) was calculated using equation (3-3 and 3-4). The particle density was calculated by using equation (3-5).

$$OM\% = \frac{w_i - w_f}{w_i} \times 100 \qquad \textit{Equation 3-3}$$

where: OM is the percentage of organic matter (% w w^{-1}), w_i is initial weight (g) and w_f is final weight (g).

Ash% = 100 – OM% *Equation 3-4*

$$PD = \frac{1}{\frac{OM}{1.55} + \frac{Ash}{2.65}} \qquad \textit{Equation 3-5}$$

where: *PD* is the particle density (g cm^{-3}), *OM (%)* is the percent of organic matter and *Ash* is the present of ash (%)

3.3.3 Porosity and total pore volume

The porosity is the percentage of air and water filled pores (Baker et al. 1998). It is a function of the bulk and particle density (Agnew and Leonard 2003). The porosity was calculated using the following equation:

$$porosity\% = \left(1 - \frac{BD}{PD}\right) \times 100 \qquad \textit{Equation 3-6}$$

where: *BD is* the dry bulk density and *PD* is the particle density.

The total pore volume of the packed columns was calculated by using the following equation:

$$\gamma = \frac{\%porosity}{100} \times volume\ of\ colmun \qquad \textit{Equation 3-7}$$

3.3.4 Total Carbon and Nitrogen

Nitrogen and carbon in manure and compost are in two forms. Organic forms include protein and amino acids and inorganic forms (mineral) including ammonium/ammonia and nitrate compounds or bicarbonate. Total carbon and total nitrogen were analysed using a PrimacsSNC Carbon-Nitrogen/Protein Analyzer (TN/TC) (Skalar, Netherlands). Approximately, 500 mg or less of dry manure/compost was dried at 60^{o}C for 48 hr and then placed in a glass container and burnt at 550^{o}C. The results were expressed as % w.w^{-1} for both total carbon and total nitrogen.

3.3.5 Inorganic elements in material

To determine the total cations (Ca, Mg, K and Na) as well as total P and Cl in the manure and compost, a representative sub-sample was digested at high temperature of 120^{o}C with nitric acid and hydrogen peroxide (Huang and Schulte 1985, Campbellm and Plank 1992). A 0.5-1.0 g (± 0.001) sample of manure or compost, dried at 55^{o}C for 72hr, was digested with 5 mL concentrated HNO_3 (70% w w^{-1}) in Folin-Wu tubes placed in an electrically heated block for 1/2 hr at 60^{o} C. Then 3 mL of Hydrogen peroxide (30% w w^{-1}) was added in 1 mL doses and heated to 120^{o}C for an additional 2hr until the solution became yellow or clear. The solution was moved to a 100mL flask and distilled water added to complete the volume. Determination of the quantities of K, Na, Ca, Mg, was made by using a SHIMADZU Atomic Absorption Spectrophotometer (AAS, AA-7000 connected with auto-sampler). The concentration of total P and Cl was measured by using a DIONEX Ion Chromatography System (ICS-2000), where the flow rate was 1 mL min^{-1} and the run time was 18 min per sample. The results were presented in mg g^{-1} dry material.

3.3.6 Electrical conductivity (EC), pH and soluble elements

Electrical conductivity is a measure of salinity and the pH is a measure of the acidity or alkalinity of the manure/compost. The EC, pH and soluble elements (cations and anions) of samples were measured in 1:10 manure/compost: distilled water basis by mass (Peters et al. 2003).

A 4 g oven dried (±0.01) sample of manure or compost was put into a 50 mL centrifuge tube and 40 mL of distilled water was added. This suspension was then agitated on a shaker for 30 min. The pH of the suspension was measured using a pH-240 pH meter (Radiometer Copenhagen, Denmark). The solution was then filtered using (Whatman no 4) filter paper, and then the EC was measured using a TPS MC-84 EC meter.

The solution was filtered using a 0.45µ fiberglass filter. The cations (Ca, Mg, Na, K) were measured using a SHIMADZU Atomic Absorption Spectrophotometer (AA-7000 connected with auto-sampler); and a DIONEX Ion Chromatography System (ICS-2000), where the flow rate was 1 mL min^{-1} and the run time was 18 min per sample to measure soluble P and Cl.

3.3.7 Cation exchange capacity (CEC)

The CEC was determined for both chicken manure and compost using an adapted form of a method that was described by Chapman (1965). This method was considered applicable due to the pH of manure and compost ranging from 7 to 7.6. The method involved four steps; the first was the saturation of the sample with sodium (Na) by washing it with *1N* sodium acetate (Na-OAC) solution, the second was the removal of excess Na-OAC by washing with alcohol, the third was the removal of Na ions from the surface by washing with *1N* ammonium acetate, and the final step involved measuring the Na concentration in the solution using a SHIMADZU Atomic Absorption Spectrophotometer (AAS-7000 connected with auto-sampler).

A 5 g (±0.01) oven dried (at 60^{o}C) manure /compost sample was put in a 50 mL centrifuge tube and shaken with 33 mL of 1 *M* Na-OAC buffered at pH=7. This action was repeated twice more and then the sample was washed with 33 mL of alcohol three times. 33 mL of *1N* NH_4-OAC was then added to the sample and shaken, with the solution being collected in a 100 mL flask. This action was repeated two more times to collect a total 99 mL and then the volume was increased to 100 mL by adding distilled water. The concentration of Na in the solution was obtained using an AAS. The results were presented as cmol kg^{-1} dry material.

3.3.8 Exchangeable cations

The exchangeable cations in manure and compost samples were determined by the method described in section 3.3.7 except ammonium acetate was used in the first step instead of Na-OAC (Chapman 1965). The results were presented as cmol kg^{-1} dry material. A 5 g oven dried sample was shaken with 33 mL of 1*M* ammonium acetate (buffered at pH 7) and the solution collected. This action was repeated two more times. Total solution collected was 99 mL and 1 mL of distilled water was applied. The solution was filtered and the cations (Ca, Mg, Na and K) were determined using a SHIMADZU Atomic Absorption Spectrophotometer (AAS-7000 connected with auto-sampler).

3.3.9 Pathogens

Many studies have shown that chicken manure contains pathogens such as *Escherichia coli O157:H7* and *Salmonella spp.* In this study, *E.coli* and *Salmonella spp* were identified in the chicken manure/ compost samples by using selective media. The selective media was a solid form which included a substance to inhibit the growth of nonspecific bacteria, while allowing the growth of target bacteria. MacConkey's media is a selective media where the material "cow manure" was adjusted by adding sodium taurocholate (bile salt) to allow the growth of gram-negative bacteria such as *Escherichia coli*, while inhibiting the growth of gram-positive bacteria such as *Salmonella and Shigella* species (Kohare 2008). However, adding tetrathionate broth to a liquid media promotes the growth of gram-positive bacteria *Salmonella spp* and inhibited gram-negative bacteria *Escherichia coli* (Kohare 2008).

MacConkey media plates were created to detect *E coli* by dissolving 25 g of MacConkey Agar in 500 mL of distilled water. The suspension (media) was heated to boiling and stirred gently to dissolve the agar completely. The media was then sterilized by autoclaving at 121^{o}C for 20 minutes at 15 lbs pressure, being careful to avoid overheating. After that the media was cooled to 45 - 50^{o}C and poured into Petri dishes. The surface of the medium was dry when inoculated.

To detect *Salmonella spp* an enriched media was created by suspending 30 g of agar in 500 mL of distilled water. The media was heated to boiling for one minute with frequent agitation to allow for complete dissolution. Care was taken not to over-heat the media, which would have altered its selective properties. Subsequently, the media was cooled to 45^{o} to 50^{o}C and then poured into petri dishes. The petri dishes were storage in a refrigerator at 5^{o}C.

3.3.9.1 Isolation of Escherichia coli and Salmonella spp

To isolate *E* coli and *Salmonella* spp from the fresh manure or compost, the fresh weight equivalent (4 g dry weight) of manure or compost was mixed with 10 mL of sterilised peptone water [0.87% (w v^{-1}) sodium chloride NaCl] in a centrifuge tube. The tube was then placed on a shaker for 30 min and subsequently centrifuged at 1500 rpm for 10 min. The supernatant was then used to inoculate the agar plates.

3.3.9.2 Full Dilution Series and Inoculum E coli and Salmonella spp

The cell count concentration of *E.coli and Salmonella spp* in the leachate or extraction solution from manure or compost were measured from 5-fold serial dilutions, where appropriate of the manure or compost leachate or extraction solution. The procedure of cell count with up to 5-fold dilution series for is summarised by the following steps:

1) Five nutrient agar plates which were prepared for both *E. coli* and *Salmonella spp* left under room temperature.

2) 0.9 mL of the saline water was (3.3.9.1) added to 5 centrifugal tubes with 1.5 mL volume, and the tubes were numbered.

3) 0.1 mL of leachate was added to the first centrifugal tube.

4) After vortexing the mixture from step 3 vigorously, 0.1 mL of the mixture was transferred to tube 2. The same procedure was repeated for the tubes 2, 3, 4 and 5 fold dilutions.

5) After adequate vortexing, 0.1 mL of each tube was added to an agar plate, and then spread using a customised (L) slipped spatula.

6) The prepared plates were incubated at 37°C for 18-24 hours and then counted.

7) The colonies on each plate were counted using Suntex 570 colony counter with electronic register as shown in Figure 3-2. The results of pathogen colony counts were presented in Log_{10} CFU g^{-1} dry material.

Figure 3-2 SUNTEX colony counter

3.4 Characteristic of chicken manure/compost leachate

3.4.1 Measurement of soluble nutrients in leachate

Small particles of dissolved solids were observed in the leachate from both the manure and compost. Thus, a 0.45µ fiberglass filter was used prior to measuring soluble nutrients in the leachate. After the filtration was completed, the leachate was stored at 4^{o}C and the analysis was completed within 72 hours.

Soluble cations (Ca, Mg, K and Na) were measured by using a SHIMADZU Atomic Absorption Spectrophotometer (AA-7000 connected with auto-sampler) while soluble P and Cl were measured by using a DIONEX Ion Chromatography System (ICS-2000), where the flow rate was 1 mL min^{-1} and the run time was 18 min per sample. The results were presented in mg g^{-1} dry material. The resultant measure of soluble nutrients in both manure and compost leachate was presented as cumulative released mass by using the following equation:

$$CR_{aj} = \frac{\left(\sum_{j=n}^{j} C_a \times V\right)}{m} \qquad \textit{Equation 3-8}$$

where: CR_{aj} is the cumulatively released mass of solute species a per unit volume, C_a is the concentration of released solute a in j^{th} water loading event (mg L^{-1}), j is the number of water loading events (j=1, 2, …10), V is the volume of distilled water applied to columns (0.15 L and 0.3 L for 50 and 100 mm columns, respectively) and m is the dry weight (g) of manure /compost in the column.

3.4.2 Pathogens in leachate

The pathogens in chicken manure /compost leachate were detected by using the same media as detailed in the manure and compost section (3.3.9). The leachate was diluted 100 times and 0.1 mL of diluted leachate was inoculated into the agar plates and incubated for up to 48 hr at 37^{o}C. The results were presented in Log$_{10}$ of colonies formation unit (CFU g^{-1}) per dry material.

CHAPTER 4: PRELIMINARY EVALUATION OF LEACHATE FROM CHICKEN WASTE

4.1 Introduction

In recent years there has been a significant increase in the interest and use of chicken compost as a source of plant nutrients. Chicken manure contains a high concentration of essential plant nutrients (Kelleher et al. 2002) and the positive effects on soil chemical, physical and biological properties due to animal manure application are well documented (Shafqat and Pierzynski 2010). However, over-application of chicken manure to agricultural land can lead to various environmental issues: contamination of surface water by phosphorus in runoff (Field, Reneau, and Kroontje 1985, Sharpley, McDowell, and Kleinman 2004); contamination of ground water by nitrate; and release and spread of pathogens (Bitzer and Sims 1988, Moore et al. 1995). Researchers have shown that composting animal manure could reduce hazards to the environment (Eghball and Power 1999, Vervoort et al. 1998).

Loss of nutrients is one disadvantage of the composting process. Nitrogen loss can occur through volatilisation of ammonia (NH_3) which can be significant during the composting process (Ogunwande et al. 2008). Martins and Dewes (1992) found that up to 75% of the total nitrogen present in the material was lost via volatilisation of ammonia. Jiang et al. (2011) reported that 1.5 – 7.3% of initial total nitrogen was lost as N_2O and 32% of total nitrogen was lost as NH_3. Nitrogen losses can also occur through nitrate (NO_3^-) leaching (Eghball et al. 1997a). Seymour and Bourdon (2003) found that nitrate lost by leaching was greater than the amount lost in runoff. However, in a laboratory study, the loss of phosphate in runoff was found to be higher than the amount of phosphate by leaching (Webber et al. 2011).

While it is generally agreed (Van Horn et al. 1995, Polprasert 2007) that composting significantly reduces the pathogen hazard, the fate of pathogens during composting is uncertain due to differing composting processes. For example, the impact of the composting process on pathogens such as *Escherichia coli* O157: 7H and *Salmonella. spp* is not clearly understood due to the wide range of conditions in which composting is performed (Himathongkham and Riemann 1999).

Guo, Labreveux, and Song (2009) reported that a high concentration of plant available and required nutrients were released from poultry litter, which suggests that chicken manure or compost may be a good source of agricultural nutrients. However, contamination and pathogen concerns remain and applying manure or compost in solid form usually means that nutrients are applied at the start of the crop growing season. Application of leachate derived from compost piles, or directly from manure, to agricultural land could increase the efficiency of nutrient recycling due to the ability to apply leachates throughout the season. According to Webber et al. (2011), nutrient losses increase as compost matures. Similarly, the pathogen count is affected by composting period. For example, the number of *E coli* was found to decrease from $\log_{10}$ 7.86 cells g^{-1} to $\log_{10}$ 1.69 cells g^{-1} after 14 day of composting (Larney et al. 2003). Thus, the age of compost may affect leachate quality in terms of both amount of soluble nutrients and pathogens.

This study was conducted to: (1) evaluate the chemical composition of leachate originating from a range of chicken composts ages, and (2) investigate the fate of

Escherichia coli and *Salmonella spp* both in the compost and leachate as affected by the composting process.

4.2 **Methodology**

4.2.1 Description of compost samples

The chicken manure and compost samples were collected as in section 3.2. The compost contained approximately 20 % (v v^{-1}) of sawdust and 80 % (v v^{-1}) of layer chicken manure. For sampling, each pile was nominally divided into five sections of 20 m. Five subsamples were subsequently taken from each section and bulked to provide a representative sample for each section. The samples were collected from three different piles which had been composting for one, six and eighteen weeks respectively. All samples from the same compost pile were mixed together to make a single homogenous compost-age sample before storage at 4^{o}C. Five subsamples of 500 g each were taken for chemical, physical and biological analyses, which were conducted within 72 hr of collection, using the methods outlined in section 3.3.

4.2.2 Column preparation and leaching

Five PVC columns (dimensions: 150 mm height and 87.5 mm inner diameter) were prepared for each age of compost. The bottom of the columns was covered with a rigid plastic net which was attached to the column using tape. A filter paper (Whatman No. 4) was placed on the net to prevent loss of compost. The chicken manure or compost was packed into the column to a depth of 100 mm and the column gently dropped five times from a height of 50 mm to settle the material. The column was then topped up to 100 mm with the appropriate chicken waste and dropped a further five times. The process of topping up and dropping was then repeated a third time. After packing, the average dry bulk density of the week 1 compost was 0.428 g cm^{-3} and for the week 6 and 18 compost was 0.535 g cm^{-3}. A filter paper (Whatman No. 4) was placed on top of the compost.

A series of 10 leaching events were then applied to the top of each column using 300 mL of distilled water per application. Each application represented 1.17 cm^3 of distilled water per g of dry matter (0.62 pore volume) for the week 1, and 0.93 cm^3 of distilled water per g of dry matter (0.67 pore volume) for both the week 6 and 18 treatments, respectively. A total of 3000 mL of distilled water was applied to each column.

During each water application, the leachate from each column was collected in individual plastic containers. The leachate was immediately measured for pH and electrical conductivity (EC) and then stored at <4^{o}C to measure soluble nutrients at a later time. The chemical and biological characteristics of the leachate were measured using the procedures outlined in section 3.3.7 and 3.3.9, respectively. Pathogen testing was undertaken on only the first, second, fifth and tenth event leachates. The cation ratio of soil structural stability (CROSS) of the leachate was calculated using the equation described by Rengasamy and Marchuk (2011):

$$CROSS = \frac{Na+(0.56K)}{\sqrt{\frac{(Ca+(0.6Mg))}{2}}} \qquad \text{Equation 4-1}$$

where: *Na*, *Ca*, *Mg and K* is the concentration (meq L^{-1}) in the leachate.

4.2.3 Statistical analyses

This study was conducted using a complete randomised design where the experiment contained two factors (type of material and leaching events). Data are expressed as the mean of five replicate values for each measured parameter. Statistical analyses were undertaken using Statistical Package for the Social Sciences (SPSS) v19 for Windows 7 (Cramer 2004). Analyses involved univariate ANOVA. Two-Way ANOVA was used to study the effects of interactions between material type and leaching events. One-Way ANOVA was used to study the effect of water application on soluble nutrients concentration in leachate. Least significant difference (LSD) was used to compare the means with a probability level of 5%.

4.3 Results

4.3.1 Manure and compost properties

Table 4-1 shows the changes during composting period in selected chemical, physical and biological properties. The electrical conductivity (EC) varied significantly (P=0.001) over the 18 weeks of composting. The EC of the fresh manure (11.1 dS m^{-1}) was significantly higher than the week 1 manure (7.3 dS m^{-1}) due to the addition of the sawdust in the week 1 manure. However, the EC was then observed to increase to 10.9 dS m^{-1} by week 6 of composting before decreasing to 8.6 dS m^{-1} after 18 weeks of composting. The pH decreased during composting from an initial 8.7 in fresh manure to reach 7.6 after 18 weeks. There were no pathogens observed in the compost after 4 weeks or more of composting (Table 4-1).

Table 4-1 Chemical, biological and physical properties of fresh chicken manure and compost

Compost age (week)	Moisture content* (%)		$EC_{1:10}$ (dS m^{-1})	pH (1:10)	Log CFU g^{-1} dry material	
	-----wet-----	-----dry-----			E.coli	Salmonella
Fresh	52.3^{c} (±2.5)	112.1^{d}(±12.5)	11.1^{c} (±0.9)	8.7^{a} (±0.1)	6.2^{a} (±0.3)	5.0^{a} (±0.1)
1	37.5^{ab} (±0.2)	65.1^{c} (±2.5)	7.3^{a} (±0.2)	8.1^{b} (±0.1)	6.0^{b} (±0.2)	4.7^{a} (±0.1)
4	44.3^{bc} (±0.6)	65.7^{c} (±2.7)	9.2^{b} (±0.3)	8.0^{b} (±0.1)	n.d	n.d
6	42.2^{abc} (±0.2)	59.3^{b} (±4.5)	10.9^{c} (±0.1)	7.8^{c} (±0.1)	n.d	n.d
18	32.6^{a} (±0.5)	48.2^{a} (±1.2)	8.6^{b} (±0.1)	7.6^{c} (±0.1)	n.d	n.d

Standard deviation in brackets
** Moisture content based wet weight and dry weight*
Lowercase superscript shows significant differences within columns.
n.d = below lower limit of detection.

4.3.2 Leachate electrical conductivity (EC) and pH

Both the number of leaching events (i.e. water applied) and the age of compost were found to significantly affect the cumulative total dissolved salts (TDS) extracted from chicken waste (Figure 4-1). Most of the dissolved salts were extracted in the first three leaching events (~3 cm^3 water applied per g^{-1} dry matter) with no significant increase in cumulative TDS observed after 8.2 and 6.5 cm^3 water applied per g^{-1} dry matter to the week 1, and week 6 and 18 compost treatments, respectively (Fig 4-1). The week 1 and 6 compost leachates had similar TDS levels (maximum of

52.7 and 45.5 mg g^{-1}) and were significantly higher than the week 18 TDS levels (maximum 19.7 mg g^{-1}).

The pH of leachate was >8.8 for all compost ages (Figure 4-1). There was generally no significant difference in leachate pH with water applied except for the week 6 compost that increased from 8.8 in the first leaching to >9 for the subsequent leaching events.

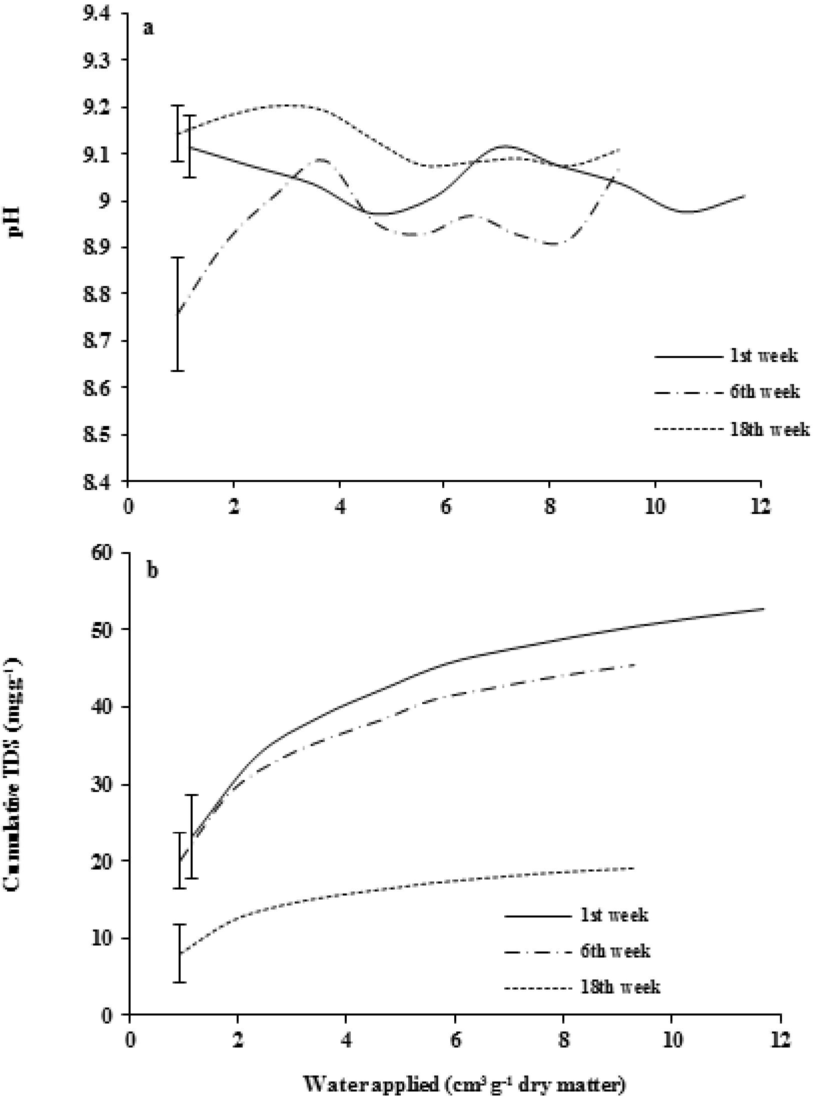

Figure 4-1 Effect of compost maturity and water application on leachate (a) pH and (b) cumulative total dissolved salts (TDS) extracted from chicken waste. Bars on plotted are LSD (α=0.05) to compare water applied within each compost age.

4.3.3 Macronutrients in leachate

4.3.3.1 Nitrogen

Cumulative total dissolved nitrogen (TDN) extracted from chicken waste was significantly affected by both water application and compost age (Figure. 4-2). Most of the TDN had been leached from the week 1 compost after 5.8 cm^3 water applied per g^{-1} dry matter. However, the rate of TDN extraction from the week 6 and 18 compost did not change throughout the ten leaching events (i.e. 9.3 cm^3 of water applied per g^{-1} dry matter). TDN extraction was higher from the week 18 compost (3.5 mg g^{-1}) compared to the week 1 (2.2 mg g^{-1}) and week 6 (1.5 mg g^{-1}) compost. The major forms of TDN were dissolved organic nitrogen (DON) and inorganic nitrogen. The inorganic-N nitrite (NO_2^-) and nitrate (NO_3^-) was found to comprise approximately 20% of the TDN in the week 18 compost leachate compared to approximately 50% and 65% of the week 1 and 6 compost leachates, respectively (Figure. 4-2 b and c). The final concentration of NO_3^- leached was similar between all treatments (approximately 0.6 mg g^{-1}). However, there were significant differences in NO_2^-extracted with the lowest value from the week 18 compost (0.12 mg g^{-1}) and the highest from week 6 compost (0.4 mg g^{-1}).

4.3.3.2 Phosphorus

Compost maturity was found to significantly affect the dissolved phosphorus contained in the leachate (Figure 4-3). Higher phosphorus levels were obtained from week 1 (1.7 mg g^{-1}) and week 18 (1.9 mg g^{-1}) columns compared with week 6 compost leachate which contained a lower level (0.5 mg g^{-1}) of phosphorus. This suggests that phosphorus was being converted to an organic form during the initial stage of composting but then remobilised during the later stages of composting. Most of the phosphorus was leached during the first water application events. There was no significant difference in cumulative phosphorus extracted after 4.65 cm^3 water applied per g^{-1} dry matter to the week 18 compost or after 5.6 and 5.8 cm^3 water applied per g^{-1} dry matter to the week 6 and week 1 compost, respectively (Figure 4-3).

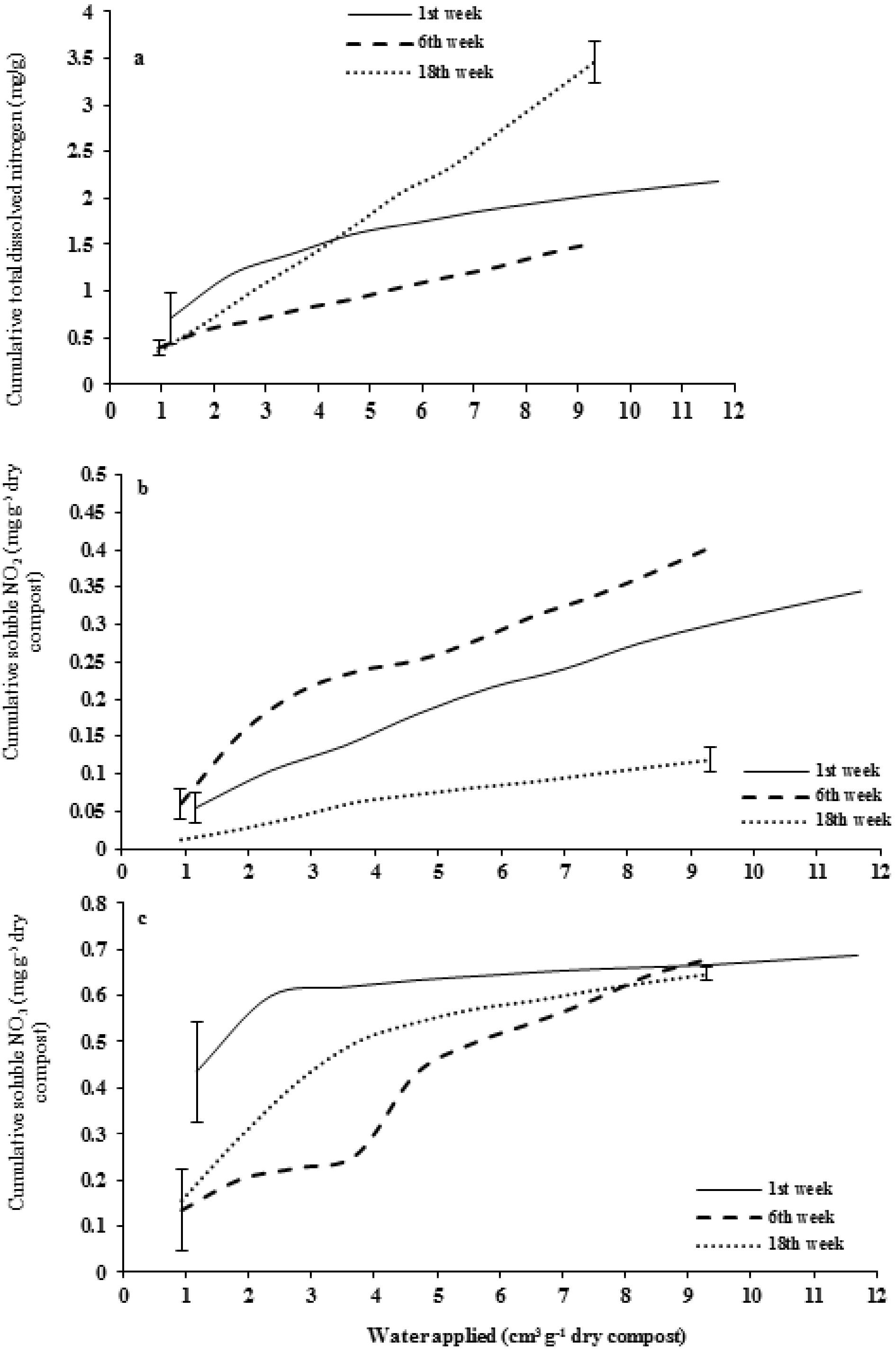

Figure 4-2 Effect of compost maturity and water applied on cumulative (a) total dissolved nitrogen TDN, (b) nitrite and (c) nitrate in compost leachate extracted from chicken waste. Bars are LSD (α=0.05) to compare between leaching events for each compost age.

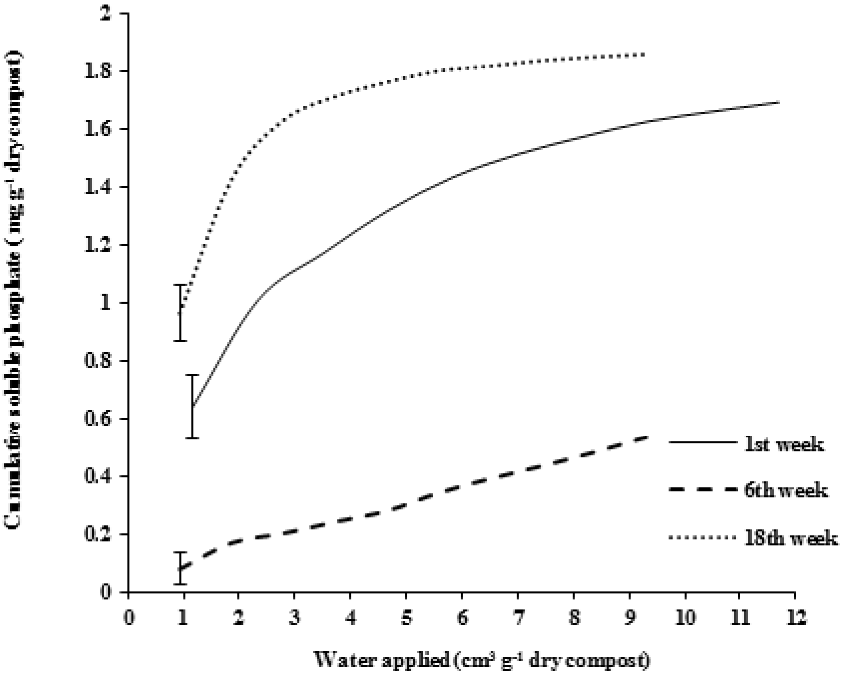

Figure 4-3 Effect of compost maturity and water applied on cumulative soluble phosphate in leachate extracted from chicken waste. Bars are LSD (α=0.05) to compare between leaching events for each compost age.

4.3.3.3 Potassium

The leachate also contained high levels of soluble K (Figure 4-4). The week 1 and 6 compost leachates were not significantly different (~8.3 mg g^{-1}) but did have significantly higher amounts of K than the mature compost leachate (2.9 mg g^{-1}). Most of the K was extracted by the initial water applications with no significant increase in cumulative K extracted after 3.5, 2.8 and 1.9 cm^3 water applied per g^{-1} dry matter to the week 1, 6 and 18 treatments, respectively.

4.3.3.4 Sodium and Chloride

The cumulative sodium (Na) and chloride (Cl) extracted by water application ranged from 0.84 (week 18) to 2.98 (week 6) and from 4.5 (week 18) to 7.29 (week 6) mg g^{-1} dry weight basis, respectively (Figure. 4-5). Increasing water application was found to have a significant impact on the amount of leached Na and Cl. The Cl was leached more rapidly than Na. More than 80% of the maximum Cl was leached from the compost after approximately 2 cm^3 water applied per g^{-1} dry matter to the compost (Figure 4-5). However, approximately 70% of Na was leached after 3 cm^3 water applied per g^{-1} dry matter to the compost.

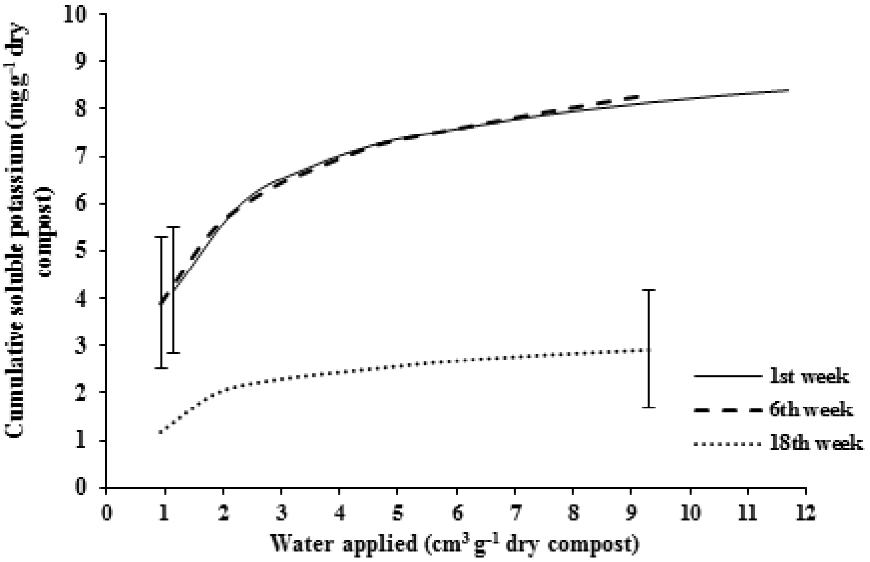

Figure 4-4 Effect of compost maturity and water applied on cumulative soluble potassium (K) in leachate extracted from chicken waste. Bars are LSD (α=0.05) to compare between leaching events for each compost age.

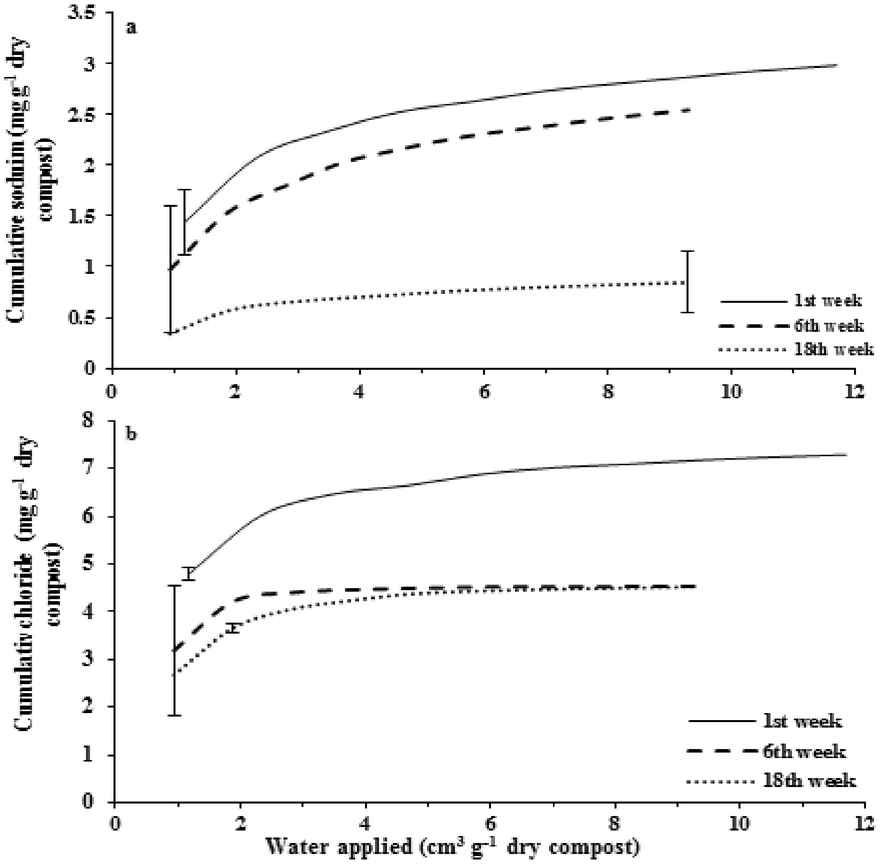

Figure 4-5 Effect of compost maturity and water application on cumulative (a) sodium, and (b) chloride in leachate extracted from chicken waste. Bars are LSD (α=0.05) to compare between leaching events for each compost age.

4.3.3.5 Cation Ratio of Soil Stability

The change in CROSS with increasing water application is presented in (Figure. 4-6). CROSS decreased with water applied for each compost treatment age. Week 1 and 6 compost leachate had significantly higher CROSS (initially ~50) than week 18 leachate (initially ~20) when < 3 cm^3 water applied per g^{-1} dry matter. However, there was no significant difference in CROSS (< 10) between compost treatment ages when > 5.5 cm^3 water applied per g^{-1} dry matter.

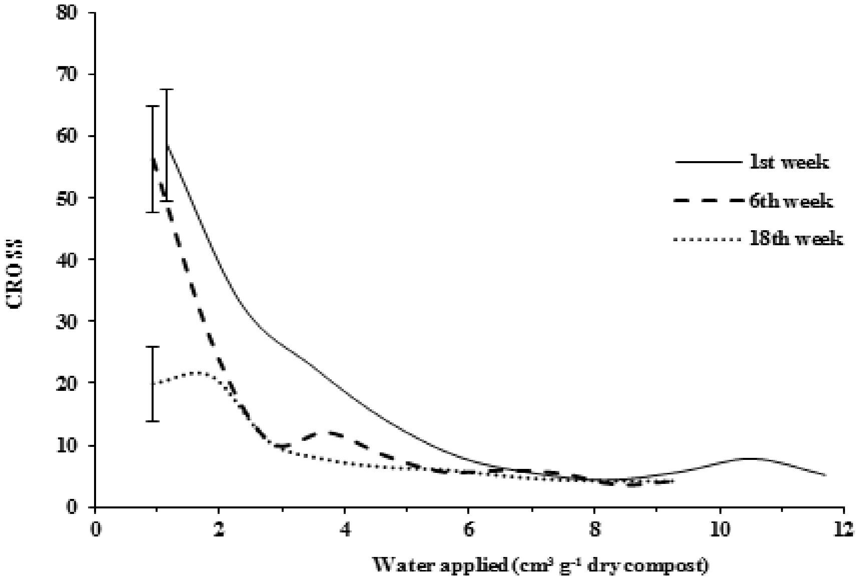

Figure 4-6 Effect of compost maturity and water applied on cation ratio of soil stability of leachate extracted from chicken waste. Bars are LSD (α=0.05) to compare between leaching events for each compost age.

4.3.4 Pathogens in leachate

The population of *Escherichia (E.coli O157: H7*) and *Salmonella ssp* in leachates obtained from the fresh manure and compost treatments is presented in Table 4-1. The number of colonies decreased significantly (*P= 0.001*) after one week of composting. *E.coli* and *Salmonella ssp* colonies were observed in fresh manure and week 1 leachate while no pathogens were detected in the week 4, 6 and 18 compost (Table 4-1) or leachates.

Increasing the water applied to the columns was found to significantly decrease the number of *E.coli* colonies in fresh manure and week 1 compost leachate (Figure. 4-7a). The number of *Salmonella ssp* colonies also decreased significantly in the fresh manure treatment with increasing water applied (Figure. 4-7b). However, there was no significant difference in *Salmonella spp* colonies with increasing water application to the week 1 compost treatment (Figure. 4-7b).

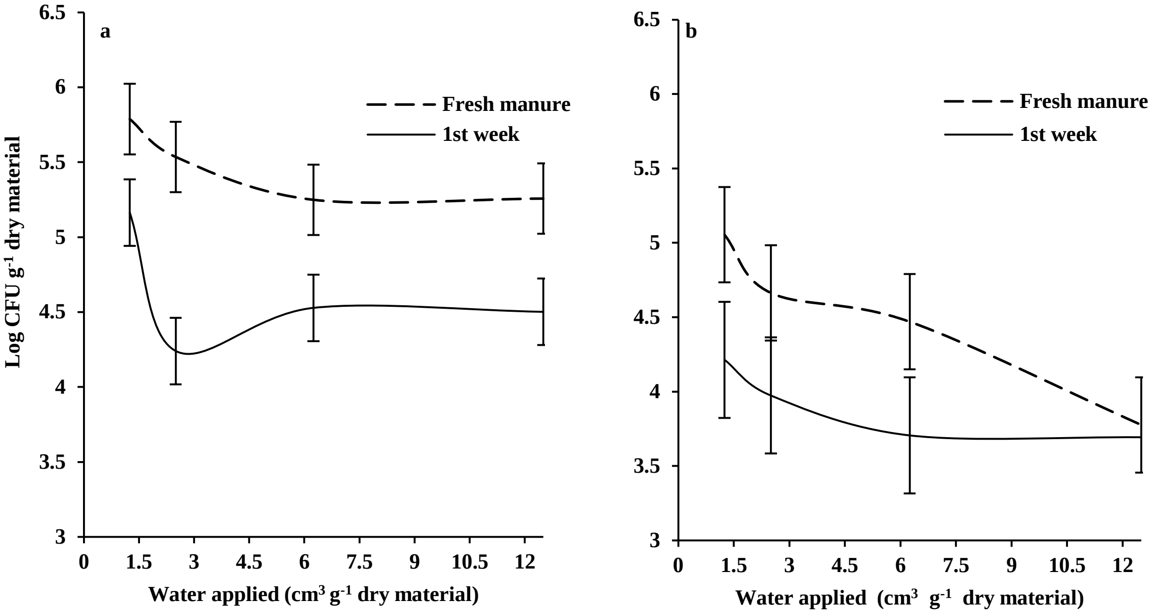

Figure 4-7 Effect of compost maturity and water applied on number of colonies of (a) E coli, and (b) Salmonella spp in leachate extracted from chicken waste. Bars are LSD (α=0.05) to compare between leaching events for each compost age.

4.4 Discussion

4.4.1 Effect of compost age and nutrient in leachate

This preliminary experiment has shown that the leachate from chicken compost contains relatively high soluble ions. However, the concentration of ions in the leachate varies with compost age and amount of water applied. The variations in leachable ions with compost age were consistent with microbial mineralisation and immobilisation processes (Bernal et al. 1996). Also, Sommer and Dahl (1999) suggested that turning compost piles increased microbial activity, which could enhance immobilisation of nitrogen. In particular, TDN and phosphate decreased in the leachate from week 1 to week 6 as microbes immobilised these soluble ions. However, subsequent mineralisation produced both TDN and phosphate levels that were higher in the mature compost leachate than the week 1 leachate. In contrast, the concentration of ions which were not consumed by microbes in large quantities (e.g. K^+ and Na^+) and are highly mobile (Cl^-) generally decreased as the age of compost increased due to leaching from the pile (Pittaway 2002). This is consistent with Milligan, Bomke, and Temple (2008) who reported that the losses of K and Na during composting were around 75% and 80% respectively, and between 2% and 3% for calcium and magnesium, respectively.

4.4.2 Effects of compost age on pathogens in leachate

The composting process significantly reduced the number of *E.coli* and *Salmonella.ssp* colonies in the leachate with no pathogens detected in the week 4 and subsequent columns. This is consistent with other studies showing that well-constructed composting with elevated pile temperature kills pathogens. The temperature of the compost pile exceeded 70^o C after 2 weeks of composting (Fig. 3-1) due to aerobic microbial activity. The increase in temperature in the pile was due to respiration by microbes (Tiquia, Tam, and Hodgkiss 1996). *E.coli* and *Salmonella ssp* populations in animal manures are commonly affected by temperature increase during aerobic composting. Larney et al. (2003) showed that the number of *E.coli* in beef cattle manure reduced from $\log_{10}$ 7.86 cells g^{-1} dry weight to $\log_{10}$ 1.69 cells g^{-1} dry weight during 14 days of windrow composting. Similarly, Hassen et al. (2001) found that *Salmonella* and *E.coli* populations were affected by compost temperatures 55 – 65°C.

4.4.3 Effect of extraction volume on leachate

Increasing the volume of water applied generally increased the cumulative quantity of ions extracted. The extraction rates of the various ions differed potentially due to exchange interactions with charged surfaces, differing solubility of the solid forms, interactions with other ions to form complexes and diffusion processes between pore flow paths. In general, the efficiency decreased rapidly as leaching volume increased after 3 – 6 cm^3 of water was applied per g^{-1} dry matter. In these cases the ions are most likely mainly in solution and there is little exchange reserve or solubilisation of solid forms. However, for some treatments (e.g. TDN from week 6 and 18) the rate of extraction is constant over the 10 leaching events suggesting that exchange processes or solubilisation play a more significant role. The change in CROSS with water applied (Figure 4-6) also confirms that there are differences in the relative rate of specific ion extraction, presumably due to exchange processes.

4.5 Conclusion

This study has shown that compost age significantly affects the concentration of soluble ions in the compost leachate. In general, the week 1 and 6 compost leachates were found to have higher concentration of soluble macronutrients ions compared with the week 18 compost. However, the week 1 compost also had higher levels of sodium and chloride than the week 18 compost. The number of *E coli* and *Salmonella spp* in the week 1 leachate was also high; thus, the use of leachate from week 1 compost may only be viable if treated to reduce CROSS and eliminate pathogens. On the other hand, both the 6th and 18th week compost leachates were pathogen free. While the concentrations of soluble nutrients in the week 6 and 18 compost leachates were lower than the week 1 leachate, they could be used to supplement nutrients applied via chemical fertilisation.

Additional experiment work is required to optimise the design and management of systems to extract soluble nutrients from chicken waste. Similarly, if fresh waste or compost of <4 weeks age are used, additional research is required to produce a pathogen free leachate.

CHAPTER 5: FACTORS AFFECTING NUTRIENT EXTRACTION FROM CHICKEN WASTE

5.1 Introduction

Brentnall (2008) suggests that the future supply of mineral fertilisers will be insufficient to sustain the required growth in production given current usage rates. Demand for nitrogen, phosphorus and potassium is forecasted to increase by about 2.5% per year on average to 2020 (Heffer and Prud'homme 2010), which will impact on fertiliser prices. Also, due to the limited availability of phosphate reserves, it is critical to ensure that phosphorus is recycled to a large extent (Herring and Fantel 1993, Weikard and Seyhan 2009, Dawson and Hilton 2011). Thus, it is apparent that unconventional sources of fertiliser, such as chicken manure, will provide a valuable source of plant nutrients and significantly contribute to the mitigation of demand for mineral fertilisers. Gross et al. (2008) indicated that total nitrogen and phosphate (P2O5) content in chicken manure is typically about 4%, and 1.5% to 2.5%, respectively, while DEFRA (2010) showed that the total and available nutrients in poultry manure make chicken manure a worthwhile nutrient source for agricultural use (Table 2-1).

As the price of urea is US $310 per ton (FAO 2012), and the amount of nitrogen produced around the world about 4.34 million ton per year, the urea-based price of nitrogen is around $670 per ton. Therefore, approximately $2.9 billion per year is lost worldwide due to a failure to capitalise on chicken manure nutrients.

To decrease environmental issues, such as excessive nitrogen leaching (Bitzer and Sims, 1988), phosphorous runoff (Kingery et al., 1994, Moore et al., 1995), spread of pathogens (Chapter 4) and production of plant phytotoxic substances (Kelleher et al., 2002) due to direct application of chicken manure , alternatives to frontloading of chicken manure as a cropping system organic fertiliser are sought. One possible solution is controlled application of manure nutrients in liquid form. The application of animal manure through irrigation systems has been shown to encourage crop health, provide nutrients and reduce the need for pesticide application (Alcantara and dela Cruz, 2005). Different strategies of applying manure/compost as liquid have been used, including manure/ compost tea or water extract which is used to suppress soil-borne diseases (Scheuerell and Mahaffee, 2004, Santos et al., 2011). However, these methods typically use processes that are only viable on a small-scale. Thus, there is benefit in developing a method that considers the efficacy of nutrient leaching with the intention for use on a commercial scale.

Further consideration is that Chapter 4 showed compost age to affect nutrient leaching whereby nutrients that are known to be mineralised or immobilised by microbes were decreased (Bernal et al. 1996; Sommer and Dahl 1999) and those not associated with such processes were likely lost due to leaching during the composting process (Pittaway 2002; Milligan et al. 2008). Chapter 4 also found that the nutrient concentration was a function of the volume of water applied. Hence, this current chapter reports on an experiment to investigate the factors affecting nutrient extraction efficiency, with the specific objectives of: (a) evaluating the effect of column length and (b) investigating the effect of packing density of chicken waste columns on nutrient extraction.

5.2 Materials and Methods

5.2.1 Manure and compost sampling

Samples of fresh chicken manure (FCM) and mature chicken compost (MCC) were collected as in section 3.2. Individual samples (fresh manure and mature compost) were homogenised using a rotary drum and five representative sub-samples (approximately 500 g each) were taken for analysis of physical and chemical properties.

5.2.2 Chemical and physical properties

The chemical and physical properties were characterised using the methods outlined in section 3.3. The organic matter (OM; % w w^{-1}) and ash (% w w^{-1}) were determined on three replicates in a muffle furnace using the method of Thompson et al. (2001). The electrical conductivity (EC) and the pH were determined as outlined in section 3.3.6. The cation exchange capacity (CEC) was determined using ammonium acetate as described by Chapman (1965) in (Hendershot, Lalande, and Duquette 2007). Exchangeable cations were determined using 1 *M* of ammonium acetate (section 3.3.7). The concentrations of total and soluble inorganic elements (N, P, K, Ca, Mg, Na and Cl) were determined as described in sections 3.3.4 to 3.3.6. The moisture content, particle density (D_p), total porosity and pore volume (η), were calculated as described in section 3.3.1 to 3.3.3.

5.2.3 Column preparation and extraction of soluble nutrients

The chicken manure or compost was packed into PVC columns (87.5 mm inner diameter) of two lengths (50 and 100 mm), as made in the preliminary study (Chapter 4) and three packing densities (0.2, 0.3 and 0.4 g cm^{-3} for fresh manure and 0.4, 0.5 and 0.6 g cm^{-3} for mature compost). To avoid excessive compaction, the materials were progressively compacted to the treatment bulk density in 25 mm segments using a steel compaction dolly. Filter papers (Watman No. 45), mesh and PVC caps were placed on both ends of the columns to avoid loss of material.

5.2.4 Saturated hydraulic conductivity and collection of leachate

Saturated hydraulic conductivity ($KSat$) was determined using the constant head method (Klute 1965) under controlled laboratory conditions at 20°C. Inverted plastic bottles were used to apply a constant water head of 50 mm to the top of each column. The outflow was captured in plastic containers of 0.5 L capacity placed at the bottom of each column. The outflow leachate was sequentially captured in 150 mL (50 mm column) and 300 mL (100 mm column) for separate analysis. These sequential increment will be referred to as leaching events numbered sequentially from one to ten (LE_1 to LE_{10}), respectively. Therefore, the total volume captured as leachate was 1500 mL and 3000 mL for each of the 50 and 100 mm columns, respectively. Table 5-1 shows the amount of water applied per unit mass of chicken waste for each column length and packing density.

Table 5-1 The amount of water applied per unit of mass of chicken waste (cm^3 g^{-1} dry matter) for each column length and packing density.

Column Length (mm)	**Volume applied per event (cm^3)**	**Bulk density (g cm^{-3})**				
		0.2	**0.3**	**0.4**	**0.5**	**0.6**
50	150	2.5	1.7	1.2	1	0.8
100	300	2.5	1.7	1.2	1	0.8

A leachate efficiency index (LEI) was calculated by applying the following equation (Carrión et al. 2004):

$$LEI = 100 \times \left(1 - \frac{x}{x_0}\right) \qquad \textit{Equation 5-1}$$

where: x = ion concentration (mg L^{-1}) in material (manure/compost) after leaching, x_0 = ion concentration (mg L^{-1}) in material before leaching.

The chemical characteristics of the leachate were measured using the procedures outlined in section 3.3.7 and 3.3.9, respectively. The cation ratio of soil structural stability (CROSS) of the leachate was calculated using the equation described by Rengasamy and Marchuk (2011):

$$CROSS = \frac{Na + (0.56K)}{\sqrt{\frac{(Ca + (0.6Mg))}{2}}} \qquad \textit{Equation 5-2}$$

5.2.5 Experimental design and statistical analyses

The study was conducted using a complete randomised design where the experiment contained three factors (packing density, column length and water applied). Data are expressed as means of three replicates for each parameter measured. Statistical analyses were undertaken using the Statistical Package for the Social Sciences (SPSS) v19 for Windows 7 (Cramer 2004). Analyses involved univariate ANOVA. Two-way ANOVA was used to study the effects of both bulk density and column length through each water application and one way ANOVA was used to study the effect of leaching events for each value of packing density and column length. Least significant difference (LSD) was used to compare the means with a probability level of 5%.

5.3 Results

5.3.1 Chemical and physical properties of chicken waste materials

Selected properties of both the fresh chicken manure and mature chicken compost are used in the trial shown in Table 5-2. There were significant differences in the chemical properties of the fresh and composted materials due to both the addition of sawdust and the composting processes. In general, the nutrient leached was lower in the compost than in the fresh waste. Total nitrogen and potassium were ~ 50% lower in the compost while total phosphorus was 35% lower and magnesium 25% lower.

However, calcium was higher in the compost presumably due to high concentration of calcium in the sawdust. The soluble ion concentrations were all lower in the compost due to rainfall and irrigation pile where nitrogen lost in form nitrate by leaching and phosphorus lost in runoff from the compost pile. However the cations exchange capacity was higher in the compost. Total carbon was lower in the compost as compared to the manure.

*Table 5-2 Selected properties of chicken waste**

Parameter	Fresh manure	Mature compost	Difference (%)
	Value	Value	
Moisture content (% w w^{-1})	65.7 (± 0.3)	36.4 (±0.85)	-
Total carbon (% w w^{-1})	57.5 (± 0.2)	28.5 (±0.1)	-50.4
Total Nitrogen (% w w^{-1})	8.6 (± 0.8)	4.5 (±0.1)	-47.8
Total Phosphorus (mg g^{-1})	82.4 (± 2.1)	53.2 (±3.7)	-35.3
Total Potassium (mg g^{-1})	79.2 (± 3.1)	40.2 (±1.1)	-49.3
Total Calcium (mg g^{-1})	30.6 (± 5.9)	55.6 (±0.9)	81.8*
Total Magnesium (mg g^{-1})	26.7 (± 2.2)	19.8 (±4.9)	-25.9
Total Sodium (mg g^{-1})	15.2 (± 0.7)	11.0 (±0.3)	-27.9
Total Chloride (mg g^{-1})	76.2 (± 8.5)	34.2 (±12.3)	-55.1
pH(1:10)	8.8 (± 0.02)	7.5 (±0.02)	-
EC $_{(1:10)}$ (dS m^{-1})	11.8 (± 0.3)	6.2 (±0.01)	-47.5
Soluble ions			
Potassium (mg g^{-1})	46.7 (± 0.7)	28.4 (±0.2)	-39.2
Calcium (mg g^{-1})	1.1 (±0.02)	0.9 (±0.1)	-18.2
Magnesium (mg g^{-1})	5.6 (± 0.9)	1.2 (±0.2)	-78.6
Sodium (mg g^{-1})	3.8 (± 0.1)	2.3 (±0.1)	-39.5
Chloride (mg g^{-1})	7.4 (± 0.3)	5.3 (±0.01)	-28.4
Phosphate (mg g^{-1})	13.3 (± 1.3)	7.9 (±0.2)	-40.6
Sulfate (mg g^{-1})	0.3 (±0.02)	>0.01	-96.7
Cation Exchange Capacity (CEC) (cmol kg^{-1})	47.4 (±1.3)	330 (±6.7)	556.2
Exchangeable Cations			
Potassium (cmol kg^{-1})	10.5 (±0.3)	5.3 (±0.2)	-49.5
Calcium (cmol kg^{-1})	55.8 (±1.7)	101.4 (±3.1)	81.7
Magnesium (cmol kg^{-1})	12.7 (±0.4)	9.4 (±0.3)	-26.0
Sodium (cmol kg^{-1})	6.4 (±0.2)	4.6 (±0.1)	-28.1

*based on dry weight.

5.3.2 Effects of compost packing density and column length on hydraulic conductivity

The Saturated hydraulic conductivity (K_{sat}) of the fresh manure was generally higher than the mature compost K_{sat} (Figure 5-1 a). In all cases, the K_{sat} was initially high but decreased rapidly as more water was applied (Figure 5-1). There was generally a significant difference in K_{sat} after 10, 6.8 and 4.8 cm^3 water applied per g^{-1} dry matter to 0.2, 0.3 and 0.4 g cm^{-3} fresh manure columns, respectively. Saturated hydraulic conductivity significantly (P<0.05) decreased with increasing packing density (R^2 = -0.88), which was observed for all materials and column lengths. The final K_{sat} for the low packing density (0.2 g cm^{-3}) fresh manure treatment was significantly higher (3.3 mm hr^{-1}) in the 50 mm column compared with (2.3 mm hr^{-1}) in the 100 mm column. For the fresh manure, there was no significant difference (P=0.453) in the K_{sat} for the 50 and 100 mm columns and the interaction of column length × packing density was not significant (*P-value* =0.073) (Figure 5-1 a and b).

The K_{sat} of the mature compost was affected (P<0.01) by both packing density and column lengths. For example, the packing density for 0.4 to 0.6 g cm^{-3} in the 50 mm column decreased the initial K_{sat} from 561.2 mm hr^{-1} to 349.3 mm hr^{-1}. Similarly increasing the column length generally reduced the $K_{sat.}$ However, the K_{sat} observed for the 0.4 g cm^{-3} in 100 mm column length treatment was significantly lower than for the 0.5 and 0.6 g cm^{-3} treatments (Figure 5-1 d). It is not clear why this occurred. However, it was also noted that the packing densities of the 0.4, 0.5 and 0.6 g cm^{-3} in the 50 mm mature compost columns increased to 0.67, 0.625 and 0.66 g cm^{-3} treatments; respectively after 1.2, 1 and 0.8 cm^{3} g^{-1} dry matter of water was applied to the columns. Similarly, the bulk densities of the 0.4 and 0.5 g cm^{-3} treatment in the 100 mm columns were observed to increase to 0.73 and 0.58 g cm^{-3}, respectively. However, there was no change in the bulk density of the 0.6 g cm^{-3} mature compost treatment in the 100 mm column.

5.3.3 Effect of bulk density and column length on total dissolved salts

The effect of column length on the rate of TDS extraction varied between the two chicken waste materials. Increasing the column length in the fresh manure generally reduced the rate of TDS leaching. For example the cumulative TDS after applying 12.5 cm^{3} water applied per g^{-1} dry matter to the 0.4 g cm^{-3} 50 mm column was 139.6 mg g^{-1} but was only 77.1 mg g^{-1} where the same percolating volume was applied to the 0.4 g cm^{-3} 100 mm columns. However, the effect on TDS of increasing column length was less evident as density decreased with no significant difference in TDS extraction observed between the 0.2 g cm^{-3} treatments.

Increasing the column length for the mature compost significantly increased the rate of TDS extraction for all bulk densities (Figure 5-2 c, d). In all cases, there was no significant increase in TDS extracted after ~ 6 cm^{-3} g^{-1} dry matter of water had been applied. However, increasing the column length for the 0.6 g cm^{-3} density treatment increased the cumulative TDS extracted after 6 cm^{3} water applied per g^{-1} dry matter from ~ 33.8 to 61.5 mg g^{-1}. Similar, results were obtained for the 0.4 and 0.5 g cm^{-3} treatments.

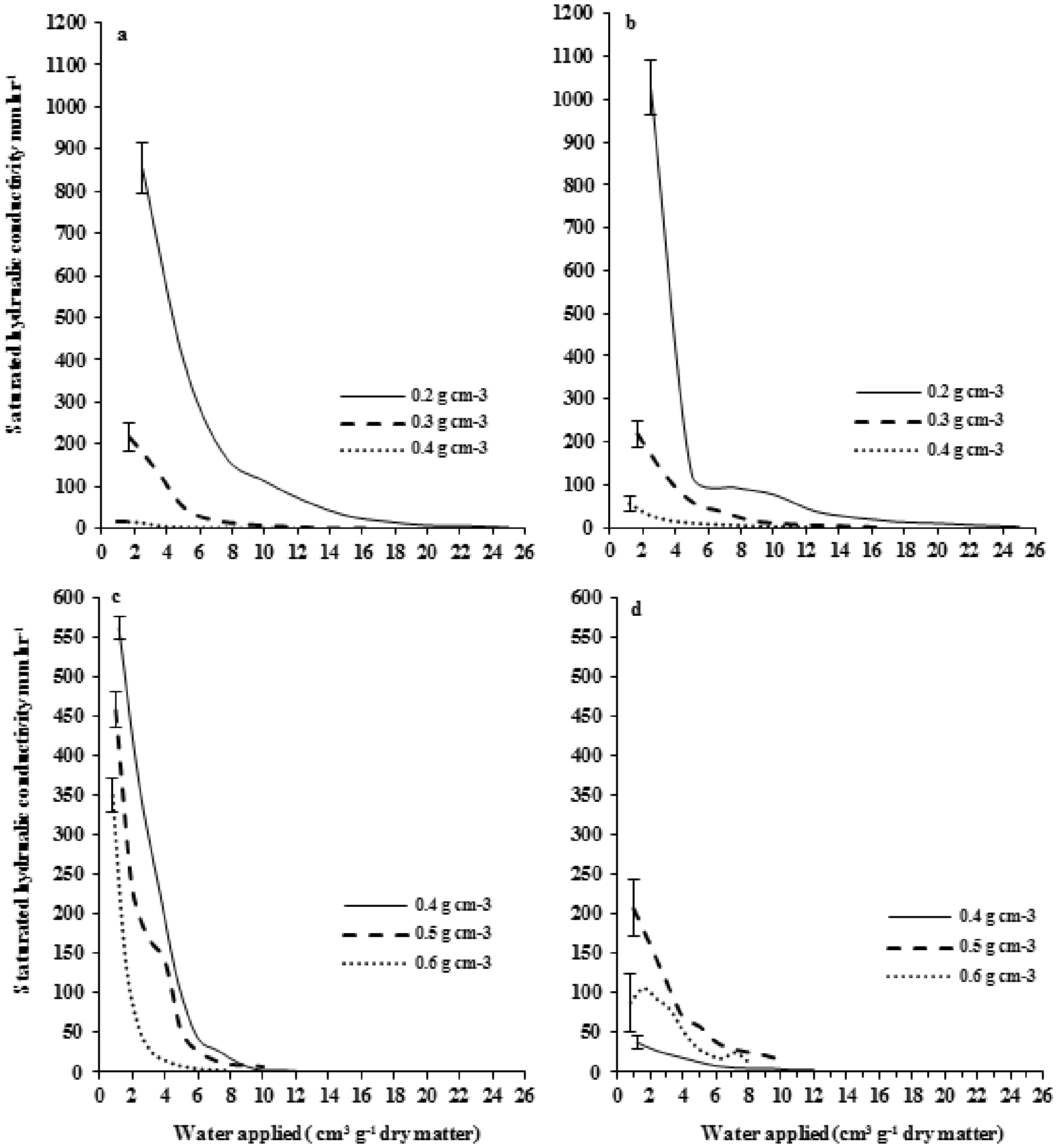

Figure 5-1 The effect of packing density of chicken manure on saturated hydraulic conductivity in (a) 50 mm column and (b) 100 mm columns of fresh manure, and (c) 50 mm and (d) 100 mm columns of mature compost. Bars are LSD (α=0.05) comparing water applied for all packing density.

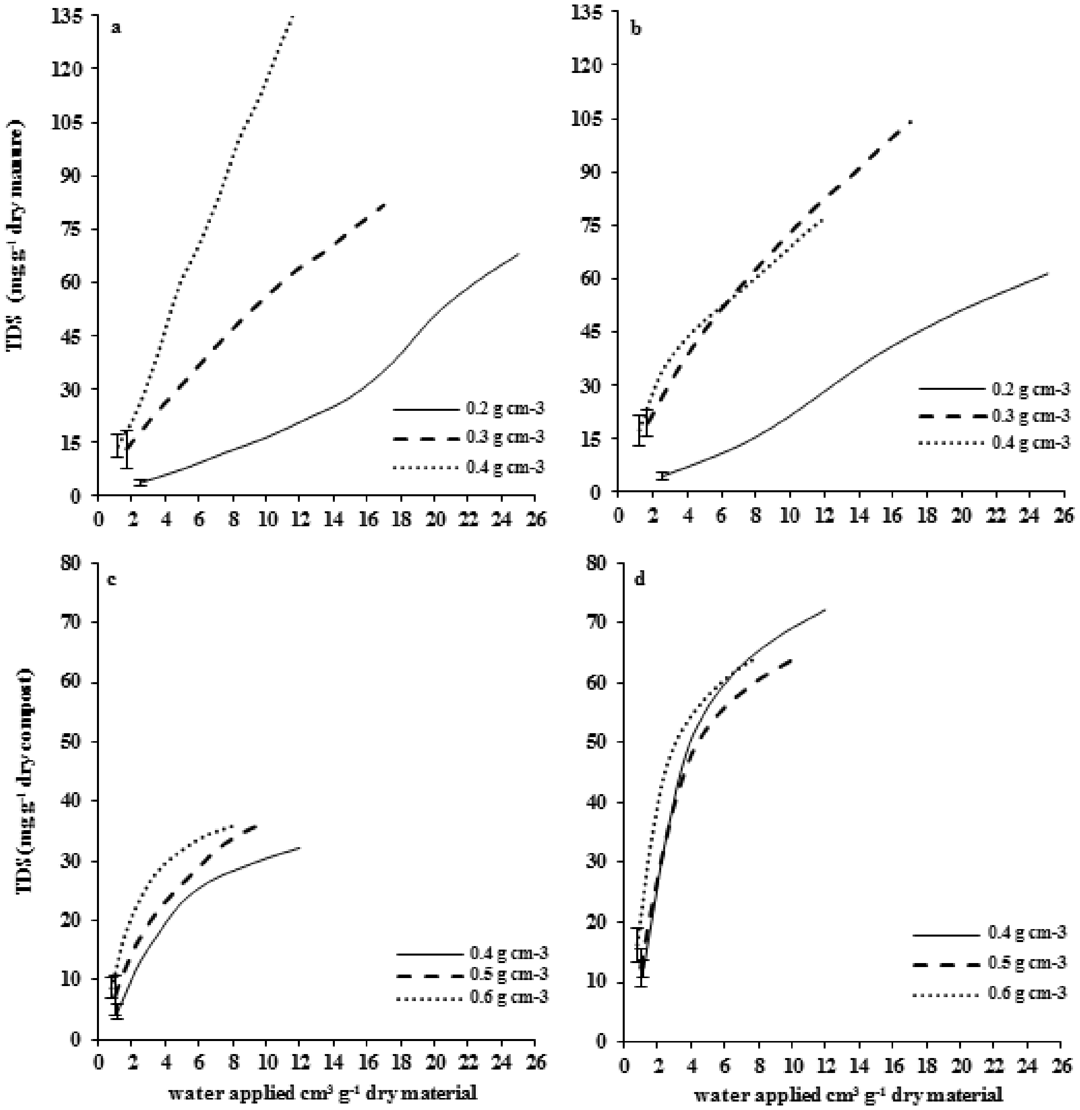

Figure 5-2 The effect of packing density on cumulative total dissolved salts (TDS)extracted from chicken manure in (a) 50 mm and (b) 100 mm columns, and mature compost in (c) 50 mm column and (d) 100 mm columns. Bars are LSD (α = 0.05) comparing water applied for each packing density.

5.3.4 Effects of bulk density and column length on ions extraction

5.3.4.1 Nitrogen

Total dissolved nitrogen (TDN) extraction was generally higher from fresh manure than compost (Figure 5-3). The packing density also significantly affected the TDN in the leachate extracted from chicken waste (Figure 5-3). The highest cumulative TDN (15.1 mg g^{-1}) was extracted from the 100 mm fresh manure column packed at 0.4 g cm^{-3} after 12 cm^3 water applied per g^{-1} dry matter.

Increasing the packing density was found to have a large significant effect on TDN extraction in the fresh manure but produced smaller (and often non-significant) increase in the mature compost. The higher density in fresh manure (0.4 g cm^{-3}) produced high TDN in both column lengths (Figure 5-3 a, b). However, the column length had little significant effect on TDN in both fresh manure and mature compost treatments.

Increasing water application significantly affected the cumulative TDN in all treatments (Figure 5-3). Cumulative TDN shows a sign of plateauing after 6.4 to 9.6 cm^3 g^{-1} dry matter of water was applied in both the 50 and 100 mm mature compost columns. However, this effect was less evident for the 50 and 100 mm fresh manure columns where the change in cumulative TDN was significant until 12 to 25 cm^3 water applied per g^{-1} dry matter.

Leaching efficiency index (LEI) of nitrogen was significantly higher in long columns for fresh manure than short columns (Figure 5-4). The highest LEI for N (98.6%) was found for the 100 mm column at bulk density 0.4 g cm^{-3}. However, the short columns were significantly more efficient to extract N than long column in mature compost (Figure 5-4). There was no significant effect on LEI for N due to increasing packing density in long columns for either material. However, increasing the density in the short columns for the fresh manure did significantly increase the LEI for N (Figure 5-4 a).

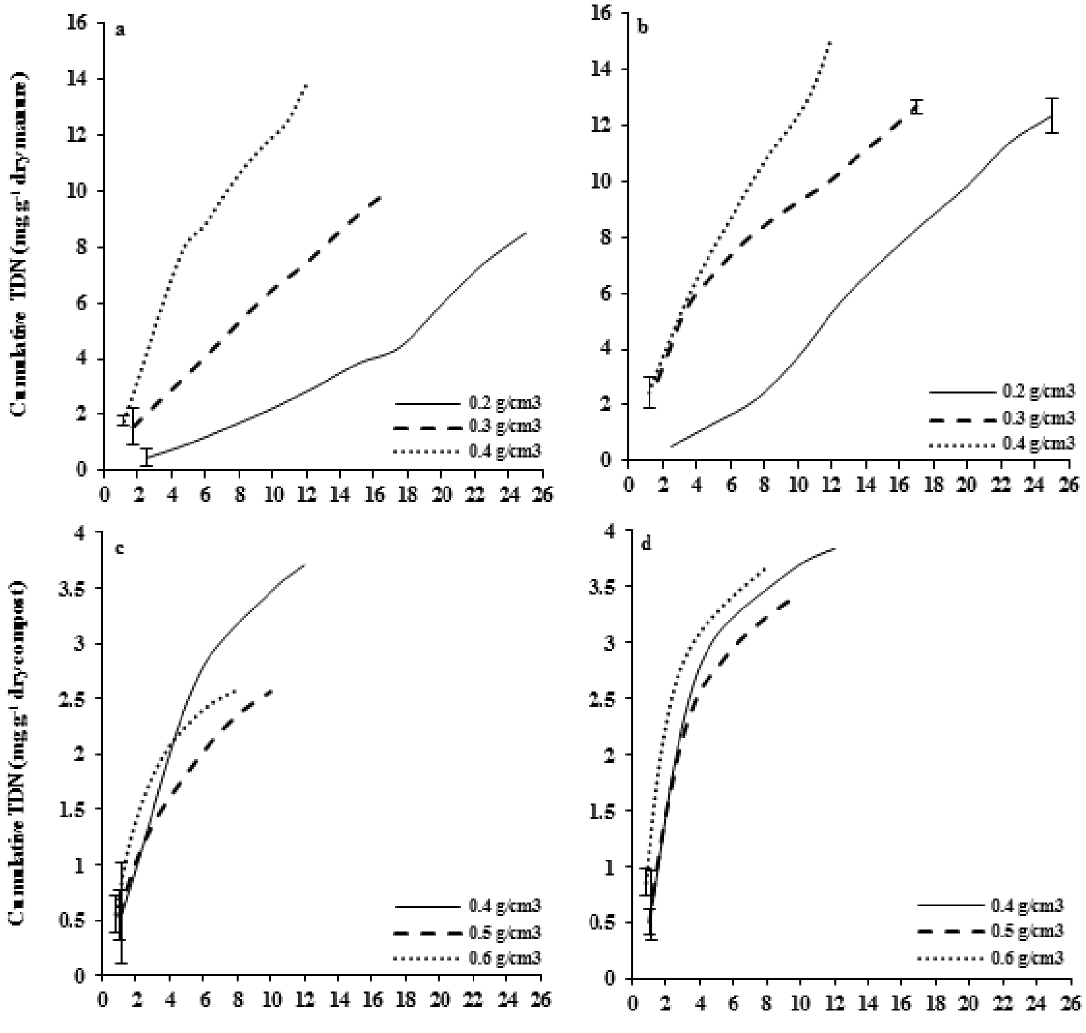

Figure 5-3 The effect of packing density on cumulative total dissolved nitrogen (TDN) extracted from chicken manure in (a) 50 mm and (b) 100 mm column, and mature compost in (c) 50 mm column and (d) 100 mm column. Bars are LSD (α = 0.05) comparing water applied for each packing density.

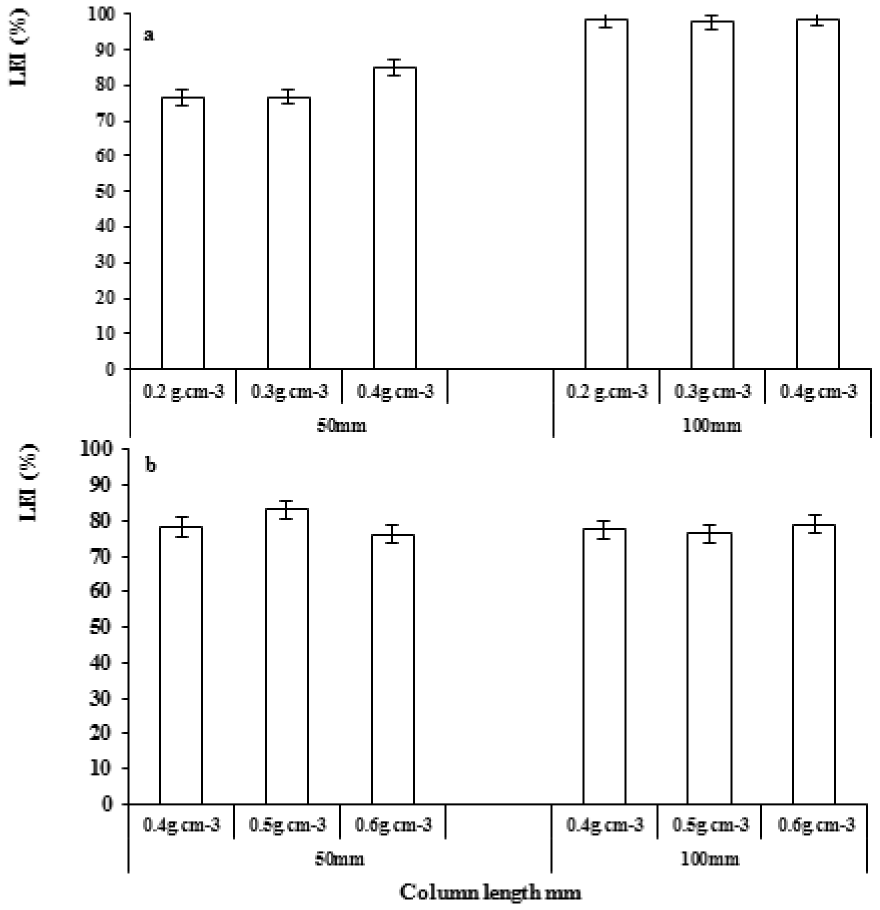

Figure 5-4 Effect of column length and packing density on leaching efficiency index for total dissolved nitrogen in (a) fresh manure and (b) mature compost. Bars are LSD (α=0.05) comparing with treatments.

5.3.4.2 Phosphorus

Soluble phosphorus (P) extraction was generally higher from the fresh waste than from the mature compost (Figure 5-5). The highest cumulative soluble P (13.2 mg g^{-1}) was extracted from the 100 mm fresh manure column at density (0.4 g cm^{-3}) compared to the mature compost (5.3 mg g^{-1}) (Figure 5-5 b, d). Both packing density and column length had a significant effect on soluble P extraction (Figure 5-4). Increasing packing density generally increased the soluble P in leachate extracted from both materials. However, the effect was greater in the fresh manure than in the mature compost.

The column length affected soluble P extraction in both materials. For fresh manure, there was no significant difference in soluble P extraction between the 50 and 100 mm columns when packed at either 0.2 or 0.3 g cm^{-3} (Figure 5-5 a and b). However, when packed at 0.4 g cm^{-3}, increasing the column length increased the soluble P extraction for the mature compost (Figure 5-5 c and d). There was no significant effect of column length on soluble P extraction after 2.4 to 3.6 cm^3 water applied per

g^{-1} dry matter. However, significantly more soluble P was extracted from the 100 mm columns than the 50 mm when more water was applied.

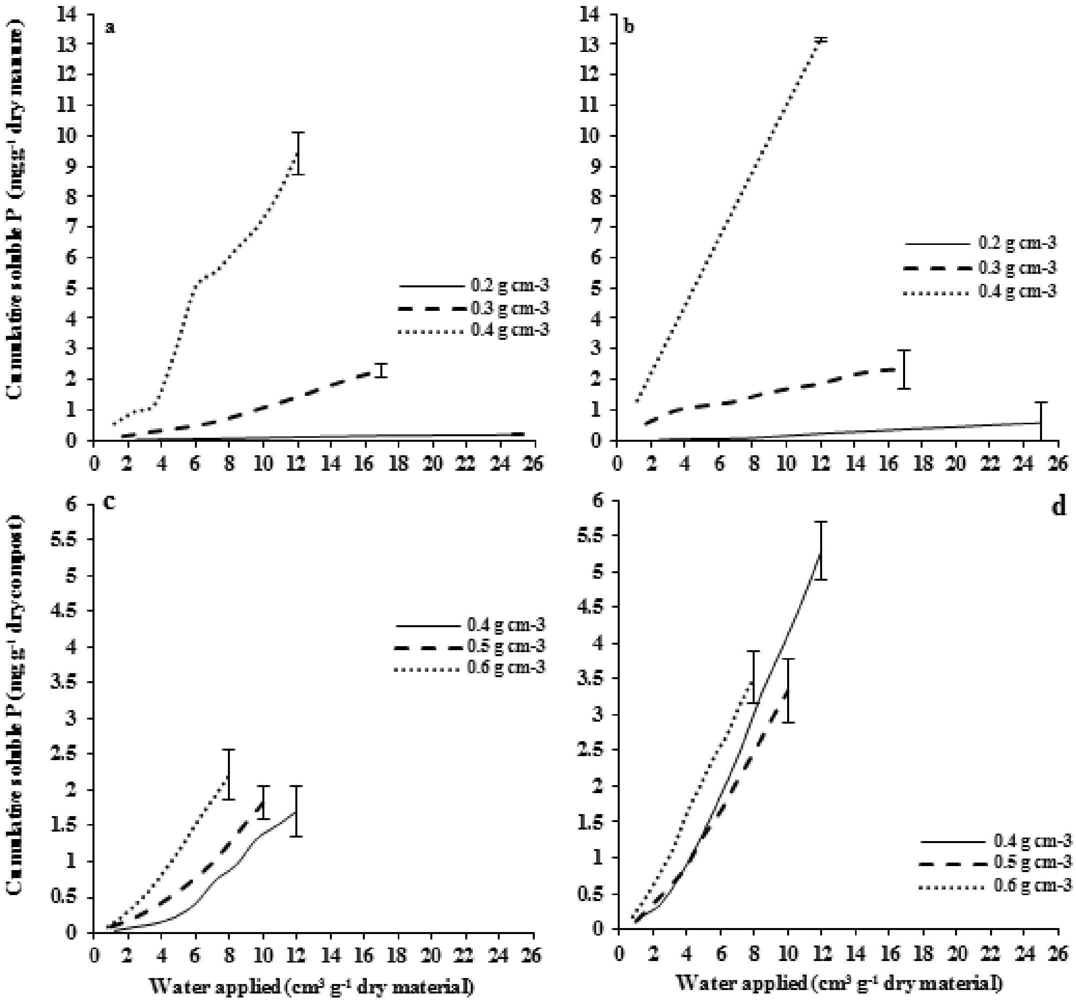

Figure 5-5 The effect of packing density on cumulative soluble phosphorus extracted from chicken manure in (a) 50 mm and (b) 100 mm columns, and mature compost in (c) 50 mm column and (d) 100 mm columns. Bars are LSD ($\alpha = 0.05$) comparing water applied for each packing density.

The long column was more efficient at extraction of soluble P from fresh manure compared with the short column (Figure 5-6). The highest LEI for P was 98.8% in the 100 mm column packed at 0.4 g cm^{-3} but there was no significant effect of packing density in the fresh manure. However, there was no significant effect of column length (*P=0.86)* and bulk density (*P*=0.649) on LEI for P in mature compost.

5.3.4.3 Potassium

The leachates extracted from both fresh manure and mature compost generally contained high concentrations of soluble potassium (K) (Figure 5-7). The highest concentration of soluble K (23.5 and 18.4 mg g^{-1}) were extracted from the 50 mm fresh manure column packed at 0.4 g cm^{-3} and the 100 mm mature compost column packed at 0.5 g cm^{-3}. Column length significantly affected soluble K extraction in both materials with long columns generally more effective than short columns. The exception was the short columns of fresh manure packed at 0.4 g cm^{-3} where K extraction was higher than longer columns (Figure 5-7).

Increasing the packing density significantly increased the rate of soluble K extraction. However, the effect of density was greater in the short columns compared to the long columns for both waste materials.

The rate of soluble K extraction from the fresh manure columns showed a linear relationship with water application, but for mature compost the relationship plateaued after ~ 3 to 4 cm^3 water applied per g^{-1} dry matter (Figure 5-7).

The LEI of K was significantly affected by bulk density (*P*=0.012) in fresh manure (Figure 5-8). Increasing packing density decreased the LEI for K in fresh manure particularly for the longer column length. The highest LEI (97.4%) was observed for the low density (0.2 g cm^{-3}) 100 mm column treatment (Figure 5-8a). However, the LEI for K in the mature compost was significantly affected by column length only (*P*>0.05*).* Increasing column length decreased the LEI, with the highest LEI (99.5%) observed in the 50 mm column.

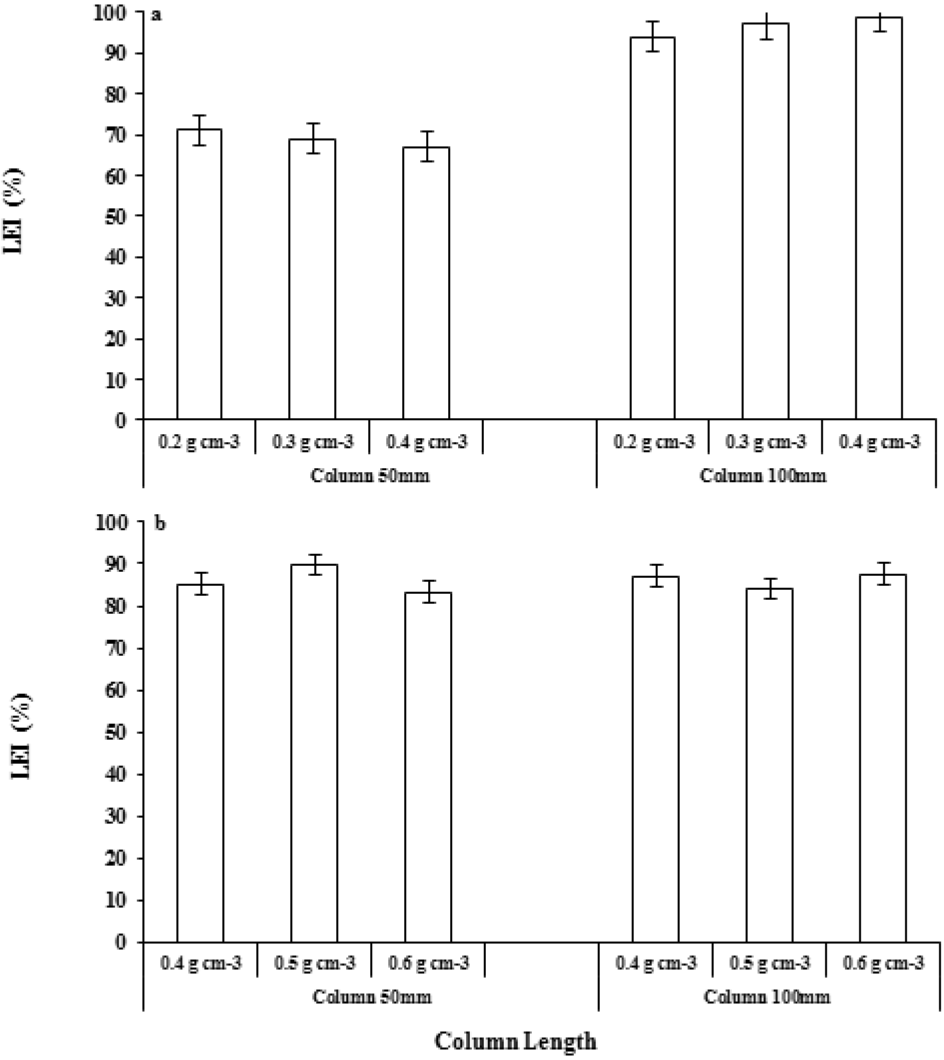

Figure 5-6 Effect of column length and packing density on leaching efficiency index for soluble phosphorus in (a) fresh manure and (b) mature compost. Bars are LSD (α=0.05) comparing within treatments.

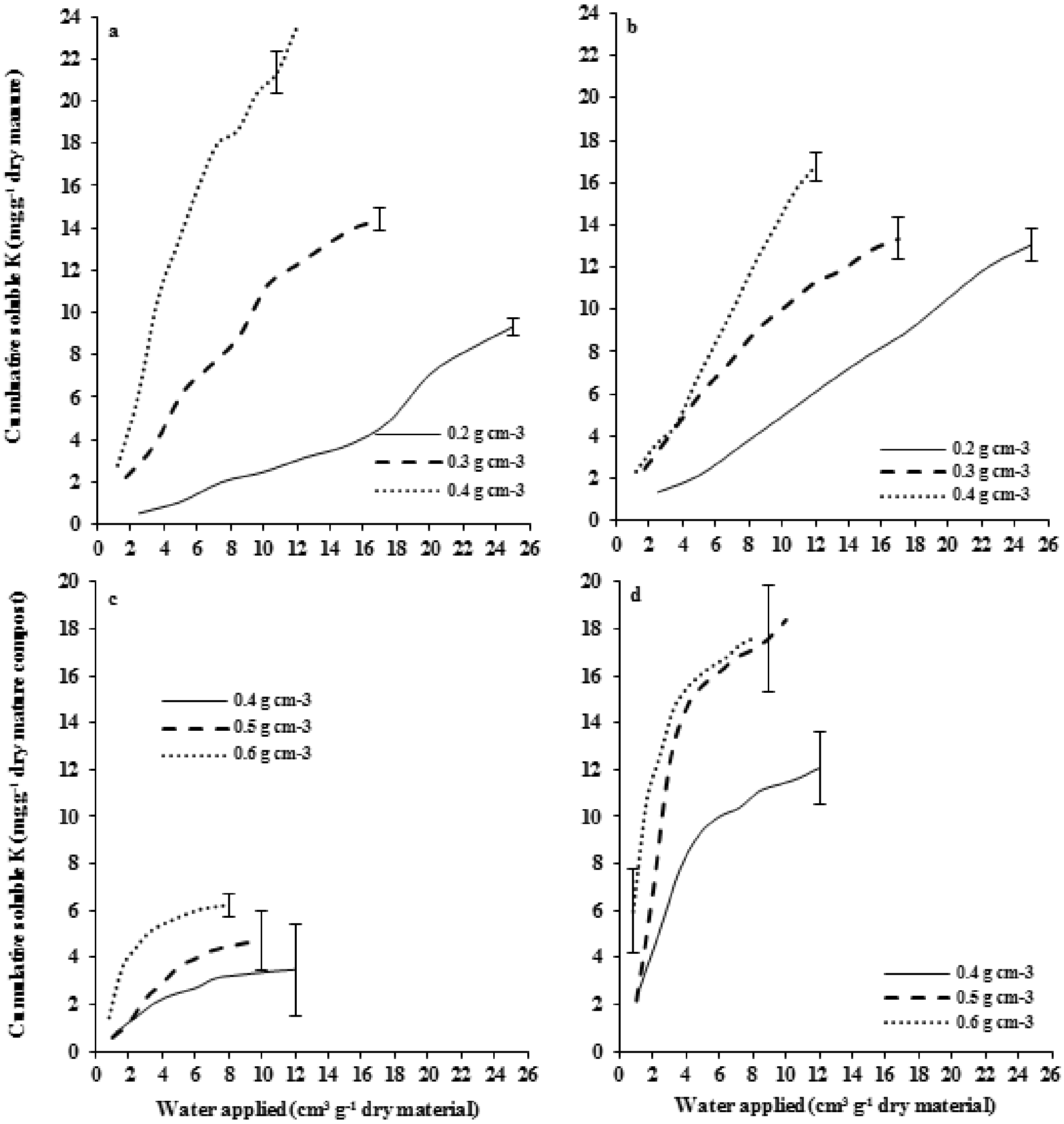

Figure 5-7 The effect of packing density on cumulative soluble potassium extracted from chicken manure in (a) 50 mm and (b) 100 mm columns, and mature compost in (c) 50 mm column and (d) 100 mm columns. Bars are LSD (α = 0.05) comparing water applied for each bulk density.

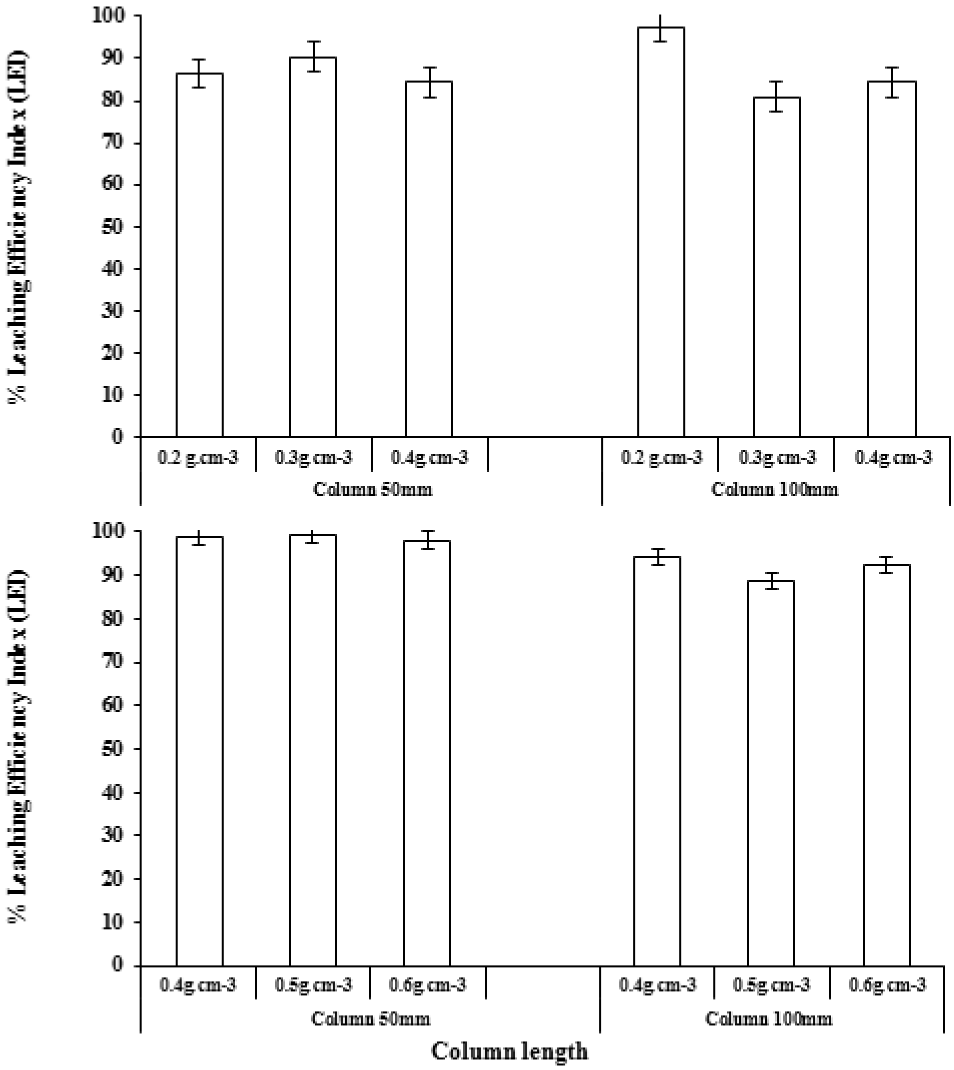

Figure 5-8 Effect of column length and packing density on leaching efficiency index of soluble potassium in (a) fresh manure and (b) mature compost. Bars are LSD (α=0.05) comparing within treatments.

5.3.4.4 Sodium and chloride

More soluble sodium (Na) was generally extracted from the fresh manure than the mature compost (Figure 5-9). The effect of packing density on soluble Na extraction was significant compared with the effect of column length. For fresh manure, the rate of soluble Na extraction was constant with increasing water application. However, for the mature compost, most of the soluble Na was extracted in the first 4 to 6 cm^3 water applied per g^{-1} dry matter.

Column length and packing density also significantly affected chloride (Cl) extraction (Figure 5-10). The Cl was leached more rapidly than Na. Approximately 71 - 96% of the maximum Cl was leached from the fresh manure after approximately 8.4 to 17.5 cm^3 water applied per g^{-1} dry matter, while approximately 53-96% of the Cl was leached from the mature compost after 3.2 to 4.8 cm^3 water applied per g^{-1} dry matter (Figure 5-10). However, approximately 53-96% of Na was leached from the fresh manure columns after 8.4 to 17.5 cm^3 water applied per g^{-1} dry matter, while for the mature compost approximately 68 – 95% of Na was leached after 5.6 to 8.4 cm^3 g^{-1} dry matter of water was applied.

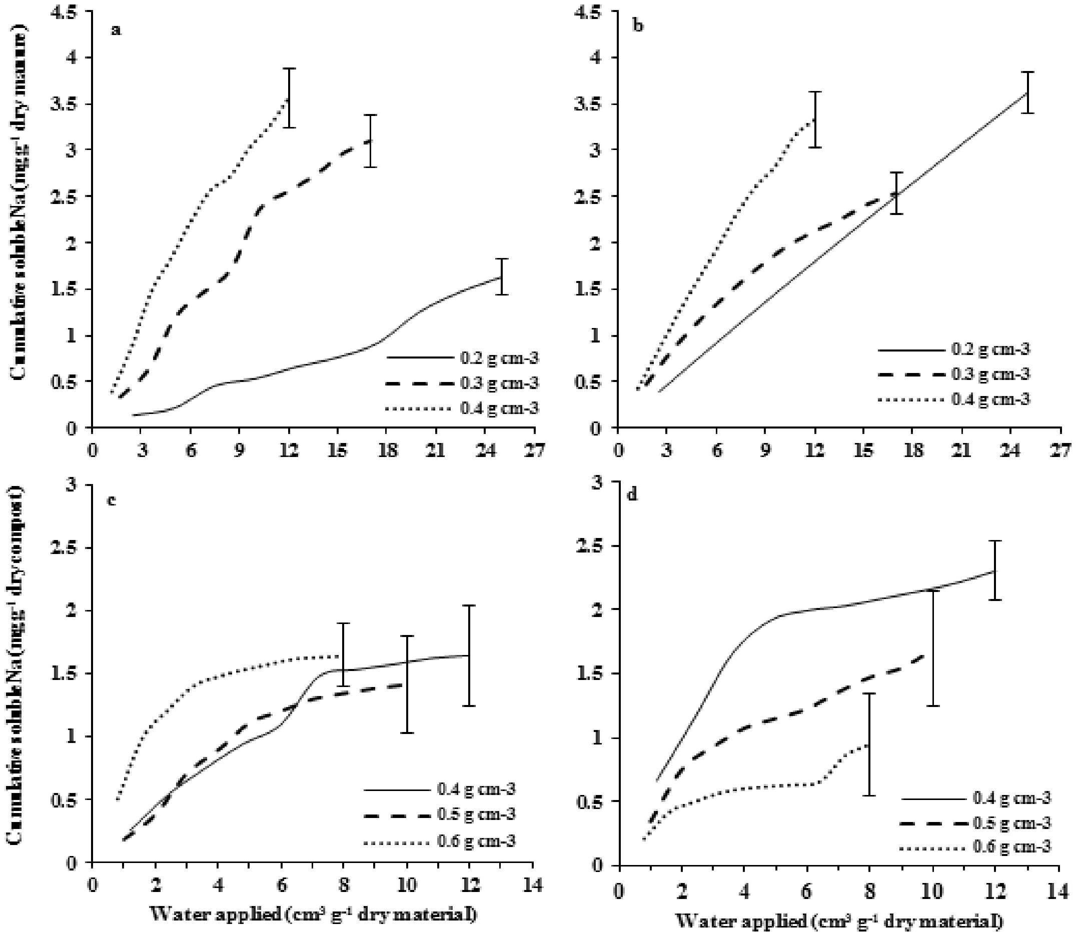

Figure 5-9 The effect of packing density on cumulative soluble sodium extracted from chicken manure in (a) 50 mm and (b) 100 mm columns, and mature compost in (c) 50 mm column and (d) 100 mm columns. Bars are LSD (α = 0.05) comparing water applied for each bulk density.

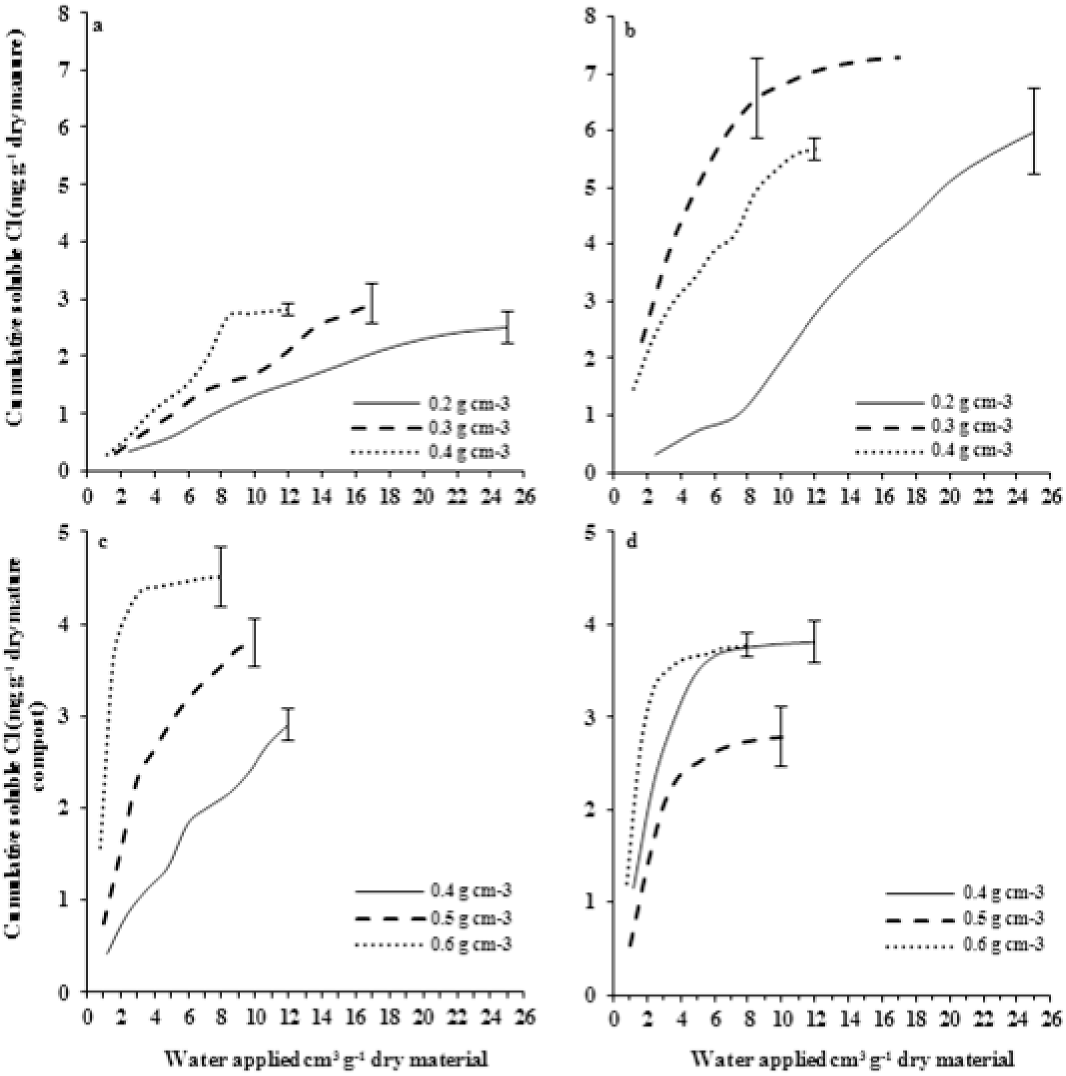

Figure 5-10 The effect of packing density on cumulative soluble chloride extracted from chicken manure in (a) 50 mm and (b) 100 mm columns, and mature compost in (c) 50 mm column and (d) 100 mm columns. Bars are LSD (α = 0.05) comparing water applied for each bulk density.

5.3.5 Cation ratio of soil stability (CROSS)

The change in CROSS of fresh manure and mature compost leachate with increasing water application is presented in Figure. 5-11. In general, the CROSS decreased with water applied for all treatments and there were few significant differences between density treatments after ~ 10 cm^3 water applied per g^{-1} dry matter. However, increasing packing density was found to have a significant effect on initial CROSS. The CROSS of leachate decreased with increasing bulk density in all treatments, except fresh manure in 50 mm columns (Figure 5-11 a).

The long columns in mature compost had significantly higher initial CROSS than the short columns (Figure 5-11a, b). However, in fresh manure the short columns generally had significantly higher initial CROSS in short columns than long columns

(Figure 5-11 a, b). The long columns in the mature compost had significantly higher initial CROSS than the short columns (Figure 5-11, c, and d).

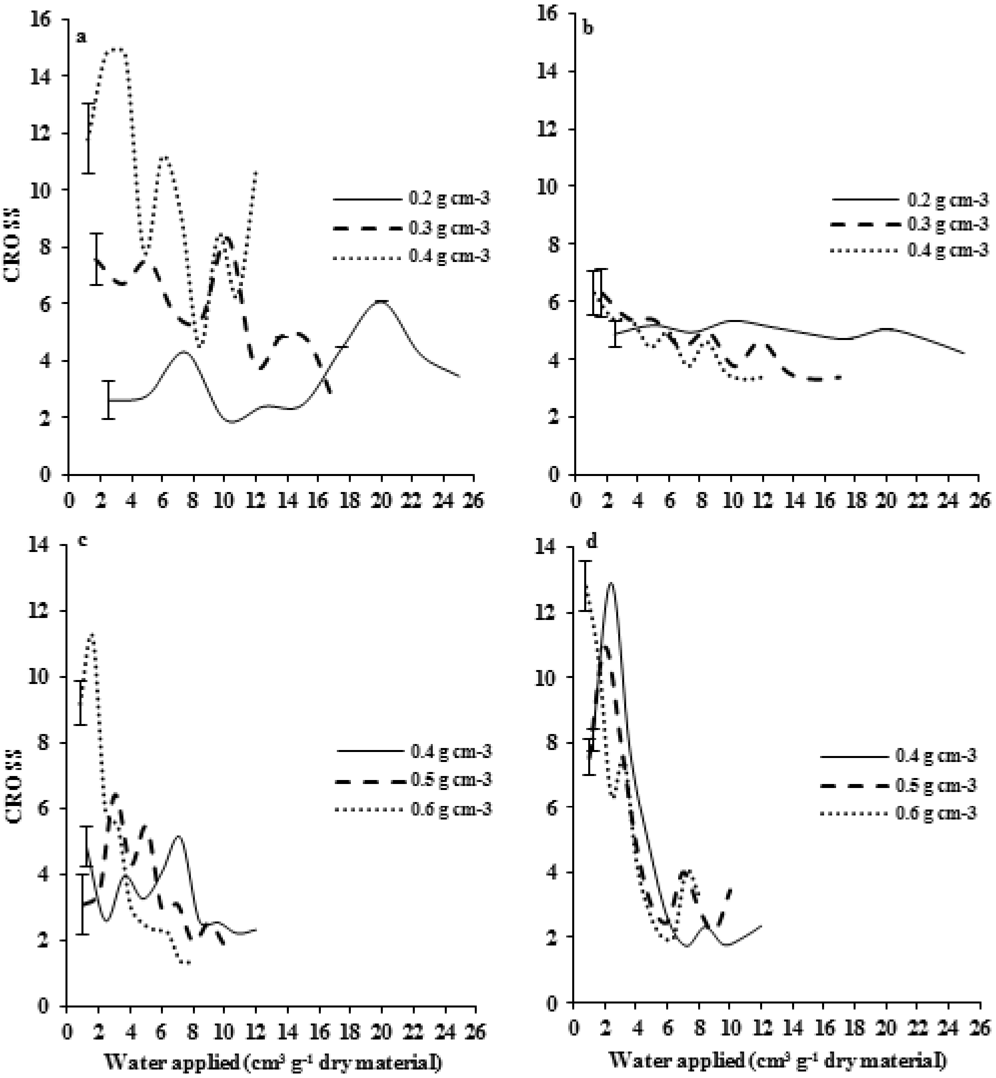

Figure 5-11 The effect of packing density on leachate CROSS extracted from chicken manure in (a) 50 mm and (b) 100 mm columns, and mature compost in (c) 50 mm column and (d) 100 mm columns. Bars are LSD (α = 0.05) comparing water applied for each bulk density.

5.4 **Discussion**

5.4.1 Comparison of fresh manure and mature compost leachates

The composting process affects the soluble nutrient content in both the chicken waste (Table 5-1) and leachate. Relatively higher nutrient concentrations were observed in the fresh manure compared with the mature compost due to either addition of sawdust or uncontrolled nutrient leaching during the composting process. Fresh manure appears to be a more viable source of soluble nutrients compared to mature compost due to the relatively higher concentrations of N, P and K extracted.

The loss of nitrogen (~48%) during composting is similar to the 47 to 62% reported by Kithome, Paul, and Bomke (1999) for chicken layer manure and Ogunwande et al. (2008) who reported that the average nitrogen loss from chicken litter composting ranged from 70.7 to 88.2%.

The EC clearly indicated loss of nutrient through composting evidenced by the fall in EC from before composting (11.8 dS m^{-1}) to after composting (6.2 dS m^{-1}). Similar results were found in a study using cattle feedlot manure (Eghball et al. 1997b).

The fresh manure leachate had high concentrations of macronutrients (N, P and K), but also had a high concentration of potentially toxic ions such as Na and Cl. As a result, the extraction process also took longer to complete in fresh manure compared to mature compost. The leaching time ranged from 0.3 to 2.5 days for the fresh manure short columns, while the long columns took between 1 day and 3 days. By contrast, the leaching time for mature compost ranged from 5 to 19 hr and from 0.3 to 2 days for the short and long columns, respectively.

Extraction of nutrients from fresh manure may be problematic due to both the time required to complete the leaching process and the high sodium and chloride concentrations in the leachate. Munns and Tester (2008) illustrated that increased NaCl concentrations in the soil above 40 m*M* could lead to a reduction in shoot growth because of an increase in external osmotic pressure and the slow accumulation of Na in the leaves. This could reduce crop yield significantly (Tavakkoli, Rengasamy, and McDonald 2010) because of the effects on critical biochemical processes and water uptake by plants.

While fresh chicken manure is a potentially valuable source from which to extract soluble nutrients, there are three major issues that may affect its commercial viability:

1) The long leaching time due to K_{sat} reducing significantly during leaching, presumably as dissolution of the soluble salts of the solid phase particulate occurs leading to cementation and expansion of the diffuse double layer surrounding the charged surfaces of organic materials and exchange surface, which may limit leachate production output.
2) The concentration of Na and Cl contained within the leachate is high and may cause concern for soil aggregate stability (Sumner 1993) and plant physiology responses (Tavakkoli, Rengasamy, and McDonald 2010), Na causing decline in soil stability, and Cl increasing the soil ionic strength that counters the effect of Na as well as being taken up by plants and increasing the osmotic gradient governing plant water occurs (Ayers and Westcot 1985), and
3) The leachate derived from un-composted fresh chicken manure may contain pathogens such as *Escherichia coli* or *Salmonella spp* (Gross et al. 2008).

There is a need for further research on advantages of nutrient extraction from fresh chicken manure compared to composted material and the disadvantages of using fresh manure when the leaching time was long and may contain pathogens.

5.4.2 Selection of the optimum column length and packing density

For the mature compost, using a long column is more effective for extracting nutrients than a short column, as a higher concentration of soluble nutrients, except soluble K, was obtained irrespective of packing density. This difference in concentration of soluble nutrients could be attributed to the contact time between distilled water and material in columns whereby the contact time was longer per unit volume using the long column than for the short column. Similar results were observed by López Meza et al. (2010) who investigated demolition waste and found that a prolonged contact time caused a significant increase in the release of contaminants into the percolating solution, compared to when contact time was shorter (López Meza et al. 2010).

Although the 100 mm column was found to have the highest concentration of soluble nutrients at all density treatments for mature compost, the packing density of material in the 100 mm column was also found to have a significant impact ($P \leq 0.05$) on the amount of these nutrients and the type of salts. The primary effect was on the quantity of salts, where the increase in mature compost packing density led to an increase in the leachate cumulative TDS. This increase in TDS may be attributed to (a) increased contact time between the percolating solution and the solid phase, and (b) a change in the solid to solvent ratio. Although the initial bulk density of the mature compost 0.4 g cm^{-3} column is the lowest density used, it had the highest TDS as a result of the consolidation that occurred in the columns due to gravity, constant water head, particulate slumping/settling, and the dissolution of soluble structural bonds. The packing density also affected the quality of leachate in terms of the type of cations or anions that predominate, due to the difference in mobility of the ions.

The CROSS of the leachates is another factor that will affect the optimum extraction method. Solutions with high CROSS values are more likely to cause dispersion of clay within the soils they are applied to (Rengasamy and Marchuk 2011), which can result in soil pore blockage, decreased soil infiltration and ultimately decreased vegetation productivity (Sumner 1993). The leachate derived from the various bulk density treatments had different CROSS values, with the lowest CROSS being 1.4 for the mature compost 0.6 g cm^{-3} in 50 mm column treatment, and the highest CROSS being 13.7 from the fresh manure 0.4 g cm^{-3} in 50mm treatment.

Considering the factors above, the optimum extraction design for mature compost is a 100 mm column packed at an initial density of 0.6 g cm^{-3}, while for fresh manure the high density (0.4 g cm^{-3}) and 100 mm column. Extraction columns with these characteristics were observed to extract the most nutrients (particularly nitrogen and phosphorus), whilst limiting the sodicity of the leachate. For fresh manure, the optimal nutrient extraction column characteristics are also 0.4 g cm^{-3} consolidated within a 100 mm column, where it was observed that 98.6 and 98.8% of total dissolved nitrogen and soluble phosphorus were extracted, respectively.

5.4.3 Treatment of leachates for CROSS

The CROSS of a solution is a measure of the Na concentration as a ratio of the Ca and Mg concentration, taking into account the dispersive potential of K and Mg (Eq 5-1). Some studied have reported that increases in exchangeable K in soils can cause clay swelling and dispersion (El Swaify, Ahmed, and Swindale 1970, Chen, Banin, and Borochovitch 1983, Rengasamy and Marchuk 2011). Similarly, Emerson and Bakker (1973) and Rengasamy and Sumner (1998) reported that clay swelling and dispersion can occur due to increase in exchangeable Mg under specific conditions. Therefore, if sodium can be removed from the solution and Ca retained, CROSS can be effectively decreased. Fractionating the leachate, by volume, is one possible method to achieve this. For example, considering the CROSS values in section 2.6.1, disposal of the first and second leachate volumes collected from the mature compost (packing density 0.4 g cm^{-3} and 100 mm column length) leachate will decrease the CROSS of the leachate from 4.6 to 2.8 (Figure 5-12). Similarly, in fresh manure (density 0.4 g cm^{-3} and 10mm column length) the CROSS of collected leachate could be decreased from 7.0 to 5.9 if the first two leachate volumes are disposed. The decrease occurs as the majority of Na is leached within the first and second leaching fractions, while Ca and Mg are released more slowly. Unfortunately, the essential nutrients (N, P and K) will also decrease, but there should still sufficient nutrients (Figure 5-12) to be considered appropriate as a fertiliser supplement for plants.

An alternative method to retain nutrients but decrease CROSS would be to apply an external source of calcium. This would increase the denominator of Eq. 5-1 and effectively decrease the impact of leachate containing Na. Gypsum ($CaSO_4$) is a common source of Ca used in agriculture to address the issue of excess sodium in both solution and soil. Considering the CROSS of the obtained leachates, it can be seen from Table (5-3) that the addition of gypsum to the leachate may also be a feasible treatment method. However, applying gypsum to leachate would increase the salinity of leachate where when applied 1.4 g per L^{-1} produces solution with salinity 2.2 dS m^{-1}. Depending on the solution alkalinity, it might also be of advantage to apply an acid into the solution, or a sulphur source into the intended soil (Johnston, Vance, and Ganjegunte 2008). The applying acid uses as an amendment to decrease pH and enhance calcite dissolution to release calcium (Amezketa, Aragüés, and Gazol 2005).

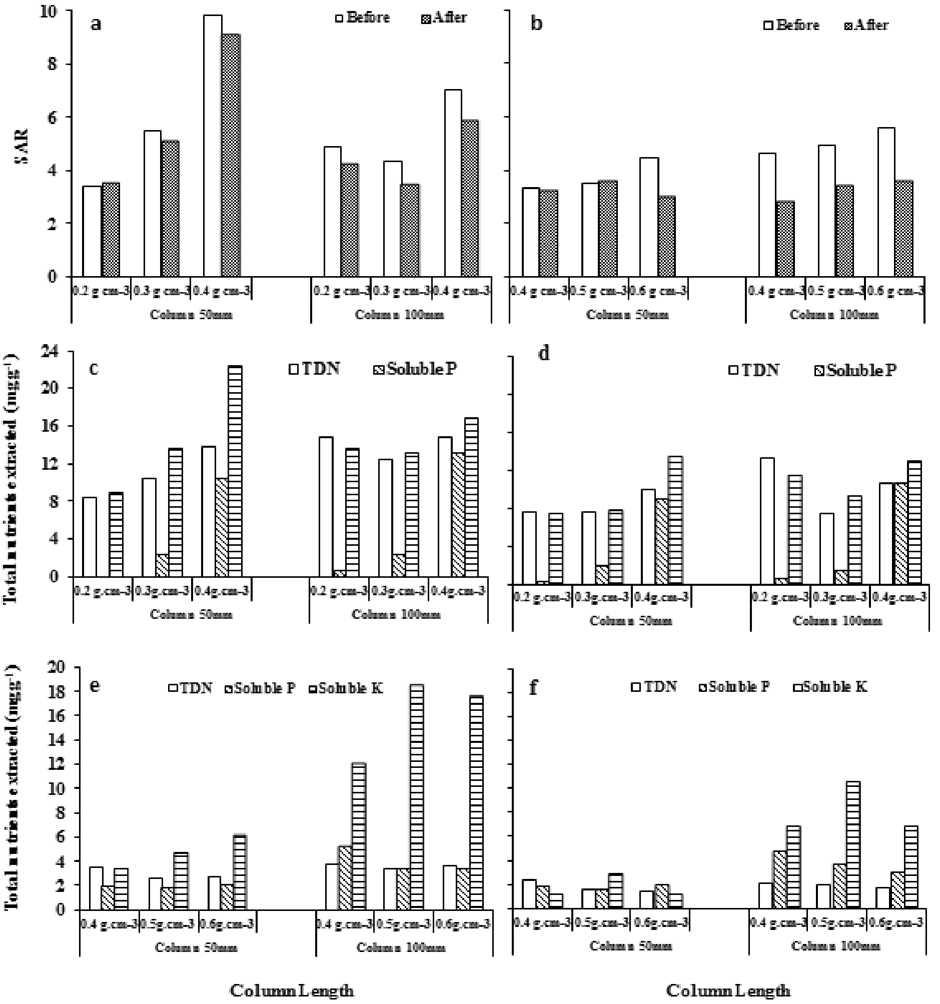

Figure 5-12The effect of fractionating on SAR in (a) fresh manure leachate (b) mature compost leachate and nutrients in fresh manure leachate (c) before and (d) after partition, and nutrients in manure compost leachate (e) before and (f) after partition.

Table 5-3 Reduction in cation ratio of soil stability after applying gypsum to the leachate derived from chicken manure by using bulk density 0.4 g cm^{-3} and 100 mm column length.

Leaching Event	**$CaSO_4$ weight g L^{-1}**	**Ca meq L^{-1}**	**CROSS**	**% Reduction**
LE 1	0	0	8.86	
	0.7	9.59	7.89	10.92
	1.4	19.18	7.18	18.90
	2.1	28.77	6.64	25.07
	2.8	38.36	6.20	30.01
LE 10	0	0.00	17.98	
	0.7	9.59	17.74	1.34
	1.4	19.18	17.50	2.64
	2.1	28.77	17.28	3.90
	2.8	38.36	17.06	5.10

5.5 Conclusions

This study aimed to determine the efficiency of extraction of soluble nutrients from both fresh manure and mature compost. The results showed those soluble nutrients are more reality extracted from fresh chicken manure. However, fresh manure is usually highly heterogeneous even from the same source and the variation between leachates may reduce the commercial viability of this medium. Leachate extracted from fresh chicken manure may require treatment to reduce the CROSS of the final solution. The Ksat of fresh manure is also relatively slow compared with mature compost at the same density 0.4 g cm^{-3} in both 50 mm and 100 mm columns and, hence a long time is needed to complete the leaching process. This further reduces the commercial attractiveness without further engineering solutions. Due to the increased contact time between the percolating solution and the mature compost medium, a 100 mm column of compost was observed to provide greater macronutrient (N, P and K) extraction per unit volume compared to the 50 mm column. Packing density was also found to have a significant impact on the quality of the leachate with a packing density of 0.4 g cm^{-3} and 100 mm column for fresh manure columns and 0.6 g cm^{-3}, 100 mm column for mature compost, identified as optimum for nutrient extraction. Finally, to avoid the negative effects of high solution CROSS on agricultural soil permeability, fractionation of extracted leachate was identified as a possible strategy by which to reduce sodium concentration and thus leachate CROSS.

CHAPTER 6: CONTROLLING PATHOGENS IN CHICKEN WASTE LEACHATE

6.1 Introduction

Fresh chicken manure leachate has been shown (Chapter 5) to be a viable source of organic liquid fertiliser. However, leachate extracted from chicken fresh manure contains pathogens such as *Escherichia coli* and *Salmonella spp* (Chapter 4). Previous studies (Himathongkham and Riemann 1999) have also suggested that fresh chicken manure contains enteric microorganisms including *Escherichia coli* and *Salmonella spp.* The risk of pathogen transmission from untreated manures applied to the soil into the food chain is well documented (Gerba and Smith 2005). Pathogen contamination could be caused by transmission from animal manure applied as either solid or in liquid (effluent or leachate). Applying solid manure could lead to contamination of fresh produce in the field or surface water when the application of manure is excessive (Gerba and Smith 2005). However, application of liquid manures may also contaminate groundwater. For example, coliform bacteria were found to move through soil when applied as municipal sewage effluent (Guan and Holley 2003).

Composting animal manures before application to agricultural land is an effective method to reduce pathogen load (Tiquia and Tam 2002). However, the composting process can result in losses of nutrients (up to 40% for nitrogen, and 12% for phosphorus) either by leaching or in runoff (Eghball et al. 1997a, Mahimairaja et al. 1994). Therefore, many farmers prefer to apply fresh manure as the cost per unit of nutrient in the fresh manure is lower than in the composted manure (Wilkinson 1979). However, the use of fresh manures can affect human health due to the presence of pathogens (Gross et al. 2008). USDA (2000) requires a wait period between manure production and incorporation of manure into the soil where this period should be at least 120 days, or more. However, Natvig et al. (2002) reported that incorporation manure in early summer may cause pathogens to be present on plant roots and leaf. To mitigate this problem, liquid fertilisers derived from fresh animal manure should undergo treatment so that pathogens are eliminated.

Cheremisinoff (2001) classified the common methods used to treat wastewater as chemical or physical treatments. Chemical treatments involve the use of germicidal agents such as chlorine or hydrogen peroxide, which are added to the wastewater to reduce pathogen load. An alternative chemical method includes the use of ozone which is effective but also energy-intensive (Richardson et al. 2000, Oppenländer 2003). Physical methods may involve the use of heat; wastewater is subjected to boiling for about 5 to 10 min which results in pathogen destruction (Cheremisinoff 2001), or Ultra violet (UV) light.

While there are a range of sterilisation techniques available, it is not clear which technology and methodology is appropriate for use on fresh chicken manure leachate. Hence, the objectives of this chapter were to: 1). evaluate different methods to sterilise *E.coli and Salmonella spp* in fresh chicken manure leachate, and 2) determine whether the sterilisation method influenced the concentration of soluble nutrients in the leachate. It was hypothesised that using ozone would be more appropriate than the addition of chemical agents because using ozone should eliminate pathogens without affecting the soluble nutrients concentration.

6.2 Materials and methods

6.2.1 Manure and compost sampling

Samples of the fresh chicken manure were collected as in section 3.2. All samples were homogenised using a rotary drum and five representative sub-samples (approximately 500 g each) were taken for analysis of physical and chemical properties.

6.2.2 Chemical and physical properties of manure

The chemical and physical properties of the manure were measured as outlined in section 3.3. The organic matter (OM) and ash were determined on three replicates in a muffle furnace using Thompson et al. (2001). The electrical conductivity (EC) and the pH of samples were determined as outlined in section 3.3.6. The cation exchange capacity (CEC) was determined by using ammonium acetate as described by Chapman 1965 (Hendershot, Lalande, and Duquette 2007). Exchangeable cations were determined using 1 *M* of ammonium acetate (section 3.3.7). The concentrations of total and soluble inorganic elements (N, P, K, Ca, Mg, Na and Cl) were determined as described in sections 3.3.4, 3.3.5 and 3.3.6.

The moisture content, particle density (D_p), total porosity and pore volume (η), were calculated as described in section 3.3.1, 3.3.2 and 3.3.3. Table 6-1 shows some selected properties of the fresh chicken manure and in this trial.

6.2.3 Column preparation and extraction of soluble nutrients

Based on the results obtained from Chapter 5, the chicken manure was packed into PVC columns (87.5 mm inner diameter and 100 mm length) at a packing density of 0.4 g cm^{-3}. To avoid excessive compaction, the materials were progressively compacted to the treatment packing density in 25 mm segments using a steel compaction dolly. Filter papers (Watman No. 45) were placed on both ends of the columns to avoid loss of material.

6.2.4 Description of the sterilisation treatments

Sterilisation treatments were applied either during leaching or to the leachate on a post-treatment basis. Four solutions of ten pore volumes each were applied to the columns. The solutions used were: distilled water (control), hot water at 75°C, 0.01*M* acetic acid and 0.01 *M* hydrogen peroxide. The temperature of the hot water was chosen based on the results of a preliminary trial (Appendix A.1). A 50 mm water head was maintained above the columns.

Post-treatments applied to the leachate used ozone, heating, 0.01 *M* acetic acid or 0.01 *M* hydrogen peroxide. Ozone (O_3) was generated by using an ozone generator (Model ZXA-200, Zox Ozonatord Pty Ltd, Australia) with an output of 200 mg h^{-1} producing 10.41 mg L^{-1} of O_3 at an oxygen flow rate of 3.20 L min^{-1} at ambient temperature. The results of chapter 4 showed that the leaching volume did not have a significant ($P<0.05$) effect on the number of *Escherichia coli* and *Salmonella spp* in the leachate. Thus, the first and second pore volumes of leachate from distilled water treatment were mixed and 50 mL of mixture was taken. The ozone treatment was applied for 30 min directly into leachate after extraction or leaching. The time of sterilisation using ozone was chosen based on the results of a preliminary trial

(Appendix A.2). The heating treatment (or pasteurisation) was applied on 50 mL of leachate derived from the distilled water treatment. The temperature applied was from 55 to 65°C for 15 min. For the acetic acid and hydrogen peroxide treatments, 1 mL of 0.01 *M* acetic acid or hydrogen peroxide was applied to 10 mL of leachate derived from the distilled water treatments. Three replicates of each treatment were used.

Table 6-1 Some selected chicken fresh manure characteristics

Parameter	Unit	Value[*]
Moisture content	(%w w^{-1})	64.7 (± 0.2)
Solid material	(%w w^{-1})	35.5 (± 0.2)
Organic matter O.M	(%w w^{-1})	25.7 (± 0.4)
Total Nutrients		
Carbon	(mg g^{-1})	359.2 (± 24.2)
Nitrogen	(mg g^{-1})	127.2 (±9.8)
Phosphorus	(mg g^{-1})	13.5 (±0.1)
Potassium	(mg g^{-1})	53.5 (±3.0)
Calcium	(mg g^{-1})	60.4 (±3.3)
Magnesium	(mg g^{-1})	2.3 (±0.6)
Sodium	(mg g^{-1})	8.7 (±0.03)
Chloride	(mg g^{-1})	10.9 (±0.01)
Soluble Nutrients		
Electric conductivity (EC)	(dS m^{-1})	12.3 (±0.1)
pH (1:10) (mass: volume)	-	7.9 (±0.1)
Phosphorus	(mg g^{-1})	0.4 (±0.1)
Potassium	(mg g^{-1})	23.9 (±0.2)
Calcium	(mg g^{-1})	1.7 (±0.1)
Magnesium	(mg g^{-1})	0.13 (±0.005)
Sodium	(mg g^{-1})	1.2 (±0.06)
Chloride	(mg g^{-1})	3.7 (±0.6)
Exchangeable Cations		
Potassium	(cmol kg^{-1})	4.0 (±0.7)
Calcium	(cmol kg^{-1})	17.7 (±2.9)
Magnesium	(cmol kg^{-1})	1.1 (±0.2)
Sodium	(cmol kg^{-1})	10.7 (±1.9)
Cations Exchangeable Capacity	(cmol kg^{-1})	39.7 (±1.0)
Pathogens		
Escherichia coli	(Log CFU g^{-1})	6.5 (±0.4)
Salmonella spp	(Log CFU g^{-1})	5.2 (±0.3)

[*] *Standard deviation in brackets*

6.2.5 Leachate properties

The chemical and biological characteristics of the leachate were measured using the procedures outlined in section 3.3.7 and 3.3.9, respectively. Pathogen analyses were conducted using a 1 mL aliquot of each leachate extracted from each treatment. Serial dilutions (10^{-1} to 10^{-5}) using [0.87 % (w v^{-1})] of sterile peptone water (NaCl) were prepared. Petri plates were inoculated by spreading 0.1 mL of diluted leachate on the surface of selective media, *Salmonella and Shigella* Agar (SS agar) and MacConkey agar for *E.coli*. The petri plates were incubated at 37°C for 24 to 48 hours. *Salmonella spp* and *E. coli* were determined by counting black colonies and pink colonies developed, respectively.

The cation ratio of soil structural stability (CROSS) of the leachate was calculated using the equation described by Rengasamy and Marchuk (2011):

$$CROSS = \frac{Na+(0.56K)}{\sqrt{\frac{(Ca+(0.6Mg))}{2}}} \qquad \textit{Equation 6-1}$$

where: *Na, Ca, Mg and K* is the concentration (meq L^{-1}) in the leachate.

6.2.6 Experimental design and statistical analyses

This study was conducted as two experiments; treatments applied during leaching and treatments applied post-leaching. Each experiment was conducted using a complete randomised design. Data are expressed as the mean of three replicates. Statistical analyses were undertaken using Statistical Package for the Social Sciences (SPSS) v19 for windows 7 (Cramer 2004). Analyses involved univariate ANOVA. Two-Way ANOVA was used to study the effects of both treatment and leachate pore volume; and One-Way ANOVA was used to compare the effect of leaching volume for each treatment. Least significant difference (LSD) tests were used to compare the means at a probability level of 5%.

6.3 Results

6.3.1 Pathogen control

6.3.1.1 Treatments during leaching

The pathogen count was significantly influenced by the type of solution used for the extraction ($P<0.05$). *E coli* count in the first pore volume of leachate was reduced from about 10.5 Log_{10} 100 mL^{-1} in distilled water to about 8.6 and 8.4 log_{10} CFU 100 mL^{-1} manure in hot water (75°C) and 0.01 *M* acetic acid (AcA), respectively. After 6.5 pore volumes of hot water applied, *E. coli* counts were reduced to a level which was below that obtained (<6 1 log_{10} CFU 100 mL^{-1}) in the peroxide treatment (Figure. 6-1a). The most effective solution after ten pore volume was hot water, where the count of *E coli* was < 1 log_{10} CFU 100 mL^{-1} (Fig. 6-1a).

The presence of *Salmonella spp* in the leachate was also significantly ($P<0.05$) affected by the type of solution applied to the column (Figure 6-1b). *Salmonella spp* declined from about 9.5 $\log_{10}$ CFU 100 mL^{-1} in distilled water to 8.1 $\log_{10}$ CFU 100 mL^{-1} in hot water while the use of acetic acid did not reduce the number of *Salmonella spp* significantly (P>0.05). The value recorded with acetic acid was 9.31 $\log_{10}$ CFU 100 mL^{-1} (Fig. 6-1b). The use of hot water produced significant reductions in the number of *Salmonella spp* and values were below detection limits (< 1 $\log_{10}$ CFU 100 mL^{-1}) after ten pore volumes were applied.

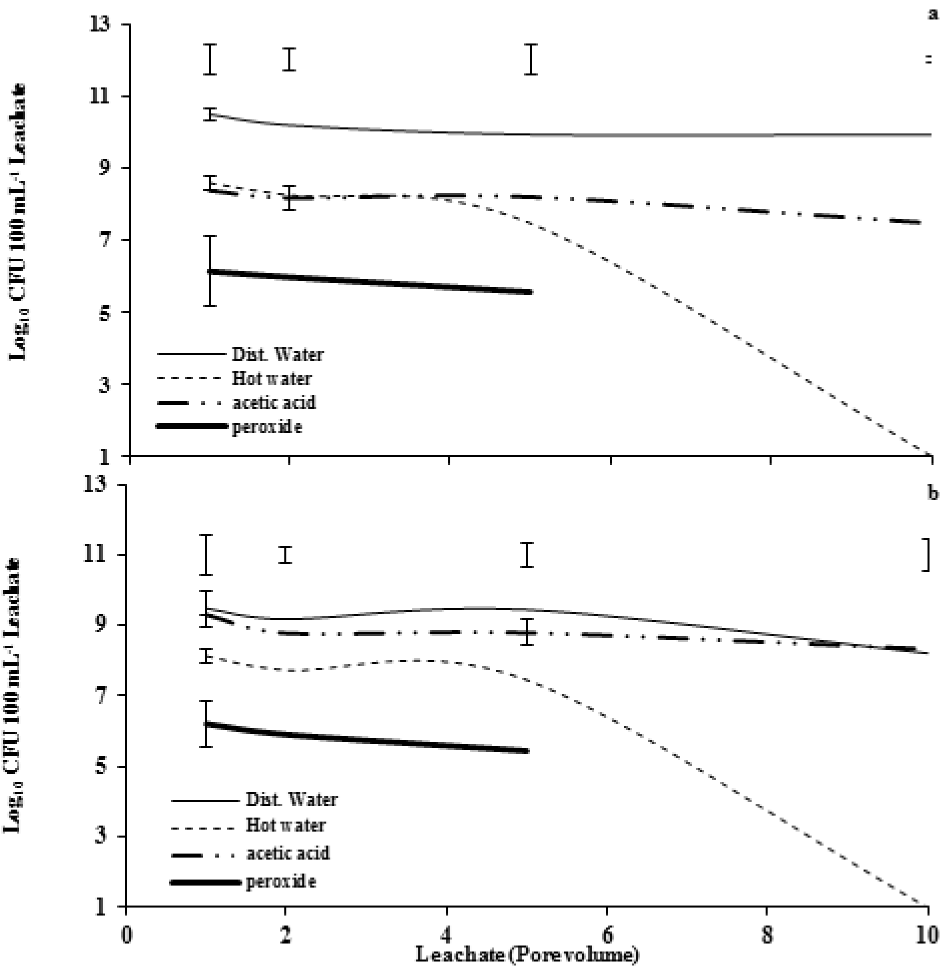

Figure 6-1. Effect of extraction solution on (a) E coli and (b) Salmonella spp in manure leachate. Bars at top of graph are LSD (α=0.05) comparing solutions extraction at respective pore volume. Bars on plotted lines are LSD (α=0.05) comparing within treatments.

The acetic acid treatments did not produce significant ($P<0.05$) reductions in pathogen counts with increasing leachate volume. The number of colonies recorded for *E. coli* and *Salmonella ssp* were relatively high (8.4 $\log_{10}$ CFU 100 $mL^{-1}$and 9.3 $\log_{10}$ CFU 100 mL^{-1}), respectively, compared with the tolerance levels (< 4 $\log_{10}$ CFU 100 mL^{-1}) given in irrigation water standards (Fan et al. 2009).

The hydrogen peroxide treatments produced a significant reduction for *E. coli* and *Salmonella ssp* in leachate compared to the other treatments after 1 pore volume. However, the 0.01 *M* hydrogen peroxide treatment blocked the columns after three pore volumes had been applied. This is most likely due to dissolution of inorganic and organic salts in the chicken manure, decreasing the flow path diameter and blocking flow paths with released particulate. Increasing leaching events was found to have no significant effect on the number of either *E coli* or Salmonella spp. The number of colonies recorded for E. coli and Salmonella ssp in leachate derived from distilled water after application of the first pore volume was relatively high at 8.2 and 6.21 $\log_{10}$ CFU 100 mL^{-1}, respectively. Similarly, after ten leaching events, *E. coli* and *Salmonella spp* colonies were 9.92 and 8.21 $\log_{10}$ CFU 100 mL^{-1}, respectively, (Figure 6-1).

6.3.1.2 Post-leaching treatments

The effect on pathogen counts of the different treatments applied to the leachate is shown in Table 6-2. The use of heat was effective in reducing pathogen load from leachate. The application of heat at 65°C for 15 minutes, or ozone (O_3) for 30 minutes yielded the lowest pathogen loads. These treatments reduced *E coli* and *Salmonella spp* from 7.44 and 7.94 $\log_{10}$ CFU 100 mL^{-1} respectively, to less than 1 $\log_{10}$ CFU 100 mL^{-1} after the treatment was imposed (Table 6-2). The application of peroxide did not reduce pathogen load significantly ($P>0.05$). The application of acetic acid produced a small but significant reduction in the number of *E coli* and *Salmonella spp* in the leachate when compared with the distilled water control treatment (Table 6-2).

Table 6-2 The effect of post-sterilising treatment on the number of pathogens in chicken manure leachate

Treatment	***E.coli* (*Log₁₀ CUF 100 mL⁻¹*)**	***Salmonella.ssp* (*Log₁₀ CUF 100 mL⁻¹*)**
Distilled	6.34^a (±0.01)	6.84^a (±0.15)
Acetic	4.47^b (±0.20)	5.12^b (±0.03)
Peroxide	6.24^a (±0.13)	6.57^a (±0.16)
Heating	$< 1^c$	$< 1^c$
Ozone	$< 1^c$	$< 1^c$

Lowercase superscript shows significant differences within columns.
< 1 is less than detection limit.

6.3.2 Soluble ions extraction

6.3.2.1 Treatments applied during leaching

Total dissolved salts and pH

The total dissolved salts (TDS) and pH of the leachate extracted from chicken fresh manure were both significantly influenced by the type of sterilisation applied to the columns ($P<0.05$) (Figure 6-2). For all treatment, most of the TDS were leached by seven pore volumes with no significant increase in cumulative TDS observed after eight pore volumes of solution was applied (Figure 6-2a). The hot water treatment produced a maximum cumulative TDS level of 210.5 mg g^{-1}, significantly higher than the maximum TDS levels extracted by using either distilled water or 0.01 *M* acetic acid (132.5 and 147.1 mg g^{-1}, respectively). However, the hydrogen peroxide solution blocked the column after three pore volumes and produced a maximum cumulative TDS of only 85.8 mg g^{-1}.

The first pore volume of leachate extracted using hot water, 0.01 *M* acetic acid and 0.01 *M* hydrogen peroxide all had higher pH values (8.3, 8.3 and 8.8, respectively) than leachate extracted by distilled water (Figure 6-2 b). The leachate pH remained high with increasing in pore volume for the distilled, hot water and hydrogen peroxide treatment, while in acetic acid the pH value declined as buffering capacity was exhausted (Figure 6-2b).

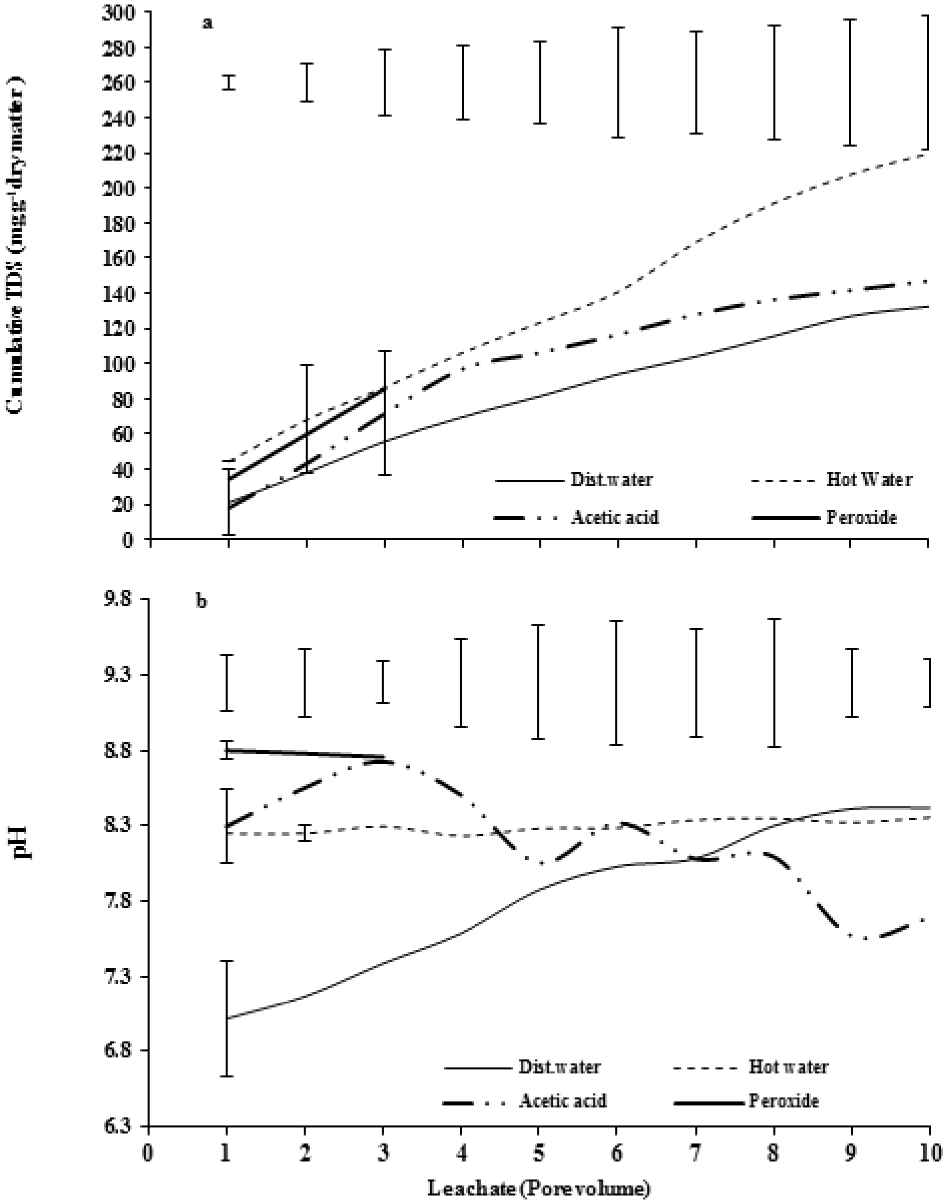

Figure 6-2 The effect of leaching chicken manure column by different sterilisation solutions on (a) total dissolved nitrogen and (b leachate pH. Bars at top of graph are LSD (α=0.05) comparing treatments at respective pore volumes. Bars on line plots are LSD (α=0.05) comparing volumes with each treatment.

Macronutrients

The highest cumulative TDN of 49.6 mg g^{-1} manure was extracted by distilled water while the lowest cumulative TDN of 27.7 mg g^{-1} manure was extracted by using 0.01 *M* acetic acid after ten pore volumes were applied (Figure 6-3a). Similarly, the highest cumulative soluble P 2.4 mg g^{-1} was extracted with distilled water while the columns treated with hot water and acetic acid recorded 0.9 mg g^{-1} (Figure 6-3b). The highest potassium extracted was 20.7 mg g^{-1} using 0.01 *M* acetic acid , while only 12.6 mg g^{-1} were extracted with hot water (Figure 6-3c). However, extraction by hydrogen peroxide is misleading. Ten pore volumes could not be applied due to the column becoming blocked at three pore volumes.

Overall, the concentration of soluble Ca and Mg encountered in the leachate was relatively low (Figure 6-4) regardless of the solution used for extraction, and despite the relatively high content of Ca (60.4 mg g^{-1}) and Mg (2.3 mg g^{-1}) found in the chicken manure (Table 6-1). Hot water was found to be more effective in extracting Mg and less effective to extracting Ca than the other treatments (Figure. 6-4). The addition of distilled water resulted in 1.8 mg g^{-1} of Ca and 0.13 mg g^{-1} of Mg extracted whereas the addition of hot water extracted 0.91 mg g^{-1} of Ca and 0.19 mg g^{-1} of Mg. The addition of acetic acid resulted in 1.79 mg g^{-1} of Ca in the leachate (Figure 6-4).

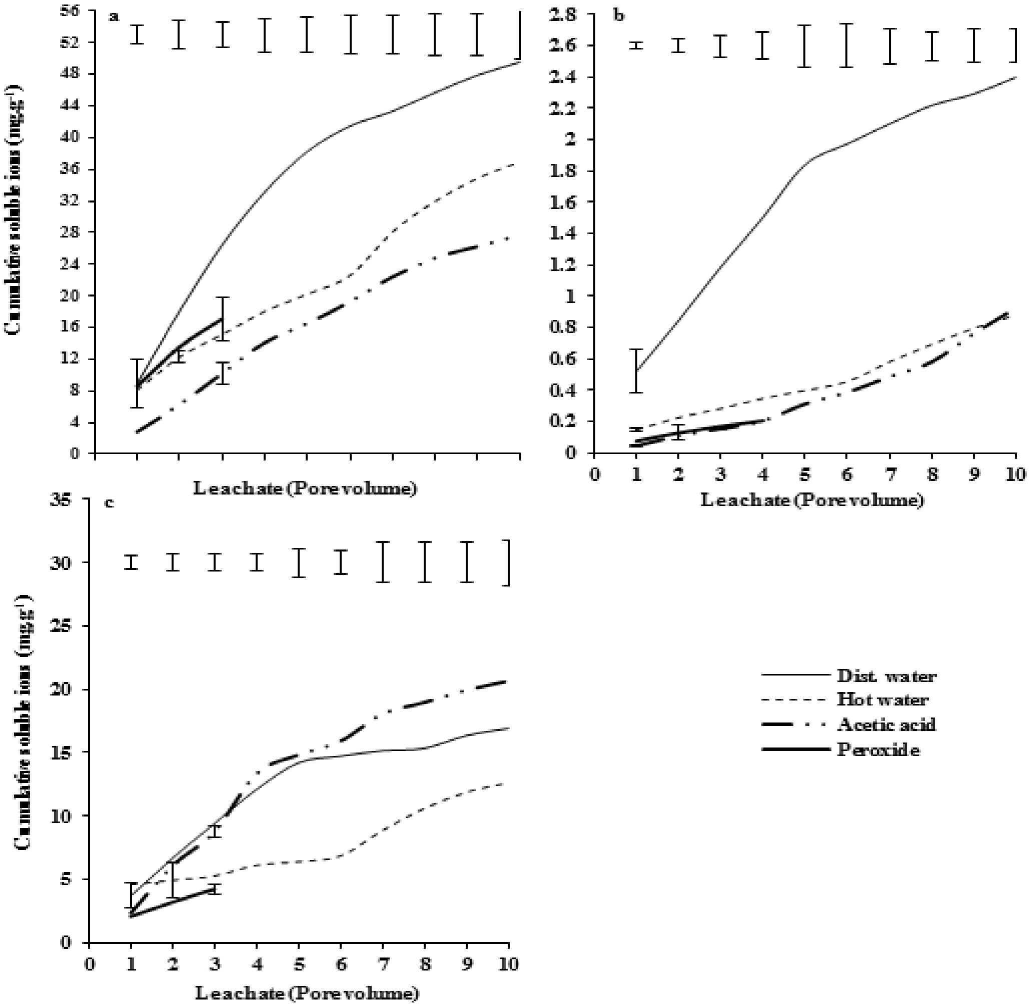

Figure 6-3 The effect of leaching chicken manure columns with different sterilisation solutions on (a) total dissolved nitrogen (b) soluble phosphorus and (c) soluble potassium. Bars at top of graph are LSD (α=0.05) comparing treatments at respective pore volume. Bars on line plots are LSD (α=0.05) comparing volume within each treatment.

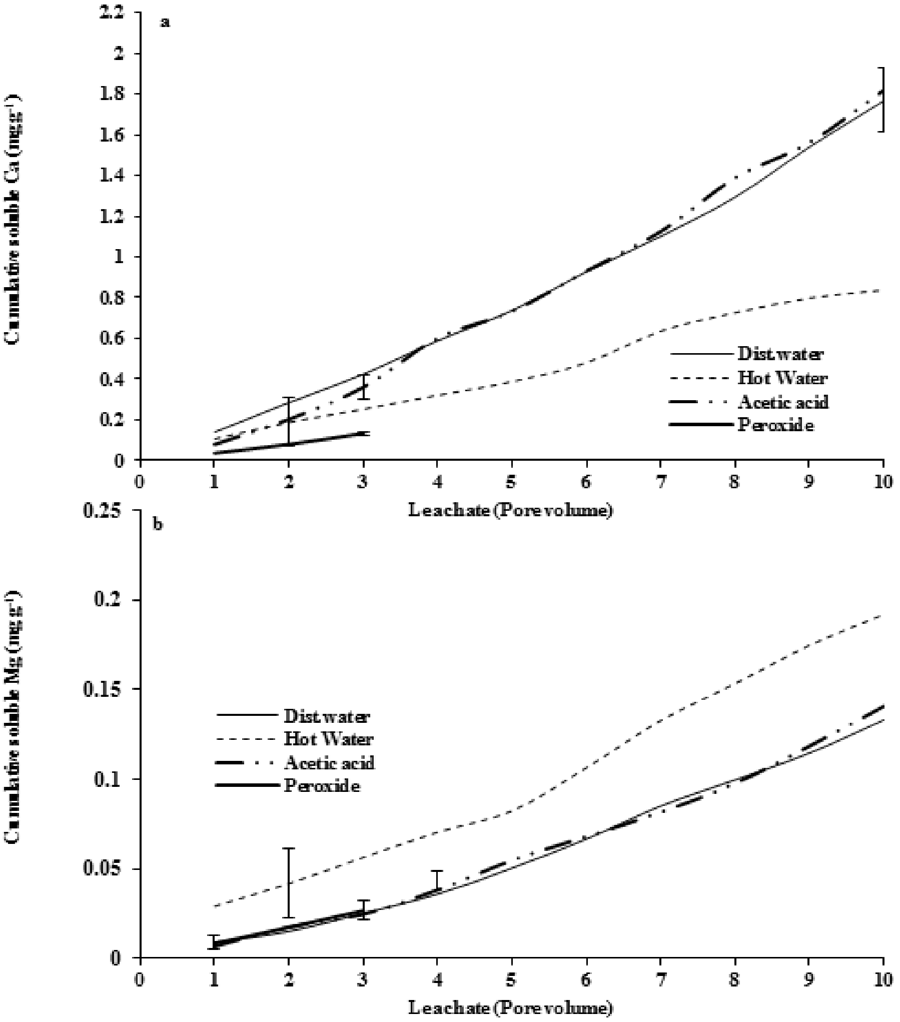

Figure 6-4 The effect of leaching chicken manure column by different solutions on (a) soluble Ca and (b) soluble Mg. Bars at top of graph are LSD (α=0.05) comparing treatment at respective pore volumes. Bars on line plots are LSD (α=0.05) comparing volume within each treatment.

Sodium and Chloride

The application of acetic acid was relatively more effective than hot water in extracting Na from the columns (Figure 6-5a). Mean values of cumulative Na content in the leachate were 6.9 mg g^{-1} in the former and 3.7 mg g^{-1} in the latter treatment, respectively. By contrast, cumulative Cl concentration increased in the hot water treatment compared with the acetic acid treatment (Figure 6-5b). The highest value of cumulative Cl (9.4 mg g^{-1}) was extracted by distilled water (Figure 6-5) while the lowest value of Cl was 6.02 mg g^{-1} extracted by using acetic acid.

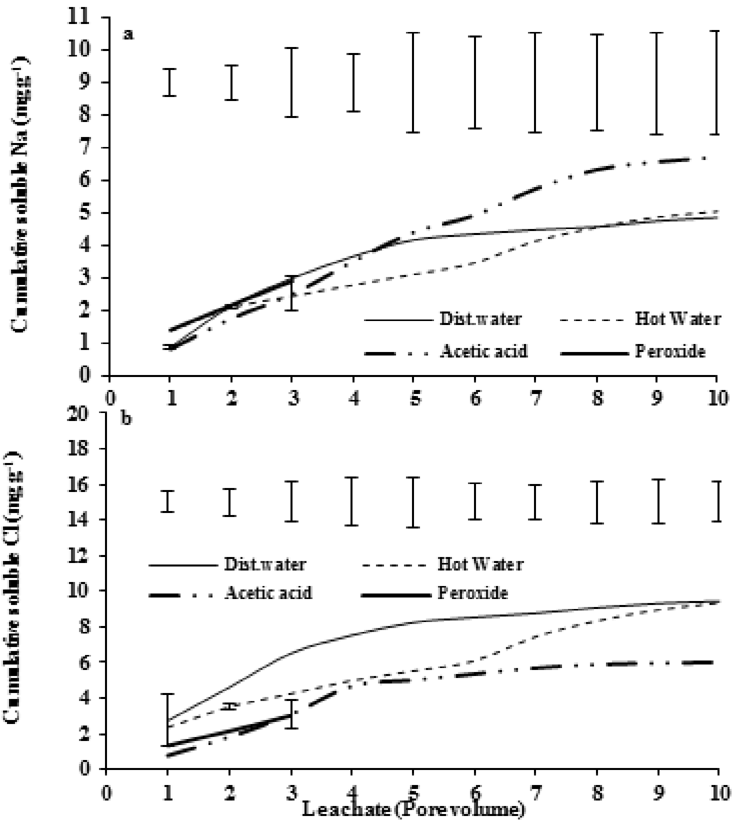

Figure 6-5 The effect on soluble nutrients of applying different sterilisation treatments to chicken manure leachate. Bars on plotted are standard error for each nutrient.

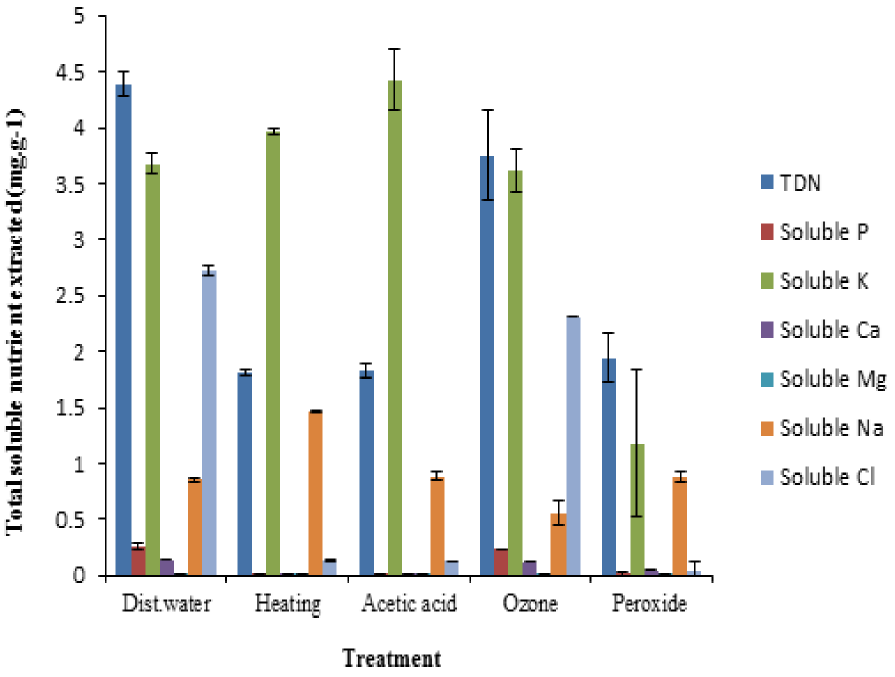

Figure 6-6 The effect on soluble nutrients of applying different sterilisation treatments to chicken manure leachate. Bars on plotted are standard error for each nutrient.

6.3.2.2 Post-leaching treatments

Post-leaching treatments intended to reduce the pathogen load significantly ($P<0.05$) affected the concentration of soluble nutrients in the leachate. Overall, the use of heat and acetic acid reduced the concentration of TDN and phosphate compared with using ozone (Figure 6-6). However, the concentration of soluble potassium was increased as a result of applying acetic or hot water (Figure 6-6). Soluble Cl, Ca and Mg were significantly reduced by heating or applying acetic acid (Figure 6-6). On the other hand, the soluble Na was significantly ($P<0.05$) increased with heating or acetic acid (Figure 6-6).

Applying ozone was found to not significantly impact on TDN in the leachate. Similarly, the ozone treatment did not affect the soluble P concentration in the leachate. However, the soluble K was significantly reduced by ozone. Soluble K also decreased as a result of adding hydrogen peroxide, while heating and applying acetic and led to an increase in the soluble K in leachate (Figure 6-6). Ca and Mg were not significantly affected by heating, acetic acid or ozone, but applying hydrogen peroxide did increase the Ca and Mg in the leachate (Figure 6-6).

Soluble Na decreased where applying ozone but increased when the leachate was heated. However, soluble Cl was significantly affected by all treatments. The soluble Cl decreased from 2.73 to ≤ 0.13 mg g^{-1} when the leachate was heated or acetic acid or hydrogen peroxide application increased, but decreased to only 2.3 mg g^{-1} where ozone was applied (Figure 6-6).

6.3.3 Cation ratio of structural stability

The CROSS of the leachate was generally high (16.8 – 50.6) and varied depending on the extracting solution with significant ($P<0.05$) differences were only observed after the second pore volumes of leaching (Figure 6.7). The CROSS decreased progressively with increasing leaching for all treatments (Figure 6-7). This effect was expected as the Na in the columns was rapidly leached.

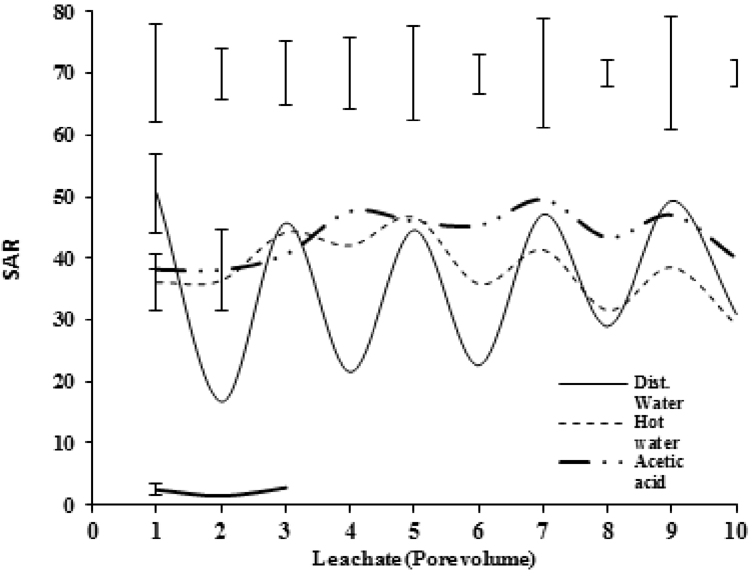

Figure 6-7 The effect of leaching chicken manure column by different sterilisation solutions on sodium adsorption ratio. Bars at top of graph are LSD (α=0.05) comparing treatments at respective pore volume. Bars on line plots are LSD (α=0.05) comparing volume within each treatment.

6.4 Discussion

6.4.1 Effects of treatments on pathogen control

The use of hot water (at 75^{o}C) was relatively more effective than applying acetic acid in eliminating pathogens in chicken manure leachate. However, significant reductions in pathogens with the use of hot water were not observed until after the fifth leaching event. This was due to the increase in temperature recorded in the columns which eventually exceeded 55^{o}C. Fallik et al. (2007) and Bari et al. (2008) have shown that the rate of pathogens survival where exposed to hot water is related to the time of exposure. Bari et al. (2008) demonstrated that *E. coli* and *Salmonella spp* were reduced from about 6.1 and 5.3 $\log_{10}$ CFU g^{-1}, respectively, to below detection limits when the microorganisms were exposed to water at 90°C for 90 seconds. Fallik et al. (2007) found that a 20 second exposure was sufficient to reduce the number of *E. coli* from 3.60 $\log_{10}$ CFU g^{-1} to an undetectable level when fresh fruit was soaked in hot water at 70^{o} C. In the present study, the chicken manure initially had 6.45 ±0.38 and 5.15 ±0.31 $\log_{10}$ CFU g^{-1} of *E coli* and *Salmonella spp*, respectively. However, it was found that the temperature inside the 100 mm chicken manure column only reached 55^{o}C ±1.39 after four pore volumes of hot water passed through the column. If the temperature is required to be reached sooner, then strategies for pre-treating the column should be investigated. It should be noted that increasing temperature affects both bacteria cells and indirectly affects manure properties. For example, the pH of the leachate with hot water treatment was relatively high, which suggests losses of nitrogen in the form of ammonia as a result of increased temperature in the columns. Himathongkham and Riemann (1999) reported that an elevated level of ammonia in chicken manure led to a reduction in *E. coli* and *Salmonella spp* < 2 $\log_{10}$ CFU g^{-1}.

The use of acetic acid was found to significantly reduce pathogens. However, the level of pathogens was still high compared to the irrigation water standard. Fan et al. (2009) reported that the acceptable level of pathogens in irrigation water should be not exceed 4 $\log_{10}$ CFU 100 mL^{-1}. Thus, using 0.01 *M* acetic acid was not an effective treatment to eliminate pathogens in the leachate. Parish et al. (2003) reported that using organic acids (such as acetic acid) is a common practice to eliminate pathogens in food where microorganisms generally cannot grow at pH below 4.5. Even though the acetic acid in this study had a pH $\leq$ 3.37, the collected leachate had high a pH. The alkalinity of the leachate was most likely produced from dissolved carbonate salts in chicken manure derived from feeding the birds $CaCO_3$, K_2CO_3 and Na_2CO_3 (Guo and Song 2009). The CaCO3 buffers the pH for the first 3 or 4 pore volumes of drainage. The buffering capacity is then exhausted; allowing the pH to fall. The number of pathogens in the leachate obtained from the acetic acid treatment was lower than the leachate derived from distilled water because of the relative high pH and the nitrogen losses in the form of ammonia.

The use of either heat (pasteurisation) or ozone applied to the leachate, post leaching, was shown to be an effective method to reduce pathogen load in the liquid. Pasteurisation at 65^{o}C for a period of 15 minutes was sufficient to reduce pathogens content in the liquid to a safe level. This is a cooler and shorter, method as compared to the method of Sahlström et al. (2008) who reported that the suitable temperature and time for pasteurisation of chicken manure is 70^{o}C and 30 min.

Using ozone to eliminate pathogens does not have negative effects on manure leachate chemical qualities. The use of 10.41 mg L^{-1} ozone for 30 min was enough to produce a safe leachate product in this study. This result agrees with results for black pepper, where pathogens were eliminated after exposure to 6.7 mg L^{-1} ozone for 60 min without any significant effects on pepper quality (Zhao and Cranston 1995). Guzel-Seydim, Greene, and Seydim (2004) also illustrated the mechanism of ozone treatment to eliminate pathogens, where the ozone attacks the bacteria membrane, whether glycoproteins or glycolipids.

6.4.2 Effect of treatments on leachate nutrient composition

The use of acetic acid resulted in leachates of pH >8.3 during the first leaching event, and reduced the amount of Ca and P leached, most probably due to dissolved carbonate salts from manure and subsequent precipitation of Ca and P in the form $Ca_3(PO_4)_2$ which is sparingly soluble. Total carbonate content in the leachate was 150.8 mg g^{-1} as HCO_3 chicken feeding programs contain $CaCO_3$, $NaHCO_3$ and $KHCO_3$ to balance osmotic relationships and pH through the body (NRC 1994). According to Guo and Song (2009) the leachate pH was increased after the second water leaching event due to carbonate salts being dissolved. Therefore, when acid is applied to raw manure, the solubility of carbonate salts increases, HCO_3^- and CO_3^{-2} become solubilized and the availability of Ca^{+2} is increased.

Karukstis and Van Hecke (2003) reported that the solubility of calcium carbonate was increased with the addition of acetic acid. Therefore, both Ca and P could likely form octacalcium phosphate ($Ca_3(PO_4)_2$) which has a slow solubility. Barker and Pilbeam (2010) reported that octacalcium phosphate could be formed when an acidic fertiliser solution is added to a calcareous soil. In this case, the solubility of $CaCO_3$ increases, resulting in increased availability of Ca and precipitation of dicalcium phosphate dehydrate ($CaHPO_4.2H_2O$) as a first reaction product, followed then by octacalcium phosphate ($Ca_8H_2(PO_4)_6.5H_2O$) or by one of the apatites ($Ca_5(PO_4)_3$ –F, Cl, OH).

Similarly, Ca and P were observed to decrease in leachate when hot water at 70^oC applied. The pH of the hot water leachate obtained showed a very slight progressive increase with the amount of leaching, due to precipitation of carbonate salts; a process that was enhanced by the increasing temperature inside the columns. In addition, the increase in pH of leachate derived from hot water or acetic acid, as compared to the initial medium pH, was due to dissociation of calcium carbonate and a decrease in total dissolved nitrogen (TDN) in leachates. The decrease in TDN might be attributed to ammonia volatilisation where the pH of leachate was high. Increases in pH and temperature lead to an increase in the loss of NH_3 by volatilisation (Logan and Donovan 1983). Feigin, Ravina, and Shalhevet (1991) reported that the concentration ratio of NH_3 to NH_4 in solution depended on the pH of solution. They found that the relative concentration of NH_3 to NH_4 in different pH solutions of 5, 7 and 9 were 0.0036, 0.36 and 36%, respectively.

Similar results were found in the post-leaching treatments where the TDN, soluble P and Ca were decreased after heating at 65^oC or application of acetic acid. However, soluble Na was significantly increased in leachate treated by heating or application of acetic acid, due to dissolution of sodium bicarbonate salts.

Nitrogen, phosphorus and calcium are important for soil and plant health. Therefore, using acidic or hot water leachate treatments to eliminate pathogens has not maximised nutrient values. However, ozone was observed to be an effective treatment to deactivate pathogens. Unlike other treatments, the use of ozone to eliminate pathogens did not have any residual effect on leachate chemical characteristics.

6.5 Conclusion

This study has shown that although the application of hot water or post-leaching heating deactivated *E coli* and *Salmonella spp*, it had negative effects on nutrient solubility. Applications of acid reduced pathogens marginally but did not reduce the count sufficiently to be used for irrigation. There was also a negative effect on the availability of nutrients, due to acetic acid. Whilst, the use of hydrogen peroxide may be viable, the chicken manure columns were blocked after three pore volumes had been applied. However, applying ozone to leachate was found to be an efficient and viable treatment as it deactivated the pathogens and does not significantly alter leachate characteristics. Hence, it is summarised that ozone is used to treat the leachate prior to use as fertiliser.

CHAPTER 7: EVALUATION OF INCREASING LEACHING SOLUTION PRESSURE IN AN INITIAL NUTRIENT EXTRACTION SYSTEM FOR CHICKEN WASTE

7.1 Introduction

The common practice for disposal of chicken manure is direct application to agricultural land (Vervoort and Keeler 1999a) on the basis it contains most of the plant essential nutrients (Sekar, Karthikeyan, and Iyappan 2010, Kelleher et al. 2002). In addition, chicken manure has low C: N ratio (Singh 2000, Mason 2003) which may affect the nutrient availability process. Syers and Craswell (2004) reported that availability of nutrients from animal manure could be slow, as it depends on organic matter breakdown. However, applying this material under poor management could lead to contaminated ground water via nitrate leaching (Bitzer and Sims 1988), contaminated surface water through phosphorus in runoff (Sharpley, McDowell, and Kleinman 2004) and release and spread pathogens (Bitzer and Sims 1988, Moore et al. 1995). Rasnake, Thom, and Sikora (2004) added that the crop type (nutrient demand) and method of applying manure to land also affect nutrient availability dynamics (often front loaded and surface applied/ incorporated). Hence, applying chicken manure through irrigation systems (fertigation) may be a reasonable solution for these issues, as the nutrient is immediately available in soluble format and can be applied through controlled release during the growing season, addressing peak demands. The amount and type of soluble nutrients in liquid manure depend on the type of material used (Alcantara and dela Cruz 2005). Recently, there has been interest in a number of means to prepare manure water extract within the agricultural industry, although these means range from a domestic suitability to a commercial level.

Brewed manure in water has been shown to yield an aerated nutrient suspension, by use of an air pump, which is a common practice for smaller manure water extract operations in Beer – Sheva Israel (Gross et al. 2008). However, Alcantara and dela Cruz (2005) reported that this type of liquid organic fertiliser has low concentrations of soluble nutrients. Manure or compost leachate is another form of manure or compost water extract. Diver (2002) defined leachate as a solution leaching from the bottom of manure or compost piles containing high concentration of soluble nutrients.

In previous chapters, the effect of leaching different maturity composted material and fresh manure for nutrients was investigated (Chapter 4) and it was shown that fresh chicken manure yielded more concentrated manure leachate with greater efficiency, as compared to mature compost. Furthermore, the results obtained from Chapter 5 showed that the different column characteristics led to effects on quality of leachate in terms of concentration of soluble ions, where long columns produced leachate with higher concentrations of soluble ions compared to short columns.

However, using fresh manure has some disadvantages in that pathogens appear in the leachate (Chapter 4) and the leaching time can be lengthy due to highly soluble constituents dissolving and altering flow paths. To address the pathogens (*E coli* and *Salmonella* spp), use of ozone as a post-treatment for leachate was shown as an efficient treatment to eliminate pathogens (Chapter 6). Consequently, the time of leaching fresh manure is still considerable (more than 2 days to collect 3 L of leachate from 601 cm^3 manure column). Therefore, this study hypothesised that increasing leaching solution pressure will reduce the time of leaching and produce a leachate with comparable nutrient concentration gravity fed leaching (like that used in Chapter 4). Hence, the objective of this chapter was to develop a conceptual, but laboratory functional, chicken fresh manure nutrient extraction system and evaluate the effect of increasing leaching solution pressure on nutrient extraction viability. It is important to note that this system has been suggested on the basis of previous work in this thesis and is not being suggested as the ultimate practical system suitable for commercial scale. Discussion on commercial scale systems will be briefly presented based on the above evaluation.

7.2 Material and methods

7.2.1 Manure and compost sampling

Samples of the fresh chicken manure were collected as in section 3.2. All samples were homogenised using a rotary drum and five representative sub-samples (approximately 500 g each) were taken for analysis of physical and chemical properties.

7.2.2 Chemical and physical properties

The chemical and physical properties were conducted as outlined in section 3.3. The organic matter (OM) and ash (%) were determined on three replicates in a muffle furnace using Thompson et al. (2001). The electrical conductivity (EC) and the pH of samples were determined as outlined in section 3.3.6. The cation exchange capacity (CEC) was determined by using ammonium acetate as described by Chapman (1965) cited by Hendershot, Lalande, and Duquette (2007). Exchangeable cations were determined using 1 *M* of ammonium acetate (section 3.3.7). The concentrations of total and soluble inorganic elements (N, P, K, Ca, Mg, Na and Cl) were determined as described in sections 3.3.4, 3.3.5 and 3.3.6.

The moisture content (%), particle density (D_p), total porosity (%) and pore volume (η), were calculated as described in section 3.3.1, 3.3.2 and 3.3.3. Table 6-1 shows the properties of the fresh chicken manure used in this study.

7.2.3 Column preparation and extraction of soluble nutrients

Based on the results obtained from Chapter 5, the chicken manure was packed into PVC columns (87.5 mm inner diameter and lengths 100 mm) at bulk density 0.4 g cm^{-3}. To avoid excessive compaction, the materials were progressively compacted to the treatment bulk density in 25 mm segments using a steel compaction dolly. Filter papers (Watman No. 45) were placed on both ends of the columns to avoid loss of material.

7.2.4 Pressure treatments, system construction and leachate collection

Four pressure levels were used, which were 0.49, 35, 70 and 105 kPa. To produce 35, 70 and 105 kPa, a small electrical pump was used, while to produce 0.49 kPa, a 50 mm constant water head was used. The pump was connected to a 50 L tank to provide the column with water. A flow meter was set up in the system before the column to control the level of pressure. To avoid overload pressure, a bypass was set up (Figure 7-1), at an adequate pipe distance after flow meter to ensure the flow meter could function properly.

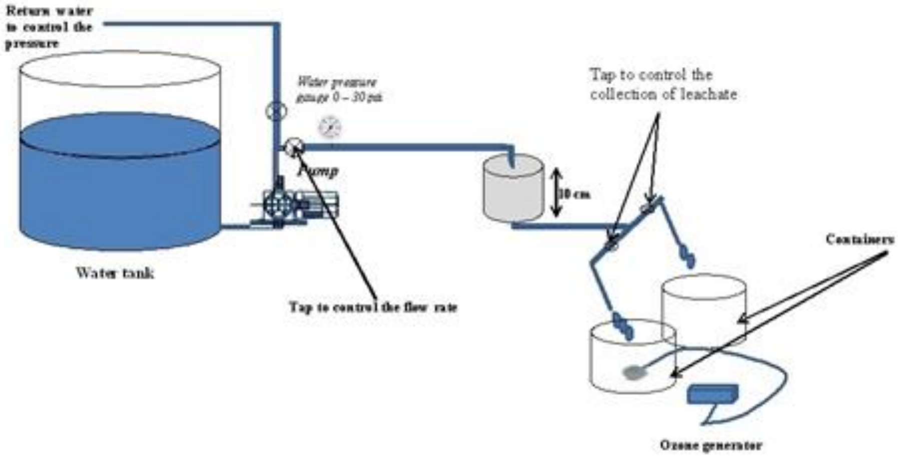

Figure 7-1 The schematic design for extraction of soluble nutrients from chicken fresh manure using pressure

Leachate from the system was collected after each pore volume (461.5 mL) until pore volume 10, where collection caused for treatment 0.49 kPa, where the time was more than 2 days. However, for treatment 35 kPa the leachate was collected as a single pore volume until the tenth pore volumes, then 5 pore volumes were collected together up until 20 pore volumes. For 70 and 105 kPa treatments, the leachate was collected as a single pore volume until pore volume 20 and subsequently as 5 pore volume units.

The temperature in the water tank and leachate was monitored by using three thermometers. Two thermometers were installed in the water tank and a third thermometer was used to measure the leachate temperature. The temperature was measured at the beginning of the experiment, as well as after 5, 10, 15, 20, 25 and 30 pore volumes was applied.

7.2.5 Elimination of pathogens

All leachates derived from all treatments were subjected to Ozone post-treatment. The Ozone gas was generated using an ozone generator (Model ZXA-200, Zox Ozonatord Pty Ltd, Australia). The output of the generator was 200 mg per hour, where the ozone generator produces 10.41 mg L^{-1} Ozone at an oxygen flow rate 3.20 L per minute at room temperature. Previous results in Chapter 6 showed that the appropriate time to eliminate pathogens by Ozone was 30 min with 50 mL of leachate, although this was scaled up to use 100 mL of leachate, with treatment time increased to 60 min at the same Ozone flow rate.

7.2.6 Characteristics of leachate

The chemical and biological characteristics of the leachate were measured using the procedures outlined in section 3.3.7 and 3.3.9, respectively. Pathogen analyses were conducted using 1 mL of leachates derived from different treatments. Serial dilutions with 0.87% (w v^{-1}) of sterile peptone water (NaCl) were made from 10^{-1} to 10^{-5}. Inoculation of petri plates was done by spreading 0.1 mL of diluted leachate on the surface of selective media, *Salmonella and Shigella* Agar (SS agar) and MacConkey agar for *E.coli*. The petri plates were incubated at 37°C for 24 to 48 hours.

Salmonella spp and E. coli were determined by counting black colonies and pink colonies developed, respectively.

The potential of the leachate to affect structure of soil it might be applied to was calculated using the cation ratio of soil structural stability (CROSS) equation described by Rengasamy and Marchuk (2011):

$$CROSS = \frac{Na+(0.56K)}{\sqrt{\frac{(Ca+(0.6Mg))}{2}}} \qquad \textit{Equation 7-1}$$

where: *Na, Ca, Mg and K* is the concentration (meq L^{-1}) in the leachate.

7.2.7 Summary of experimental design and statistical analyses

The study was conducted to evaluate the effects of using various pressure levels on efficiency of extraction of soluble nutrients. The experiment was conducted in a complete randomised design where the experiment contained two factors. These factors were different levels of pressure (0.49, 35, 70 and 105 kPa) and pore volume. Data are expressed as a mean of triplicate values of each parameter measured. Statistical analyses were undertaken using Statistical Package for the Social Sciences (SPSS) v19 for windows 7 (Cramer 2004). Analyses involved univariate ANOVA. Two -Way ANOVA was used to study the effects of both treatment and pore volume; and One Way ANOVA was used to study the effect of leaching events for each pressure level. Least significant difference (LSD) was used to compare the means with a probability level of 5%. Furthermore, correlation and multiple regression analyses were conducted to examine the relationship between total dissolved salts, total dissolved nitrogen leached and pressure, time and pore volume.

7.3 Results

7.3.1 Effects of pressure levels on pH and total dissolved salts (TDS)

The use of different pressure levels significantly affected the pH and total dissolved salts in leachates. Leachate produced from high pressure leaching has a low pH (7.34), while the highest pH values were 8.42 and 8.49 in leachate derived from 0.49 and 35 kPa, respectively. The leachates collected at the first pore volume in all treatments had neutral pH except 0.49 kPa treatment was 7.7; however, with increase in number of pore volumes, leachate pH increased. The increase in pH value was significant until pore volume 25 where the increase in pore volume was non-significant between 70 and 105 kPa (Figure. 7-2a). In addition, the 70 and 105 kPa treatments increased initially, and then plateaus at around 7.5, while the 0.49 and 35 kPa treatments continue to increase.

Total dissolved salt (TDS) was also significantly affected by increase in the leaching pressure level. Increasing the pressure from 0.49 to 35, 70 or 105 kPa resulted in a decrease in the TDS values in first ten pore volumes, where 0.49 kPa produced leachate with (240.6 mg g^{-1}) after ten pore volumes, while 35, 70 and 105kPa treatments produced leachate with (173.4, 170.9 and 87.9 mg g^{-1}), respectively. The value of TDS after 9.2 L of water applied was 280.35 mg g^{-1} at 35 kPa whilst 70 and

105 kPa produced leachate with low TDS (211.95 and 106.18 mg g^{-1} respectively). Even though the leaching process in 70 and 105 kPa treatments continued to 30 applied pore volumes (13.83 L of water applied), the TDS values remained low compared with the 35 kPa treatment (Fig. 7-2b).

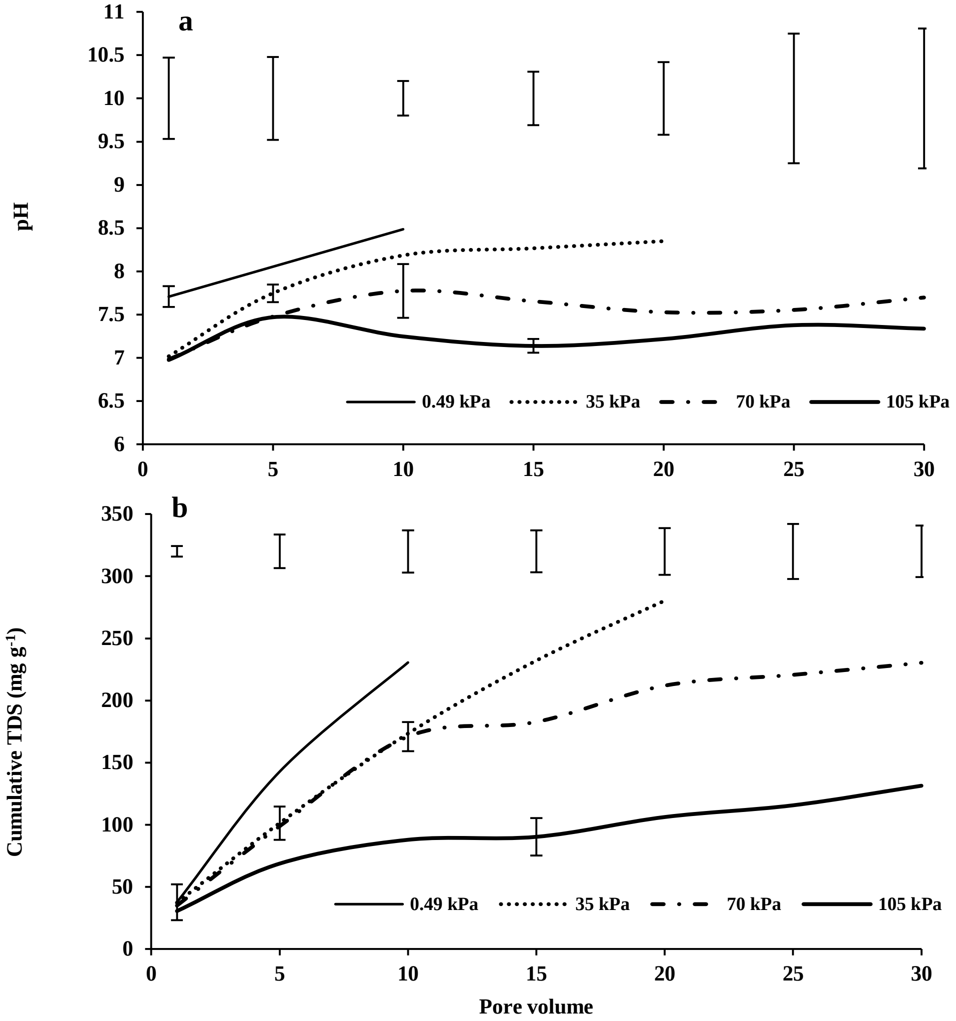

Figure 7-2 The effects of different pressure levels on (a) pH and (b) total dissolved salts (TDS). Bars at top of graph are LSD (α=0.05) comparing pressure levels at respective pore volume. Bars on plotted lines are LSD (α=0.05) comparing variability within treatments.

7.3.2 Effects of pressure on soluble nutrients

Increasing the leaching solution pressure during extraction of soluble nutrients from chicken manure was shown to decrease the rate of solubilisation of nutrients (N – P – K) and, consequently, the total concentration for the comparative volume of leached water. Change in pressure from 35 to 70 kPa significantly (*P-value* < 0.05*)* decreased soluble K by around 25% with a further reduction of ~25% at 105 kPa, while extrapolation of the very low pressure (0.49 kPa) K curve suggests that all higher pressures tested caused significantly lower rates of K leaching (Fig. 7-3). Increasing leaching pressure from 35 to 70 or 105 kPa led to decrease of soluble P in leachate by around 35 and 49%, respectively. Considering reductions in nutrient leaching at 105 kPa, TDN and soluble K were decreased by 51 and 40%, respectively, as compared to the 35 kPa leachate, which is greater than reductions observed for soluble P. The rate of extraction under pressure for all nutrients was generally slower after 10 PV with observations of leachate concentrations after 30 PV generally not of practical increase for the water volume passed. That is, increasing leaching pore volumes generally did not significantly increase the concentration of soluble nutrients in leachate with medium and high pressure (70 and 105 kPa).

Similarly, soluble Ca and Mg solubility rates were significantly decreased by increasing the leaching pressure level. The soluble Ca and Mg were decreased by 50 and 61%, respectively, when the pressure increased from 0.49 to 35 kPa and comparing the last measured pore volume for each pressure (10 and 20 pore volumes, respectively). However, under high pressure (105 kPa), soluble Ca and Mg were significantly (*P-value* <0.05) increased in leachate compared with the other pressure treatments (35 and 70 kPa) (Figure 7-4).

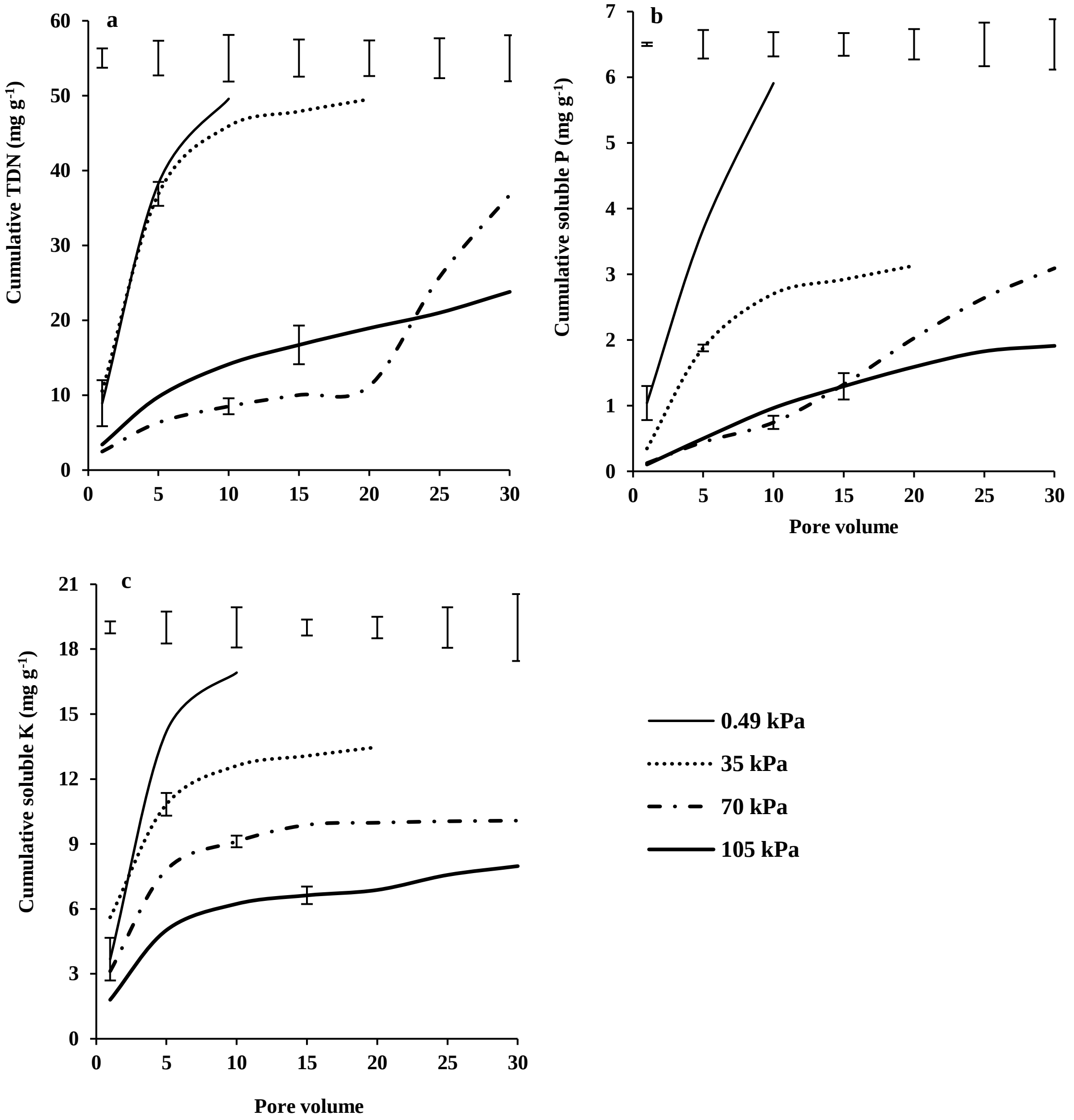

Figure 7-3 The effects of different pressure level on (a) Total dissolved nitrogen, (b) soluble P and (c) Soluble K. Bars at top of graph are LSD (α=0.05) comparing pressure levels at respective pore volume. Bars on plotted lines are LSD (α=0.05) comparing variation within treatment.

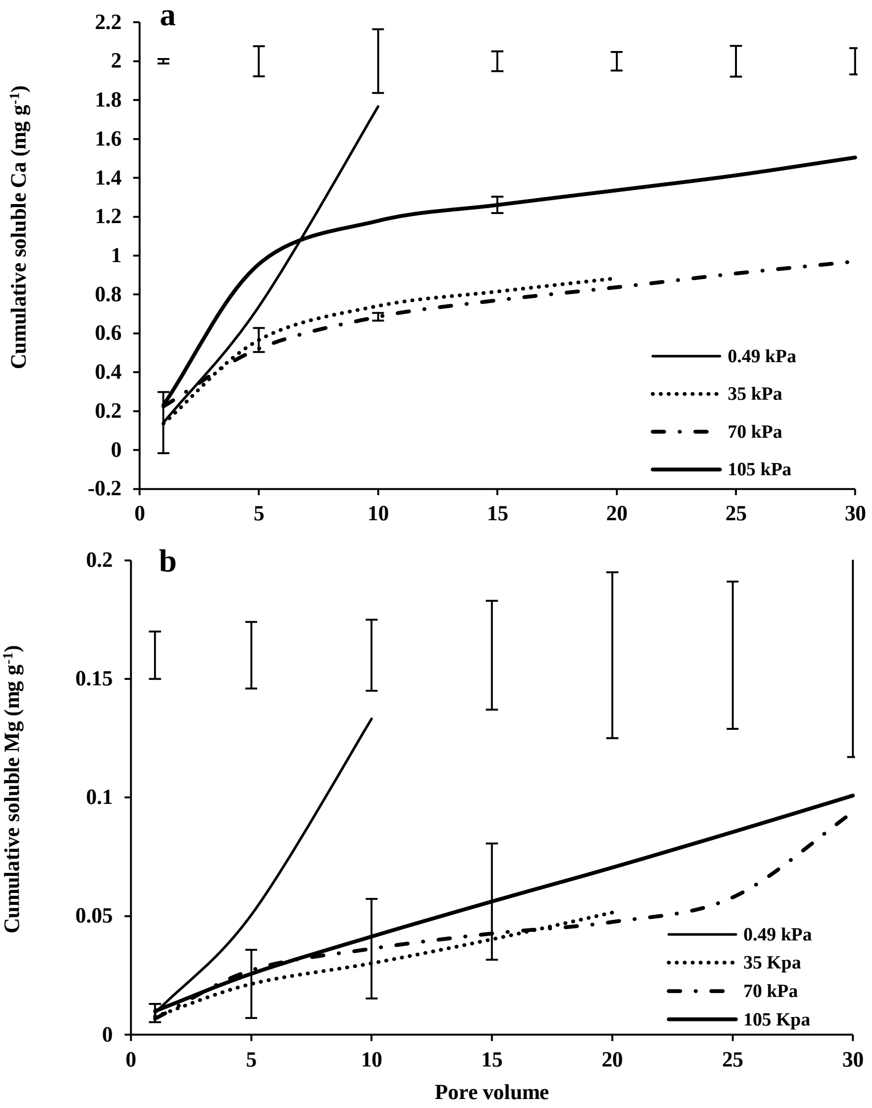

Figure 7-4 The effects of different pressure level on (a) Soluble Ca and (b) soluble Mg. Bars at top of graph are LSD (α=0.05) comparing pressure levels at respective pore volume. Bars on plotted lines are LSD (α=0.05) comparing variation within treatment.

7.3.3 Sodium and Chloride

As soluble Na and Cl in leachate affect the quality of leachate in terms of soil and plant health when leachate is applied to land, both soluble Na and Cl were studied. The increase in pressure during extraction of nutrients led to decreased concentration of these elements in leachate. The soluble Na and Cl were decreased by more than 50% when using high pressure 105 kPa (Figure 7-5). Similarly, use of low pressure (35 kPa) was found to decrease soluble Na and Cl around 50 and 30% respectively.

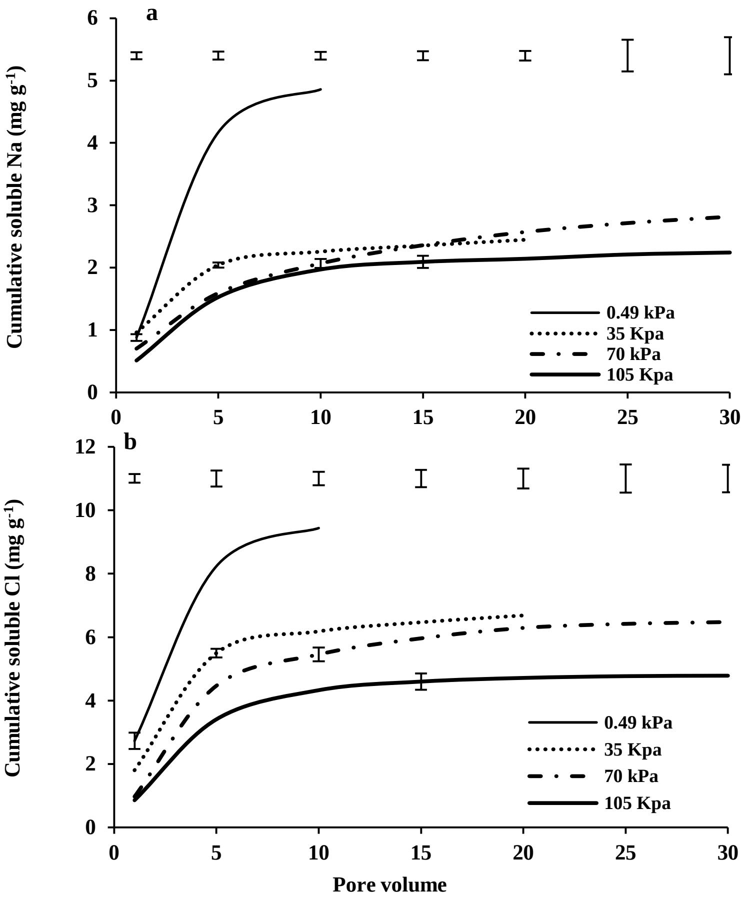

Figure 7-5 The effects of different pressure level on (a) Soluble Na and (b) soluble Cl. Bars at top of graph are LSD (α=0.05) comparing pressure levels at respective pore volume. Bars on plotted lines are LSD (α=0.05) comparing variation within treatment.

7.3.4 Temperature of water and leachate

During the experiment, it was noted that the water temperature increased, reaching 55°C ±1.2 at high pressure (105 kPa), while other treatments exhibited temperatures between 30°C ±2.3 and 41°C ±0.9 for 35 and 70 kPa, respectively. However, the water temperature of the very low pressure 0.49 kPa treatment was approximately 20°C ±1.1. The leachate temperature was almost stable and ranged between 19 and 27.5°C for low and high pressure, respectively (data not shown). An increase in temperature may be required cause to eliminate pathogens (section 6.3.1).

7.3.5 Cation Ratio of Soil Structural Stability (CROSS)

Using high pressure to extract soluble nutrients from chicken manure resulted in decreased CROSS of the leachate. High pressure 105 kPa produced leachate with relatively lower CROSS, as compared to 0.49 kPa results, while the 35 kPa produced leachate with comparable CROSS after the first pore volume applied (Fig. 7-6). Subsequent pore volumes exhibited reduced CROSS values of the leachate where CROSS values after 20 pore volume were 5.22, 4.73 and 0.95 for 35, 70 and 105 kPa, respectively. Comparatively, use of a small pressure head (0.49 kPa) resulted in a very gradual decrease in CROSS over the ten pore volumes measured (CROSS 50 to 30.94).

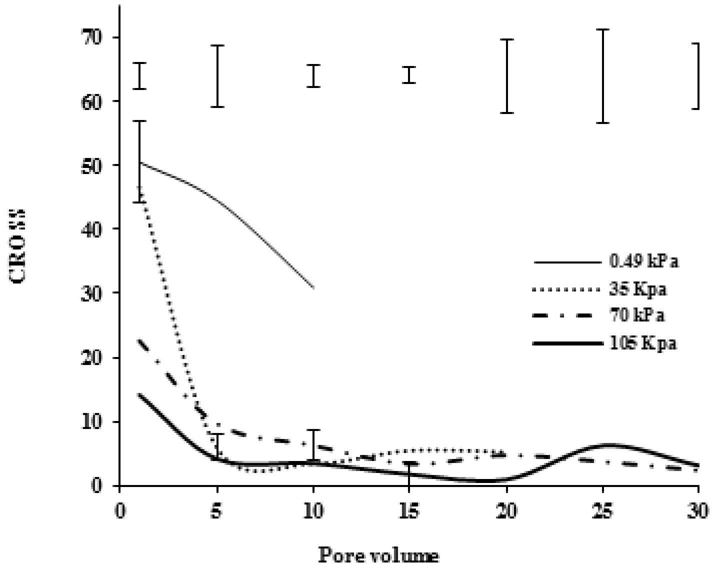

Figure 7-6 The effects of different pressure level on CROSS of leachate. Bars at top of graph are LSD (α=0.05) comparing pressure levels at respective pore volume. Bars on plotted lines are LSD (α=0.05) comparing variation wihtin treatment.

7.3.6 Pathogens in leachate

Table 7-2 shows the number of pathogens detected in leachate as affected by use of different pressure levels. Increasing the pressure of the leachate resulted in slightly reduced number of pathogens in leachate, although this reduction was not sufficient to provide an acceptable standard of irrigation water. On the other hand, using 10.41 mg L^{-1} Ozone at an oxygen flow rate of 3.20 L per minute was found to eliminate pathogens within the leachate after 60 min. As expected, 30 min of ozone treatment was insufficient given the increased treatment volume (100 mL, as opposed to 50 mL) (Table 7-2).

Table 7-1 The number of pathogens in leachates extracted by different pressure levels

Method of extraction	Non-Ozone		100 mL at 30 min Ozone		100 mL at 60 min Ozone	
	E.coli	*Salmonella. ssp*	*E.coli*	*Salmonella. ssp*	*E.coli*	*Salmonella ssp*
0.49kpa	8.77a	7.87a	5.59^{a}	5.70^{a}	n.d*	n.d
35kpa	7.46b	6.69b	4.78^{b}	4.62^{b}	n.d	n.d
70kpa	7.3b	6.95b	4.83^{b}	4.52^{b}	n.d	n.d
105kpa	7.21b	6.04c	4.95^{b}	4.51^{b}	n.d	n.d

*n.d is referred to non-detection of pathogens in leachate.

7.4 Discussion

7.4.1 Effect of pressure on nutrient extracted

It was found that the use of different pressure levels to reduce the time of leaching resulted in a decrease in the concentration of soluble nutrients in manure leachate. The effect of pressure was indirect where the pressure decreased the contact time between the leaching volume and the raw manure, which in turn decreased the rate of nutrient leaching, and decreased the leachate pH, due to decrease contact time; i.e. the Ca, Mg and K carbonates were not provided sufficient time to dissolve in leachate. Also, increasing pressure leads to increase in the column and leachate temperature. This affects the solubility of salts in different ways; thus applying different pressure on manure columns produced different leachate characteristics in terms of concentration of soluble nutrients in the obtained leachates. The relationship between total dissolved salts (TDS) and increase in pressure was significantly negative ($R^2 = -0.344$); also, the relationship between increased pressure and contact time was significantly negative ($R^2 = -0.739$). Hence, the reduction of soluble nutrients in leachate was likely due to reduced contact time between water and manure in columns resulting from increased leaching velocity. These results are consonant with López Meza et al. (2010) and Dijkstra, van der Sloot, and Comans (2006) where they found that contact time controlled the release of soluble salts such as NaCl and KCl from municipal waste columns. In this experiment, the contact time declined from 1 hr and 30 min to 23 min at the first pore volume collected when pressure increased from 35 to 105 kPa, respectively. Similarly, after 20 pore volumes, the contact time for high pressure 105 kPa was less than 35 kPa where total time to obtain the 20 pore volume leachate was around 8 hr compared to 3 days, respectively. Conversely, under the very low pressure head (0.49 kPa) contact time is greater, increasing the likelihood of the leaching volume to interact with the organic colloids and break them down, or dissolve them, during the leaching process. During the first pore volume at 0.49 kPa, it was observed that the colour of collected leachate was a dark colour as a result of leached organic colloids, which was not observed to the same extent in higher pressure treatments. Also, during preparation of 0.49 kPa leachate samples for analyses, high sediment load was found with particle diameter more than 0.45 μm as measured with glass filter paper.

As leaching solution pressure increased, the temperature of both the column and leachate was increased where the relationship between pressure and temperature is a positive relationship. The increasing temperature of the medium leads to increased salt solubilisation (Verma 2012), but also affects the availability of ions by changing their available form. For example, increasing the temperature should increase the solubilisation of carbonates and increase available calcium, but increased calcium in solution could precipitate with phosphorus in the form of dicalcium phosphate dehydrate $CaHPO_4.2H_2O$ (Chapin et al. 2011). In addition, the increased temperature will affect the nitrogen form and subsequently the nitrogen loss. Logan and Donovan (1983) suggested that the ammonia volatilisation increased due to increasing the temperature of solution.

Furthermore, while increasing the pressure has reduced leachate pH, it has not increased solubility of organic compounds within the manure as the reduced pH in the leachate is a function of low solubility of carbonates, rather than an increase in leaching solution acidity. Hence, low pressure levels produced more alkaline leachate due to the increased contact time between leaching solution and medium, allowing increased time for carbonate salt dissolution. Guo and Song (2009) suggest that an increase in leachate pH might be likely produced from dissolved salts of carbonate in chicken manure, derived from feed containing carbonates such as $CaCO_3$, K_2CO_3 and Na_2CO_3. Consequently, the leached Ca, K and Mg are generally reduced as pressure increased, which further supports contact time as the driving mechanism.

7.4.2 Preliminary analysis of system viability

In this study, the total dissolved salts (TDS) and total dissolved nitrogen (TDN) leached from fresh chicken manure were studied as a function of pore volume over different pressure levels. In addition, as there is negative correlation between time and pressure, TDS and TDN were investigated as a function of time and pore volume.

$$\boldsymbol{ln\,TDS = 2.175 - 0.521\,ln\,Pre + 0.576\,ln\,Pv}. \qquad \boldsymbol{R^2 = 0.918} \qquad \textit{Equation 7-2}$$

$$\boldsymbol{ln\,TDS = 0.629 + 0.217\,ln\,T + 0.343\,ln\,Pv}. \qquad \boldsymbol{R^2 = 0.899} \qquad \textit{Equation 7-3}$$

$$\boldsymbol{ln\,TDN = 2.356 - 1.170\,ln\,Pre + ln\,Pv}. \qquad \boldsymbol{R^2 = 0.771} \qquad \textit{Equation 7-4}$$

$$\boldsymbol{ln\,TDN = -0.425 + 0.555\,ln\,T - 0.023\,ln\,Pv}. \qquad \boldsymbol{R^2 = 0.80} \qquad \textit{Equation 7-5}$$

where: *Pre* is level of pressure in kPa, PV is the pore volume of the leaching medium and T is time of leaching in hours.

To extract 100% of nitrogen from chicken manure used in this study, at an applied pressure of 35 kPa, 249 pore volumes are required, which is around 115 L of water applied through a 100 mm column (Φ 87.5 mm internal diameter) over 77.72 hr. While increasing pressure might reduce the required leaching time, it increases the number of pore volumes required to leach the same nutrient concentration and therefore actually marginally extends the leaching time; predicted required pore volumes for 100% nitrogen removal at 70 and 105 kPa are 1024 and 2351 over 82.43 and 85.32 hr, respectively. Even at 35 kPa, these results may not be economically practical in terms of the amount of water required if the system were to be scaled up.

Furthermore, after the leaching process, the solid material becomes a system waste, which should be disposed of it in a safe way that maximises the use. Composting of this material for reincorporation would appear to be a viable method. However, some nitrogen in this material is required to allow microbial breakdown of the material during composting, if an external nitrogen source is not added (which would be counter intuitive to the nutrient extraction process). While not measured in this study, 50% of nitrogen remaining in the residual material may be beneficial to the composting process. Response (1994) suggested that microorganisms require sufficient concentrations of specific nutrients such as N, P and K to progress composting efficiently. Hence, to extract 50% of total nitrogen, it is required to leach approximately 74, 304 or 698 pore volumes when applying pressure levels of 35, 70 and 105 kPa, respectively. The time of leaching would be decreased to less than one day at all pressure levels (35, 70 and 105 kPa). That said, this is still a large amount of water required for leaching at the commercial scale, which could be difficult to obtain in industries, such as agriculture, where fresh water resources are already stretched.

7.5 Limitations and future directions

This experiment has not produced a prototype system; it has investigated a conceptual system for the purpose of understanding leaching solution pressure on nutrient extraction. The component characteristics of the system would benefit from further analysis to determine column characteristic dynamics under pressure. For example, with decreased contact time and increased solution leaching rate, it might be possible that the column length be extended to expose solution to more medium without significant detriment to the leaching time.

From the results, it would appear that the use of pressure significantly decreases the potential of the leached solution to affect soil structural stability by decreasing the CROSS of initial and subsequent pore volumes when under pressure above 35 kPa. However, these results are not truly indicative of this. While the CROSS is decreased under increased solution pressure at any given pore volume compared to very low pressure (0.49 kPa), this is a function again of contact time. It is likely that if CROSS graphs were extrapolated to the pore volume required to extract nutrients to the extent of 50% total nitrogen, for example, then the CROSS value of the final solution would likely be comparable to that obtained under the very low pressure (0.49 kPa) for the same extracted nitrogen concentration. Hence, further treatment of leached solution and/ or land resource the leachate is to be applied to would still be required using the pressure based system. For example, as Na is highly soluble, an initial leaching fraction could be extracted and discarded, then subsequent fractions collected to provide a high nutrient, low CROSS leachate.

The conceptual system tested during this experiment was entirely linear. Future research should consider solution saturation concentrations for the various required nutrients and investigate a feedback loop system whereby leachate is transferred from the leachate reservoir to the percolate reservoir for further leaching use. While the water used in the investigated system had passed the column, it was not nutrient saturated and hence was not precluded from further leaching. However, to evaluate the effect of pressure it was not sensible to feed the leachate back through the column, as chemical analysis of the various nutrients is not a short and autonomous process. The required water volume to leach a given concentration of nutrients could be reduced though a feedback loop, but the dynamics of solubilisation are affected by the ionic concentration of the solution and this would require further analysis; i.e. the above relationship equations would not hold for prediction of the required pore volumes to leach a given nutrient concentration.

7.6 Conclusion

It was concluded from this study that use of pressure to reduce the time of leaching observed under very low pressure (0.49 kPa) was successful, but required significantly more leaching solution volume to obtain the same concentration of nutrients. Furthermore, that this volume of solution is unlikely to be economically viable at the commercial scale if this exact system were to be used. A further system that feeds low concentration leachate back through the leaching medium would benefit from further research in addressing both a short leaching period and high nutrient extraction

Increasing leaching solution pressure indirectly affected the concentration of soluble nutrients in terms of an increase in leaching solution required, leaching medium (in this case fresh chicken manure) temperature and decreased contact time between the solution and medium. Contact time was found to be the overriding mechanism, masking any increased compound solubility effect from increased temperature.

CHAPTER 8: GENERAL DISCUSSION, CONCLUSION AND FUTURE WORK

8.1 General discussion

This chapter considers the information from the outcomes of the study (Chapter 4, 5, 6 and 7) regarding the optimisation of the extraction of soluble nutrients from chicken manure and compost. This section contains three main subsections. Section 8.1.1 provides a brief review of the major issues this research is based upon, principally concerned with application of chicken manure though irrigation systems. The important outcomes of this study are discussed in section 8.1.2 while section 8.1.3 discusses the strategies used to optimise total dissolved nitrogen. Finally, section 8.1.4 considers the viability of implementing the findings of this study in the field.

8.1.1 The viability of using fresh chicken manure or mature compost as a source of soluble nutrients to produce liquid organic fertiliser

Applying compost leachate via irrigation systems as liquid organic fertilizer was identified as safe for application as it was free of pathogens. However, the quality of this product was less valuable than leachate from fresh manure as the concentration of the soluble nutrients was substantially lower. The reduction in concentration of soluble nutrients in compost leachate resulted from the reduction of the nutrient content in compost during the composting process. The percentage of nitrogen loss from chicken manure during the 107 days of composting is up to 77% of the initial nitrogen found in manure (Martins and Dewes 1992). Most nitrogen would be lost during composting in form of ammonia NH_3^+ (Jiang et al. 2011, Guo and Song 2009). The loss of NH_3 would be due to high air supply by frequent turning. Ogunwande et al. (2008) found that turning compost piles every 2 days produced largely losses of TN due to NH_3 volatilisation. Also, low C/N ratio is the main reason for increased loss of nitrogen in form of NH_3^+ (Jiang et al. 2011, de Bertoldi, Vallini, and Pera 1983, Rynk et al. 1992). Therefore, it is recommended to decrease nitrogen loss by increasing the carbon content of manure by adding a high carbon source such as sawdust. The recommended C/N ratio is between 25 to 30 (Rynk et al. 1992) and this is based on the assumption that aerobic microorganisms require from 15 to 30 units of carbon to metabolise one unit of nitrogen (de Bertoldi, Vallini, and Pera 1983).

The aeration and moisture content of the raw material or the compost pile affects the composting process as these factors affect microorganisms that are responsible for the breakdown of organic matter. Both aeration and moisture content are interrelated because the pores of compost should be filled with appropriate portions of water and air. Also, these properties are affected by the bulk density and particle size distribution of the raw material or the compost pile. The recommended percentage of moisture content in a compost pile is between 70-75%, while other spaces should be filled by 25-30% air (de Bertoldi, Vallini, and Pera 1983) where low moisture leads to early dehydration of the pile and arrests the biological process.

Therefore, the decrease in the concentration of the total dissolved nutrients in the compost leachate in this study could be attributable to the management of the composting process. It was observed that the compost pile was turned once per week, causing the moisture to remain below the desirable threshold (Pittaway 2002, Rynk et al. 1992, Misra, Roy, and Hiraoka 2003). In addition, the C/N ratio was low (10:1).

While chicken manure produced high quality leachate in terms of the content of soluble nutrients, it had a high value of cation ratio of soil stability (CROSS). Furthermore, high CROSS affects soil chemical and physical properties after application.

Research has previously been conducted on different methods of eliminating pathogens through chemical treatments. The common chemical agents include hydrogen peroxide, ozone and chlorine (Richardson et al. 2000, Oppenländer 2003). Such methods could be suitable for manure treatment prior to leaching, during leaching or as post-treatment on manure leachate. These chemical oxidants work by invading the cells of microbes in both manure and leachate and damaging these cells. Himathongkham and Riemann (1999) found the number of *E coli* and *Salmonella spp* in chicken manure decreased below detection when using gassing with ammonia. Physical treatment, in contrast, involves the direct application of force, such as application of increased temperature (e.g. pasteurisation) for an adequate period (Kim, Shepherd Jr, and Jiang 2009), which was further demonstrated by (Sahlström et al. 2008) that heating mixed bio-waste at 70°C for 30 min significantly decreased pathogens. As manure leachate is a liquid, the methods used for wastewater may be suitable for eliminating pathogens through post-treatments or during the leaching process of manure. Cheremisinoff (2001) outlined the methods used to eliminate pathogens in wastewater which may be used as chemical treatments for manure. Many studies found that the application of chemical compounds such as chlorine, hydrogen peroxide, acetic acid or ozone to wastewater eliminated the pathogens in the wastewater (Richardson et al. 2000, Oppenländer 2003). On the other hand, the results of these studies were focused on elimination of pathogens, and did not consider possible negative effects on the nutrient availability in manure or leachate. Furthermore, Richardson (2003) showed that the use of these chemical compounds have negative effects on humans during handling. This research studied the effects of hydrogen peroxide, acetic acid and ozone as chemical treatments on the chemical properties of leachate. Similarly, it compared the use of heating as a post-treatment, or applying hot water as the leaching solution, on the availability of soluble nutrients in manure leachate. The results for this study showed that applying ozone to manure leachate as a post-treatment effectively eliminates pathogens and does not affect the availability of soluble nutrients in the leachate. However, other chemical or physical treatments were not feasible because heating and hot water affected the pathogens but also reduced the availability of nutrients in the leachate. In contrast, acetic acid and hydrogen peroxide did not affect the pathogens and reduced the availability of nutrients. Therefore, while this range of treatments has been investigated, further investigations should be made to identify other potential treatments and their effect on both pathogen elimination and nutrient availability.

This study showed that the leachate derived from fresh chicken manure has a high CROSS value where it can be reduced by using fractionation. Thus, to use manure leachate as an organic liquid fertiliser, it is requires treatment to reduce CROSS. The CROSS of a solution affects soil properties; Halliwell, Barlow, and Nash (2001) reported that the effects of sodicity on soil are related to two phenomena (dispersion and swelling). Applying water with moderate SAR (an alternative measure of

CROSS) value can increase the exchangeable sodium percentage (ESP) in the soil (US.Salinity.Laboratory.Staff 1954), a practice that has a negative impact on soil physical properties (Halliwell, Barlow, and Nash 2001). Frenkel, Levy, and Fey (1992) and Tarchitzky et al. (1999) reported that soils irrigated with wastewater, which have a high SAR value and less dissolved organic carbon, may likely lead to high dispersion and blockage of soil pores. Bhardwaj et al. (2007) found applying wastewater with a SAR value greater than 6 to soil led to a decrease in soil hydraulic properties compared with applying fresh water with lower SAR. The resultant situation was blockage of soil water conducting pores (macropores and mesopores) by water particle suspension and by dispersed clay.

This study considered fractioning of leached pore volumes, where the first and second pore volume will be disposed. As a result of the fractionation, the CROSS value could be decreased to a generally acceptable level. However, the level of other nutrients was significantly decreased, as the first and second leachate pore volumes contained the majority of highly soluble nutrients. Thus, further treatment methods are required to produce a manure leachate that has both a high nutrient content and a low CROSS value.. A solution with a high sodicity is likely to cause dispersion of clay within the soil and result in soil pore blockage, decreased soil infiltration and ultimately decreased vegetation productivity. While this effect on soil structure is dependent on the solution EC and the soil threshold electrolyte concentration (Ezlit et al. 2013, Quirk and Schofield 1955), the CROSS values for leachate observed in this study should cause caution in consideration of the leachate for direct land application without further treatment. In addressing the CROSS of the leachate, an additional calcium source would be required, usually in the form of gypsum; application of gypsum to land to ameliorate, or mitigate, sodic conditions is well documented (Valzano, Murphy, and Greene 2001, Qadir et al. 2006, Bennett et al. 2014).

Hence, while fresh manure is a valuable source from which to extract soluble nutrients and create a liquid organic fertiliser, further treatment of leachate is required to treat pathogens and likely required to reduce the effects of a high concentration of sodium within the leachate.

8.1.2 Optimising nutrient extraction for manure and compost

The primary aim of a nutrient leaching strategy should be to maximise extraction of total dissolved salts (TDS) and total dissolved nitrogen (TDN), as these nutrients are of primary importance for most crops relative to other nutrients (Havlin et al. 2005). However, phosphorus is a relatively sorbed element in the soil (Havlin et al. 2005). Thus, optimising phosphorus extraction could lead to the contamination of surface water through runoff and sediment transport under poor management practices. Sharpley, Kleinman, and Weld (2004) reported that the increase in the over-application of chicken manure has resulted in contamination of surface water by phosphorus in runoff. The discussion below is based on a system similar to that used in Chapter 7; further adaptions to the system, such as a leachate feedback loop from the leachate reservoir to the percolate reservoir, will change the optimisation relationships presented below and the optimisation strategies.

Packing chicken manure into 100 mm columns at 0.4 g cm^{-3} bulk density was found to extract a high concentration of TDS and TDN in manure leachate relative to 50 mm columns. The increase in contact time resulted in the increase in the concentration of soluble nutrients. Dijkstra, van der Sloot, and Comans (2006) found an increase in leaching time led to an increased release of elements. The leaching time of 100 mm columns ranged from 1 day to 3 days, depending on the bulk density. The concentration of soluble nutrients in the leachate was observed to increase as the bulk density of column increased, due to an increase in contact time, although the bulk density of column was weakly correlated (R^2 = 0.351 and 0.289) with TDS and TDN respectively.

One suggested strategy is the use of pressure to reduce leaching time without affecting the concentration of soluble nutrients. The outcome of this study showed the use of 35 kPa could reduce leaching time from 3 days to 1.5 days. However, the concentrations of TDS and TDN decreased when pressure was increased above 0.49 kPa. The relationship between TDS and pore volume was medium (R^2 = 0.647), while that between TDN and pore volume was a strong positive (R^2 = 0.830). The concentration of total dissolved nitrogen (TDN) in manure leachate was found to be a function of the bulk density of manure in the column, the running pressure of the systems, the time of leaching, and the amount of water applied (leaching event) (Eq. 8.1, 8.2 and 8.3).

$$\ln TDN = 2.953 + 1.0057 \ln BD + 0.865 \ln Pv. \qquad R^2 = 0.84 \qquad \text{Eq 8-1}$$

$$\ln TDN = 2.856 - 1.170 \ln Pre + 0.571 \ln Pv. \qquad R^2 = 0.77 \qquad \text{Eq 8-2}$$

$$\ln T = 1.739 + 0.931 \ln Pv - 0.945 \ln Pre + 0.035 \ln TDN. R^2 = 0.86 \qquad \text{Eq 8-3}$$

where: TDN is total dissolved nitrogen (mg g^{-1}) manure, BD is bulk density of manure in column (g cm^{-3}), Pv is pore volume “amount of water collected (mL)”, Pre is running pressure kPa and T is time of leaching (min).

The cumulative TDN release is predicted at 63 mg g^{-1}, representing 50% of total nitrogen in chicken manure when packing chicken manure at 0.4 g cm^{-3} in 100 mm column length and an applied 23 pore volumes (equivalent 10603 mL) is used. However, to collect of this amount of leachate requires a time of more than 5 days. Thus, the use of pressure may decrease the time of leaching, where Eq 8.3 describes the required time to release 50% of the total nitrogen from chicken manure after applying 16 pore volumes. The potential leaching time is predicted to be 15 hrs when using a running pressure of 35 kPa. On the other hand, the cumulative TDN may decrease as time decreases, where the relationship between pressure and cumulative TDN is negative (R^2 = - 0.663). Eq. 8.2 shows that the TDN may decrease at a rate of 1.170 units when pressure is increased by one unit due to the decreased contact time between water and manure. In this case, to extract 50% of TN, the pore volume needs to be increased. Furthermore, increase in bulk density (BD) may increase the TDN in the leachate as result of the increased leaching time, optimising TDN. The change in cumulative TDN with an increase in BD is predicted by Eq. 8.1, where packing chicken manure in a column of 100 mm at BD of 0.5 g cm^{-3} would require applying 18 pore volumes (8298 mL water) to extract 50% of the TN in chicken manure.

Hence, the characteristics of the system would differ according to which characteristic of the system is most important (e.g. Time, water, TDN). If the time is important and there is no problem with water, the use of low bulk density and high pressure could be appropriate. However, if the water is limited, increasing bulk density and pressure would be efficient.

8.1.3 Approaching the design of a nutrient extraction system for irrigation purposes

In Australia, the layer chicken industry produces approximately 738,000 tonnes of manure per year (Turnell et al. 2006) which could be used for soil improvement or as a source of important nutrients such as nitrogen, phosphorus and potassium. The average nitrogen content in chicken manure is 2.45% (DEFRA 2010), implying that each year chicken manure could provide the soil with 1808 tons of nitrogen. As the price of urea (46% - N) is around AUD $475 per ton (DEFRA 2010), the urea-based price of nitrogen is around $1032.60 per ton. Therefore, the Australian poultry industry loses approximately $1.86 million per year because chicken manure is not utilised as a nutrient source. However, chicken manure also has numerous characteristics that make it unsuitable to apply as a solid material. For example, chicken manure has a high water content of around 55 – 70 % (Wilkinson 1979), which leads to an increase in the cost of handling and spreading per unit. The cost of transportation of chicken manure for around 40 km is around $50 – 75 per ton based on Australia prices (personal communication), so the application of 10 tons per hectare will cost around $550 – 700 excluding spreading cost. Furthermore, chicken manure contains high salinity due to increased concentrations of nitrogen, potassium, sodium and chloride (Kelleher et al. 2002, Guo and Song 2009, Guo, Labreveux, and Song 2009). Hence, the main goal of this research was to extract nutrients from chicken manure in the form of soluble ions that may be then be applied through irrigation systems in a controlled fashion as compared to bulk application of raw manure directly to land. Moreover, the application of soluble nutrients through irrigation water may also be more energy efficient and the efficiency of nutrient uptake by the plant may be increased.

There are two general strategies for the extraction of soluble nutrients; 1) using a system of leaching like the column leaching used in this study and, 2) through a composting process where a collection system is set up under the compost pile. The difference between both strategies is the moisture condition of leaching. The moisture condition of the leaching unit is saturated while the moisture condition of the compost pile during composting is unsaturated. This difference could affect the concentration of soluble nutrients in the leachate. Guo and Song (2009) simulated, in a laboratory column, the loss of nutrients during composting under unsaturated conditions where around 1684.98 mL of distilled water was applied, and found the amount of total dissolved nitrogen, soluble phosphorus and potassium collected over 190 days was 13.54, 0.372 and 40.52 mg g^{-1} manure. These amounts represent about 36, 2.74 and 100%, respectively, of the total nutrients in manure. The advantage of using a leaching column system is that it provides flexible control of the amount of nutrients extracted and minimises the loss of nitrogen by volatilisation when leached from fresh manure. However, this study has demonstrated that it is possible to extract more than 50% of the total nutrients (N, P and K) by applying significant water volume while reducing the leaching time to around 2 days.

The major issue associated with extraction of soluble nutrients from chicken manure is residual material (solid material) after the leaching process is complete. This material was found to have sufficient level of nutrients; thus, composting could be a potential solution to dispose of this residual material. In addition, the compost produced from this material would be low salinity as a result of leaching prior to composting. Based on the outcomes of this study, the electrical conductivity (EC) of chicken manure in (1:10) (mass: volume) was decreased from 11.80 to 2.46 dS m^{-1}

after ten pore volumes were applied. In obtaining high quality compost, sufficient nitrogen, phosphorus and potassium are required within the manure upon completion of the extraction process. Rynk et al. (1992) reported that nitrogen, phosphorus and potassium are essential nutrients for microorganisms to breakdown organic matter. Hence, further investigation of a residual nutrient content for economically viable compost, alongside economically viable leachate for fertigation, would be warranted.

8.2 General conclusion

From the outcomes and evaluation of this study, the following general conclusions are drawn:

Some conclusions regarding the viability of the use of chicken manure or mature compost as a source of soluble nutrients to produce organic liquid fertiliser “leachate” are:

- Mature compost is a valuable raw material to produce pathogen-free compost leachate; however, it has a relatively low concentration of soluble nutrients compared with leachate derived from fresh manure, due to the loss these nutrients during the composting process.
- Fresh manure produced leachate “liquid fertiliser” had a relatively high concentration of soluble nutrients, but it also contained pathogens such *E coli* and *Salmonella spp*.
- To use fresh manure as source for the production of liquid organic fertiliser, requires some treatments, such as the elimination of pathogens by the application of ozone as a post-treatment, as ozone was not found to have any significant effects on the availability of soluble nutrients compared with pasteurisation.
- Fractionating (i.e. disposal of first and second leaching events, or “pore volumes”, could be a viable treatment to reduce the effects of increased CROSS of leachate, but is in conflict with high soluble nutrients that would also be discarded in these volumes.

Some conclusions regarding the optimization of soluble nutrients from chicken manure where the main focus is on total dissolved nitrogen (TDN) as the essential element for the majority of plants are:

- The use of a long column was found to extract more TDN compared with a short column, due to increased contact time (leaching time).
- Compacted chicken manure at a high packing density caused an increase in the concentration of TDN. However, the leaching time was increased to more than 5 days compared to low packing densities.
- Using pressure to decrease leaching time led to a decreased concentration of TDN in the leachate. To obtain 50% of the total nitrogen in manure, the number of leaching events “pore volumes” needed to be significantly increased to the extent that water resource availability may be a concern of a commercial scale.

8.3 Future work direction

As a result of the work undertaken in this thesis, numerous directions for future work have been identified below.

Due to the lack of information about the use of manure leachate as source of soluble nutrients for plants, further work is needed to approach a viable commercial system. This work investigates the effect of bulk density and column length on leaching.

However, further investigation of soluble nutrients extraction under different bulk densities and column lengths is needed to understand the optimal column length to bulk density ratio. The potential to improve nutrient extraction efficiency by applying a salt solution rather than water should be investigated. Furthermore, while increasing the pressure of the leaching solution results in time savings, only a single column configuration was investigated. The relationship between column characteristics is likely to change under the influence of pressure and this has not been tested in this study.

This study has demonstrated that manure leachate contains high cation ratio of soil stability (CROSS). Further work is required to study the effects of manure leachate on soil chemical and physical properties, considering soil threshold electrolyte concentrations and appropriate land conditioning strategies (e.g. gypsum as a source of calcium and a sulphur bentonite as a source of sulphur to address leachate alkalinity). It may be more economically viable to treat the manure with a calcium and sulphur source prior to leaching, but this could affect the solubilisation and form of available nutrients, which would require further investigation. In terms of adapting a commercial system to address Na concentration onsite, options such as zeolites might be explored as a system component. While this study has shown ozone treatment of leachate to be highly effective at removing pathogens, and likely inexpensive, the treatment suite explored in this work was not exhaustive and further options may exist. However, future evaluation of methods should be subject to an initial economic evaluation against the demonstrated ozone performance in this work.

A system for extraction of nutrients from raw manure was explored during the experimentation on leaching solution pressure and its effect on nutrient extraction. This work demonstrated clearly that the nutrient content of the leachate was reduced when the pressure of the solution was increased, primarily due to decreased contact time between solution and medium. Initial economic calculations based on relationships developed from the experiment showed that in order to extract 100% or 50% of the nutrients from the manure, increased water was required relative to nil (0.49 kPa) pressure extraction. However, this evaluated pressure based leaching system was completely linear by necessity of the experimental design. Extension of this work should consider leaching of nutrients by providing a feedback loop from the leachate reservoir to the percolating reservoir. Solution ionic saturation will need to be considered. Also, extraction under pressure with a controlled exit flow rate, with pressure rising after the pores are filled to create a static pressure could be investigated. It may help to increase contact time, high pressure, and may be not bed compaction.

After leaching manure there is a residual solid material that is required to be disposed of. A complimentary process would be to compost this and apply it to the field as an organic amendment. However, it has been identified that, while low salinity, it is also low in nutrient that is required for microbial processes. In this work a 50% contained nitrogen requirement was used as a reasonable arbitrary value on which to demonstrate calculations. However, optimal nutrient concentrations for valuable compost product and adequate composting process should be further explored. The compost source used in this work was identified as potentially low in nutrient due to the frequency of turning. An understanding of commercial, compost production procedures would be of benefit to the industry; from which specific compost management protocols could be developed based on optimal composting processes.

The assessment of nutrient value of mature compost leachate should then be reassessed.

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Appendix A: Additional Preliminary Trial for Chapter 6

Appendix A.1: Identifying the appropriate hot water temperature to eliminate pathogens

A.1.1 Introduction

This work aimed to identify the appropriate temperature of water to eliminate pathogens in chicken manure leachate.

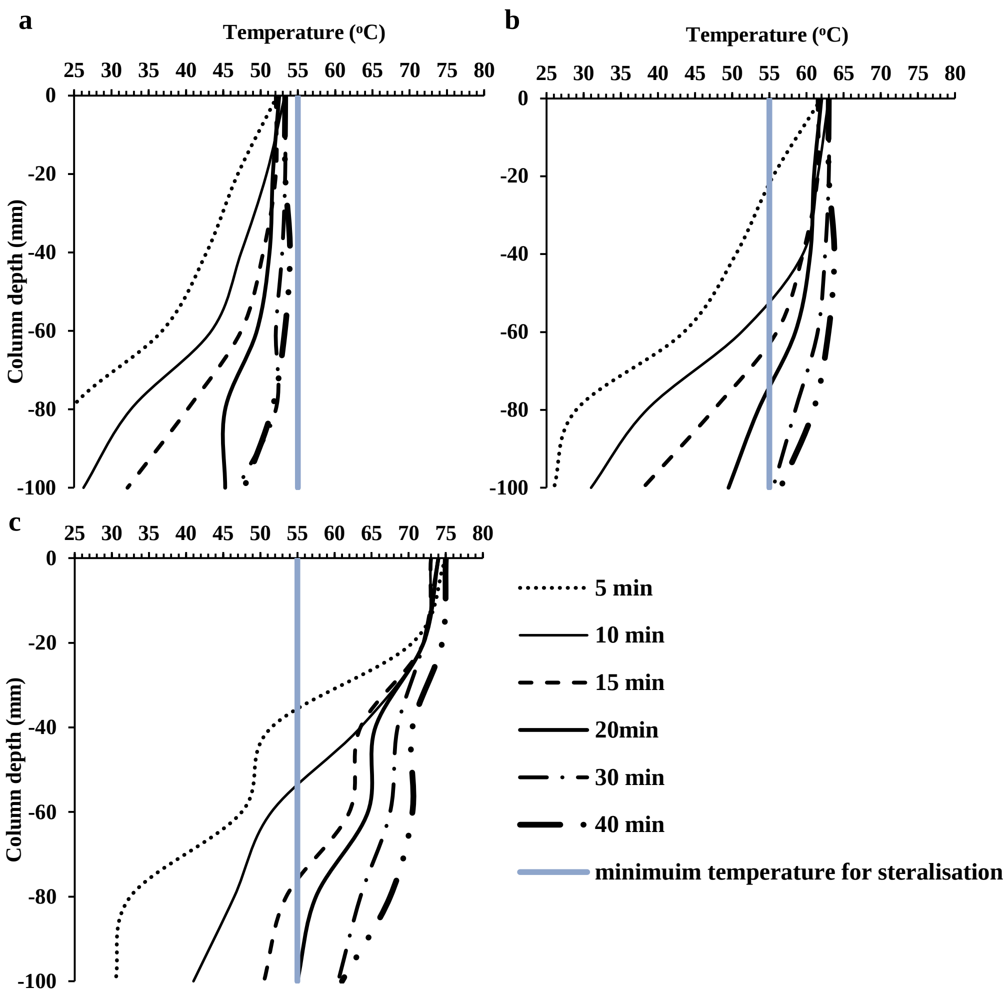
A.1.2 Measurements and treatments

A column trial using chicken manure was described in methods of chapter 6. Based on the previous studies, the optimum temperature to eliminate pathogens in chicken manure was 55^{o}C for 30 min. However, this temperature was applied directly to manure. Hence, to choose feasible hot water temperature in terms of elimination of pathogens in leachate, three levels of water temperature were investigated (55, 65 and 75^{o}C). A thermostat regulated unit for boiling water was used to produce these temperatures. Four thermometers were installed in columns at 20, 40, 60 and 80 mm to record the temperature of manure during leaching. The leachate was collected each 5 min; the overall time was 50 min. The pathogen assessment was done immediately to detect (*E. coli and Salmonella spp*).

A.1.3 Results

The results showed that where 75^{o}C water was applied to the columns, *E. coli and Salmonella spp* colonies were non-detectable after the third leaching at this temperature (75^{o}C). The following figure (A.1) shows the distribution of temperature inside the manure column at different hot water temperature.

Figure A-1 Average of temperature inside manure column when 55, 65 and 75^{o}C hot water is applied to extract soluble nutrients from chicken manure. Time refers to time after initial application



8.4 Appendix A.2: Identifying the appropriate period of ozone application to eliminate pathogens

A.2.1 Introduction

This work aimed to identify the appropriate application period for ozone in order to eliminate pathogens in chicken manure leachate.

A.2.2 Measurements and treatments

A 50 mL aliquot of chicken manure leachate was subject to 10.41 mg L^{-1} ozone for 10, 20 and 30 min. A 0.1 mL aliquot of chicken manure leachate, treated by ozone, was incubated in petri dishes with MacConkey agar and *Salmonella and Shigella* Agar (SS agar) for *E.coli* and *Salmonella spp,* respectively. The petri dishes were put in incubation for 24 – 48 hr at 37°C. The number of colonies was reported in Log_{10} CFU mL^{-1}.

A.2.3 Results

Table A.2.1 shows the effects of applying ozone at different periods. The results showed that both *E coli* and *Salmonella spp* were not detectable after applying ozone for 30 min. However, the 10 and 20 min treatments showed that the number of *E coli* and *Salmonella spp* in leachate post treatment was still relatively high compared with irrigation water standards.

Table A.2.1 The number of colonies in leachate treated by ozone for different periods

Period (min)	**number of colonies (Log 10 CFU mL-1)**	
	E coil	**Salmonella spp**
0*	7.23	7.45
10	5.06	5.22
20	1.45	1.49
30	n.d**	n.d**

[*] 0 is leachate was not treated by ozone

[**] n.d is non-detectable which is less than limited detection.

Glossary

Aerobic	An adjective describing an organism or process that requires oxygen (for example, an aerobic organism) (Rynk et al 1992).
Anaerobic	An adjective describing an organism or process that does not require air or free oxygen (Rynk et al 1992).
Bedding	Dry absorbent materials used to provide a dry lying surface for livestock. Bedding materials such as sawdust and straw absorb moisture from livestock wastes, the soil, and the environment (Rynk et al 1992).
Compost	A rich source of organic matter, dark and easily crumbled, with an earthy aroma (not an aroma of decaying organic material.
Compost Extract	Compost watery extract—made from compost suspended in a barrel of water for 7 to 14 days, usually soaking in a burlap sack— a centuries-old technique. The primary benefit of the extract will be a supply of soluble nutrients, which can be used as a liquid fertilizer (Diver 2002).
Compost Leachate	Compost windrow leachate — the dark-colored solution that leaches out of the bottom of the compost pile—most likely will be rich in soluble nutrients; but, in the early stage of composting it may also, contain pathogens. It would be viewed as a pollution source if allowed to run off-site.Compost leachate needs further bioremediation and is not suitable or recommended as a foliar spray (Diver 2002).
Compost Tea	Compost tea, in modern terminology, is a compost extract brewed with a microbial food source molasses, kelp, rock dust, humic-fulvic acids. The compost-tea brewing technique, an aerobic process, extracts and grows populations of beneficial microorganisms (Diver 2002).
Composting	Transformations of raw organic materials into biologically stable, humic substances suitable for a variety of soil and plant uses (Cooperband 2000).
Chicken manure	A mixture of excreta, waste food, broken eggs and feathers (Kelleher et al. 2002, Tiquia and Tam 2002).

Manure Tea	Manure – based extracts – a soluble nutrient source made from raw animal manure soaked in water for 7 to 14 days (Diver 2002).
Slurry manure	Has a near liquid consistency. It can be handled with conventional, centrifugal manure pumps and equipment, but the solids content may be too high for irrigation equipment. (Rynk et al 1992).