The Production of Vermicompost from Dairy Sludge
and its Value as a Plant Growth Medium

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DEDICATION

This project is dedicated to my parents, Kenneth and Jean; and brothers, Winston and Allen.

DECLARATION

I confirm that the enclosed is all my own work, with acknowledged exception.

Signed: _Percy Foster_

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ABSTRACT

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Dairy sludge generated at Glanbia Ingredients Ltd., Kilkenny has up until now beeen landspread. This study investigated the feasibility of using earthworms to vermicompost the sludge as an alternative method of treatment. It was found that high levels of ammonia in the sludge led to earthworm fatality but that by manually aerating the sludge the ammonia could be volatilised or by adding zeolite the ammonia could be absorbed, thus solving the problem.

In a medium scale trial, the earthworm species *Dendrobaena veneta* and *Eisenia fetida* dominated the polyculture. Earthworms grew and generated cocoons during vermicomposting. During vermicomposting no leachate was generated.

Nutrient changes took place during vermicomposting. There were high levels of nitrate, increased calcium and sulphate in the vermicomposted dairy sludge. The amount of magnesium, potassium and chloride did not change, while phosphate was undetectable after vermicomposting. The levels of nitrate and phosphate were good indicators of the extent of vermicomposting.

The vermicomposted dairy sludge provided improved growth and yields of radishes and barley compared to the dairy sludge and control. Compared to the vermicompost, the dairy sludge provided heavier ryegrass yields and more marigolds with larger flower diameters.

Generally, it is the amount of phosphate in dairy sludge that dictates how much can be applied as a fertiliser on land. Vermicomposting reduced the amount of phosphate to an undetectable level but on the other hand created a problem of high nitrate levels. In a pot trial with grass grown in vermicompost the nitrate leached from the vermicompost. In field conditions the leaching of nitrate might occur and could cause an increased risk of contamination of groundwater and watercourses.

Table of Contents

APPENDICES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1 General introduction

The processing of milk into dairy products produces significant amounts of wastewater the treatment of which in turn produces a large amount of sludge requiring disposal. Current methods of disposal are becoming increasingly unacceptable. For example the disposal of dairy sludge to landfill is not good practice because its high nitrogen content creates the risk of nitrate contamination of groundwater. Incineration of dairy sludge is not an attractive option as it releases carbon and nitrogen oxides into the atmosphere.

Alternative environmentally friendlier ways of management of organic wastes are becoming increasingly established. In the last ten years there has been a dramatic increase in the recycling of organic wastes into compost, which has a potential resource as fertiliser with economic value. One potential means of recycling organic wastes such as dairy sludge is by vermicomposting. Vermicomposting is the use of earthworms to consume organic materials and generate castings known as vermicompost. Vermicomposting of sludges such as sewage sludge is practised in large scale vermicomposting facilities in Australia. However the use of dairy sludge as a substrate for earthworms in vermicomposting is relatively new and only a small number of successful laboratory-scale trials have been conducted. In this study, vermicomposting was scaled up from laboratory-scale and investigated as a means of treating the dairy sludge generated at Glanbia Ingredients Ltd., Kilkenny.

1.2 Dairy sludge management in Ireland

The volume of dairy sludge in Ireland has increased over the years (Figure 1). The results of a survey carried out in 1997 (Anon., 1998*a*) showed that an average of 23,989 tonnes of dairy sludge were generated in 1997. A national survey by the Environmental Protection Agency (EPA) found approximately 75,000 tonnes of dairy sludge was generated in 1998 (Anon., 1998*b*). The two surveys have conflicting results and this discrepancy remains unexplained. A more recent survey on the quantity of dairy sludge generated in 2003 was conducted by this author with the assistance of the Irish Dairy Industry Association, who sent the questionnaire to all its members. The respondents (58%) generated approximately 91,664 tonnes of dairy sludge. The survey showed that the quantity of dairy sludge produced had increased. The survey showed that landspreading of sludge is the dominant

method of treatment but other methods of composting, landfill and land injection were also used. In the survey, four companies had researched other methods of treatment; anaerobic digestion, drying, composting and vermicomposting.
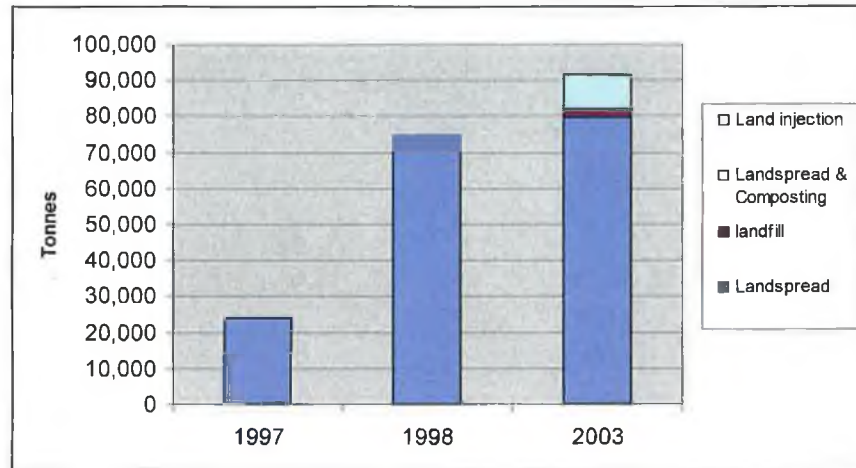
Figure 1. Tonnes of dairy sludge generated in Ireland and method of disposal/treatment

1.3   Sludge generation and management at Glannbia Ingredients Ltd.

Glanbia Ingredients Ltd. is a multinational company that processes milk. The Ballyragget, Co. Kilkenny plant is the largest dairy processing facility in Ireland and one of the largest in the world. The plant processes approximately 945 million litres of milk per year. The focus of this study is on the dairy sludge generated at Glanbia's wastewater treatment plant in Ballyragget, Kilkenny. Milk washings are generated from the cleaning of tanks and pipelines used in processing milk. These washings are treated in the wastewater treatment plant; and from this, sludge is generated. Sludge is generated all year round, but the quantity produced in the winter months is generally lower compared to the summer months.

The current use of the dairy sludge by Glanbia, is by landspreading it as fertiliser on agricultural land during dry weather conditions from Spring to Autumn (Figure 2). Farmers are not paid to take the dairy sludge, but instead Glanbia organises and pays for nutrient management plans and for the agricultural contractors to spread it.

The dairy sludge is landspread onto various crops such as grass (*Lolium multiflorum*), maize (*Zea mays*), sugar beet (*Beta vulgaris*), cereals, lupins (*Lupinus*), and potatoes

2

(*Solanum tuberosum*). The use of dairy sludge has a beneficial effect in that it reduces the farmers' need for artificial fertiliser. The dairy sludge was certified 'organic' by the Organic Trust in June 2003, and is also used by organic growers. The organic farmers pay Glanbia €1 per tonne; which covers the cost of registration to the Organic Trust.

During the winter months the dairy sludge cannot be landspread due to wet weather conditions and it is therefore stored on-site from November to February. This is in compliance with the conditions of Glanbia's Integrated Pollution Control (IPC) licence from the EPA. If weather conditions are dry, landspreading of the sludge can occur earlier. Over a short number of years Glanbia has dramatically increased the volume of sludge generated (Figure 3). This was partly due to the merger in the late 1990s of Waterford and Avonmore dairies. In the past three years the volume has stabilised to approximately 17,000 tonnes of dairy sludge per annum.
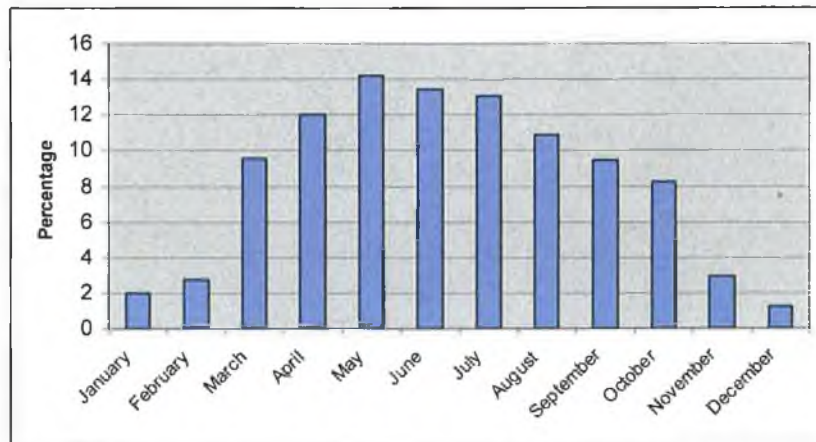
Figure 2. Mean percentage of dairy sludge landspread by Glanbia monthly from 1997-2004
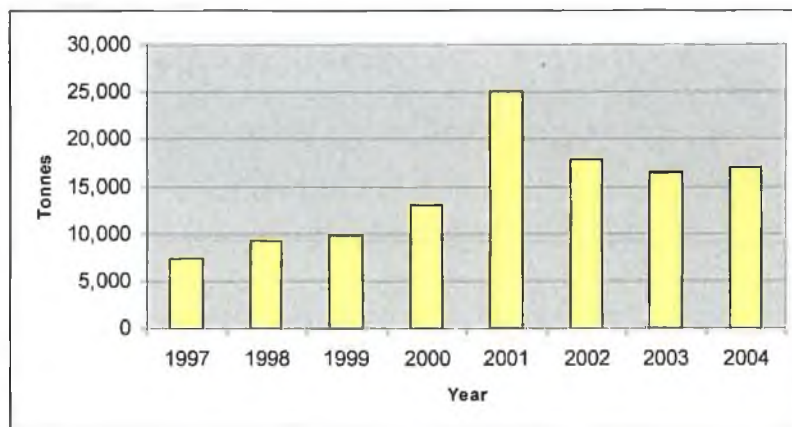
Figure 3. Quantity of dairy sludge generated at Glanbia each year from 1997-2004

In 2001 (Figure 3) the increase in the quantity of sludge was due to phosphate limit in sludge was reduced by fifty percent. To achieve the new limit twice as much of aluminum sulphate was added to the wastewater treatment process to reduce phosphate levels. This in turn was meant to keep the aluminum content in sludge low; a higher quantity of sludge generated to dilute the metal concentration.

The volume of dairy sludge has increased both nationally and at Glanbia. Future legislation (revision of the Sewage Sludge Directive) will require the sludge to be treated; this has stimulated this research on the use of vermicomposting as a sustainable means of treating dairy sludge.

1.4   Aims of this Research

The aims of this study were to:

(1) Investigate if earthworms consume, live, grow and maintain sustainable populations in dairy sludge.

(2) Investigate how to optimise conditions in the vermicomposting process.

(3) Conduct chemical, biological and physical analysis on dairy sludge, vermicompost and its leachate.

(4) Produce a vermicompost.

(5) Conduct plant growth trials to compare the performance of vermicompost and dairy sludge.

(6) Assess the suitability of vermicomposting as a tool for sustainable management of dairy sludge.

(7) Investigate if vermicomposting complies with the proposed revision of the EU Sewage Sludge Directive (Working Document on Sludge, 3rd Draft, 2000).

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of vermicomposting

Composting is an increased bio-oxidation of organic waste going through a thermophillic stage (45–60°C) during which microorganisms release heat, carbon dioxide and water. The organic material is changed into stabilised humus by turning or aerating the material (Dominguéz et al. 1997). In comparison to composting, vermicomposting is the breakdown of organic material that involves the joint action of earthworms and microorganisms at temperatures below 35°C and does not involve a thermophilic stage (Dominguéz et al. 1997).

It has been known for a long time that earthworms play an important role in improving the fertility of agricultural land (Jensen, 1993). In the last 200 years, it has been shown that some species of earthworms prefer to live in environments rich in decaying organic matter and this is best illustrated by observing certain species in compost heaps.

Earthworms obtain their nutrition from the microorganisms that grow upon the organic materials (Edwards, 1995). They consume leaf litter and organic wastes such as food waste, animal wastes and sludges (Dominguéz et al. 1997), breaking it down into finer particles by passing it through a gizzard that grinds the material. Consequently, the earthworms act as agents of mixing, fragmentation and aeration (Dominguéz et al. 1997).

A by-product of this activity is earthworm castings (vermicompost) which can be used as a soil conditioner (Dominguéz et al. 1997). During this process the earthworms promote enhanced microbial activity in the organic material. The faecal material or castings (vermicompost) generated by earthworms is more fragmented and microbially active. The processing of organic material by earthworms makes nutrients more available for plants (Edwards, 1998). The vermicomposting process is shorter than composting and produces better quality compost compared to traditional thermophilic composting (Ghosh, 1999).

2.2 Dairy wastewater

Milk is processed into various dairy products ranging from pasteurised milk, butter and cheese to yoghurts. The dairy processing industry consumes between 2 and 6 m^3 of water

per tonne of milk entering the plant (Gendebien *et al.* 2001). Most of this is used for cleaning and washing, which in turn generates a large volume of wastewater.

Dairy wastewater consists mostly of wash water, cleaning agents, residual milk and milk products. Most dairy processing facilities have a wastewater treatment plant to treat the milk washings, from which dairy sludge is generated.

Dairy wastewaters are rich in biodegradable organics and nutrients (Poompavai, 2002; Ramasamy *et al.* 2000). They contain a relatively high concentration of carbon, mainly in the form of lactose and milk proteins and therefore ammonium (from amino acids) and phosphate (from caseins) (Cameron *et al.* 2002; Carta-Escobar *et al.* 2002; Perle *et al.* 1995). Dairy wastewater has an pH value between 6.6 and 12.2 which is due to the presence of washing products (Gonalez *et al.* 1982).

2.3 The nutrient content in dairy sludge

Dairy sludge is rich in organic matter and mineral nutrients, particularly nitrogen and phosphorus (Garcia López *et al.* 1999; Brown *et al.* 1990; Gendebien *et al.* 2001). It comprises 60-80% of readily degradable organic material as shown by its carbon to nitrogen ratio, which is usually around 5:1. Dairy sludge is rich in nitrogen which is usually present in amounts of around 6.5%. Approximately 85-90% of the nitrogen is in organic form and 10-15% in ammoniacal form (Gendebien *et al.* 2001). When dairy sludge is used a fertiliser, it is estimated that 60-90% of the nitrogen is assimilated in the first year (Gendebien *et al.* 2001). The phosphoric anhydride level (P_2O_5) is high at around 7-8% of the total sludge. The potassium levels are negligible with values around 0.4% of the sludge (Gendebien *et al.* 2001). The primary value of dairy sludge residues in its nutrient content, mainly nitrogen and phosphorus (Table 1).

Table 1. Composition of dairy sludge (International Dairy Federation; Anon., 2000*a*)

pH range	5.6-6.9
Organic Matter	67 %
Total Nitrogen	8 %
Total Phosphorus	9.8 %
Total Potassium	1.3 %
Total Calcium	8.2 %
Total Magnesium	1.8 %

2.4 Metals, pathogens, organic compounds and odour associated with dairy sludge

Dairy sludge generally has low metal content, low concentrations of organic compounds and far fewer pathogens than sewage sludge (Anon., 2000*a*; Garcia López *et al.* 1999; Gendebien *et al.* 2001; Brown *et al.* 1990). In an investigation to examine pathogens in dairy sludge, 63 samples from different wastewater treatment plants were examined for *Samonella* and *Brucella* (Anon., 2000*a*) and results showed that *Brucella* was not present in any of the samples and *Salmonella* was isolated in only two samples. Dairy sludge is attractive as a fertiliser because it has a low content of metals, organic compounds and pathogens (Brown *et al.* 1990).

Dairy sludge can be particularly odorous because it is rich in poorly stabilised organic matter and has a low carbon to nitrogen ratio. It can therefore cause an odour nuisance during storage and landspreading.

2.5 Legislation requirements for dairy sludge management

The spreading of sludge on agricultural land may result in leaching of nitrates into surface and groundwater (Anon., 2000*a*). According to the Nitrate Directive (91/676/EEC) nitrate vulnerable zones require nutrient management plans for wastes applied onto land, and this influences the availability of land for the application of dairy sludge.

On a European level the main legislation on the management of dairy sludge is governed by the proposed revision of Sewage Sludge Directive 86/278/EEC (Anon., 2000*b*)

The Sewage Sludge Directive is an initiative to improve the present situation for sludge management. The Sewage Sludge Directive states that sludge should undergo a treatment process such as composting, anaerobic digestion or lime stabilisation; the full list of treatment options are included in Annex I of the working document. However the treatment process may not apply to dairy sludge which is listed in Annex VIII because the directive states "The competent authority may decide that the obligation of treatment does not apply to those industrial sludges listed in Annex VIII that do not contain potentially pathogenic micro-organisms".

The European Commission announced in 2004 that the revised Sewage Sludge Directive and Biowaste Directive (for composting) would now not be separate pieces of legislation, but instead would be an integral part of the 'Soil Thematic Strategy' which has to be announced.

At a national level in Ireland the management of sludge is governed by integrated pollution control licences (IPC) enforced by the EPA. At a local level by some county byelaws e.g. Laois County Council has byelaws which ban the importation of waste into the county for landspreading.

2.6   Earthworm species used in vermicomposting

There are approximately 3000 species of earthworms (Sims and Gerard, 1999) of which only 10-12 species are used for vermicomposting worldwide (Titov, 2004). 26 species have been recorded in Ireland (Sims and Gerard, 1999), but only 5 are used in vermicomposting. Earthworm species can be categorised into three groups. These morpho-ecological groups, described by Bouché (1977), are based on different parameters such as size, shape, colour, position in the soil profile, source of food, reproduction and burrow construction. The three groups are described below:

(a) Epigeic (litter dwellers) - these species normally do not live in mineral soil and are found in rich organic matter. They have a small body size, tend to be red-pigmented, normally live on the surface of rotting matter and have a high reproduction rate (e.g. *Eisenia fetida* known as the tiger, or the brandling worm, *Lumbricus rubellus*).

(b) Endogeic (horizontal burrowers) - these have horizontal branching burrows in organic mineral layers and live below the surface. They have a variable body size, and are weakly pigmented (e.g. *Allobophhora chlorotica, Aporrectodea caliginosa)*.

(c) Anecic (deep burrowers) - these live deep in the soil and have deep burrows. They have a large body size, are strongly pigmented, have a surface feeding and casting behaviour and a slow reproduction rate (e.g. *Lumbricus terrestris* known as the lob/common worm).

The earthworms used in vermicomposting are usually the epigeic species that grow and reproduce quickly in habitats that are rich in organic matter (Werner, 1997).

Research on the suitability of different earthworm species for vermicomposting was conducted in Rothamsted research station in the early 1980s (Edwards, 1998). *Eisenia fetida, Eisenia andrei, Dendrobaena veneta* and *Lumbricus rubellus* were identified as potentially useful species for vermicomposting. The survival, growth and reproduction of these species was studied in a range of organic wastes including, pig, duck, turkey, poultry, potato, brewery and paper wastes; and activated sludge. It was found by Edwards (*ep. citi*) that all the earthworms survived and grew in different organic wastes with some species growing faster than others.

2.7   Life cycle of epigeic species

The life cycle of only a few earthworm species has been studied in detail. The life cycle of the epigeic *E. fetida* is well documented. This species has been subject to much laboratory based research assessing its potential for use in vermicomposting. The life cycle of the common species in vermicomposting varies and is shown in Table 2.

All earthworms are hermaphrodites. Reproduction happens when two earthworms join together (copulate) by secreting mucus from their clitellum and as the mucus dries it helps to hold them together (Sims and Gerard, 1999). During copulation spermatozoa are exchanged. When an earthworm is about to lay a cocoon, the clitellum generates a 'slime tube/band, which moves forward by movement of the earthworm's body. As the band passes over the female pore, ova are collected, and then as it passes over the male pore, sperm is released and fertilisation occurs. The band continues moving forward until it is released at the end of the body to form a cocoon (Sims and Gerard, 1999). A cocoon may contain up to twenty eggs, but it is unusual for more than one egg to hatch and develop. There is a correlation between the number of cocoons produced by any species and its exposure to environmental conditions. Species exposed regularly to environmental stresses generally produce more cocoons to enable them to survive e.g. *E. fetida* which is used in vermicomposting and lives near the surface (Edwards and Bohlen, 1996). Endogeic species

can avoid environmental stresses by moving deeper from the surface and produce fewer cocoons.

The time taken for cocoons to hatch varies (Table 2). This depends on species type, environmental conditions and population density. Edwards and Neuhauser (1988) reported that it took between 32 and 72 days for cocoons of *E. fetida* to hatch, and between 53 and 76 days for hatchlings to reach sexual maturity, under controlled laboratory conditions. These results are misleading if they are extrapolated to large-scale vermicomposting, as field conditions are different. There are many factors that influence the life cycle of *E. fetida,* such as temperature, moisture, pH and oxygen, which are discussed in greater detail in section 2.10.

Table 2. Comparison of some aspects of the biology of the earthworm vermicomposting species found in Ireland (Dominguéz, 2004)

Species	*Eisenia fetida*	*Eisenia andrei*	*Dendrobaena rubida*	*Dendrobaena veneta*	*Lumbricus rubellus*
Colour	Brown and buff bands	Red	Reddish purple	Reddish and purple bands	Reddish brown
Size of adult worms (mm)	4 to 8 x 50 to 100	4 to 8 x 50 to 100	3 to 4 x 35 to 60	5 to 7 x 50 to 80	4 x 70 to 150
Mean weight of adults (g)	0.55	0.55	0.25	0.92	0.80
Time to maturity (days)	28-30	21-28	54	65	74-91
Number of cocoons day^{-1}	0.35-0.5	0.35-0.5	0.20	0.28	0.07-0.25
Mean size of cocoons (mm)	4.85 x 2.82	4.86 x 2.64	3.19 x 1.97	3.14 x 1.93	3.50 x 2.46
Incubation time (days)	18-26	18-26	15-40	42.1	35-40
Hatching viability (%)	73-80	72	85	20	60-70
Number of worms cocoon^{-1}	2.5-3.8	2.5-3.8	1.76	1.10	1
Self fertilisation	+	+	+	?	-
Life cycle (days)	45-51	45-51	75	100-150	120-170
Optimal and limits Temp (^{0}C)	25 (0-35)	25 (0-35)	?	25 (15-25)	?
Optimal & (limits) moisture %	80-85 (70-90)	80-85 (70-90)	?	75 (65-85)	?

2.8 Polyculture and monoculture

In nature, different earthworm species may live in the same area of soil, each having a different niche and using different substrates as a food source (Neuhauser *et al.* 1988). An investigation to examine if a mixture of different species (polyculture) might achieve greater stabilisation in vermicomposting when compared to a single species (monoculture) was carried out by Neuhauser *et al.* (1988). Using sewage sludge as a food source, five species; *Dendrobaena veneta, Eisenia fetida, Eudrilus eugeniae, Perionyx excavatus* and *Pheretima hawayana* (with different combinations of species together) were studied. At the end of this very short study (four weeks) Neuhauser *et al.* (1988) concluded that there were no obvious advantages of polyculture over monoculture.

While studying the suitability of different earthworm species for vermicomposting at Rothamsted Edwards (1998) studied the growth of different combinations of earthworm species in a polyculture. The total earthworm biomass was usually greater in polyculture, but the results were not conclusive.

Elvira *et al.* (1996) studied the growth and reproduction of *Lumbricus rubellus* and *Dendrobaena rubida* in cow manure and in mixed cultures with *Eisenia andrei*. They determined that the mixed cultures did not show any advantage over pure cultures. However, *E. andrei* had higher growth rates in mixed cultures. In mixed cultures, the growth rate of *L. rubellus* and *D. rubida* decreased slightly compared to growth rate in pure cultures. In the mixed cultures the competition for food seemed to be the factor that affected results.

Wright (2002) studied large scale vermicomposting of biodegradable municipal waste. Wright wanted to simulate conditions in a large scale vermicomposting facility in Wales and thus used a polyculture of *E. fetida* and *D. veneta*. It is almost impossible to keep a monoculture pure in field conditions. This was confirmed by Wright (2002) who showed mixed species are found in vermicomposting.

2.9 Predators and parasites

According to Edwards (1995) "earthworms do not have many serious natural enemies, diseases or predators". But the author noted there is concern in Australia about predation on earthworms by terrestrial flatworms (planaria), which could affect ground based vermicomposting systems (Edwards and Steele, 1997). In Ireland the introduced planarian e.g. *Arthurdendyus triangulatus* (New Zealand Flatworm) is now widespread and could be a potential threat to vermicomposting beds.

Earthworms are an essential part of the diet of many vertebrate predators such as some species of birds. They are also part of the diet of some mammals, such as badgers and rats. Exclusion of birds and mammals from a vermicomposting bed is therefore necessary.

2.10 Factors affecting the vermicomposting process

In field conditions, the growth and survival of earthworms is influenced greatly by environmental factors such as temperature, bed moisture and rainfall. Temperature and moisture content are considered to be the most important environmental factors (Giraddi, 2000). The optimal conditions of both can accelerate the feeding activity of earthworms. The optimal conditions for breeding the earthworm species *E. fetida* are summarised in Table 3. These conditions do not change much for other species of earthworms (Edwards and Neuhauser, 1988).

Table 3. Optimal conditions for breeding *E. fetida* in animal and vegetable wastes (Edwards and Arancon, 2004)

Condition	Requirements
Temperature	15-20 C (limits 4- 30 C)
Moisture Content	80-90% (limits 60 –90%)
Oxygen Requirement	Aerobic
Ammonia Content of Waste	Low: <0.5mg/g
Salt Content of Waste	Low: <0.5%
pH	>5 and <9

2.10.1 Temperature

The temperature of the earthworm's environment is one of the most important conditions for vermicomposting (Dominguéz *et al.* 1997). There are lower and upper temperature

limits for earthworm survival (Table 3). At $10°C$ earthworm activity decreases and at $4°C$ production of cocoons and the development of juveniles cease (Pincince and Donovan, 1981). Few earthworm species can survive below $0°C$ (Curry, 1998). Various authors have reported that at $30°C$ earthworm activity decreases and temperatures above $35°C$ are lethal to earthworms (Pincince and Donovan, 1981; Edwards, 1998; Loehr, 1985). The high temperatures may not kill earthworms directly. It may be that the warm temperatures increase microbial activity in the substrate and therefore reduce the volume of oxygen available to the earthworms (Dominguéz, 2004). Epigeic species can tolerate higher temperatures than endogeics and anecic species (Edwards, 1995).

The same species of earthworms involved in vermicomposting are found on different continents. Based on reported information from around the world the optimum temperature range for feeding and conversion of wastes to castings varies slightly, but appears to be optimal around $20°C$. Different ranges that have been reported to be the optimal are $13°C$ to $22°C$ for *E. fetida* and *L. rubellus* (Pincince and Donovan, 1981); $15-20°C$ for *E. fetida* (Edwards, 1998; Edwards and Arancon, 2004) and 20 and $25°C$ for *E. fetida* (Hartenstein, 1981; Vishnaykov *et al.* 2002).

However, providing the optimal temperature continually has a negative effect. Giraddi (2000) studied the influence of seasons on vermicomposting and discovered that continual high temperature has a negative affect on the growth of earthworms, even when all other conditions are favourable.

Temperature also affects the number of cocoons produced and their viability. At $25°C$ the species *E. fetida* and *D. veneta* generated the most cocoons (Loehr, 1985; Aston, 1988). However at $25°C$, the viability of cocoons and number of hatchlings per cocoon decreased significantly. At $20°C$, *E. andrei* generated more cocoons with higher viability than *E. fetida*, but *E. fetida* generated a higher number of juveniles per cocoon (Haimi, 1990).

2.10.2   Moisture
A large proportion (75-90%) of earthworm body weight consists of water (Grant, 1955). The survival of earthworms is dependant on the prevention of water loss. They can do this

by two means; by either moving to an area more moist or by estivating (a summer diapause, in which the earthworm is in a dormant state). If earthworms cannot avoid dry conditions, they are able to survive even if they loose a large proportion of their body water content. Grant (1955) estimated that earthworms can sustain a water loss of around 50%. Earthworms lack a mechanism to enable them to maintain constant internal water content. So their water content is influenced greatly by the water potential of their environment (Edwards and Bohlen, 1996). Lack of moisture can cause earthworm species to become quiescent (earthworms response to adverse conditions such as drought or high temperatures by going into a non active state and only becomes active again when conditions are optimal). Earthworms also can go into diapause (a non active state during which the earthworm has an empty gut and stays tightly coiled in mucus lined cell within the habitat to protect it against adverse environmental conditions).

Earthworm species that live in compost or dung heaps tend to prefer moister conditions than most soil dwelling earthworms (Edwards and Bohlen, 1996). The optimal moisture content of organic waste for earthworms varies slightly from different authors but generally the range is from 80-90%, with the average range around 85% moisture content (Edwards, 1998; Edwards and Arancon, 2004 and Loehr, 1985). A lower limit of 60% and an upper limit of 90% moisture content hinders earthworm activity (Edwards, 1998).

The moisture content influences not only feeding activity but sexual development. In a trial by Dominquez and Edwards (1996) it was found that as the moisture content increased up to 90%, there was clear evidence that sexual maturity was accelerated. At a lower moisture content range of 65-75%, not all of the earthworms developed a clitellum, even after 44 days. Dominquéz (ep. citi) also found that moisture content affected biomass. At higher moisture content earthworms had heavier biomass compared to earthworms at lower moisture contents.

2.10.3   Oxygen
Earthworms obtain oxygen by diffusion from air through their body wall (Dominguéz, 2004). Earthworms require aerobic conditions and cannot live in anaerobic environments (Edwards, 1988). If earthworms are in an environment that becomes too moist, they can

experience low oxygen levels. This can cause mass migration of earthworms from their burrows in order to seek atmospheric oxygen (Edwards and Bohlen, 1996). The depletion of oxygen or the increased volume of carbon dioxide and hydrogen sulphide can also cause mass migration of *E. fetida* (Dominguéz, 2004). Vermicomposting beds can be maintained at aerobic conditions by maximising the surface area and protecting against bed saturation. Mechanically turning the bed at regularly may also help (Pincine and Donovan 1981).

Masciandaro *et al.* (2000) found that earthworms avoid anaerobic sludges. Ceccanti and Masciandaro (1999) and Hartenstein (1981) showed that sludges that were 100% anaerobic caused immediate death of the earthworms. When the sludges were between 25-50% anaerobic, the earthworms survived for 10 to 20 hours. Anaerobic sludges can be made amenable to earthworms by mixing with bulking agents that are rich in lignin (Reinecke, 2000). Only activated sludges that are aerobic are suitable for vermicomposting (Hartenstein, 1981). In thermophilic composting, the waste material has to be kept aerobic by mechanical turning which can involve expensive machinery. With vermicomposting the earthworms keep the material aerobic by turning it. This is an important factor for earthworms as they can only survive in aerobic conditions.

2.10.4 pH

Earthworms have an optimum pH range of between >5 and <9. Pincince and Donovan, 1981 and Lee, 1985). There is a slight variation for different species. The optimum range for *E. fetida* is between pH 5 and pH 9 (Edwards, 1998; Edwards and Arancon, 2004). An optimal pH of 5-8 for vermicomposting was reported by Vishnaykov *et al.* (2002). Earthworms died within a week at pHs above 9 and below 5. Although some authors have conflicting results, it is generally agreed that during vermicomposting, the pH decreases (Moreno *et al.* 2000; Ndegwa and Thompson 2000).

2.10.5 Ammonia

The amount of ammonia in biodegradable wastes is important for the vermicomposting process. All earthworms are sensitive to ammonia and will not survive in wastes with high levels of ammonia such as fresh chicken waste (Dominquez, 2004; Edwards and Arancon, 2004; Edwards, 1998; Edwards and Bohlen, 1996).

The lethal limit of ammonia was found to be >0.5mg/g for *E. fetida* (Edwards and Arancon 2004; Edwards 1998; Edwards and Bohlen 1996 and Phillips 1988). Edwards (1988*a*) reported a lethal limit of >1mg/g of ammonia for *E. fetida*.

There are several ways that wastes high in ammonia can be made more suitable for earthworm survival. The ammonia can be removed by either washing it out, composting or by aerating for one day (Edwards and Arancon, 2004; Domínquez, 2004; Reincke, 2000; Edwards 1998; Edwards, 1988 and Mahimainathan, 1996). If wastes with high ammonia content are stored outdoors for a period, the ammonia content will fall to levels acceptable for earthworms (Phillips, 1988).

Zeolite is a mineral which can trap ammonia through ion exchange and has been used to avoid toxic levels for the earthworms. Zeolite is a natural mineral present in rocks in environments ranging from deep oceans to shallow water of lakes. It has been used to remove ammonia from sewage wastewater (Harben and Bates, 1990). In a composting trial by Bernal *et al.* (1993) the zeolite clinoptilolite was effective in trapping the ammonia lost during composting. There are no published papers on the use of zeolite in vermicomposting, but is used extensively in large scale vermicomposting in Australia (John Dorman pers. comm.).

2.10.6   Salt content

Wastes that contain a high amount of inorganic salts of >0.5% salt are lethal to earthworms (Edwards and Bohlen, 1996). However, if a waste has high salt content, it can be removed by washing out or by pre-composting for 2/3 weeks (Reinecke, 2000). Electrical conductivity is a measure of the salt content of waste. Perez *et al.* (2000) reported that high electrical conductivity made dairy sludge toxic to earthworms.

There are various reported toxic limits of electrical conductivity; Neuhauser *et al.* (1988) reported >3500µS/cm (3.5mS/cm); Anon., (1999) reported a tolerance limit of 1000µS/cm (1mS/cm) and Flack and Hartenstein (1984) reported 8mS/cm was detrimental to the growth of *E. fetida*.

2.10.7   Carbon to nitrogen ratio

Carbon and nitrogen play an important part in the cell growth, synthesis and metabolism of all living organisms. To have the correct nutrition for earthworms during vermicomposting, carbon and nitrogen have to be present in the waste in the correct ratio (Ndegwa and Thompson, 2000). The usual way to correct an imbalance is to add a material rich either in carbon or nitrogen. Thermophilic composting requires a carbon to nitrogen ratio (C/N) between 15 and 35:1. This range is also considered to be similar for vermicomposting (Gilbert *et al.* 2001). The C/N ratio affects the microorganisms present which the earthworms feed on.

Most of the vermicomposting studies reviewed have used a qualitative method more readily than a quantitative approach to balancing C/N ratios in wastes. The usual method has been to add a carbonaceous material to a waste high in nitrogen. It would be more accurate to use quantitative methods to adjust C/N ratios.

The effect of C/N ratio is not fully clear. One study on different C/N ratios on vermicomposting activated sewage sludge by Ndegwa and Thompson (2000) found that the best earthworm growth (*Eisenia fetida)* was at the low ratios (10:1 and 15:1), but the C/N ratio of 25:1 had the highest reduction in percentage volatile solids. This was the greatest reduction of N and the only ratio where there was a reduction of soluble phorphorus. However Naddafi *et al.* (2004) studied the effect of different C/N ratios on vermicomposting activated municipal sludge with the earthworms (*E. fetida*). They found the same C/N ratio of 15:1 provided the best earthworm weight gain and maximum reduction of total kjeldahl nitrogen (TKN) and organic to mineral phosphorus.

Table 4. Total organic carbon, total kjeldahl nitrogen and carbon to nitrogen ratio of dairy sludge reported by various authors

Parameter	López-Mosquera *et al.* (2000)	Gratelly *et al.* (1996)	Elvira *et al.* (1998)	Nogales *et al.* (1999)
	% value	g/kg^{-1}		
TOC	38.30	339	234	340
TKN	6.9	75	43	75
C/N ratio	5.55	4.5	5.4	4.5

As shown in Table 4, dairy sludge has a high nitrogen content of around 5.5%. Compared to municipal biosolids, dairy sludge has higher nitrogen and lipid content and lower C/N ratio (Nogales *et al.*1999).

Various authors have investigated dairy sludge as a substrate in vermicomposting using different species of earthworms. Hatanaka *et al.* (1983); Kavian and Ghatnekara (1991); Nogales *et al.* (1999) and Gratelly *et al.* (1996) determined that dairy sludge alone could support the growth of earthworms.

Hatanaka *et al.* (1983), Kavian and Ghatnekara (1991) Elvira *et al.* (1998) and Elvira *et al.* (1999) found that the addition of a carbonaceous material to dairy sludge improved earthworm growth. However not all authors concurred with this. Nogales *et al.* (1999) found that earthworm growth and reproduction was better in the dairy sludge alone (C/N ratio of 4.5) than with the addition of a carbon source.

Of the publications reviewed above, five authors found that dairy sludge (without any other material) over a bed of vermicomposted waste was a good substrate for vermicomposting. Only two authors did not find that dairy sludge was a good medium for vermicomposting.

Of the six publications mentioned above, four authors found the addition of another organic waste/carbon source, improved earthworm growth. However two authors did not find any improved earthworm growth.

2.10.8 Pre-composting

Traditional thermophillic composting as a technology to treat organic waste has a number of problems. It is a long process (3-6 months) which requires regular turning of the wastes, shredding of large particles of wastes, the loss of nutrients in leachate and a prolonged curing stage. However it does reach the high temperatures necessary to kill pathogens. In vermicomposting the earthworms do the role of turning and maintaining aerobic conditions.

An approach of integrating the two technologies would combine the best features of each. It would provide a product that was pathogen free with good characteristics at a faster rate

than either of the individual technologies alone (Ndegwa and Thompson, 2001). By combining the two technologies for sewage sludge and green waste the stabilisation time was shorter and the end compost product had improved quality (Ndegwa and Thompson, 2001; Frederickson *et al.* (1997). However, Frederickson *et al.* (1997) reported that precomposting had a negative effect on earthworm growth and reproduction. It was recommended by Frederickson *et al.* (1997) that precomposting should only be conducted for a short period of time before vermicomposting.

2.10.9   Stocking density and processing rate

Stocking density is the weight of earthworm biomass per some measure of area or volume. It is an important parameter as it helps determine the rate of vermicomposting. Most studies on stocking density have been focused on the effect on earthworm growth and reproduction than on the treatment of waste by vermicomposting. In practice the earthworms will populate a substrate and stabilise it at their own 'stocking rate'.

A study by Ndegwa *et al.* (2000) determined that for vermicomposting biosolids with paper mulch, the optimal stocking density was 1.60kg-worms/m^2 with a feeding rate of 0.75 kg-feed/kg-worm/day. This resulted in the highest conversion of waste into vermicompost. Neuhauser *et al.* (1980) determined the optimal stocking density of *E. fetida* was 0.8 kg-worm/m^2 in horse manure and 2.9 kg-worm/m^2 on activated sludge. Frederickson (2003) in an outdoor vermicomposting trial, determined that in a heated bed, a stocking density of earthworm (*Dendrobaena veneta*) of 4 kg per m^2, processed 3.2kg waste (potato slurry) per m^2 per day. The large scale reactors in America have an earthworm density of 9kg per m^2 (Edwards and Arancon, 2004).

Domínguez and Edwards (1996) demonstrated that earthworm density effects growth and reproduction. It was shown with *Eisenia andrei* that earthworms grew faster at lower population densities. At the lower stocking rate all the earthworms reached maturity over a longer period of time. When compared to the higher stocking densities, they reached maturity in a shorter time. At higher densities not all of the earthworms reached maturity.

A stocking rate of 1:10 of earthworm biomass to organic waste biomass is recommended to achieve maximum productivity (Edwards, 1988). Wastes for vermicomposting have to be moister (70-90%) than traditional composting (50-60%), because earthworms need to live in environments of that moisture range. Moisture is liberated into the atmosphere in composting by the high temperatures but in vermicomposting this does not happen. Gilbert *et al.* (2001) stated that the end product vermicompost is moist and often the conversion of waste to vermicompost results only in a small weight reduction of approximately 10%.

2.10.10   Metals

Dominguéz *et al.* (1997) found that after two months' vermicomposting of biosolids and pig manure there was a decrease between 35% and 55% in the level of the bioavailable metals. Total amounts of metals tend to increase by around 25% and 30% due to loss of carbon during vermicomposting. During the vermicomposting process, heavy metals form complex aggregates with the humic acids (Dominquez, 2004). The decrease in the bioavailable metals means that less is available for plants to take up from the vermicompost.

2.10.11   Nitrification in vermicomposting

Nitrification is not a factor that affects the vermicomposting process. However it is a factor that gives a good indication to the extent of vermicomposting. The quantity of nitrates in the leachate from the vermicomposting process is an indication of aerobic conditions in the vermicomposting bed. A decrease in nitrate is an indicator that the vermicomposting process has failed. It is essential that vermicomposting beds are kept aerobic for earthworms, as mentioned in section 2.10.3. With aerobic conditions nitrification will take place. If anaerobic conditions happen in the bed, the earthworms will die and the vermicomposting process will fail.

Loehr *et al.* (1988) noticed a pattern between the loading rate of sewage sludge and the amount of nitrate and ammonia in the leachate from the vermicomposting bed. With an increased rate of sludge addition, the nitrate levels decreased in the leachate. Also with the beds that failed, the highest levels of ammonia were recorded.

In a trial using sewage sludge as a substrate in vermicomposting, Benitez *et al.* (1999) observed a decrease in ammonium. At the end of the trial large amounts of nitrate was present. This indicated that there was increased nitrification by the earthworms, which is representative of an aerobic vermicomposting process (Benitez *et al.* 1999; Parkin and Berry, 1994). Dominguéz (2004) found that *E. andrei* provided conditions that increased nitrification in pig manure.

Near the end of the vermicomposting process, when the earthworms are active and bigger, there was a high nitrification rate. The nitrification rate was 50-65% higher in the vermicomposting bed than in that of the controls (Dominguéz *et al.* 1997). Subler *et al.* (1998) also noticed that vermicomposts have very low concentrations of ammonium nitrogen and very high concentrations of nitrate nitrogen. Edwards (1988*b*) reported that most samples of vermicompost had relatively high levels of available nitrogen.

2.11   Vermicompost

Organic waste that has been processed by earthworms is referred to as 'vermicompost'. Depending on the parent material vermicompost is generally a finely divided peat-like material with high porosity, water holding capacity, good aeration and good drainage (Edwards and Burrows, 1988). When vermicomposts are compared to their parent material they have reduced amounts of soluble salts, greater cation exchange capacity, and increased total humic acid content (Albanell *et al.* 1988). During vermicomposting important changes takes place. Many of the nutrients are released and converted through microbial action into forms that are more soluble and available for plant uptake compared to parent materials. These nutrients include nitrate, ammonium nitrogen, exchangeable phosphorus, soluble potassium, calcium and magnesium (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh *et al.* 2001; Nedgwa and Thompson, 2001). Table 5 shows examples on the effect of earthworms on the nutrient content in some organic wastes.

The nutrient content of vermicompost varies depending on the parent material, but when compared to other commercial plant growth media to which nutrient have been added, vermicompost tends to have more of the mineral elements needed by plants (Edwards, 1998). In vermicomposts, there tends to be a deficiency of magnesium, although this can be

rectified by adding magnesium sulphate (Edwards, 1998). Most vermicomposts are generally on the alkaline side of the neutral pH 7, though many plants tend to prefer a pH on the acidic side of neutral pH. The mixing of vermicompost with an acidic medium such as peat may be necessary. Subler *et al.* (1998) compared traditional composts and vermicomposts. They showed that vermicomposts have a slightly lower pH, and slightly higher concentration of nutrients, especially nitrogen, when compared to other composts. They also noticed that vermicomposts have very low concentrations of ammonium nitrogen and very high concentrations of nitrate nitrogen. This is the generally the opposite scenario for many other composts.

Table 5. Effect of earthworms on nutrients in organic wastes (Edwards and Arancon, 2004)

Organic Waste	Nitrate Nitrogen	Readily Soluble P	Exchangeable (% d.m.)		
	(ppm)	(% d.m.)	K	Ca	Mg
Cattle waste unworked	8.8	0.11	0.19	0.35	0.05
Cattle waste worm worked	259.4	0.18	0.41	0.59	0.08
Pig waste unworked	31.6	1.05	1.49	1.56	0.45
Pig waste worm worked	110.3	1.64	1.76	2.27	0.72
Potato waste unworked	74.6	0.19	1.94	0.91	0.24
Potato waste worm worked	1428.0	0.22	3.09	1.37	0.34

2.11.1 Stability of vermicompost

Stabilisation determines the extent to which readily biodegradable organic matter in organic waste has been decomposed. Mature vermicompost is less likely to cause problems for plant growth, thus the use of plant growth tests indicate stability/maturity of compost. Stability is closely associated with reduced phytotoxicity and increased availability of plant nutrients e.g. nitrate (Short, 1999). Zucconi *et al.* (1981) used a phytotoxicity (germination) bioassay to monitor stabilisation of a composting process. Moreno *et al.* (2000) conducted an experiment on vermicomposting olive oil wastewater sludge. The initial substrate of the olive oil sludge had a low germination index. However after vermicomposting for 56 days, the germination index of the final sludge was high >80%. This indicated that there was a reduction in toxic organic substances. It was reported by Zucconi *et al.* (1981) that when the germination index is greater than 60% there is no phytotoxicity.

Earthworms increase the rate of volatile solids reduction, a parameter which can be used to indicate the rate of stabilisation (Neuhauser *et al.* 1988). Reductions in volatile solid content after vermicomposting have been found (Neuhauser *et al.* 1988; Frederickson *et al.* 1997; Hartenstein and Hartenstein, 1981; Kim *et al.*, 2000; Ndegwa and Thompson, 2000).

2.11.2   Plant growth trials with vermicompost

In recent years, there has been increased scientific evidence which demonstrates that vermicompost can influence greatly the growth and production of plants (Edwards, 1998). One of the first studies on the effect of vermicompost on plant growth was done in the 1980s in Rothamsted station, UK. Twenty five types of vegetables, fruits and ornamentals were grown with vermicompost. It showed that vermicompost provided better growth than compost or commercial potting mixtures (Edwards, 1995). There have been an increasing number of investigations and plant growth trials studying the effect of different vermicomposts (Table 6).

Table 6. Plant trials using vermicompost

Plant Type	Author
Tomatoes	Atiyeh *et al.* 2001; Atiyeh *et al.* 2002*a*; Atiyeh *et al.* 2000*a*; Subler *et al.* 1998; Atiyeh *et al.* 2000*b*; Arancon *et al.* 2002
Cucumbers	Atiyeh *et al.* 2002*a*
Peppers	Subler *et al.* 1998; Edwards, 2002; Arancon *et al.* 2002
Batchler button	Subler *et al.* 1998
Marigolds, Petunias and Poinsettias,	Atiyeh *et al.* 2002*b*; Subler *et al.* 1998; Atiyeh *et al.* 2000*b*; Klock-Moore *et al.* 2000
Raspberries	Subler *et al.* 1998; Atiyeh *et al.* 2000*b*
Grapevines	Porter, 1999
Rice	Jeyabal and Kuppuswamy, 2001
Radishes	Buckerfield *et al.* 1999, Tomati *et al.* 1988
Strawberries	Arancon *et al.* 2004*b*
Wheat	Tomati *et al.* 1988

Atiyeh *et al.* (2000*a*) investigated the effect of pig manure vermicompost on the growth and yield of tomatoes under greenhouse conditions. The results showed that the largest marketable yield of tomatoes was with 20% vermicompost. At this proportion of vermicompost, the average weight of the tomato fruit was 12.4% greater than that of the control. Substitution with 10%, 20% and 40% vermicompost reduced the proportions of fruits that were non-marketable. At 10% vermicompost, the dry weight of tomato seedlings increased by 30.8% compared to the controls in 'Metro Mix 360'. However at the highest

proportion of vemicompost (100%), the seedlings were much shorter, had less leaves and weighed less than the controls. These results agree with Subler *et al.* (1998), that the subsitution of vermicompost at low volumes like 10% and 20% produce the best plant growth. While at higher volumes of vermicompost, there is decreased plant growth.

Higher concentrations of vermicompost can have a negative effect on plant growth as demonstrated in the study by Atiyeh *et al.* (2000*a*). The growth and yield of the tomatoes at the higher concentrations of vermicompost declined above 60% vermicompost and 40% Metro Mix 360 soilless media. The decrease in plant growth at the higher concentrations was possibly due to high soluble salt content, heavy metal toxicity, poor aeration and or plant phytoxicity in the vermicompost (Atiyeh *et al.* 2000*a*; Arancon *et al.* 2004*a*).

When plants were unfertilised, increased plant growth was correlated with increased concentrations of vermicompost (Atiyeh *et al.* 2001). The greater proportion of vermicompost does not always provide the better growth (Subler *et al.* 1998). It has been discovered that even substitution with 5% vermicompost provides the best plant growth. This has led researchers to believe that the improved plant growth is not completely due to nutrients, despite equivalent nutrient levels being applied. This is possibly due to the presence of plant growth regulators/hormones in the vermicompost (Subler *et al.* 1998).

Even with nutrients supplied, vermicompost acted better than the control media. This indicated the vermicompost had properties, other than nutrients, that provided superior plant growth. Increased plant growth with vermicompost was possibly due to increased physical structure, improved microbial activity, plant growth factors, and/or humic acids (Arancon *et al.* 2004*a*).

The effect of humic acids from vermicompost on plant growth was investigated by Atiyeh *et al.* (2002*a*). Humic acids were extracted from pig manure vermicompost and were mixed with a soil-less medium called 'Metro Mix 360'. Tomato and cucumbers seedlings were grown. The plants were watered daily with a liquid containing nutrient to ensure all the plants were given the same amount of nutrients. This eliminated nutrients as a source of increased plant growth. At the end of the trials the plant growth was assessed. There was a

significant increase in plant growth in the plants with humic acids. Plant growth increased with increasing humate acid content up to a certain limit. However this limit varied between plant type, vermicompost and soil-less medium (Atiyeh *et al.* 2002*a*). It was discovered that the best plant growth was in the range of 50–500 mg/kg humic acids, and plant growth decreased in the range of 500–1000 mg/kg. Atiyeh *et al.* (2002*a*) concluded that plant growth responses were either due to the ability of humic acids to act as plant growth regulators/hormones and or because the humates may have plant growth hormones adsorbed onto them.

2.12   Vermicomposting dairy sludge

There were early studies on vermicomposting of dairy sludge by Hatanaka *et al.* (1983) and Kavian and Ghatnekara (1991). In recent years two colleges, the University of Vigo and Estación Experimental del Zaídín, have investigated the vermicomposting of industrial dairy sludge (Elvira *et al.* 1999; Elvira *et al.* 1997*a*; Gratelly *et al.* 1996; Gratelly, 1995; Nogales *et al.* 1999; Elvira *et al.* 1997*b*). The Technical Institute of Snow Brand Milk, Japan (Hatanaka *et al.* 1983) investigated the growing of *Eisenia fetida* in dairy sludge. Kavian and Ghatnekara (1991) determined that dairy sludge alone could support the growth of the earthworm *Lumbricus rubellus*. Both investigations found that when a cellulose material (rice straw or sawdust) was added to the sludge, the earthworm biomass increased.

Nogales *et al.* (1999) investigated the vermicomposting of dairy sludge alone and with bulking agents of cereal straw or wood shavings, using the species *Eisenia andrei*. The earthworms added to these substrates all died within 48 hours. Gratelly *et al.* (1996) assessed the feasibility of using *E. andrei* earthworms to stabilise dairy sludge. It was found that after 24 hours all the earthworms were dead in the dairy sludge. The death of the earthworms was probably due to the high ammonia concentration of the sludge (Gratelly *et al.*, 1996). To overcome the toxic effects of the dairy sludge, it was placed upon a layer of vermicomposted sheep manure. The sheep manure vermicompost acted as a microbial inoculum and resulted in suitable habitat for the survival and growth of the earthworms.

Elvira *et al.* (1999) investigated the suitability of dairy and paper sludges for the growth and reproduction of the epigeic earthworm species *E. andrei*. They found the dairy sludge

alone did not appear to be a good substrate. However when the dairy sludge was mixed with paper sludge and cow manure, there was better growth than with the paper and dairy sludge substrates combined.

Elvira *et al.* (1998) carried out pilot-scale laboratory trials ($2m^2$ beds), using *E. andrei* with dairy and paper industry sludges mixed with cattle manure. The findings of the trial indicated that the best results occured when earthworms were fed cattle manure alone. This was expected because it was known as one of the best natural food sources (Elvira *et al.* 1998). *E. andrei* also grew well in mixtures of manure with paper and dairy sludge.

Pérez *et al.* (2002) showed that by pretreating (co-composting) wastes, earthworm mortality can be avoided, especially with dairy and fish wastes. These wastes have high electrical conductivity and ammonia levels, which can cause the death of earthworms. Pérez *et al.* (2000) examined the co-composting of dairy sludge. This comprised an initial aerobic fermentation phase inside a bioreactor, followed by a maturation phase, and lastly a vermicomposting phase. Two kinds of sludges were used with bulking agents, a fatty fraction of milk and a residual sludge. The fatty fraction was vermicomposted while the high electrical conductivity made the residual sludge toxic to the earthworms.

Generally from the reports about vermicomposting dairy sludge, it was determined that the level of ammonia in the dairy sludge can be lethal to earthworms (Nogales *et al.* 1999; Gratelly *et al.* 1996). However, it is a good substrate for vermicomposting, when placed on a bed of vermicompost (Elvira *et al.*, 1999; Gratelly *et al.* 1996; Nogales *et al.* 1999).

2.13   Large scale vermicomposting

The use of vermicomposting as a means to treat organic materials has been proposed for more than 30 years. Vermicomposting during this time remained a small process around the world, with very few facilities capable of processing large volumes of organic materials. Some of the reasons for this included the barriers to developing large facilities such as engineering, capital cost, perceived risk with working with earthworms and failed facilities. Vermicomposting facilities failed because of the misbelief that it is a natural process that needs little management, or due to lack of knowledge of the ecology of earthworms or poor

marketing (on the benefit of vermicompost) (Dominguéz *et al.* 1997). People focused a lot of attention on the technologies of vermicomposting, forgetting that earthworms were a livestock and that vermicomposting is really about raising biological organisms (Sherman, 2002*a*). Also the growing of earthworms was traditional for fish bait market and it was never fully exploited in the area of waste management.

The basic rule for a successful vermicomposting system is to add wastes in thin layers at regular periods to the surface of the bed. It is important that the earthworms are allowed to move into the new layer of waste and maintain an aerobic environment. The earthworms will always move into areas that provide new food source and will continue to move into successive layers. The key to having an efficient system with maximum production of vermicompost and optimum growth of earthworms is to have **optimal temperature** and **moisture conditions, maintain aerobic conditions** and **avoid using wastes with high levels of ammonia and salts** (Edwards and Arancon, 2004).

2.13.1   Types of large scale vermicomposting technologies

There are different approaches to large-scale vermicomposting, from relatively simple and labour intensive technology to highly automated continuous flow reactor systems. These different types of vermicomposting systems can be grouped into categories; windrow, wedge, batch and continuous reactor systems.

Windrows
Windrows are usually up to 0.6-0.9m high, and waste is applied in thin layers of 5cm to 8cm inches weekly (Plate 1). This type of system is extensively used both outdoors and indoors (Sherman, 2002*a*). The system has some drawbacks as it requires a large area of land or large buildings. Harvesting of the vermicompost is labour intensive and time consuming because the earthworms have to be separated from the vermicompost, usually with a screening system. In this case a mechanical harvester is often used. This system is also affected by climatic conditions when used outdoors. The initial cost of setting up a windrow is low, but large areas of land are required, labour costs are high and the rate of processing is slow over a period of between 6 and 12 months (Edwards and Arancon, 2004). However the system does not require high capital costs.

Plate 1. Windrow vermicomposting beds-US EPA sewage sludge trial (Eastman, 1999)

Wedge

The wedge system overcomes some of the problems of the windrow system. It is less labour intensive, requires less land and there is no need to separate the earthworms from the vermicompost. This wedge system is a modified windrow. Waste is placed in layers against a wall or windrow at a 45-degree angle. The system is started with a layer of vermicompost containing 9kg of earthworms per square metre to a depth of 15 cm (Edwards and Arancon, 2004). Gradually the windrow is continued by applying wastes at a 45-degree angle. Earthworms gradually migrate laterally to the fresh food, leaving the vermicompost behind them, which can then be taken away by a loader or tractor (Sherman, 2000*a*). The processing time is shorter than the windrow system, taking around 3-4 months (Edwards and Arancon, 2004).

Batch System

This system is composed of trays/containers containing waste and earthworms. The containers can be stacked vertically on shelves, thus taking up less space. This system is usually used within a building or an enclosed area (Edwards, 2000). It has drawbacks as it requires considerable handling and lifting as the trays have to be removed from the shelves for feeding (Sherman, 2002*a*). It can be difficult to maintain constant moisture levels and the earthworms also have to be separated from the vermicompost (Edwards, 2000).

28

Reactors

Reactors are raised beds with mesh bottoms. The waste is added in thin layers to the surface of the bed using a mobile gantry. The vermicompost is then removed by scraping with a breaker bar above the mesh. This can be manual or fully automated with a hydraulic system. Vermicompost that falls onto the floor beneath the bed can be brought out to one end with a hydraulic driven flap scraper (the type used to remove cow manure from dairy cows in sheds) (Edwards and Arancon, 2004).

The continuous flow reactor was originally developed at the National Institute for Agricultural Engineering in Silsoe, England. This design has since been adopted by large vermicomposting facilities in America and Australia and can be up to 40m long, 2.8m wide and 1m deep (Dominguéz *et al.* 1997). The earthworm population in these systems tends to reach an equilibrium biomass of about 9kg per m^2. These 1m deep reactors can fully process the waste in about 30-45 days (Edwards, 1995).

The reactor overcomes the drawbacks of both the windrow and wedge system. It must be kept indoors to maintain optimum environmental conditions. It requires a small area, which prevents leaching and with fully automated reactors, it requires low labour maintenance. It is generally constructed indoors, so it can be built in any climate (see Figure 4).However the reactors have a high capital cost e.g. a system costing \$35,000 to \$50,000 can process 3 tonnes of waste per day. It should be noted that reactor capital cost can be recovered in 1-2 years (Edwards and Arancon, 2004).

loading gantry

track for gantry

successive layers of waste

high density worm inoculation

heating cables

weight bearing grid
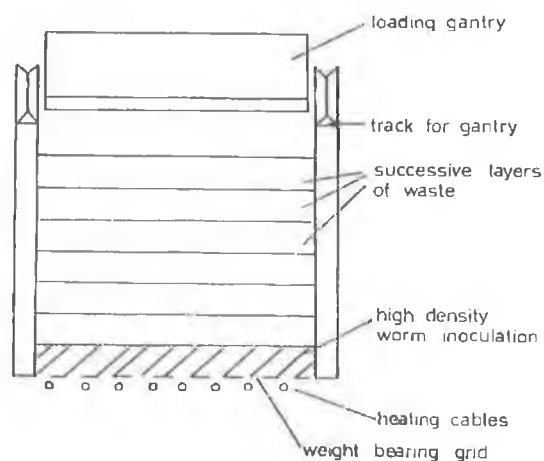
Figure 4. Design of continuous flow reactor system by C.A. Edwards (Edwards, 1998)

29

2.13.2   The development of vermicomposting systems worldwide

There is no large scale dairy sludge vermicomposting facility in the world. The nearest type of facility processing dairy sludge is sewage sludge vermicomposting facilities developed in Australia and operated by Vermitech Ltd. This type of facility handles a large volume of sewage sludge. Sewage sludge has similar characteristics to dairy sludge such as high nitrogen content and ammonia. The Vermitech system at Brisbane was selected in 1997 for treating $200m^3$/week of sludge from sewage and water treatment plants. After competition from other companies which used either lime stabilsation or composting. Vermitech was awarded the tender from the local authority. This was because of lower cost, better processing and greater end product potential. Using a continuous flow reactor, from the start up to 1999, the plant had processed 10,700 tonnes of sludge into Grade A stabilised vermicompost called BioVerm™ (Anon., 1999).

In recent years vermicomposting has become more popular in tropical regions of the world, such as China, Hong Kong, India, Indonesia, Japan, Philippines and Taiwan. Limited information is available because companies are keeping their activities confidential for commercial reasons. The types of waste processed include animal wastes, wastes from agriculture (straw, weeds & fruit rind) and food waste from homes to tea leaf waste (Gunadi, 2000). Kim *et al.* (2000) reported that there are over 80 vermicomposting facilities treating over 110,000 tonnes of organic waste per year in Korea.

Coffee pulp is a by-product of the coffee industry and is a huge waste problem in coffee growing regions. In 1985 vermicomposting of coffee pulp was tried in Mexico. With the success of this trial, vermicomposting of coffee pulp is now a common part of the coffee industry in South America. There are currently more than 15 countries that have vermicomposting facilities in operation (Barois and Aranda, 2000).

In Russia, Green-Pik Ltd is a very successful vermicomposting company and each day is producing over 3000 tonnes of vermicompost and 30,000 tonnes of liquid fertiliser. The latter being their main product. The company has a facility, like a drinks manufacturer, which bottles the liquid fertiliser into one litre bottles (Joha, 2005)

The Moraka Foundation in India is the largest producer of vermicompost in the world. It has over 400 employees including over 100 scientists who carry out research on vermicomposting for the benefit of agricultural development. The foundation has delivered its training programme to over 100,000 farmers. They vermicompost animal manures and food waste creating a combined capacity of over 500,000 tonnes of vermicompost per year using *E. fetida* (Anon., 2004).

In America the in-vessel (reactor) systems are required in most regions due to extreme weather conditions of hot summers and cold winters. However on the West Coast of America outdoor windrows are common. American Resource Recovery in California is the largest vermicomposting facility in America. It was established in 1993 and uses outdoor windrows that process 75,000 tonnes of waste with an estimated 23 tonnes of earthworms annually. The facility spans over 28 hectares and the earthworms are fed with paper pulp, tomato residuals, manure and green waste in three-foot wide windrows (Sherman, 2000). Yelm Earthworm and Castings Farm, Washington, USA uses the wedge system consisting of approximately fifteen tonnes of earthworms that are used to process animal manure, green waste, paper and wood-chips (Sherman, 2000). The Pacific Garden Company operates a continuous flow reactor system for cow manure.

There are few large-scale vermicomposting facilities in Europe. There was a facility in Montelemar located in southern France. Sovadek Industries built it in 1991. Municipal waste from the local town was handsorted and the organic waste was thermophilically composted in beds for 30 days and then was vermicomposted in large bins for around 60 days (Edwards, 1995; Edwards and Arancon, 2004).

A facility in Wales operated by WormTech Ltd was established in 2004. It processes green waste and cardboard in a two-stage process. The thermophilic composting takes place first over about three months and is then followed by vermicomposting for one month. In Wales, there is a large vermicomposting facility covering a area of 10,000m^3 (Fredickson, 2000).

In Ireland, local authorities with small volumes of sludge have shown interest in vermicomposting. Small trials have been conducted at Moyvane, Co. Kerry, Mullinahone and Toomevara Co. Tipperary (Walsh, 1996) and Carlow (Healion, 1996). These trials have since been abandoned due to varying degree of success and the amount of labour involved. Mayo County Council in 2002 carried out a trial at Bangor Erris sewage treatment plant and recommended vermicomposting should be established at the facility (Tomas, 2002). In 1996, the Aquatic Services Unit in University College Cork carried out a small trial on vermicomposting of sewage sludge at Cork County Council's Ballincollig sewage treatment plant. The findings from this trial indicated odour neutralization and reduction of pathogens in the sludge. Since then vermicomposting has not been established at the facility (Walsh, 1996).

In August 2002, Shannon Vermicomposting Ltd was granted a waste permit from Tipperary North Riding County Council for $1,000m^3$ on site or twenty tonnes weekly intake of sludges, slurries and foodwaste to their facility. The process combined composting in windrows followed by vermicomposting. This facility is no longer in operation as the local authority closed the facility due to poor management. Wonder Worms Ltd in Cork has a waste permit from Cork County Council. They process horse manure and food waste.

There have been many trials on vermicomposting over the years in Ireland. It is only now that large scale vermicomposting plants are being planned and developed. This is due to many reasons, such as: (1) the cold climate conditions in Ireland does not favour vermicomposting in winter; (2) the economic incentive to vermicompost, as it is cheaper than landfilling; (3) legislation requires organic wastes to be diverted away from landfill. (4) high premiums can be achieved for vermicompost.

For large scale vermicomposting to be a viable waste management alternative, it must be economically sustainable and this has been proved around the world with facilities in Russia, America, India, Cuba and Australia. Large scale vermicomposting of sludge is possible and economically viable as shown by Vermitech Ltd. in Australia.

CHAPTER THREE

VERMICOMPOSTING

3.0 Introduction

Vermicomposting is the processing of organic materials by earthworms generating vermicompost. In this study this was examined with dairy sludge from Glanbia Ingredients Ltd., Kilkenny. This thesis is a broad study of earthworm survival, growth, the conversion of dairy sludge into vermicompost (chapter 3) and plant growth trials using the vermicompost and untreated dairy sludge (chapter 4).

In this chapter three, vermicomposting was studied with different experiments;

- Firstly by determining the physical and chemical properties of dairy sludge,
- Examining the growth of 10kg of earthworms in 1800kg of dairy sludge (windrow trial),
- Determining when the dairy sludge is vermicomposted,
- Conversion of 15kg dairy sludge into vermicompost with 0.8kg of earthworms,
- Investigating the processing rate with smaller quantities of dairy sludge and earthworms,
- Determining the effect of different polyelectrolyte concentrations on earthworms survival,
- Reducing ammonia levels in dairy sludge for earthworm survival.

A limitation of this study is that due to time constraints, some work in this chapter may not be statistically repeatable.

3.1. Characterisation of Dairy Sludge

To determine main characteristics of dairy sludge, it was analysed for a range of typical parameters, metals and pathogens. Details on the methods of analysis are in Appendices G and K.

Table 7. Composition of dairy sludge

Parameter	Value
% Moisture Content	85 (0.13)
% Volatile Solids	68.4 (0.4)
pH	7.86 (0.09)
Electrical Conductivity ms^{cm-1}	0.263 (0.03)
Total Kjeldahl Nitrogen (%)	5.50 (0.52)
Organic Carbon (%)	39.2 (0.14)
Carbon to Nitrogen Ratio	7:1

Values in parentheses are standard deviation of 3 samples.

Table 7 showed that the dairy sludge had a high content of total Kjeldahl nitrogen, volatile solids and moisture content. The carbon to nitrogen ratio was low. The electrical conductivity was low and the pH was mildly alkaline.

Table 8. Metals in dairy sludge with a comparison to the draft Sewage Sludge Directive

Metal	Cadmium	Chromium	Copper	Mercury	Lead	Niekel	Zinc
mg/kg	<0.01 (0)	8 (3)	11 (4)	<1.1 (0)	6 (4)	15 (1)	181 (44)
Sludge Directive Limit (mg/kg)	10	1000	1000	300	10	750	2500
Metal	Aluminium	Manganese	Cobalt	Molybdenum	Boron	Iron	
mg/kg	402 (557)	56 (8)	1 (0)	2 (1)	24 (9)	930 (244)	

Values in parentheses are standard deviation of 3 samples. Aluminium to Iron are not in Sludge Directive

The metal concentrations in the dairy sludge (Table 8) were below the limits in the draft Sewage Sludge Directive (Anon. 2000*b*). This is the future legislation affecting the management of dairy sludge.

Table 9. Pathogen content in dairy sludge with a comparison to the draft Sewage Sludge Directive

Draft Sewage Sludge Directive	Sample 1	Sample 2	Sample 3
Salmonella (None present)	None present	None present	None present
E.coli (500 cfu)	<3 cfu	430 cfu	2620 cfu

The sludge was sampled three different times for pathogens in relation to the draft Sewage Sludge Directive (Table 9). *Salmonella* was not present in any of the dairy sludge samples. *E. coli* was below the limit in two samples. However, on the third sample there was a substantial increase to 2,620 cfu of *E. coli*. A more intensive sampling schedule is required to get an accurate account of the pathogen content in the dairy sludge. The increased *E. coli* was possibly due to human sewage generated on site being treated in the dairy wastewater treatment plant

3.2 Vermicomposting

3.2.1 Introduction

Vermicomposting trials can be done at three levels of scale, (1) small (2) medium and (3) large commercial scale. Small scale size is laboratory trials involving petri dishes, ice-cream tub size containers. Most of the academic research is done on this scale.

Large scale vermicomposting is usually done with large amounts of organic materials e.g. >50 tonnes.

Results from research done on a small scale cannot be scaled up to large scale due to different conditions and the 'edge effect' on earthworms. When an edge is formed in any natural ecosystem, the surrounding area outside the boundary is disturbed and the natural ecosystem can be gravely affected for some distance from the edge. Usually small scale is done under controlled optimal environmental conditions. However these conditions may not be achievable in large scale as it is usually done outdoors and conditions are dependant on the weather. In small scale the 'edge effect' may affect earthworm activity and thus the outcome of the trial. If the small scale trial was extrapolated to large scale, different results would be obtained because the edge effect may not affect earthworm activity. This statement has been confirmed by both academic researchers (Dr. Hartenstein of the State University of New York (Hartenstein, 1981), Meyer (2004), Dr. Jim Frederickson of the Open University (Gilbert *et al.* 2001) and commercial vermicomposting systems operators (Stanley, 2002). This was reinforced by Elvira *et al.* (1996) who scaled up research from petri dishes to pilot scale and reported a significant difference in the growth rates and cocoon generation of earthworms.

The ultimate aim of the overall project is that, based on this study, Glanbia might conduct a large scale vermicomposting of dairy sludge under Irish climatic conditions. Considering this, it was decided in this study to conduct a research trial on a medium scale ($6m^2$) along with smaller trials. Medium scale might have similar conditions to large scale vermicomposting and would be physically manageable because of the smaller volume of sludge required. This was a crude experiment in order to simulate conditions to a large scale trial. The earthworm population was monitored for growth and survival by sampling using cores. The sludge was analysed for environmental conditions and stability.

3.2.2 Aims

- To carry out a medium scale vermicomposting trial in a polytunnel under Irish climatic conditions.
- To investigate the survival and growth of earthworms in dairy sludge.

- To monitor the environmental conditions and stability of the dairy sludge during vermicomposting.

- To investigate if dairy sludge can be vermicomposted.

3.2.3 Design of trial

Prior to and during this study, it was necessary to learn about the operation, maintenance of the vermicomposting process and the care of earthworms to facilitate the design of trials. In order to acquire this knowledge different members of the research team of Glanbia and IT Sligo visited the following vermicomposting facilities (1) Vermitech, Brisbane, Australia (2) Oregon Soil Corporation, Oregon, USA (3) Irish Earthworm Company facility in Donegal (4) McGrath's earthworm farm in Tipperary (5) Mullinahone and (6) Toomevara, Tipperary sewage vermicomposting systems (7) Mayo County Council, Bangor – Erris sewage sludge vermicomposting trial.

Vermicomposting can be done in outdoors beds in a field. However this is a slow process and prone to possible waterlogging with heavy rainfall. Vermicomposting can also be done indoor in a shed, but sheds are expensive to build. Vermicomposting can be conducted in a polytunnel, which is cheap and easily built. A polytunnel provides a warm environment. In Ireland other vermicomposting trials have been conducted in polytunnels (Healion, 1996; Tomas, 2002). Vermicomposting companies have also conducted their processes in polytunnels (Shannon Vermicomposting Ltd in Ireland and Oregon Soil Corporation in the USA) (Riggle and Holmes, 1994). It was decided to conduct the trial in a polytunnel in Sligo located convenient to the college.

Construction and setting up of the beds

The vermicomposting bed was a simple design. Factors that influenced the design were (1) convenience, (2) allow the option to study and monitor earthworm behaviour, so an improved bed could be designed based on the findings (3) low costs, if Glanbia conducted a large scale trial the cost of construction of the beds would be low. A bed in the design of a windrow (see section 2.13) was built contained by plywood sides. It had 1.19m wide, 4.89m long and 0.6m high plywood sides. The top of each bed was covered with a breathable tarpaulin cover; which prevented loss of moisture from the sludge. A anticrawl device which is a strip of plastic on the edge of the plywood on the top, was used to prevent the earthworms escaping.

Plate 2. The vermicomposting and control windrow beds

5500 kg of dairy sludge were collected from Glanbia and transported on the same day to Sligo. On the same day the sludge was moved into the beds by use of a wheel barrow. Approximately 1800 kg of sludge at a depth of 25cm were placed into each bed.

Choice of earthworms species

Having established the bed, the earthworms had to be added. It did not matter which species of earthworms were used, as long as they were used in vermicomposting. A mixture of earthworm species of *Dendrodrilus rubidus, Eisenia fetida, Lumbricus rubellus* and *Dendrobaena veneta* (clitellated and non-clitellated) were obtained from a vermiculture farm which used horse manure as a food source. The literature stated that the density of earthworms used in vermicomposting depends on the nature of organic waste (see section 2.10.9). Gilbert *et al.* (2001) states that in large scale vermicomposting a density of 1-4kg/m^2 is used. A ratio around the middle of stated density (1.7kg/m^2) was used to stock the bed with earthworms. In total 10kg of earthworms were added to the vermicomposting bed. The earthworm biomass was of mixed age and species of earthworms. The earthworms were placed on the surface of the sludge with a small amount (3kg) of vermicomposted material which the earthworms were grown in. This material acted as a habitat to which the earthworms could retreat to, if conditions in the sludge were not favourable. A control bed had sludge but no earthworms.

3.2.4 Sludge analysis

Sludge samples were taken from the earthworm sampling cores and cores from the control bed in a stratified random pattern (this was where the bed was first divided into

37

three strata and within each strata cores were taken at random). Details of each analytical method are found in Appendix G. The sludge was analysed for ammonia using standard wastewater method (APHA, 1989), moisture content (APHA, 1989) on the day of sampling or kept at 4°C until analysed within 24 hours. Samples were kept at 4°C until analysed for pH (Erhart and Burian, 1997) and electrical conductivity (Atiyeh et al. 2001) within one week of collection of samples. The sludge colour was compared to a soil colour chart (Anon., 1970). The temperature was measured using a datalogger (Grant) and spot readings using a probe. The sludge was analysed for total kjeldahl nitrogen (TKN) according to Bremner (1965) and total organic carbon TOC (Schumacher, 2002) at the start of the trial. The stability of the sludge was examined at each sampling period by examining the sludge with a slight modification to biological oxygen demand (BOD), chemical oxygen demand (COD) methods and percentage volatile solids content (APHA, 1989). The vermicompost was sampled for its metal content, details of metal analysis in Appendix K. There is no pH, electrical conductivity, ammonia, BOD, COD and % volatile solids results for the control bed on sampling period 4, because worms had escaped from the vermicomposting bed and entered into it.

3.2.5   Earthworm sampling

Sherman (2002b) reported that large scale operators allow earthworms to settle into their new environment for a few weeks. In this trial the earthworms were allowed a period of six weeks to settle in before a sampling programme was initiated to monitor their progress. The method of earthworm sampling (weight, length and identification) is given in Appendix G. The earthworms were sampled by taking cores in a stratified random pattern.

3.2.6   Statistical analysis

Statistical tests were done using Sigma Stat software. Data was analysed using a T-test and significant level was set at 0.05. T-tests were performed to evaluate if there was difference in means between the two groups. One way ANOVA (analysis of variance) and the Tukey Test were conducted to determine the different between concentric cores.

3.2.7   Earthworm results

The earthworm population was sampled by taking cores from the bed four times; sampling 1 (week 6), sampling 2 (week 14), sampling 3 (week 23) and sampling 4

(week 31). After each sampling period the earthworm results were analysed. Each sampling period determined the next sampling regime.

weeks 1-6

During the six week period some earthworm fatalities were noted. After the first 2/3 days approximately 90 earthworms escaped out of the bed and died. After the first week approximately 100 earthworms were dead on surface of the bed. On week 5 (28th March), approximately 30 dead earthworms were on the surface of the bed. Usually there were no dead earthworms, except after a few days at the start.

sampling 1 (week 6)

Table 10. Number of earthworms in the three cores

	Core 1	Core 2	Core 3	Mean
No. of Earthworms	13	33	9	13

Cores with a large diameter might damage the bed and take longer to sort. It was decided to use a small cores size of 11cm for the first sampling. There was a large variation in the results (Table 10), it was determined that more replicates were needed in the sampling period 2. When an edge is created in an ecological system, in this case the edge was created with the cores. The area outside the boundary is disturbed for some distance. Sampling with three core sizes determined what size had the least edge effect. There was also an ammonia odour associated with the bottom layer of the cores, possibly due to build up in this layer. The majority of earthworms occupied the top layer (Table 11), likely because of more aerobic conditions.

Table 11. Percentage of earthworms in top and bottom of the cores

	Core 1	Core 2	Core 3	Mean
Top of the Core	77	85	67	77
Bottom of the Core	23	15	33	23

sampling 2 (week 14)

Table 12. Est. No. of earthworms in the entire vermicomposting bed using three replicates of concentric cores

	Estimated Total No. of Earthworms in Bed		
CORE ID	11cm	16cm	31.5cm
Mean	39,606	38,124	37,095
S.D.	21,551	23,373	24,350

Initially three concentric cores (11cm, 16cm & 31.5cm) were taken in a stratified random pattern. This was to determine how much 'edge effect' the cores had on the earthworm population and determine which core size was the most suitable to sample the earthworm population. For each core site it was found that the estimated number of earthworms was not significantly different (P>0.05) between the three different core sizes. It was then decided that the 11cm cores were of sufficient size to sample with the remaining sites. This was done, however the mean value for the 11cm core appeared to over-estimate the number of earthworms, due to the edge effect (Table 13). The 16cm and 31.5cm had relative standard deviation that ranged from 43 to 66%. This was very high and it was determined that more replicate cores were needed to lower the variation.

Table 13. Est. No. of earthworms in the bed from the cores samples (11cm, 16cm & 31.5cm) on week 14

	Estimated Total no. of Earthworms in Bed		
CORE ID	**11cm**	**16cm**	**31.5cm**
Mean	**46,918**	**38,124**	**37,095**
S.D.	20,078)	23,373	24,350
R.S.D.	43	61	66

11cm is a SD of 10 replicates. 16cm and 31.5cm are SD of 3 replicates

sampling 3 (week 23)

Table 14. Est. No. of earthworms in the bed from the core samples on week 23.

	Estimated Total No. Earthworms in Bed		
CORE ID	**11cm**	**16cm**	**31.5cm**
Mean	**46,105**	**38,926**	**33,282**
S.D.	6,857	7,349	3,004
RSD	15	19	9

S.D. of 10 Replicates

Ten replicates of concentric cores (11cm, 16cm & 31.5cm) were taken from the vermicomposting bed in a stratified random pattern. The 16cm core size was determined to be most suitable size core to sample the earthworm population. This was because it did not over estimate the population greatly, had a less edge effect than the 11cm core size, and was a manageable core size to sort through.

sampling 4 (week 31

Table 15. Est. No. of earthworms in the bed on week 31.

16cm core	Mean	SD	% RSD
Est. Total No. in Bed	**34,826**	7,723	22

S.D. of 10 Replicates

The 16cm size core estimated that there were 34,826 earthworms in the vermicomposting bed.

Number of earthworms

Table 16. Number of earthworms in the vermicomposting bed with 16cm size core over the sampling periods

	Week 14 (n=3)	Week 23 (n=10)	Week 31 (n=10)
Mean	**38,124** (23,373)	**38,926** (7,349)	**34,826** (7,723)

The earthworm population did not differ significantly (P>0.05) between the sampling periods on weeks 14, 23 and 31.

Earthworm biomass

Table 17. Mean weight (kg) of earthworms in the vermicomposting bed

	11cm Core	16cm Core	31.5cm Core
Week 0	10	10	10
Week 14	**3.96** (3.68)	**2.10** (1.24)	**2.80** (0.84)
Week 23	**11.79** (2.48)	**3.76** (1.13)	**5.26** (1.48)
Week 31	no core	**6.8** (2.71)	no core

Table 18. Density of earthworms (kg/m^2)

	11cm Core	16cm Core	31.5cm Core
Week 0	1.7	1.7	1.7
Week 14	0.7	0.4	0.5
Week 23	2.0	0.7	0.9
Week 31	no core	1.2	no core

The 16cm core showed that that the weight and density of the earthworms had decreased from the start to week 14. From week 14 to week 31 the weight and density increased to 6.8 kg of earthworms in the bed, at a density of 1.2 kg/m^2.

Cocoons

Table 19. Number of cocoons in the 16cm cores

Sampling Time	Number of Cocoons	
	Top	Bottom
Week 14	**35.1** (17)	**0.6** (1.4)
Week 23	**60.2** (17)	**0.5** (0.9)
Week 31	**55.3** (10)	**0.4** (0.7)

The majority of cocoons were located in the top part of the vermicomposting bed.

Length of earthworms
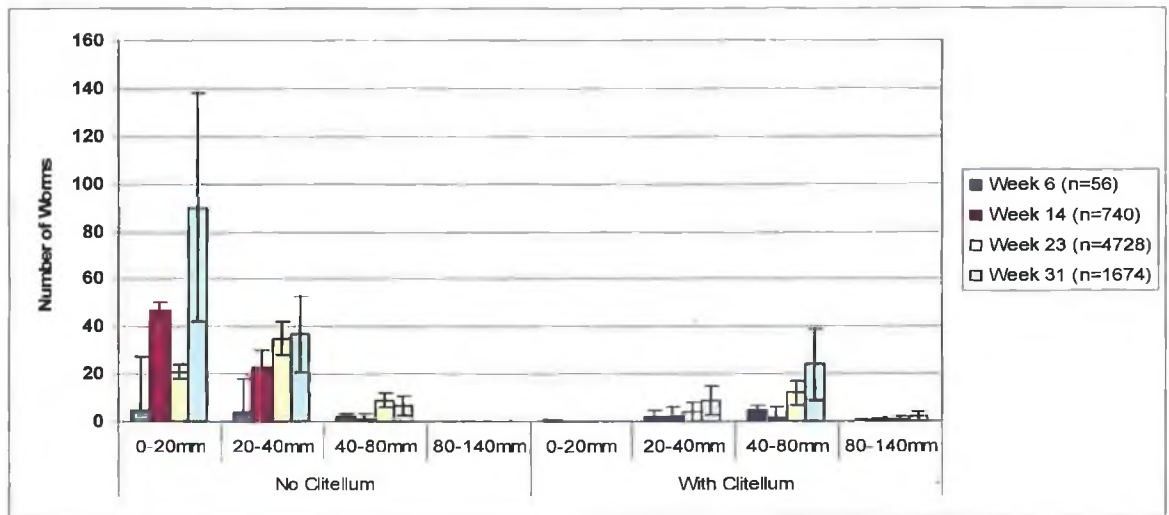
Figure 5. Number of earthworms (clitellated and non-clitellated) at different lengths

In week 14 the population was young (non-clitellated) compared to weeks 23 & 31. The number of adult (clitellated) earthworms increased in length over the sampling periods. The method of measuring the length of earthworms is described in Appendix G.

Species composition
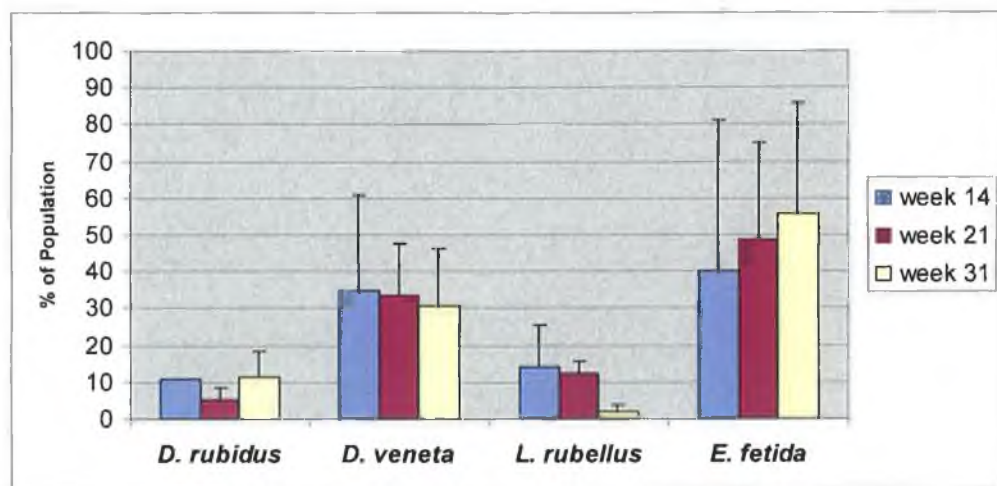
Figure 6. Species composition on sampling periods on weeks 14, 21 & 31.

On the sampling periods the earthworms with clitellums were visually identified. *D. veneta* and *E. fetida* were the dominant species of the polyculture.

3.2.8 Sludge results

Table 20. Environmental Conditions (pH, Electrical conductivity, Ammonia & moisture) and Stability (% volatile solids, BOD & COD)

Parameter	Sample Location	Week 0	Week 14	Week 23	Week 31
EC (ms/cm^{-1})	Worm Bed Top	No sample	0.4 (0.1)	0.7 (0.1)	0.9 (0.1)
	Control Bed Top	No sample	0.7 (0.1)	0.8 (0.1)	Worms in Control
	Worm Bed Bottom	No sample	0.3 (0.1)	0.7 (0.1)	0.7 (0.1)
	Control Bed Bottom	No sample	0.8 (0.3)	0.8 (0.2)	Worms in Control
pH	Worm Bed Top	No sample	7 (0.2)	6.9 (0.2)	6.7 (0.2)
	Control Bed Top	No sample	7.3 (0.1)	6.7 (0.1)	Worms in Control
	Worm Bed Bottom	No sample	7.8 (0.1)	8.4 (0.1)	8.3 (0.3)
	Control Bed Bottom	No sample	7.8 (0.4)	8.4 (0.2)	Worms in Control
Ammonia (mg/g) dry weight	Worm Bed Top	No sample	0.2 (0.2)	0.1 (0.1)	0.3 (0.4)
	Control Bed Top	No sample	0.0 (0)	0.1 (0)	Worms in Control
	Worm Bed Bottom	No sample	2.3 (0.8)	2.3 (0.8)	2.9 (1.7)
	Control Bed Bottom	No sample	2.2 (3.1)	5 (2.0)	Worms in Control
Moisture content (%)	Worm Bed Top	85 (0.3)	86 (2.4)	86 (0.6)	85 (1.5)
	Control Bed Top	86 (0.3)	87 (0.6)	86 (0.4)	Worms in Control
	Worm Bed Bottom	87 (0.3)	86 (0.4)	87 (0.5)	87 (1.0)
	Control Bed Bottom	88 (0.3)	87 (0.4)	87 (0.2)	Worms in Control
Volatile solids (%)	Worm Bed Top	68 (0)	57 (4)	53 (2)	51 (3)
	Control Bed Top	68 (0)	57 (7)	55 (1)	Worms in Control
	Worm Bed Bottom	68 (0)	62 (3)	59 (1)	56 (1)
	Control Bed Bottom	68 (0)	55 (7)	57 (2)	Worms in Control
BOD (mg/kg DS)	Worm Bed Top	64,404 (6,420)	25,677 (13,922)	20,818 (9,153)	9,599 (7,561)
	Control Bed Top	64,405 (6,420)	12,400 (5,961)	19,892 (5,080)	Worms in Control
	Worm Bed Bottom	64,406 (6,420)	51,475 (18,566)	30,434 (13,371)	28,127 (15,407)
	Control Bed Bottom	64,407 (6,420)	16,223 (10,736)	32,811 (6,589)	Worms in Control
COD (mg/kg DS)	Worm Bed Top	939,683 (86,242)	584,894 (137,765)	582,744 (180,480)	591,854 (116,868)
	Control Bed Top	939,683 (86,242)	1,225,533 (584,836)	1,370,989 (604,120)	Worms in Control
	Worm Bed Bottom	939,683 (86,242)	794,207 (291,942)	707,619 (160,793)	736,127 (172,419)
	Control Bed Bottom	939,683 (86,242)	1,370,989 (604,120)	582,479 (250,921)	Worms in Control

Table 21. Nitrogen, carbon content and C/N of dairy sludge at the start of the trial.

	Total Kjeldahl Nitrogen (%)	Organic Carbon (%)	C/N ratio
Dairy sludge	5.46 (0.06)	39.58 (0.24)	7:1

Values in parentheses are standard deviation of mean of 3 samples.

Table 22. Metals in vermicompost with a comparison to the draft Sewage Sludge Directive

Metal	Cadmium	Chromium	Copper	Mercury	Lead	Nickel	Zinc
mg/kg	**10** (0)	**9** (1)	**20** (3)	**<1.1** (0)	**7** (1)	**11** (0)	**251** (16)
Sludge Directive limit (mg/kg)	**10**	**1000**	**1000**	**300**	**10**	**750**	**2500**
Metal	**Aluminum**	**Manganese**	**Cobalt**	**Molybdenum**	**Boron**	**Iron**	
mg/kg	**<8** (0)	**166** (12)	**1** (0)	**2** (0)	**31** (2)	**2822** (109)	

Values in parentheses are standard deviation of 3 samples. Aluminium to Iron are not in Sludge Directive

The dairy sludge had a high total Kjeldahl nitrogen (TKN) content and low carbon to nitrogen ratio (Table 21).

The metal content in the vermicompost was below the limits in the draft Sewage Sludge Directive (Table 22).

On week 31, when the control bed was sampled, it was found that earthworms had escaped into the control bed. Therefore direct comparisons of pH, EC, ammonia, moisture, BOD, COD and % VS could not be made between the vermicomposting bed and control.

On week 23 the pH was significantly ($P<0.05$) greater in the top of vermicomposting bed compared to the control bed. There was no difference in the pH values of the bottom of the beds. On week 23 there was no significant difference between the EC value of the sludge in the top of the beds.

The ammonia content was significantly ($P<0.05$) higher in the bottom of the vermicomposting and control beds compared to the top of the beds (Table 20).

The moisture content of the sludge remained between 84 to 89% during the trial by spraying water upon the beds (Table 20).

From the start of the trial to week 23, there was a significantly (P<0.05) greater reduction in volatile solids in the top of the vermicomposting bed compared to the top of the control bed (Table 20).

From the start to week 23 the BOD decreased significantly (P<0.05) in both the vermicomposting and controls bed. However, neither were significantly different (P>0.05) on week 23 (Table 20).

The COD of the sludge decreased significantly (P<0.05) from the start to week 23 in the top of vermicomposting bed. It was significantly lower (P<0.05) than the COD value of the top of the control bed. There was no significant (P>0.05) difference between the COD values of the bottom of the beds on week 23 (Table 20).

Table 23. The Maximum and Minimum air temperature during the trial.

Temp(°C)	March	April	May	June	July	August	September	October
Max	26	31	37	34	39	41	40	30
Min	-1	2	7	11	11	10	12	1

The maximum and the minimum air temperatures during the trial were 41°C and -1°C.
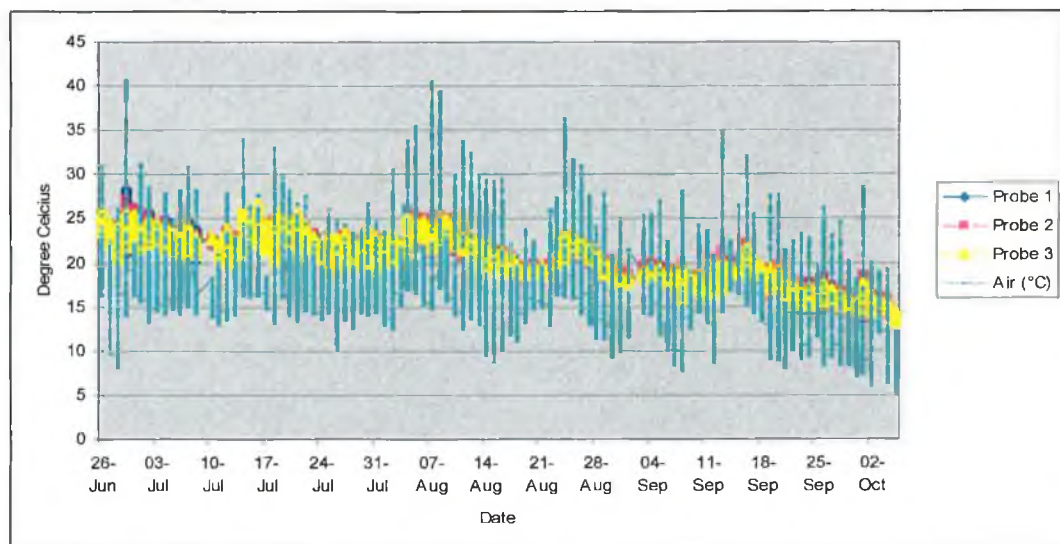
Figure 7. Temperature at three locations (7cm below surface) in vermicomposting bed and air temperature in the polytunnel

When the air temperature reached 40°C, the temperature in the bed only reached 29°C. The temperature readings show that while the air temperature fluctuated between 5 and 40°C, the temperature of the sludge varied between 12 and 29°C between June and

October (Figure 7). This appears that the vermicomposting bed had a high thermal inertia possibly due to the earthworm creating air pockets.

Table 24. Sludge colour during the trial.

Sampling Periods	Top of Cores	Bottom of Cores
Weeks 6, 14, 23, 31	4/4 Hue 7.5 YR	4/1 Hue 7.5 YR

Overall at weeks 6, 14, 23 & 31, the top cores were 4/4 Hue 7.5 YR (light brown colour) (Table 24). At the same sampling periods the bottom cores were 4/1 Hue 7.5 YR (grey/black colour) (colour chart in Appendix G).

3.2.9   Discussion

<u>**Earthworms**</u>

In this experiment to investigate earthworms converting dairy sludge into vermicompost, the earthworms were monitored on weeks 8, 14, 23 and 31. In order to assess the earthworm population over the 31 week period, cores of the bed were taken and the earthworms assessed. The earthworm numbers were estimated with a 16cm core size. This core was used because it appeared to be least effected by the edge effect and it was of a manageable size to sort through. The earthworm population did not differ significantly ($P>0.05$) between the sampling periods on weeks 14, 23 and 31 (Table 16, p41). During the sampling periods the number of earthworms in the bed appeared to have stabilised around the range of 35,000 to 39,000.

In this trial, a polyculture of earthworms was used. There are no other trials published using a polyculture with dairy sludge on a medium scale. The nearest related work is a trial using a monoculture (*E. andrei*) growing in dairy sludge in a $2m^2$ area. In that trial, the number of earthworms increased between 22 and 36-fold, after a six month period (Elvira *et al.* 1998).

The earthworm biomass had decreased significantly ($P<0.05$) from the initial 10kg at the start of the trial (Table 17, p41). However the 16cm core size showed that the biomass of earthworms had increased in the vermicomposting bed. The loss of earthworms/biomass was attributed initially to (A) earthworms escaping to the control bed (B) dead earthworms in the bottom part of the bed where there appeared to be a pattern with high ammonia levels and earthworm mortality (C) in April (before week

14) there was a short period of warm weather, when the high temperatures may have caused earthworm mortality. It has also been reported that if a vermicomposting bed is inoculated with a high density of earthworms, they will naturally thin out their population to a preferred density (Dr. Scott Subler, pers. comm.). The density of earthworms per metre square is the common parameter used for examining the population of earthworms in a vermicomposting system. The density of the earthworm is dependant on the nature of the organic material. In pig manure high densities of 7-11kg earthworm/m^2 had been reported (see section 2.10.9). However, the general range is between 1-4kg earthworms per metre square (Gilbert *et al.* 2001). The final result in this study after 31 weeks is 1.2 kg earthworms/m2, which is within that reported range (Table 18, p41). If the study was continued the earthworm density might have increased further.

No distinction of cocoons from individual species was made. The total number of cocoons present was counted. The number of cocoons (Table 19, p41) showed a clear trend demonstrating that the earthworms lay only a very small number of cocoons in the bottom of the bed. The number of cocoons increased from week 14 to week 23. The number decreased slightly in week 31. Because the cocoons counted were from a mixed population of species, the number generated could not be compared to published data because it is based on monoculture populations. However published data with monoculture species, reported that earthworms grew in dairy sludge and generated cocoons. However the cocoon production was lower in pilot scale ($2m^2$) compared to a laboratory scale trial (Elvira *et al.* 1998).

The length of earthworms at the different sampling periods show that in week 14 the population was young (non-clitellated) compared to weeks 23 & 31 (Figure 5, p42). The earthworms were gradually increasing in length and on week 31 there was a high number of non-clitellated earthworms (0-20mm). The number of adult (clitellated) earthworms increased in length over the sampling periods. This method is probably the least accurate measurement of the earthworms population as they can contract and relax their muscles.

There was no one dominant species in the polyculture, but two species dominated the earthworm population. They were *D. veneta* and *E. fetida* (Figure 6, p42). Overall regardless of sampling period, there was no significant difference ($P>0.05$) between *D.*

veneta and *E. fetida.* However, both species of *D. rubidus* and *L. rubellus,* showed a significantly (P<0.05) lower percentage composition of the earthworm population compared to *D. veneta* and *E. fetida.* There was no significant difference (P>0.05) between *D. rubidus* and *L. rubellus* in the composition of the earthworm population. For each species samples on week 14 and week 31, there was no significant difference (P>0.05) in the percentage compostition of the earthworm population. It is recommended in any future vermicomposting system, a polyculture of earthworms should be used with approximately 50% *D. veneta* and 50 % *E. fetida.*

Another feature of doing the cores in a stratified random pattern was to investigate if there were any trends in the distribution of earthworms in the bed, i.e. at the edge, in the centre or evenly distributed in the bed. From examining the earthworm core data and corresponding locations of the cores in the bed, it was determined that the earthworms were evenly distributed across the bed in the top part of the cores. However there was a clear trend between the top and bottom part of the cores. The majority of earthworms were located in the top part of the cores, compared to the bottom part. This was possibly due to high ammonia levels and anaerobic conditions in the bottom part.

Sludge

As well as monitoring the earthworms population during the vermicomposting process, the sludge was monitored. The sludge was monitored and analysed for environmental conditions that affected the vermicomposting process and the stability of the sludge.

The fresh dairy sludge in this trial (Table 21, p44) had a high total Kjeldahl nitrogen (TKN) value of 5.48% and a low carbon to nitrogen ratio (C/N) of 7:1. The TKN value of 5.48% is similar to a result of 6.9% that was obtained by López-Mosquera *et al.* (2000) for dairy sludge from a Spanish dairy processing plant. The high TKN content of Glanbia dairy sludge and Low C/N ratio is a common characteristic that is shared by other dairy sludges sampled from different dairy processing plants (Gratelly *et al.* 1996; Elvira *et al.* 1998; Nogales *et al.* 1999).

Because the dairy sludge in this trial had a high TKN content, it might have encouraged earthworm growth and reproduction. Other authors found that in substrates in vermicomposting that had a low TKN value, the earthworms did not grow well or died. For example, Elvira *et al.* (1997*a*) in a study found that the earthworm *E. andrei* fed

only with solid paper pulp mill sludge (SPPMS) did not increase in weight and died after 65 days. (SPPMS) had low total Kjeldahl nitrogen content of 0.24% and C/N value of 188:1. Elvira *et al.* (1997*a*) determined that low nitrogen content in the SPPMS was a possible limiting factor in the vermicomposting process. Wastes with low nitrogen contents need to be mixed with wastes with high nitrogen content to achieve a good medium for earthworm growth.

The vermicompost was low in metals. The metals were below the limits in the draft Sewage Sludge Directive.

The pH during the trial remained within the optimal range of 5-9 for earthworms (Edwards and Arancon, 2004). On week 23 the top of the vermicomposting bed had a significantly (P<0.05) higher pH value of 6.9 compared to the top of the control bed value of pH 6.7. This indicated that activity of the earthworms increased the pH very slightly.

There was no significant change (P>0.05) in the electrical conductivity (EC) due to earthworm activity at week 23. It has been reported (Edwards and Bohlen, 1996) that EC has a toxic effect on earthworms. Perez *et al.* (2000) reported that high EC made dairy sludge toxic to earthworms. Glanbia's dairy sludge EC was sampled on various occasions and on average was approximately 0.2 ms^{cm-1}. This was significantly lower than the cut off point reported by Flack and Hartenstein (1984) of 8ms^{cm-1}. The EC value of the Glanbia sludge did not appear to be toxic to earthworms. The source of EC value in Glanbia sludge is from sodium hydroxide used in the cleaning process. The EC of the sludge does not need to be adjusted.

Ammonia was not monitored at the start and on week 6 (1st sampling period). However at the sampling on week 6, there was an ammonia odour associated with the bottom part of the cores. The majority of earthworms were located in the top part of the cores, compared to the bottom part. This was possibly due to high ammonia levels and anaerobic conditions in the bottom part (Table 11, p39). Ammonia levels were possibly responsible for earthworm distribution. It was then decided to monitor the ammonia levels in subsequent sampling periods. Ammonia has been reported to be toxic to earthworms (see section 2.10.5) at limits of both 0.5mg NH_3/g and 1mg NH_3/g of organic waste. There was a pattern in the location of earthworms in the vermicompost

bed and the level of ammonia. Overall during the trial, the bottom content had a significantly higher ammonia content than the top (P<0.05) (Table 20, p43. The majority of earthworms were found in the top of the bed. The movement of earthworms in the top part of the bed may have allowed ammonia to be lost to the atmosphere. Enhanced levels of nitrification may also have been a factor in lower ammonia levels in the top compared to the bottom. The bottom of the control bed had a significantly higher ammonia content than the bottom of the vermicomposting bed (P<0.05). This indicated the earthworms had slightly aerated the bottom of the bed. On weeks 14 and 23 the top of the vermicomposting bed had significantly (P<0.05) more ammonia that the top of the control bed. Ammonia might have been generated as a by-product of sludge digestion by the earthworms.

The moisture levels during the 31 weeks (Table 20, p43) were kept around the optimum condition reported by Edwards and Arancon (2004) of 80-85% moisture content. As the moisture content was even throughout the vermicomposting bed it probably did not influence earthworm distribution or death. It was observed that on the surface, near the edges of the bed, there were locations that had become a bit dry. Over the duration of the trial the amount of water added to each bed was approximately 150 litres.

In April there was a brief period of warm weather, this probably caused mortality of earthworms. It was observed at warm periods that earthworms were crowded at the bottom of bed under the edge of the plywood side, probably trying to escape the warm surface of the bed. During the summer months June-September (weeks 14-31), the highest air temperature in the polytunnel was recorded to be 40°C. At this time the temperature of the bed reached around 29°C. On a warm day it was recorded that by opening the doors to the polytunnel the air temperature dropped by approximately 10°C. The data recorded with spot readings in a grid pattern determined that the temperature of the bed was affected greatly from direct sunlight. To overcome this, the bed was covered with a shading material. This did not have much of an effect and this was then covered by tinfoil. During August there was a period of warm weather. The bed was covered with tinfoil and the doors to the polytunnel were opened. With a combination of these two mitigation factors, there was no earthworm mortality observed on the surface of the bed. The temperature in the vermicomposting bed was maintained below the lethal temperature of 35°C (Edwards and Arancon, 2004) from June to the end of the trial (Figure 7, p44).

There was no leachate generated during the trial. This has positive implications for large scale vermicomposting of dairy sludge.

There was a rat infestation in the bed during the winter and the rats ate some of the earthworms. Prevention measures should be taken in large scale vermicomposting trials.

The stability of the sludge was monitored to help determine the extent of the vermicomposting process. The top part of the vermicomposting bed was the location the majority of the earthworms. It showed that the earthworms only increased the rate of volatile solid reduction by 3%. Other authors have found reductions of percentage volatile solids of 4% (Kim *et al.* 2000), 9% (Hartenstein and Hartenstein, 1981), 28% (Neuhauser *et al.* 1988) and 30% (Frederickson *et al.* 1997).

The BOD of the top of the vermicomposting bed was not significantly different ($P>0.05$) than the top of the control bed. This indicated that the earthworms did not reduce the BOD of the sludge.

The COD of the sludge decreased significantly ($P<0.05$) from the start to week 23 in the top of vermicomposting bed and was significantly lower ($P<0.05$) than the COD value of the top of the control bed. This indicated that the earthworms did reduce the COD of sludge, other than natural activity.

The earthworms did not increase the stability when the BOD results were examined. However, they did increase the rate of stability when the results of the percentage volatile solids and COD were examined. The BOD was not a useful parameter.

3.2.10 Conclusions
The study has shown that:
- A medium scale vermicomposting trial was carried out in a polytunnel under Irish climatic conditions from February 2003 to October 2003.
- Mixed earthworm species grew and produced cocoons in the dairy sludge.
- There was no one dominant species in the polyculture. However *D. veneta* and *E. fetida* dominated the earthworm population.

51

- There was no general pattern to the distribution of the earthworms in the bed. However earthworms distributed themselves to the top of the bed, where the ammonia level was low.

- The stability of the dairy sludge during vermicomposting was analysed and monitored during the trial. The BOD did not decrease due to earthworm activity. The percentage volatile solid content and COD were decreased over time which indicated stabilisation of the sludge.

- During the trial, the earthworms were observed throughout the vermicomposting bed, but not at the bottom of the bed. After 31 weeks, the sludge did not have the characteristic peat like appearance of typical vermicompost, which helps to determine when an organic material is vermicomposted. To determine if the dairy sludge was vermicomposted, it was further investigated in section 3.3.

- The pH of the sludge did not need to be adjusted because the sludge was within optimum conditions of 5-9.

- The metals in the vermicompost were below the limits in the draft Sewage Sludge Directive.

- No leachate was generated during the trial.

- Escaping of earthworms into the control bed ruined the control.

3.2.11 Recommendations

- Based on the results, this trial should be treated as a pilot trial and it is strongly recommended by this author that it should be repeated again with measures to prevent earthworms escaping and entering the control bed. Measures to prevent rats from entering the beds should be taken too.

- From the trial conducted, an insulated polytunnel might be a better environment to conduct vermicomposting. The advantages of an insulated polytunnel compared to a polytunnel with transparent plastic are (1) the use of lights to prevent earthworms migrating (2) heating the beds by heating the air (3) maintaining the desired environmental conditions.

- In any future vermicomposting system, a polyculture of earthworms should be used with approximately 50% *D. veneta* and 50 % *E. fetida.*

- Start a windrow style bed with a shallower depth to prevent ammonia conditions forming in the bottom of the bed.

3.3 Determination of Vermicomposted Dairy Sludge by Nutrient Characteristics

3.3.1 Introduction

After 43 weeks of vermicomposting dairy sludge in the windrow bed (section 3.2), the sludge had not changed into the characteristic dark black colour of typical vermicompost. Vermicompost has other characteristics which define it. During vermicomposting important nutrient changes takes place. Compared to parent materials, many of the nutrients are released and converted through microbial action into forms that are more soluble and available for plant uptake. These nutrients are nitrate, ammonium nitrogen, exchangeable phosphorus, soluble potassium, calcium and magnesium (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh et al. 2001; Nedgwa and Thompson, 2001). The process of vermicomposting results in increased amounts of plant-available nutrients in the final vermicompost. To determine if the sludge from the vermicomposting bed was indeed vermicompost, its nutrient content was compared to its parent material (fresh dairy sludge) and also to a sample of typical vermicompost (vermicomposted horse manure). Vermicomposted horse manure was easily available for this experiment. Other vermicompost samples could have been used.

3.3.2 Aims

- To determine if the dairy sludge from the windrow trial (section 3.2) had been changed into vermicompost.
- To compare the nutrient content of the vermicomposted sludge from the windrow bed to its parent material (fresh dairy sludge).
- To compare the nutrient content of dairy sludge from the windrow trial with typical peat-like vermicompost (vermicomposted horse manure).

3.3.3 Samples

Three composite samples were collected from ten locations in the windrow bed (3.2). The locations were determined in a stratified random pattern. Three composite samples of fresh dairy sludge were collected off the belt press in Glanbia on three different occasions. Three samples of typical vermicomposted horse manure were obtained from Jimmy Austin's vermicomposting farm, Kilcock, Co. Kildare.

3.3.4 Methods

Exchangeable cations (calcium, magnesium and potassium) were determined using the ammonium acetate extraction method (Thomas, 1982). Calcium and magnesium were determined with a Perkin Elmer 2380 Atomic Absorption Spectrophotometer, using an air/acetylene flame. The interference of phosphate in magnesium was overcome by the addition of lanthanum (APHA, 1989). Potassium was determined by a Corning 400 flame photometer. Cation Exchange Capacity (CEC) was determined using the method described by Chapman (1965). Water soluble inorganic ions nitrate, phosphate, chloride and sulphate (NO_3^-, Cl^-, PO_4^{3-} and SO_4^{2-}) were determined by a Dionex 100 Ion Chromatograph (APHA, 1989). Samples interferences in ion chromatograph analysis were overcome by passing samples thought solid phase extraction (SPE) cartridges (Dionex OnGuard® II H cartridges). Details of these methods are in Appendix I.

Quality Control

Samples were analysed using appropriate blanks and standard solutions. Calibrations were conducted according to the standards procedures. Linearity of response was considered acceptable by the R^2 correlation coefficients, which was acceptable if >0.995. Exchangeable cations, water soluble anions and cation exchange capacity samples were spiked with a known amount of analyte. This was carried out to verify accuracy. Recoveries of spikes are in Appendix C.

Statistical analysis

Statistical tests were done using Sigma Stat software. Data was analysed using a T-test and significant level was set at 0.05. T-tests were performed to evaluate if there was difference in means between the two groups.

3.3.5 Results

Table 25. Nutrient content of fresh dairy sludge, sludge from the vermicomposting bed and vermicomposted horse manure
(Results are expressed as dry weight)

Nutrient	Fresh Dairy Sludge	Sludge from Vermicomposting Bed	Vermicomposted Horse Manure
Cation Exchange Capacity (meq/100g)	139 (14)*	160 (14)	173 (14)
Exchangeable Potassium (mg/kg)	5,014 (1424)	5,307 (1209)	5,516 (234)
Exchangeable Calcium (mg/kg)	1,293 (70)*	3,360 (237)	2,771 (39)*
Exchangeable Magnesium (mg/kg)	105 (9)*	216 (13)	391 (6)*
Chloride (mg/kg)	838 (14)	822 (30)	548 (32)*
Sulphate (mg/kg)	1,125 (31)*	12,007 (83)	654 (49)*
Nitrate (mg/kg)	7 (4)*	21,655 (217)	2,298 (1418)*
Phosphate (mg/kg)	398 (13)*	0 (0)	623 (317)*

(Values in parentheses are standard deviation of the mean of three samples)

Fresh dairy sludge versus sludge from vermicomposting bed was compared statistically. * = P<0.05

Sludge from the vermicomposting bed versus vermicomposted horse manure was compared statistically. * = P<0.05

3.3.6 Discussion

<u>Sludge from the vermicomposting bed compared to fresh dairy sludge</u>

The cation exchange capacity content of the sludge from the vermicomposting bed was significantly ($P<0.05$) higher than the fresh sludge (Table 25). This result correlates with Albanell *et al.* (1988) who stated that when vermicomposts are compared to their parent material they have greater cation exchange capacity.

There was no significant difference ($P>0.05$) in the exchangeable potassium content of the sludge from the vermicomposting bed compared to the fresh sludge. Other authors (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh *et al.* 2001; Nedgwa and Thompson, 2001; Albanell *et al.* 1988) found that there were increased amounts of exchangeable potassium after vermicomposting. This result was not obtained in this study.

The exchangeable calcium and magnesium of the sludge from the vermicomposting bed was significantly ($P<0.05$) higher than the fresh sludge. This results correlates with various authors (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh *et al.* 2001; Nedgwa and Thompson, 2001; Albanell *et al.* 1988), who found that, after vermicomposting, there is increased plant available calcium and magnesium.

The chloride content of the fresh dairy sludge and sludge from the vermicomposting bed was not significantly different ($P>0.05$). The sulphate content in the sludge from the vermicomposting bed was significantly higher compared to the fresh dairy sludge ($P<0.05$).

The nitrate content in fresh dairy sludge was low. The samples were dried and stored for two weeks before analysis. The nitrate possibly denitrified during this period. The nitrate in the sludge from the vermicomposting bed (21,655mg/kg) was significantly higher ($P<0.05$) compared to the fresh dairy sludge (200mg/kg). The very high level of nitrate in the vermicomposted dairy sludge is a possible indicator to determine when the sludge has been vermicomposted. Subler *et al.* (1998) noticed that vermicomposts had very high concentrations of nitrate. The findings in this study correlate with Subler's findings. Increased amount of nitrate is characteristic of vermicomposts (Edwards and Burrows,

1988; Edwards, 1998; Atiyeh *et al.* 2001; Nedgwa and Thompson, 2001; Albanell *et al.* 1988).

The most interesting finding was that the phosphate content in the vermicomposted dairy sludge was not detectable. It has been stated that the level of exchangeable phosphorus increases after vermicomposting (Edwards and Burrows, 1988; Albanell *et al.* 1988). Contrary to expectations, in this study this was not the case. Bentiez *et al.* (1999) stated that an increase in the earthworm biomass might have encouraged microbial activity and caused an immobolisation of free PO_4^{3-} into microbial and earthworm tissue

Usually after vermicomposting, nutrients are more available for plant uptake compared to parent materials. These nutrients are nitrate, soluble potassium, calcium and magnesium (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh *et al.* 2001; Nedgwa and Thompson, 2001; Albanell *et al.* 1988) exchangeable phosphorus (Edwards and Burrows, 1988; Albanell *et al.* 1988). In this study similar trends were observed. There was more nitrate, CEC, exchangeable calcium, magnesium and sulphate in the vermicomposted dairy sludge compared to fresh dairy sludge. However, the levels of potassium and chloride did not change, and phosphate was not detectable in the vermicomposted dairy sludge.

Sludge from the vermicomposting bed compared to horse manure vermicompost
There was no significant difference (P>0.05) in the exchangeable potassium and cation exchange capacity content of the sludge from the vermicomposting bed compared to the vermicomposted horse manure.

The exchangeable calcium content of the sludge from the vermicomposting bed was significantly (P<0.05) higher than the vermicomposted horse manure. However, the magnesium in the sludge from the vermicomposting bed was significantly lower (P<0.05) than the vermicomposted horse manure. The vermicomposted horse manure had a lower level of chloride and sulphate. The vermicomposted horse manure had a lower level of nitrate (2,298 mg/kg) compared to the sludge from the vermicomposting bed. The level of phosphate was not detectable in the sludge from the vermicomposting bed. However, there was phosphate in the vermicomposted horse manure.

Although the vermicomposted dairy sludge did not have the dark peat like appearance of typical vermicompost (e.g. horse manure vermicompost), there were some similarities in the nutrient contents (CEC, potassium, calcium) between the horse manure vermicompost and the vermicomposted dairy sludge. There were even higher levels of nutrients (sulphate and nitrate) in the vermicompost dairy sludge compared to the horse manure vermicompost. The present findings seem to be consistent with other research (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh et al. 2001; Nedgwa and Thompson, 2001) who found that vermicomposting increases the amount of plant available nutrients.

3.3.7 Conclusions

- Due to the increased nutrient content in the dairy sludge from the vermicomposting bed compared to its parent material (fresh dairy sludge), it was determined that it was vermicompost.

- Nitrate levels are the best indicator as to when the sludge is changed into vermicompost. This parameter will also give an overall indication on the general performance of the vermicomposting process.

- There was no detectable phosphate in the sludge from the vermicomposting bed.

- Dairy sludge alone can be changed into vermicompost without the addition of any other material such as a carbon source.

- Vermicomposted dairy sludge did not have the typical peat like appearance of horse manure vermicompost, but had similar nutrient characteristics.

3.4   Medium scale vermicomposting trial

3.4.1   Introduction

The vermicomposted sludge from the medium scale (windrow) trial (section 3.2) was tested for plant available nutrients and compared to it parents material, in section 3.3. Unfortunately it could not be compared to the control sludge in that trial, because earthworms had entered into the control bed. In this trial, the plant available nutrients in the vermicomposted sludge and control sludge were determined at the start, during and at the end of the trial. In addition to this, the growth of the earthworms was monitored, the stability of the sludge were analysed and the nutrient changes during vermicomposting was assessed as a possible indicator of the extent of the vermicomposting.

This trial described below had to be repeated because all the earthworms died within 24 hours of the start of the trial. There appeared to be a pattern of earthworm mortality with high ammonia levels of 3.0 mg NH_3/g of sludge. For the repeat trial the dairy sludge was placed in a thin layer on the floor of the polytunnel. It was manually aerated with a garden rake for five days until the ammonia level had decreased to 1.2 mg NH_3/g.

3.4.2   Aims

- To investigate nutrient changes during vermicomposting.
- To determine the stability of the sludge due to vermicomposting.
- To monitor earthworm growth and reproduction.
- To identify potential parameters which indicate the extent of vermicomposting.

3.4.3   Description of trial

The trial consisted of six plywood boxes, three which contained earthworms and three which acted as controls. Each box was 60cm long by 60cm wide by 15cm high and had a surface area of 0.36m². The size of the boxes was similar to a trial done by Ndegwa and Thompson (2000). The boxes, which contained the earthworms, each had an anti-crawl device to prevent them from escaping. A black woven plastic cover was placed over each box to keep the sludge moist and allow the earthworms to crawl on the surface of the sludge. A soil heating cable was suspended over the boxes for heating. The trial was conducted in an enclosed shed. A light in the shed was left on for the duration of the trial. This helped to keep the earthworms in their boxes, as they are photosensitive.

3.4.4 Setting up of the trial

The earthworms were obtained from the Irish Organic Earthworm Company in Donegal. They were grown in a mixture of horse manure, wood shavings and peat. The majority of earthworms from this farm were *D. veneta*, with about 5% of the earthworm population made up of *E. fetida*. Approximately 800g of adult earthworms were used in each box. The density of earthworms was 2.2kg /m^2. This is in the middle of the reported range of 1-4kg earthworms/m^2 for large scale vermicomposting (Gilbert *et al.* 2001).

Earthworms were added into a bedding of 500g of vermicomposted horse manure. Initially 5kg of dairy sludge were placed upon the bedding. 24 hours later an additional 10kg of sludge were added to each box. The boxes were positioned in a randomised design. By placing the dairy sludge on a bed of vermicompost was another way (as well as aerating the sludge) to help prevent the earthworms from dying, from high ammonia levels. The bed of vermicomposted horse manure acted as a habitat where the earthworms could retreat if conditions in the sludge were unfavourable (e.g. high ammonia). Previous researchers who investigated the vermicomposting of dairy sludge have used this method of placing dairy sludge upon a layer of vermicomposted sheep manure. This overcomes the toxic effects of substrate (Gratelly *et al.* 1996; Nogales *et al.* 1999). The trial was conducted for 14 weeks.
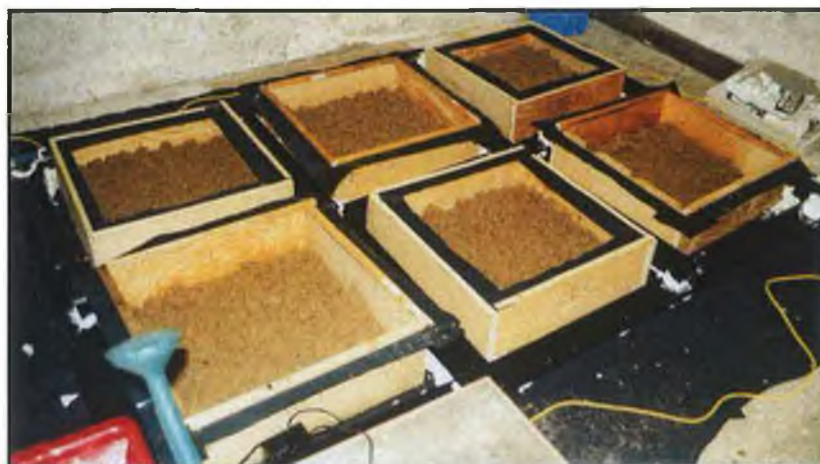
Plate 3. Overview of trial

3.4.5 Sampling
Sampling Earthworms
At the start approximately 8% of the earthworm biomass was measured for length and reproductive status (with or without clitellum). The boxes were sampled after 3, 6, 9 and 14

60

weeks. The 16cm core size that was suitable for the trial in 3.2, was also used in this trial. Each time three 16cm cores were taken from each box. After week 14, each box was dismantled and the total earthworm biomass was determined. 10% of the earthworms by biomass was measured for length and reproduction status.

Sampling Sludge

The sludge was sampled at the start of the trial and after 3, 6, 9 and 14 weeks. The samples were analysed for moisture, ammonia, pH and electrical conductivity. Stability of the sludge was monitored on weeks 0, 3, 6, and 9 using BOD, COD and percentage volatile solids methods. A phytotoxicity test was used to assess the stability of the sludge at the start and end of the trial. The nutrients analysed on weeks 0, 3, 6, 9 and 14 were chloride, nitrate, phosphate and sulphate. Potassium, calcium and magnesium were analysed at the start and end of the trial. The air temperature was monitored and spot readings of the temperature of the sludge were taken in a grid pattern at sampling periods.

Methods

The methods for moisture, ammonia, pH and electrical conductivity, BOD, COD, percentage volatile solids, chloride, nitrate, phosphate and sulphate potassium, calcium and magnesium are described in Appendix H. The phytotoxicity bioassay (cress seeds) is a slight modification of the method by Zucconi *et al.* (1981); details are in Appendix I.

3.4.6 Results
Earthworms

Table 26. Number, weight (biomass) of earthworms and cocoons generated

Parameter	Weeks				
	0	3	6	9	14
Number of Earthworms	814 (3)	1450 (241)	1426 (198)	1862 (581)	445 (77)
Earthworm Biomass (g)	818 (3)	542 (73)	805 (148)	905 (219)	768 (90)
Cocoons		1 (1)	2 (1)	7 (7)	not sampled

Note: Earthworms were only added on week 0. Cocoons were not counted on week 14

From week 0 to week 14, the number of earthworms had decreased significantly ($P<0.05$). The biomass at the end of the trial was not significantly different ($P>0.05$) than at the start. The number of cocoons had increased significantly ($P<0.05$) from week 3 to week 9.

61

Table 27. Est. percentage of the population at predefined lengths and reproduction status

Week	No Clitellum			With Clitellum		
	0-20mm	20-40mm	40-80mm	20-40mm	40-80mm	80-140mm
0	0	1	2	2	46	50
3	0	0	2	2	37	60
6	0	1	3	0	34	61
9	0	0	1	0	10	89
14	0	0	0	0	9	87

Sludge

Environmental conditions

Table 28. Mean temperature readings in boxes which contained earthworms

Week no.	Spot reading °C	Air temperature Minimum (°C)	Maximum (°C)
0	8	5	5
3	9	5	10
6	11	4	10.5
9	9	6	11
14	11	6	13

Spot temperature readings were taken in a grid pattern, half way down the sludge. These readings were taken at the same time as air temperature readings.

Table 29. Environmental conditions (pH, electrical conductivity, ammonia, moisture).

	Week 0	Week 3	Week 6	Week 9	Week 14
pH					
Earthworms	7.9 (0.1)	7.4 (0.1)	7.1 (0.3)	6.7 (0.3)	6.5 (0.02)
Control		7.4 (0.1)	7.1 (0.2)	6.9 (0.1)	6.7 (0.01)
Electrical conducivity (ms^{cm-1})					
Earthworms	0.295 (0.02)	0.313 (0.02)	0.608 (0.07)	0.881 (0.09)	1.21 (0.05)
Control		0.262 (0.01)	0.390 (0.06)	0.569 (0.05)	0.681 (0.04)
Ammonia (mg/g)					
Earthworms	1.22 (0.09)	0.03 (0.05)	0.04 (0.08)	0.42 (0.27)	0.46 (0.20)
Control		0.01 (0)	0.02 (0.01)	0.38 (0.24)	0.40 (0.18)
Moisture content (%)					
Earthworms	88 (0.0)	86 (0.0)	85 (0.0)	84 (0.01)	84 (0.01)
Control	88 (0.0)	86 (0.0)	85 (0.0)	84 (0.01)	84 (0.01)

(Values in parentheses are standard deviation of mean of nine samples)

Stability

Table 30. Percentage of germination, radicle growth and germination index of cress seeds in water extract (1:5) of the dairy sludge at the start and end of the trial.

	Germination (%)	Root length (mm)	Germination Index
Start of Trial	46	1.47	96
End : Earthworms	57	2.25	165
End : Control	42	1	60

(Values in parentheses are standard deviation of mean of five samples)

The germination index (GI) of the control sludge did not differ significantly from the start and end of the trial. The GI of the sludge with earthworms had significantly ($P<0.05$) increased at the end compared to the start of the trial, and was significantly ($P>0.05$) higher than the GI of the control sludge.

Table 31. The BOD, COD and volatile solid content of the sludge.

	Week 0	Week 3	Week 6	Week 9
Volatile Solids (%)				
Earthworms	68 (744)	64 (0.06)	67 (0.01)	64 (0.03)
Control		68 (0)	65 (0.04)	66 (0.01)
Biological Oxygen Demand (mg/kg)				
Earthworms	59,845 (5,775)	46,199 (5,224)	37,566 (6,683)	36,003 (6,303)
Control		49,236 (6,312)	38,024 (2,957)	34,432 (10,792)
Chemical Oxygen Demand (mg/kg)				
Earthworms	867,208 (53,313)	926,243 (251,229)	856,124 (145,537)	1,094,228 (160,908)
Control		947,248 (157,361)	749,351 (150,206)	1,044,526 (188,318)

(Values in parentheses are standard deviation of mean of three samples)

Table 32. Chloride, sulphate, nitrate and phosphate in the sludge during the trial.

Nutrients (mg/kg)	Week 0	Week 3	Week 6	Week 9	Week 14
Chloride					
Earthworms	1764 (744)	1790 (1006)	1,221 (262)	1,920 (954)	1,601 (371)
Control		1,829 (898)	1,161 (324)	1,792 (896)	1,497 (260)
Sulphate					
Earthworms	570 (82)	693 (171)	1071 (85)	1,469 (61)	3,992 (137)
Control		574 (25)	807 (50)	939 (123)	1,757 (182)
Nitrate					
Earthworms	148 (59)	406 (189)	3,323 (2015)	7,570 (810)	34,680 (3842)
Control		180 (45)	237 (17)	415 (236)	3,265 (463)
Phosphate					
Earthworms	693 (238)	622 (208)	602 (148)	232 (50)	0 (0)
Control		621 (73)	844 (187)	655 (364)	526 (64)

(Values in parentheses are standard deviation of mean of three composite samples)

Table 33. Exchangeable potassium, calcium and magnesium at the start and end of the trial

Nutrients (mg/kg)	Start	End- Earthworms	End- Control
Exchangeable Potassium	5,509 (317)	6,986 (874)	6,495 (1095)
Exchangeable Calcium	1,293 (70)	2,549 (446)	1,715 (244)
Exchangeable Magnesium	105 (9)	171 (34)	130 (21)

(Values in parentheses are standard deviation of mean of three composite samples)

3.4.9   Discussion

This study monitored the growth and reproduction of 818g of earthworms in 15kg of dairy sludge over a 14 week period. The stability of the sludge was assessed during this period. More importantly the nutrient changes in the dairy sludge were monitored to identify parameters which would indicate the extent of the vermicomposting process.

During the period the earthworm population was sampled with a 16cm core on weeks 3, 6, and 9. On week 14 the entire boxes were totally dismantled and the entire earthworm population sampled.

The estimated number of earthworms in the boxes at the start was 814 (Table 26). However, the number based on cores samples on weeks three, six, and nine were estimated 1451, 1427 and 1863, respectively. At week fourteen, the boxes were dismantled and 10% of the population was sampled. This result estimated that there was a mean number of 446 (+/- 77) earthworms per box. The cores appeared to have overestimated the number of earthworms and were thus unreliable. The lower number of earthworms on week 14, compared to the start of the trial, was possibly due to earthworm mortality at the start of the trial.

At the start of the trial the mean biomass in each box was 818g (Table 26). The biomass was estimated on weeks three, six, and nine based on the three cores taken from each box. The biomass decreased at week 3 to 542g and increased on weeks 6 and 9 to 806g and 905g, respectively. At week fourteen, the boxes were completely destructed and the biomass was found to be 769g. The biomass was not significantly ($P>0.05$) different at the end on week 14 compared to the start of the trial.

From the results, it appears the cores were affected by the edge effect as they were found to be inaccurate in estimating biomass. This is because the rate of cocoon and earthworm growth could not have increased at the rate it appeared to have between weeks three and six. Destruction of the boxes was the most accurate method for determining the biomass.

There was very little cocoon production (Table 26) during the trial. From the total number of cores on week 3, the average number of cocoons was 1/core. This had increased to an average of 7 cocoons/core in week nine. The slow rate of cocoon production was because the majority of the earthworm population consisted of *D. veneta*. This has a slow reproduction rate and long life cycle (Dominguéz, 2004).

The majority of the earthworms had clitellums and were 40-80mm and 80-140mm long (Table 27). There were very few/no earthworms that had no clitellum. This was indicative that the earthworm culture was not made up of a young population.

According to Dominquéz (2004) the cocoons of *D. veneta* take 42 days to hatch and viability is low (20%). The time to maturity is slow, up to 65 days. It is not a very prolific species and does not grow very fast. However it is used by a number of vermiculturalists (Dominguéz, 2004). Dominguéz (2004) stated that *D. veneta* is one of least suitable species for vermicomposting.

From the start of the trial (pH 7.9) until week fourteen, the pH had decreased significantly ($P<0.05$). It was significantly lower ($P<0.05$) in the earthworm worked sludge (pH 6.5) compared to that of the control (pH 6.7). The pH change to acidic conditions is thought to be due to the higher mineralisation of nitrogen into nitrates or nitrites and phosphorus into orthophophates (Ndegwa and Thompson, 2000). Elvira *et al.* (1998) suggested that the lowering of pH was due to the generation of carbon dioxide and organic acids by microbial activity during the bioconversion process of the waste material.

From the start of trial until week fourteen, the electrical conductivity (EC) had increased significantly ($P<0.05$). It was significantly higher ($P<0.05$) in the earthworm worked sludge ($1.21ms^{cm-1}$) compared to that of the control ($0.681ms^{cm-1}$). The increased EC during vermicomposting is possibly due to increased nutrient salts created during the process.

The level of ammonia at the start was 1.2mg NH_3/g. No dead earthworms were observed after the start. Ammonia has been reported to be toxic to earthworms (see section 2.10.5) at limits of both 0.5mg NH_3/g and 1mg NH_3/g of organic waste. From the start of the trial

until week fourteen, the ammonia content in the sludge had decreased significantly ($P<0.05$) in both the earthworm worked sludge and control sludge (Table 29). However, on week fourteen the ammonia content was not significantly different ($P>0.05$) in the earthworm worked sludge compared to that of the control. During the trial the ammonia at weeks three, six, nine and fourteen was below reported lethal limits.

The moisture content in the sludge was within the optimal conditions (Table 29) of 80-90% preferred by earthworms (Edwards and Arancon, 2004). The lower limit of 60% and upper limit of 90% moisture content, (Edwards, 1998; Edwards and Arancon, 2004) that hinders earthworm activity, were avoided during the trial.

The temperature in the vermicomposting boxes (Table 28) was low during the trial, ranging from 8°C to 11°C. The majority of the earthworm species in this trial was *D. veneta*, which has an optimal working temperature of 25°C, lower limit of 15 °C and upper limit of 25°C (Domínguéz, 2004). The temperature in the dairy sludge was below the lower limit. However, at that temperature the earthworms were active. If the optimal temperature had been provided, it would have probably increased the rate of vermicomposting.

The germination index increased significantly ($P<0.05$) of the sludge with the earthworms. This indicated a decrease in toxic organic substances in the dairy sludge (Benítez *et al.* 2002). It was reported by Zucconi *et al.* (1981) that when the germination index is greater than 60% there is no phytotoxicity.

On week nine the percentage volatile solids had significantly decreased ($P<0.05$) in both the earthworm worked sludge and the control sludge (Table 31). However, on week nine the earthworm worked sludge had a significantly lower percentage volatile solids ($P<0.05$) compared to that of the control. This showed that the earthworms increased the stability of the sludge. This finding correlates with other authors, who found that earthworms increased the rate of volatile solids reduction (Neuhauser *et al.* 1988; Frederickson, *et al.* 1997; Ndegwa and Thompson, 2000; Hartenstein and Hartenstein, 1981).

On week nine the BOD and COD were not significantly different (P>0.05) in the earthworm digested sludge compared to that of the control.

From examination of the BOD and COD results, it can be said that the activity of the earthworms did not increase the rate of stability of the sludge. However, the results of the percentage volatile solids and the phytotoxicity bioassay indicated that vermicomposting had increased the stability of the dairy sludge.

One of the main aims of this trial was to investigate if the earthworms' activity increased the availability of plant nutrients. The sludge was analysed for nutrients at the start, during the trial and at the end.

From the start of the trial to the end, the chloride levels in the earthworm digested sludge and the control had not increased significantly (P>0.05) and were not significantly (P>0.05) different from each other.

At the end of the trial, there was significantly more (P<0.05) nitrate in the earthworm digested sludge than the control. Earthworms increased nitrification by 213 times the original content. Various authors (Dominguez *et al.* 1997; Benitez *et al.* 1999) have also reported increased nitrification due to earthworms. It is mandatory that vermicomposting beds are kept aerobic for earthworms. With aerobic conditions nitrification will take place. If anoxic conditions happen in the bed, the earthworms will die and the vermicomposting process will fail. The nitrate level in the sludge can be an indicator of the overall performance of the vermicomposting process.

From the start of the trial to the end, the phosphate levels in the control decreased significantly (P<0.05) (Table 32). At the end of the trial there was no detectable phosphate in the earthworm digested sludge. No detectable phosphate, after vermicomposting, was also reported by Bentiez *et al.* (1999) who investigated the vermicomposting of sewage sludge. Bentiez stated that an increase in the earthworm biomass might have encouraged microbial activity and caused an immobolisation of free PO_4^{3-} into microbial and earthworm tissue. This circumstance where phosphate is not found in the a sludge that was

68

vermicomposted might have also been found by the commercial company Vermitech Ltd. This company adds phosphate to their vermicompost.

At the end of the trial, there was significantly more ($P<0.05$) sulphate in the earthworm digested sludge than the control sludge. At the end of the trial, the sulphate level had increased 4 times the original content.

At the end of the trial, there was no significant difference ($P>0.05$) between the potassium levels in the earthworm digested sludge and the control sludge.

At the end of the trial, there was significantly more ($P<0.05$) calcium in the earthworm digested sludge than that of the control. This result correlates with various authors (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh *et al*. 2001; Nedgwa and Thompson, 2001; Albanell *et al*. 1988), who found that after vermicomposting there is increased plant available calcium.

At the end of the trial there was no significant difference ($P>0.05$) in magnesium between the earthworm digested sludge and the control. This result did not correlate with various authors (Edwards and Burrows, 1988; Edwards, 1998; Atiyeh *et al*. 2001; Nedgwa and Thompson, 2001; Albanell *et al*. 1988), who found that after vermicomposting there is increased plant available magnesium.

3.4.10 Conclusions

The following conclusions can be drawn from this study:

- When the earthworms were placed into fresh dairy sludge, they all died. However, when the dairy sludge was aerated to reduce ammonia levels, and the sludge was placed on a small bed of vermicomposted horse manure, earthworms were able to survive in the sludge.
- Earthworm biomass was similar at the beginning and end of trial. However the number of earthworms decreased. The majority of the earthworms (*D. veneta*) had clitellums and were 40-140mm long.

- The temperature of the sludge was low ranging from 8°C to 11°C. However the earthworms were able to vermicompost at this low temperature. This is a benefit of *D. veneta* species.

- *D. veneta* is a slow growing earthworm species and generated a low number of cocoons.

- The COD and BOD showed that earthworm activity did not increase the stability. However the phytotoxicty bioassay and percentage volatile solids content showed that earthworms increased the stability of the dairy sludge.

- Vermicomposting had significantly increased the amount of nitrate, sulphate and calcium in the vermicomposted dairy sludge.

- The phosphate levels had decreased to below detectable levels.

- The potassium, magnesium and chloride levels in the sludge did not change significantly due to earthworm activity.

- The changes in nutrient content of the dairy sludge indicated that it was vermicomposted by the earthworms. Vermicomposting increased the nitrate, sulphate and calcium content of the sludge, but decreased phosphate to non-detectable levels.

- From this trial, the nitrate and phosphate levels are good indicators of the extent of vermicomposting.

- Dairy sludge alone can be changed into vermicompost without the addition of any other material such as a carbon source.

3.4.11 Recommendations

The findings of the study have a number of important implications for future practice.

- Dairy sludge needs to be manually aerated to reduce ammonia levels, so earthworms can survive in it.

- The nitrate and phosphate levels could be used as indicators of the extent of vermicomposting.

- A sustainable vermicomposting system needs a mixture of young and old earthworms of the species *D. veneta*. This is to help combat a system inoculated with adult earthworms which may experience a lull in the performance, whilst the young earthworms are growing.

3.5 Continuous Flow Reactor

Introduction

Based on the findings of the windrow trial (3.2) where the ammonia levels were high at the bottom of the bed, a small continuous flow reactor (CFR) was built based on Edwards (1998) design. This design enabled more air reaching the bottom of the bed, as the bed is supported by a mesh bottom. This might prevent the creation of high levels of ammonia in the bottom of the bed. Upon the mesh is a movable bar (beaker bar) (Plate 5) which can moved across the mesh to extract the sludge. The idea of the continuous flow reactor is that thin layers of sludge are placed on the surface of the bed, encouraging the earthworm to move upwards, leaving the vermicompost behind. Then the vermicompost is extracted with a breaker bar.

Aims

- To investigate if a mesh bottom can support a vermicomposting bed.
- To investigate if it is possible to extract vermicompost using a breaker bar.

Experimental Design

A wooden crate with the base replaced with a wire mesh (2.5cm by 7.6cm) was built (Plate 4 & 5). This was later replaced with 5cm by 5cm mesh. A breaker bar was placed upon the mesh. The CFR was placed on a slope to allow leachate drain into a collection system (Plate 4). Vermicomposted sludge and earthworms from the windrow trial (3.2) were taken and placed into the CFR. The surface of the bed was covered with a black cover to keep the sludge moist. The bed was watered to keep the sludge moist.

Plate 4.

Plate 5.

Plate 4. Small continuous flow reactor and leachate collection
Plate 5. Mesh 2.5cm by 7.6cm and breaker bar

71

Results

Plate 6.

Plate 7.

Plate 6. 5cm by 5cm mesh supported the bed
Plate 7. 5cm by 5cm mesh supported the bed after raking twice

Discussion

There was no leachate collected during the short trial. The bottom, with the 2.5cm by 7.6cm mesh, was tested and when the breaker bar was moved the bed collapsed through the mesh. The bottom was then replaced with a 5cm by 5cm mesh. The bed was rebuilt again. The breaker bar was moved and sludge came through the mesh (Plate 6). This time the mesh was able to support the bed structure (Plate 7). This result was then used to help in the design and construction of a large scale continuous flow reactor by Glanbia (Plate 8 & 9).

Conclusion

- A 2.5cm by 7.6cm mesh cannot support a vermicomposting bed. However a 5cm by 5cm mesh can support the bed.
- Vermicompost can be extracted from CFR, when a 5cm by 5cm mesh is used.

Plate 8. Glanbia's continuous flow reactor

Plate 9. Breaker bars upon the mesh bottom in Glanbia CFR

3.6   Reducing ammonia concentration in dairy sludge

Introduction

Ammonia has been reported to be toxic to earthworms (see section 2.10.5) at limits of both 0.5mg NH_3/g and 1mg NH_3/g in organic waste. It was observed during this research that there was a pattern of earthworms dying in fresh dairy sludge, which had high level of ammonia. Fresh dairy sludge can have high levels of ammonia (Table 34). The ammonia possibly caused the earthworm to blister (see Plates 10-13) and then die. Methods, such as aeration and the addition of zeolite to reduce the ammonia level in dairy sludge, were investigated.

73

Plates 10-13. Photographs of lethal effect of high ammonia concentration in fresh dairy sludge on earthworms *D. veneta* (Photographs courtesy of Anderson and Associates Forensic Scientists)

Table 34. Examples of ammonia concentration in fresh dairy sludge samples

Dairy sludge	mg/g ammonia
Fresh dairy sludge	**1.5** (0); **1.2** (0); **2.6** (0.04); **3.0** (0.11)
Sludge sealed a bucket for 6 weeks	**11** (0.04)
Old sludge left in a bucket for 3 weeks	**5.3** (0.02)
Fresh sludge stored at 5°C for 5 days	**11** (0.05)

Values in parenthesis are SD of three values

3.6.1 The ammonia content in sludge stored at different temperatures

Aim

To determine if the storage of dairy sludge results in ammonia content increasing, dairy sludge was stored at different temperatures for six weeks.

Method

Fresh dairy sludge was collected and analysed the following day for ammonia content. The sludge was analysed again after three and six weeks when it had been stored at three different temperatures (4°C, 15°C and 20°C).

74
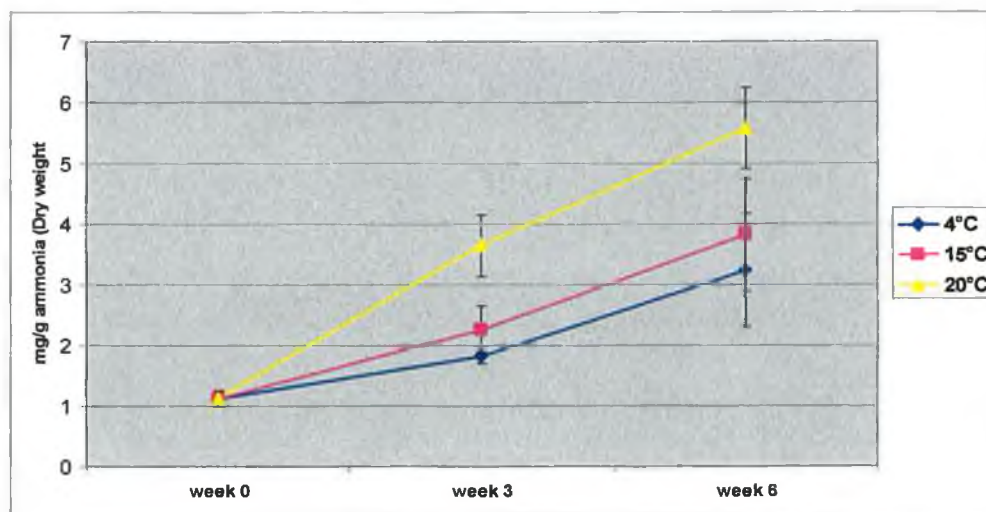
Figure 8. Ammonia concentration in fresh sludge, after three and six weeks stored at different temperatures.

Discussion

The storage of dairy sludge led to an increase in the ammonia content (Figure 8). The sludge stored at the highest temperature of 20°C had the highest increase of ammonia. The sludge stored at lowest temperature of 4°C had the lowest increase in ammonia. This showed that the increased ammonia content in dairy sludge was related to temperature.

Conclusion

This study has shown that the storage of dairy sludge led to an increase in ammonia at different temperatures.

3.6.2   Removal of ammonia

Aim

To investigate the aeration of dairy sludge and the use of zeolite as a means to reduce ammonia levels.

Aerating the sludge

Ammonia is a gas. By manually aerating the fresh sludge for a number of days this causes the ammonia to volatilise into the atmosphere. Then the sludge can be used for vermicomposting.

75

Method

Fresh dairy sludge was spread out in a thin layer (5-6cm) on a sheet of plastic located on the floor of the polytunnel. It was manually aerated with a garden fork for five days.

Results

Table 35. Ammonia concentration in dairy sludge before and after manually aerating

Dairy sludge	mg ammonia /g
Before aerating	4.1 (0.02)
After aerating for 5 days and earthworms survived in it.	1.2 (0)

Values in parenthesis are SD of three values

Discussion

The ammonia concentration was reduced from 4.1 to 1.2 mg/g sludge (Table 35). This sludge was used for a vermicomposting trial and the earthworms survived in it.

Conclusion

The process of aerating the sludge is a method of reducing the ammonia concentration.

3.6.3 Addition of zeolite to the sludge

An alternative way to remove ammonia is the addition of zeolite to the sludge (see section 2.10.5). It was recommended by Zeolite Australia Ltd that a 3-5% (w/v) is required to remove ammonia from an organic waste for vermicomposting. Zeolite clinoptilolite was obtained from the British Zeolite Corporation.

Method

The zeolite (60 Thler, <500 micron) was added to the sludge (0 hours) at various percentages (3-10% w/w) and mixed for twenty seconds and let stand for 24 hours. Then 10 earthworms (*D. veneta*) were added to the sludge with zeolite. At 48 hours the number of earthworm alive and ammonia concentration were recorded. The control received no mixing. The zeolite control received the same mixing as the zeolite percentages.

Results

The ammonia content of the sludge at the start of the trial was 1.1 mg/g of ammonia.
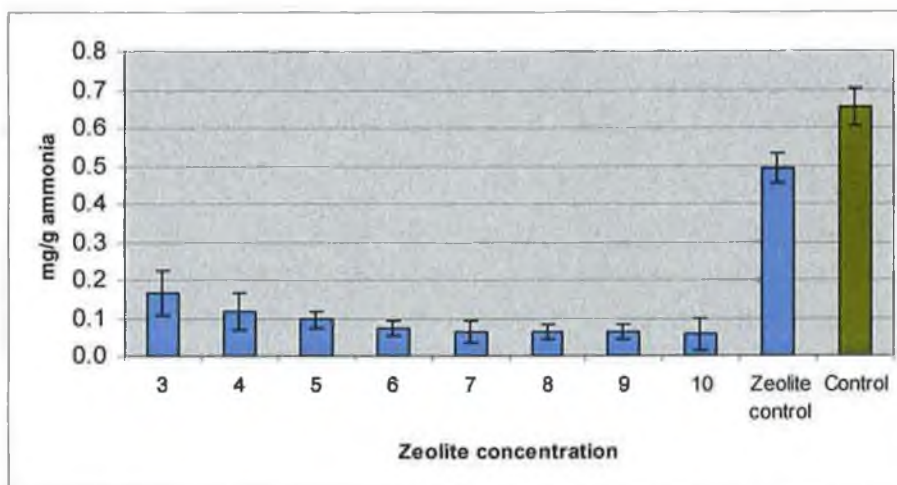
Figure 9. Ammonia concentration in dairy sludge with zeolite at 48 hours

Table 36. Number of earthworms (*D. veneta*) alive at 48 hours

Zeolite %	Number of worms alive after 24 hrs
3	10 (1)
4	10 (0)
5	10 (0)
6	10 (0)
7	10 (0)
8	10 (0)
9	10 (0)
10	10 (0)
Zeolite control	5, but worms look irritated on surface
Control (nothing done to it)	1

Values in parenthesis are SD of three values

Discussion

The results of this investigation showed that the zeolite was successful in reducing the ammonia content in the sludge (Figure 9). The 5% zeolite concentration was the range where the ability to absorb ammonia leveled off. All the earthworms survived in the zeolite concentrations (Table 36). The zeolite control had all the earthworms alive, but they appeared irritated as they were trying to escape. The control had a higher level of ammonia compared to the zeolite concentration and it had only one earthworm alive.

Conclusion

5% zeolite is recommended to be used in dairy sludge to reduce the ammonia content.

77

3.6.4 Comparing zeolite and aeration for removal of ammonia

Aim

Compare the use of zeolite to aeration for removal of ammonia.

Method

(Day 1) Sludge was analysed for ammonia. Ten earthworms (*D. veneta*) were added. **(Day 2)** Stock piles of made of (A) Control – nothing done to it (B) Sludge with 5% zeolite added (C) Aerated sludge. The 'aerated sludge-C' mixed for one minute on days 1 and 2, after sampling for ammonia. (D) Sludge with the same mixing as the zeolte (Zeolite control). The '5% zeolite -D' aerated was mixed for twenty seconds. **(Day 3)** Ammonia was analysed after setting up of piles and sub-samples of sludge were taken and 10 earthworms were added to each box. **(Day 4)** The numbers of worms alive were counted.

Results

On day 1 there was 1.6 mg/g of ammonia in the sludge. All the earthworms died.
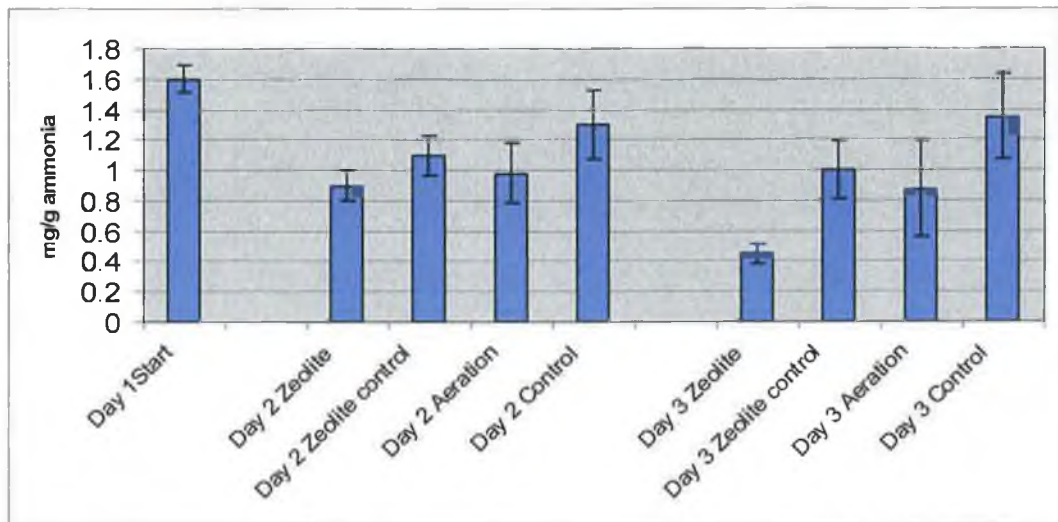
Figure 10. Ammonia content on days 1, 2 & 3 in samples

Table 37. Number of earthworms (*D. veneta*) alive on day 4 and earthworms observations

	Worms alive	Observations
5% Zeolite	10 (0)	worms not in a ball, spread out
Zeolite control	5 (5)	worms together in a ball
Aerated sludge	5 (5)	worms together in a ball
Control sludge	4 (2)	worms together in a ball

Values in parenthesis are SD of three values

78

Discussion

On day 3 the sludge with 5% zeolite had significantly lower (P<0.05) ammonia content compared to the aerated sludge, zeolite control and control (Figure 10). The 5% zeolite had all ten earthworms alive on day 4. The zeolite control, aerated sludge and control sludge had approximately five earthworms alive (Table 37). In Table 37, the earthworms' behaviour was observed and showed that the earthworms in the zeolite control, aerated sludge and control sludge were in a ball, which is a sign that they are not happy and are stressed. The earthworms in the 5% zeolite did not show any sign that they were stressed. The 5% zeolite in dairy sludge was better than aerating the sludge, because it had lower ammonia content and the earthworms did not show any signs of being stressed.

Conclusion

The findings demonstrated that the 5% zeolite in dairy sludge was better than aerating the sludge.

3.7 Polyelectrolyte Toxicity Test
Introduction

Polyelectrolyte is used in the wastewater treatment process at Glanbia Kilkenny. Dairy sludge was obtained from Glanbia, which contained the usual amount of polyelectrolyte added.

Aim

- To determine if polyelectrolyte was toxic to earthworms.

Design of trial

Five adult earthworms of *D. veneta* were added to each box. The weight of each earthworm was measured. 250 grams of dairy sludge was added to each box. The moisture content of the sludge was 83% moisture. 50ml of each polyelectrolyte concentration of 0.5%, 1% and 2% were poured onto the sludge in each box. The zeolite was mixed through the sludge with a spoon. A control with no polyelectrolyte added was used. There were three replicates of each treatment. Polyelectrolyte concentrations of 0.5%, 1% and 2% were made up in tap water from polyelectrolyte called 'Praestol®', made by Degussa and used at Glanbia. Each day the earthworms were examined for survival, growth, and reproduction.

Results

After 24 hours, all the earthworms in 1% and 2% polyelectrolyte were dead. The earthworms in the 0.5% polyelectrolyte and control were all alive. After 72 hours the earthworms were all dead in the 0.5% polyelectrolyte.

Discussion

The polyelectrolyte had a toxic effect on earthworms. These results must be interpreted with caution because it was noticed that polyelectolyte had a paste like composition when added. Because of this physical property it may have stuck to earthworms and caused their death.

Conclusion

The polyelectrolyte was toxic to earthworms.

3.8   Earthworm Sludge Consumption

Aim

- To investigate the weight reduction of dairy sludge due to vermicomposting.

Introduction

Three different trials were conducted with different ratios of earthworm and dairy sludge. The weight of sludge reduction was recorded.

Methods

The method for earthworm biomass sampling is described in Appendix G.

Method trial 1

A small laboratory scale trial was conducted over a 32 day period with 100g of earthworms and 1000g of dairy sludge. Mixed species (with clitellums) of *Dendrodrilus rubidus, Eisenia fetida, Lumbricus rubellus* were used. The weight of the sludge was measured at the start and end to determine the loss in weight.

Method trial 2

After trial 1, a trial was conducted with a ratio 200g of earthworms and 2kg sludge. Mixed species (with clitellums) of *Dendrodrilus rubidus, Eisenia fetida, Lumbricus rubellus* were used.

Method trial 3

There were three containers with 1000g of aerated fresh sludge and 100g of earthworms (*D. veneta*). The control consisted of 1000g of aerated fresh sludge. Moisture was replaced with a water spray each day. The weight and moisture content of sludge was recorded at the start and end of the trial.

Results

Trial 1 Results

The sludge was kept within the optimum moisture range of 80-85% moisture content and at room temperature. There was some mortality of earthworms in the first 23 days. At the end of the trial the biomass of the earthworms had significantly ($P<0.05$) decreased from 100g to an average of 68g.

Table 38. Loss in sludge weight

	Control	100g of earthworms
Loss in sludge (%)	3 (1)	6 (2)
Loss due to earthworms	3	

Values in parentheses are standard deviation of three samples

Trial 2 Results

After 24 hours, all the earthworms had died possibly because of high ammonia levels. The sludge had been stored in an airtight container at 4^0C and thus possibly the ammonia levels had built up in the sludge before it was used for the trial.

Trial 3 Results

Table 39. Loss in sludge weight

	Control	100g of earthworms	1000g of earthworm
Loss in sludge (%)	6 (1)	10 (1)	11 (1)
Loss due to earthworms		4	5

Values in parenthesis are mean of three samples

Discussion

In these trials the ability of earthworms to reduce the weight of dairy sludge was investigated.

In trial 1, the loss of earthworms during the first 23 days was possibly due to ammonia toxicity. There was a 3% reduction in the weight of sludge due to earthworm activity (Table 38). However, the reduction in weight by earthworms was not significantly different ($P>0.05$) from the control. This low reduction in weight corresponds to Gilbert *et al.* (2001) who stated a low reduction in weight after vermicomposting.

In trial 3, all 1000g of earthworms died after seven days, possibly due to overcrowding. The dead earthworms could not be separated from the sludge. If the weight of the container, sludge and earthworms were compared at the start and end of the trial, there was a 5% reduction in weight (Table 39). The sludge with the 100g of earthworms lost 4%. The loss of sludge between the 100g and 1000g of earthworms was not significantly different ($P>0.05$). The reduction in weight is similar to Gilbert *et al.* (2001) who stated a 10% reduction in weight.

The trials results correlate with Ndegwa *et al.* (2000) and Gilbert *et al.* (2001) who stated that feeding rate is dependant on the nature of the waste as well as the preparation/pretreatment of waste and earthworm density. These trials demonstrated that there is only a small loss in weight of the sludge. However, with a small laboratory scale trial, caution must be applied, as the findings might not be transferable to large scale vermicomposting. Further trials with larger quantities of sludge and earthworms are recommended.

Conclusions

The evidence from this study demonstrated that:

- After 32 days of vermicomposting, 100g of earthworms and 1000g of dairy sludge, there was no significant reduction on sludge weight, due to the earthworms.
- With 100g or 1000g earthworms and 1000g sludge there was a reduction of sludge weight, due to earthworm activity, of approximately 5% after five days.

3.9   Summary of Chapter Three

In this chapter, vermicomposting dairy sludge was examined. Dairy sludge was characterised by examining metals, pathogens and typical physical and chemical properties.

In a medium scale trial, it was found that initially the mixed species earthworm population decreased, but recovered and the density of earthworms increased. The two species *E. fetida* and *D. veneta* dominated the earthworm population. Unfortunately, this trial lost its control due to earthworms entering it. The vermicomposting bed was also infested with rats which ate the earthworms. It is recommended that this trial be repeated again and mitigation measures taken to prevent these circumstances reoccurring.

The sludge from the windrow trial was analysed for plant available nutrient and compared to its parent material (fresh sludge) and a typical vermicompost (horse manure). It was found that the vermicomposted sludge did have increased nutrients, notably nitrate and similar nutrient characteristics to the vermicomposted horse manure.

In the windrow trial, the earthworms had entered the control bed resulting in no sludge to compare to the vermicomposted sludge. To rectify this, a smaller trial with 15kg of sludge and 0.8kg of earthworms was conducted. At the end of the trial, it was determined that vermicomposting dairy sludge leads to increased nitrate, sulphate and calcium. An interesting discovery was that phosphate was not detectable in the vermicomposted sludge.

A small continuous flow reactor was built, which led to a larger one being built by Glanbia. The consumption rate was examined with laboratory (lab) trials and resulted in only a small reduction. A lab trial determined that the polyelectrolyte was toxic to earthworms.

There was pattern with high ammonia content in fresh sludge and earthworm mortality. When the sludge was stored, it led to increased concentration of ammonia. The elevated ammonia concentration in the sludge was overcome by either manually aerating the sludge, which vitalises the ammonia or by the addition of zeolite, which absorbed the ammonia.

Further research is warranted and experiments conducted in this study should be repeated again, notably the medium scale (windrow) trial.

CHAPTER FOUR

PLANT GROWTH TRIALS

4.1 The Growth and Yield of Plant Grown in Dairy Sludge and Vermicomposted Dairy Sludge

A series of trials using, ryegrass, radishes, marigolds and barley were conducted in a polytunnel to compare the effects of dairy sludge and vermicomposted dairy sludge on plant growth and yield. The polytunnel was the same as that used in vermicomposting trial (Section 3.2). The trials were conducted between June and October 2004. The sludges were diluted down to various concentrations with a peat/perlite medium which was low in nutrients. The materials and methods were adapted from the work by Atiyeh *et al.* (2002*b*).

4.2 Aims

- To investigate if the substitution of dairy sludge and vermicompost into a peat medium improved plant growth of grass, radishes, marigolds and barley.
- To determine which medium containing dairy sludge or vermicompost provided the best plant growth.

4.3 Plant Growth Media

4.3.3 Dairy sludge

The dairy sludge that was mixed with the peat/perlite medium was recently dewatered sludge obtained from Glanbia Ingredients on 31st May, 2004. The sludge was manually aerated with a garden rake on the floor of the polytunnel for 5 days before being mixed with the peat/perlite medium. This was done to volatilse the ammonia in the sludge; to enable it be easier to work with.

4.3.4 Vermicomposted dairy sludge

Vermicomposted dairy sludge (Plate 15) was obtained from the medium (windrow) trial described in Section 3.2. It was decided not to dry out the vermicompost. Earthworms were extracted from the vermicompost by the migration method described in Appendix H.

4.3.5 Peat/perlite media

Most vermicompost plant trials reported the use of a soil-less growing medium which was low in nutrients. The growth medium used in this research was a peat/perlite mix which was based on research by Atiyeh (2000) who used a ratio of 85% *Sphaghum* peat and 15% perlite (by volume) in vermicompost plant trials. *Sphagnum* peat moss ('ERIN') was used

in the plant trials. Plant growth is usually best between pH 5 and 6.5 (Goh and Hayes, 1977). The pH was adjusted from pH 4.5 to pH 6.5 by mixing ground limestone into the peat. 'Sinclair' standard perlite (2-5mm) was used in the plant trials.

Plate 14. Fresh sludge

Plate 15. Vermicompost

Plate 16. Peat/perlite Media

4.3.6 Properties of the media

The physical and chemical properties of the peat/perlite medium, the dairy sludge and vermicomposted dairy sludge were assessed by analysing for chloride, nitrate, phosphate, sulphate, exchangeable potassium, exchangeable calcium and exchangeable magnesium, pH, electrical conductivity and moisture content.

4.3.7 Statistical analysis

All plant growth data was graphed using Microsoft Excel (Microsoft XP 2000). Statistical tests were completed using SPSS software. Data was analysed using a two way analysis of

variance (ANOVA) and Least Significant Difference (LSD) was determined at levels 0.05, 0.01 and 0.001. Replicates were analysed using a one-way ANOVA. T-tests were performed to evaluate if there was difference in means between the two groups. In all the plant trials the replicates were not significantly different.

4.4   Experimental Design

For each of the plant trials using barley, ryegrass, marigold and radish the dairy sludge and the vermicomposted dairy sludge was mixed with the peat/perlite growth medium, respectively to give the following concentrations (by volume) 0% (control), 10%, 20%, 40%, 60%, 80% and 100%. The respective sludges were mixed at varying concentrations with the peat/perlite growth medium in a 200 litre plastic drum in approximately 50L quantities. Mixing was achieved by rolling and inverting the drum and was facilitated by including wooden baffles in the drum. The peat/perlite medium supplemented with the sludges was added directly to plastic plants pots, without drainage medium at the base. During the trials the plants were watered as required, usually every day. Throughout the growth trial the temperature in the polytunnel was recorded using a maximum/minimum thermometer.

4.4.1   Ryegrass

Perennial ryegrass seed (*Lolium perenne L.* variety Portstewart) was obtained from the Department of Agriculture, Food and Rural Development, Plant Testing Station, Backweston Farm, Leixlip, Co. Kildare. Seed was sown in seven inch plastic pots at a rate of 0.5 grams of seed per pot (Kato, 2004 used 0.5g in six inch pots) containing the peat/perlite growth medium substituted with the dairy sludge and the vermicomposted dairy sludge, respectively, as outlined above. The rate of seed per pot was based on Kato (2004) who used 0.5g in a six inch pot. Three replicates were prepared for each treatment, giving a total of 42 pots. The plant pots were placed approximately 10cm apart on a bench in the polytunnel and set out in a randomised block design. Each week for 16 weeks the height of the six highest blades of grass in each pot was measured from the surface of the growth medium to the top of the blade. The grass yield was measured every four weeks by harvesting the grass to a stubble height of 10mm and measuring the wet weight and dry weight. Dry weight was recorded after placing samples in a fan oven at 95°C for 16 hours, as described by Kato (2004).

4.4.2 Radish

Radish seeds (Scarlet Globe) were germinated and grown in trays, containing the peat/perlite growth medium substituted with the dairy sludge and the vermicomposted dairy sludge, respectively, as outlined above. A germination tray, consisting of 40 inverted pyramid cells/growth medium, was sown with a single radish seed. One tray per mix of peat media and vermicompost/dairy sludge was used. After sowing, the trays were placed in a room 15-20°C until germination occurred and were then moved to the polytunnel. 16 days later after sowing, the germinated seeds were counted. Ten radish seedlings were removed at random from the tray and transplanted into pots (10cm diameter) filled with the same proportion of vermicompost or dairy sludge growth medium in which they were germinated. The remaining seedlings were removed and the growth medium was gently washed away from the root. Then they were measured with a ruler for length of tap roots and tops (leaves) of plants per potting mixture. Wet weight of plants was determined. At week three the length and width of the first and second leaves were measured. The trial was carried out from 14th June to 28th of July 2004. After 28 days the plants were removed and measured for length of roots (bulbs) and tops of plants per potting mixture. The wet weight of plants was determined, and the total plant dry weight was determined after drying the samples for 5 days at 60°C.

4.4.3 Marigold

French marigold 'Queen Sophia' seeds were germinated and grown in trays, containing the peat/perlite growth medium substituted with the dairy sludge and the vermicomposted dairy sludge, respectively, as outlined above. Seed trays each consisting of 40 inverted pyramid cells were each sown with one marigold seed per pyramid. One tray was sown per treatment. Trays were placed in a room at 15-20°C, and moved to the polytunnel after germination. Plants were watered as required.

Thirty days after sowing, 10 plants were selected at random from each treatment. The plant height (distance from the surface of the growth medium level to the top node) and total leaf numbers (including cotyledons) of each seedling were recorded. Plants were removed from the potting mixtures and separated into shoot and root portions to determine their wet weight biomass. 10 additional marigold seedlings were removed at random from the trays

and transplanted into pots (10cm diameter) filled with same proportion of peat/perlite media and vermicompost/dairy sludge in which they had been sown. After 78 days from sowing the total number of flowers produced and the diameters of the biggest flower in each pot were recorded. On Day 101 (72 days after tranplanting into 10cm pots) the number of flowering buds per plant was recorded. On Day 110 the total number of flowers produced and the diameters of the biggest flower in each pot were recorded. The marigolds were removed from the pots and wet weights of shoots were recorded.

4.4.4   Barley

Spring barley seed (CSBC 37 11-7) was obtained from the Department of Agriculture, Food and Rural Development, Plant Testing Station, Backweston Farm, Leixlip, Co. Kildare. Seed was sown in seven inch plastic pots at a rate of 15 seeds per pot containing the peat/perlite growth medium substituted with the dairy sludge and the vermicomposted dairy sludge, respectively, as outlined above. Three replicates were prepared for each treatment, giving a total of 42 pots. The plant pots were placed approximately 10cm apart on a bench in the polytunnel and set out in a randomised block design. The plants were watered as required with water. The growth stage was monitored on day 24 and day 42 using the decimal code described by Tottman *et al.* (1979).

The number of barley plants in each pot was counted and heads were harvested on days 107 and 108 (28th and 29th of September). The heads were placed in brown paper bags and dried at 30°C for 10 days in a Gallenkamp fan oven to 9% moisture content. After drying the sheaths were removed and the number of heads (including awns) was counted and weighed. The weight per head (total weight heads/number of heads per pot) and weight of heads per plant (total weight of heads/total number plants per pot) were calculated. The heads were manually threshed by rubbing the heads between one's hands. The grains were sieved through a 2mm sieve to remove fine bits of awns. 50 grain weight was determined by weighting 50 seeds taken at random. The grain weight per plant (total grain weight/number of plants), grain weight per head (total grain weight/number of heads per pot) were determined. The percentage crude protein in barley grains was analysed using a LECO FP 328 analyser (St Joseph, MI) by F.B.A. Laboratories Ltd.

4.5   Results of characterisation of media and temperature during trials

Table 38. Water soluble chloride, nitrate, phosphate and sulphate content of media

Media	Chloride (Cl^- (mg/kg)	Nitrate (NO_3^-)(mg/kg)	Phosphate (PO_4^{3-}) (mg/kg)	Sulphate (SO_4^{2-}) (mg/kg)
Peat	**98** (23)	**74** (58)	**138** (79)	**126** (10)
Vermicompost	**3349** (271)	**33164** (944)	**0** (0)	**5811** (212)
Dairy sludge	**857** (92)	**189** (188)	**3155** (1043)	**819** (65)

Values in parentheses are standard deviation of a mean of 7 samples, except vermicompost is a mean of 9 samples

Table 39. Exchangeable potassium, calcium and magnesium content of the media

Media	Potassium (mg/kg)	Calcium (mg/kg)	Magnesium (mg/kg)
Peat	**17** (11)	**1312** (251)	**87** (24)
Vermicompost	**10752** (3796)	**5598** (632)	**427** (50)
Dairy sludge	**8428** (3207)	**1146** (248)	**169** (16)

Values in parentheses are standard deviation of the mean of 9 samples

Table 40. pH, electrical conductivity and moisture content of the media

Media	pH	Electrical conductivity ($\mu S/cm^{-1}$)	Moisture content %
Peat	**6.6** (0.14)	**110** (9)	**53** (1.03)
Vermicompost	**6.4** (0.03)	**1592** (13)	**83** (0.20)
Dairy sludge	**7.4** (0.04)	**321** (17)	**86** (0.22)

Values in parentheses are standard deviation of the mean of 9 samples

The peat media had low levels of nutrients compared to the dairy and vermicompost sludge (Tables 38 & 39). The vermicompost had very high level of nitrate and no phosphate compared to the dairy sludge which had a low level of nitrate and phosphate. The dairy and vermicompost sludge had higher levels of potassium compared to the peat (Table 39). The vermicompost had higher level of calcium and magnesium compared to the peat and dairy sludge (Table 39). The pH of the three media was close to neutral (Table 40). The vermicompost had higher level of electrical conductivity compared to the dairy sludge and peat. The dairy and vermicompost sludge had similar moisture contents. The peat media had a lower moisture content of 53%.
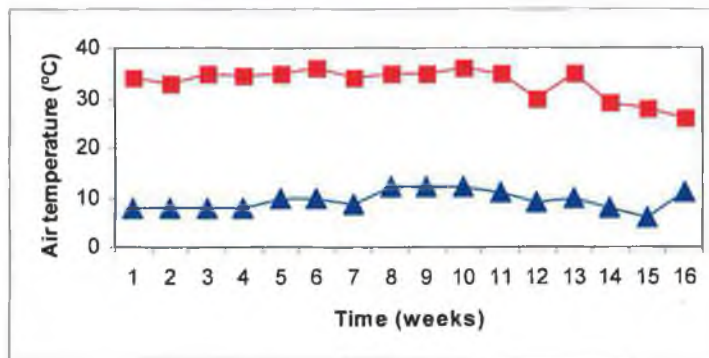
Figure 11. Maximum (■) and Minimum (▲) air temperature during the plant trials

The maximum and the minimum air temperatures during the trial were 36°C and 8°C.

4.6 Ryegrass Results

4.6.1 Height

Generally the dairy sludge produced taller blade length than the vermicompost from weeks 0-12 (Figures 12-17) at each concentration. The control had shorter grass height than any of the dairy and vermicompost treatments during the trial. From week 12-16, there was little difference in the height between the treatments except at 10% concentration. Generally blade length was greater in the growth period 4-8 weeks than other periods as the concentration of dairy sludge and vermicompost increased.
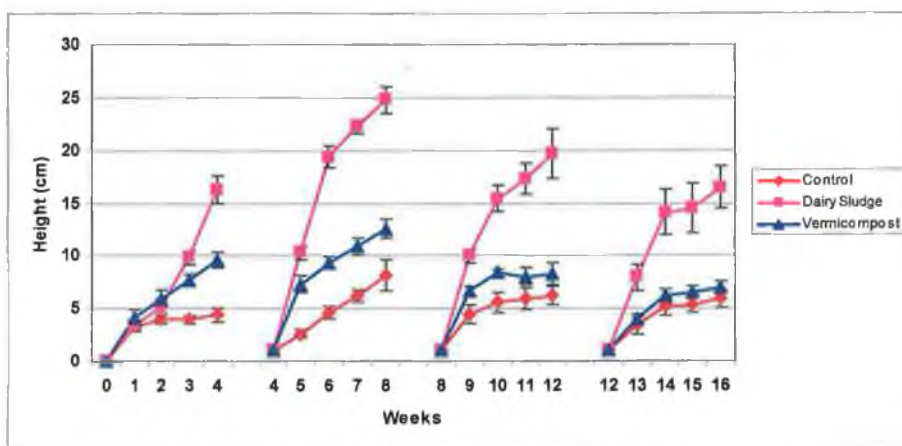
Figure 12. Grass height at 10% concentrations and control
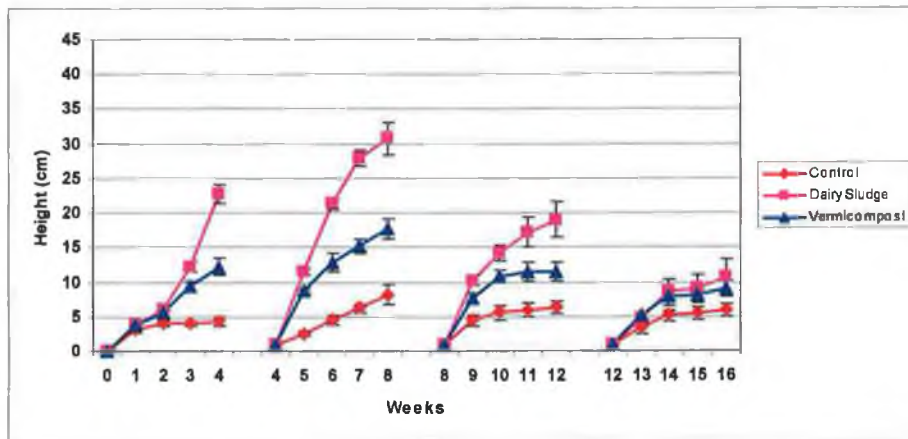
Figure 13. Grass height at 20% concentrations and control
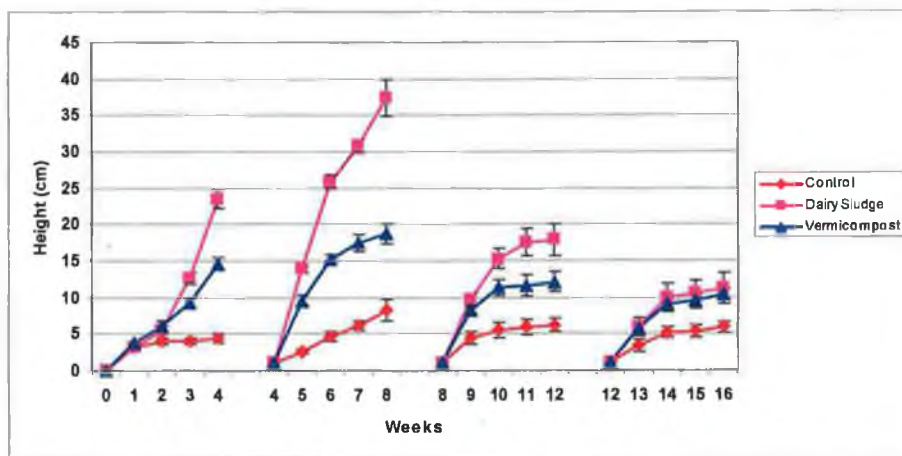
Figure 14. Grass height at 40% concentrations and control
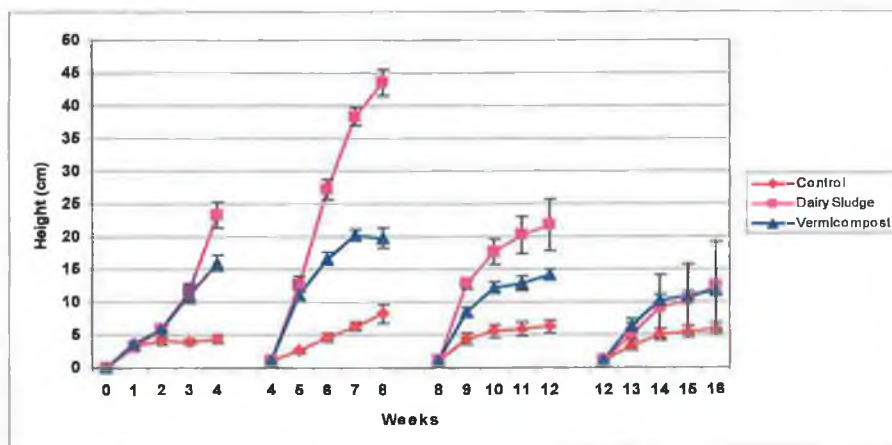
Figure 15. Grass height at 60% concentrations and control

91
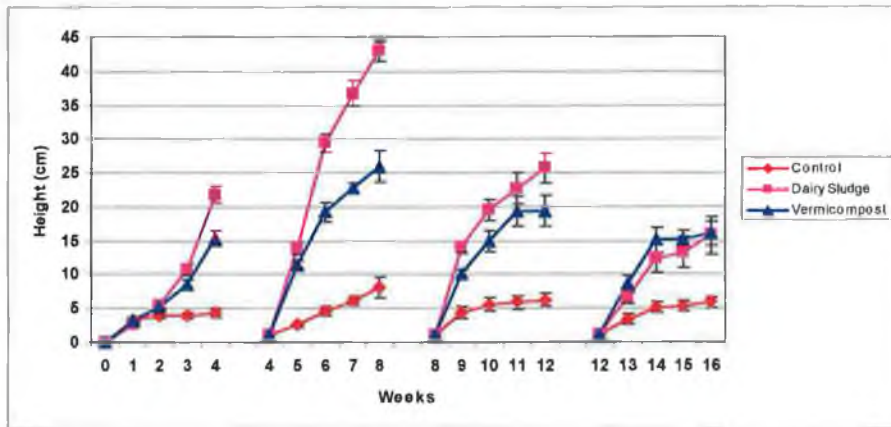
Figure 16. Grass height at 80% concentrations and control
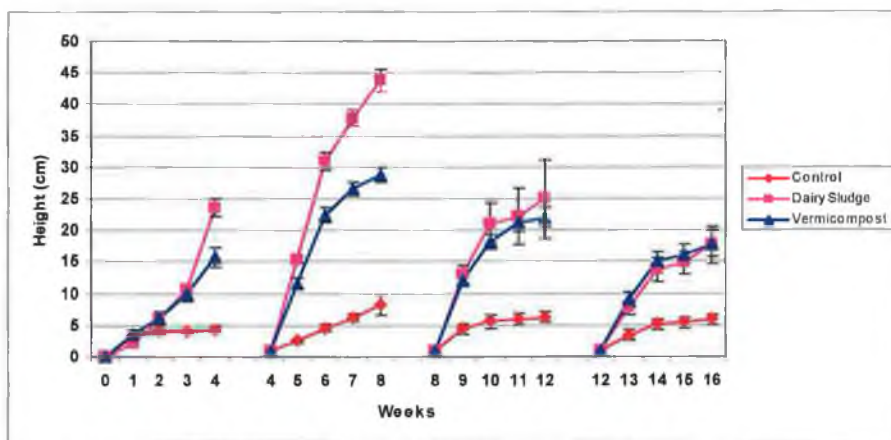
Figure 17. Grass height at 100% concentrations and control

4.6.2 Ryegrass yields

Table 41. Ryegrass wet weight (g) yield on weeks 4, 8, 12, and 16

	Control	10%	20%	40%	60%	80%	100%
				Week 4			
Dairy sludge	1 (0)	21(1)***	48(4)***	60(4)***	53(14)***	37(4)***	53(2)***
Vermicompost	2 (0)	4 (0)	9 (2)	13 (0)	16 (4)	12 (2)	18 (1)
				Week 8			
Dairy sludge	2 (1)	54(4)***	105(2)***	122(21)***	203(29)***	232(35)***	245(27)***
Vermicompost	1 (0)	7 (0)	17 (1)	27 (1)	35 (5)	47 (2)	63 (1)
				Week 12			
Dairy sludge	1 (1)	39(11)**	29 (10)	24 (10)	26 (14)***	62 (12)**	52 (32)*
Vermicompost	1 (0)	5 (0)	8 (1)	13 (2)	17 (1)	24 (2)	32 (1)
				Week 16			
Dairy sludge	3 (0)	22(8)***	8 (3)	11 (4)	10 (12)	17 (9)	16 (7)**
Vermicompost	3 (0)	5 (0)	8 (1)	13 (2)	17 (1)	24 (2)	32 (1)

* = P<0.05, ** = P<0.01, *** = P<0.001 (Values in parenthesis are SD of 3 results)

92

Table 42. Ryegrass dry weight (g) yield on weeks 4, 8, 12, and 16

	Control	10%	20%	40%	60%	80%	100%
	Week 4						
Dairy sludge	0.3(0.0)	3(0)***	6(0)***	7(0)***	7(2)***	5(1)***	7(0)***
Vermicompost	0.2(0.0)	1 (0)	2 (0)	2 (0)	2 (1)	2 (0)	3 (0)
	Week 8						
Dairy sludge	2.9(0.1)	12 (1)***	16(0)***	17(3)***	24(4)***	30(4)***	28(3)***
Vermicompost	2.8(0.1)	4 (0)	5 (0)	7 (0)	9 (0)	10 (0)	12 (1)
	Week 12						
Dairy sludge	0.2(0.1)	6 (5)***	2 (3)	2 (3)	5 (3)	6 (7)	2 (6)
Vermicompost	0.2(0.0)	1 (1)	2 (2)	3 (3)	3 (4)	5 (6)	7 (7)
	Week 16						
Dairy sludge	0.2 (0)	3 (1)**	1 (0)	1 (1)	1 (2)**	2 (1)**	2(1)***
Vermicompost	0.2 (0)	0 (0)	1 (0)	2 (0)	3 (0)	4 (0)	5 (0)

$* = P<0.05, ** = P<0.01, *** = P<0.001$ (Values in parenthesis are SD of 3 results)

In summary of the ryegrass yields, the dairy sludge treatments had significantly heavier yields (wet & dry) compared to the vermicompost treatments in the first ($P<0.001$), second ($P<0.001$) and third (dry;$P<0.05$ and wet;$P<0.001$) harvests. However at the fourth harvest, there was no significant difference ($P>0.05$) in the wet weight yields of the dairy sludge and vermicompost. But the vermicompost treatments had significantly ($P<0.05$) heavier dry weight yield compared to the dairy sludge treatment and control. The dairy sludge and vermicompost treatments showed similar trends with increased yields from 10% to 100% concentrations. The vermicompost and dairy sludge treatments had significantly ($P<0.001$) higher yields than the control. The yields at week 12 and 16 were considerably lower than those achieved at weeks 4 and 8 (Tables 41 & 42).

Overall, when the four harvest yields were combined, the dairy sludge treatments had significantly heaviest grass wet and dry weight yields ($P<0.001$) compared to the vermicompost and control treatments.

Observations

- Chlorosis was on the dairy sludge treatments 20%, 40% and 60%. It first appeared on week 6 (see Plate 17)

93

Plate 17. Left is the vermicompost 20, 40 and 60%. On the right is the 20, 40 and 60% dairy sludge, which showed signs of chlorosis.

Plate 18. Spots on grass grown in dairy sludge on week 12

After 12 weeks of growth, some of the dairy sludge treatments showed signs of brown spots on the grass blades (Plate 18). At the 3rd harvest at week 12, it was noticed that they were parasitised greenflies by wasps on grass on all the dairy sludge treatments. Whereas on the vermicompost treatments the greenflies were absent. There have been reports that vermicompost can also suppress insect attacks on plants. In greenhouse trials, tomatoes peppers and cabbage were grown in vermicompost. The number of aphids (*Myzus persicae*), mealy bugs (*Pseudococcus*), and caterpillars (*Pieris brassicae*) on the plants was significantly reduced (Arancon *et al.* 2005). The use of vermicompost as an insect repellant has become commercial business. In America, California Vermiculture was awarded a United States Patent (No. 6475, 503) for vermicompost as an insect repellant (Anon.,

2003). The conditions found on grass might have been caused by greenhouse conditions and might not exist in field trials. Further investigation into possible insect repellant in grass is warranted.

Grass at Harvest on Weeks 4, 8, 12 and 16

All Photographs show Pots (L to R) 0%, 10%, 20%, 40%, 60%, 80% & 100%

Plate 19. Week 4 Vermicompost

Plate 20. Week 4 Dairy Sludge

Plate 21. Week 8 Vermicompost

Plate 22. Week 8 Dairy Sludge

Plate 23. Week 12 Vermicompost

Plate 24. Week 12 Dairy Sludge

Plate 25. Week 16 Vermicompost

Plate 26. Week 16 Dairy Sludge

4.7 Radish Results

Table 43. Number of seeds germinated after 16 days

	Control	10%	20%	40%	60%	80%	100%
Dairy sludge	39	35	34	32	37	38	29
Vermicompost	37	39	35	34	36	23	29

There were similar numbers of seeds germinated in the two treatments (Table 43). However the 80% vermicompost had 23 seeds germinated compared to 38 seeds in 80% dairy sludge.

After 3 weeks the radishes grown in vermicompost had significantly longer first and second leaves ($P<0.001$), wider first ($P<0.05$) and second leaves ($P<0.01$) compared grown in dairy sludge. The control had significantly ($P<0.001$) shortest and thinnest leaves (Table 44).

Table 44. Leaf growth of radishes on week three

	Control	10%	20%	40%	60%	80%	100%
	Length of First Leaf (cm)						
Vermicompost	1.7 (0.3)	5(1)**	5(1)**	5 (1)	4 (1)	4 (1)	4 (1)**
Dairy sludge	1.8 (0.6)	4 (1)	4 (1)	5 (1)	5 (1)	3 (1)	3 (1)
	Width of First Leaf (cm)						
Vermicompost	0.6 (0.1)	2 (0.3)	2 (0.4)	2 (0.5)	2 (0.4)	2 (0.3)	1 (0.6)
Dairy sludge	0.7 (0.2)	2 (0.3)	2 (0.3)	2 (0.3)	2 (0.2)	1 (0.3)	1 (0.4)
	Length of Second Leaf (cm)						
Vermicompost	1.5 (0.6)	5 (1)	5(1)***	5 (1)	4 (1)	4 (1)	4 (1)*
Dairy sludge	2.0 (0.6)	4 (1)	3 (1)	4 (2)	4 (1)	3 (1)	3 (1)
	Width of Second Leaf (cm)						
Vermicompost	0.5 (0.2)	2 (0.4)	2 (0.4)*	2(0.3)**	2 (0.4)	1 (0.3)	1 (0.4)*
Dairy sludge	0.8 (0.2)	2 (0.3)	2 (0.2)	2 (0.6)	2 (0.2)	1 (0.3)	1 (0.4)

* = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$. (Values in parenthesis are SD of 10 results)

4.7.1 Harvest (week 6)

Table 45. Yields of radishes at harvest

	0%	10%	20%	40%	60%	80%	100%
	Length of Roots (cm)						
Vermicompost	12 (4)	16 (3)	16 (4)	20 (6)	17 (5)	24(8)***	27(7)***
Dairy sludge	9 (2)	15 (3)	18 (6)	18 (3)	17 (3)	16 (5)	15 (7)
	Length of Tops (cm)						
Vermicompost	3 (0)***	10(2)***	12(1)***	15 (2)**	18 (2)	18(2)***	20(2)***
Dairy sludge	4 (1)	13 (1)	16 (1)	17 (2)	18 (2)	14 (2)	10 (4)
	Number of Leaves						
Vermicompost	5 (1)	8 (1)	9 (2)	10 (1)	10 (1)*	11(2)***	12(2)***
Dairy sludge	5 (1)	8 (1)	9 (1)	9 (1)	9 (1)	7 (1)	7 (2)
	Wet Top Weight (g)						
Vermicompost	0.4 (0)	4 (1)*	6 (1)*	10 (3)*	13 (2)	15(4)***	18(5)***
Dairy sludge	0.4 (0)	6 (1)	8 (1)	12 (3)	13 (3)	7 (3)	4 (3)
	Dry Top Weight (g)						
Vermicompost	0.1 (0)	1 (0.0)	1 (0.1)	1(0.5)**	2(0.3)**	2 (0.4)*	2 (0.5)**
Dairy sludge	0.1 (0)	1 (0.2)	1 (0.1)	2 (0.3)	1 (0.3)	1 (0.8)	1 (0.2)
	Wet Root Weight (g)						
Vermicompost	0.2 (0.2)	10 (4)	19 (3)	34 (12)*	48 (9)**	31(8)***	32(8)***
Dairy sludge	0.3 (0.3)	11 (5)	22 (5)	27 (10)	32 (8)	15 (9)	6 (6)
	Dry Root Weight (g)						
Vermicompost	0.1 (0.1)	1 (0.3)	2 (0.2)	2(0.6)***	2(0.8)***	1 (0.3)**	1(0.3)***
Dairy sludge	0.1 (0.1)	2 (0.3)	1 (0.4)	1 (0.4)	1 (0.4)	1 (0.4)	0 (0.4)

* = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$. (Values in parenthesis are SD of 10 results)

After 6 weeks of growing, the radishes were harvested. Overall the radishes grown in vermicompost had significantly ($P<0.001$) more leaves and longer roots. However, there was no significant difference ($P>0.05$) in the length of the tops between the two treatments. The radishes grown in vermicompost had significantly ($P<0.001$) heavier top wet and dry weights, wet and dry root weights. The control had significantly ($P<0.001$) lowest number of leaves, shortest roots and tops, lowest top plant wet and dry weight ($P<0.01$), lightest root wet ($P<0.05$) and dry weights ($P<0.05$). Overall, the radishes grown in vermicompost had significantly ($P<0.001$) heavier wet and dry plant root weights compared to the radishes grown in dairy sludge. A trend for both treatments was that the weight increased from 10% up to 60% and decreased in weight at the higher concentration of 80% 100%. The 60% vermicompost had significantly ($P<0.001$) heaviest dry weight radishes.

Radishes at Harvest

Plate 27. 100% Dairy Sludge

Plate 28. 100% Vermicompost

Plate 29. 80% Dairy Sludge

Plate 30. 80% Vermicompost

Plate 31. 60% Dairy Sludge

Plate 32. 60% Vermicompost

Plate 33. 40% Dairy Sludge

Plate 34. 40% Vermicompost

Plate 35. 20% Dairy Sludge

Plate 36. 20% Vermicompost

Plate 37. 10% Dairy Sludge

Plate 38. 10% Vermicompost

101
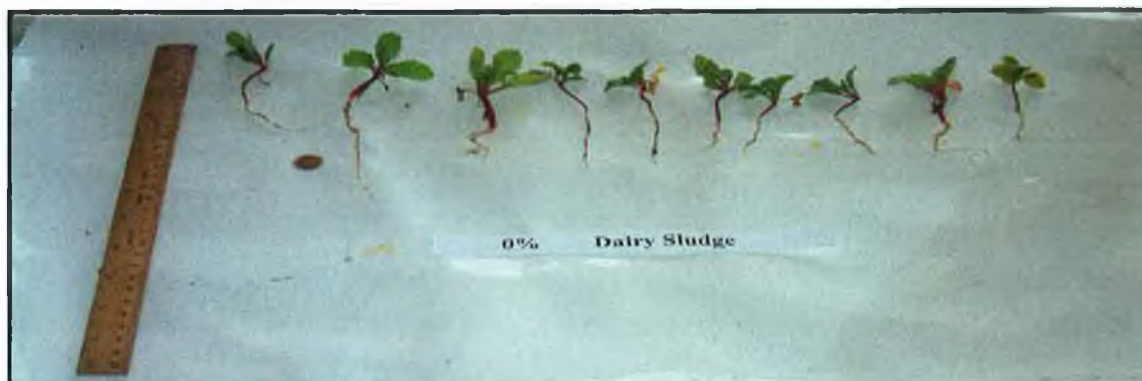
Plate 39. Control

4.8 Marigold Results

Table 46. Growth of Marigolds on days 78, 101 and 110

	Control	10%	20%	40%	60%	80%	100%
	Number of Flowers on Day 78						
Vermicompost	1 (0)	1 (0)	2 (0)	4 (2)**	4 (1)**	5 (1)	5 (1)*
Dairy sludge	0 (0)	1 (0)	2 (1)	5 (1)	6 (1)	5 (1)	4 (1)
	Number of Flowers on Day 101						
Vermicompost	0.8 (0.4)	1.8 (0.9)***	1.4 (1.1)***	3.4 (1.5)***	4.3 (1.6)***	6.9 (1.9)***	8.1 (1.6)***
Dairy sludge	0.7 (0.5)	5.6 (1.6)	8 (1.1)	10.3 (4.4)	12.6 (2.5)	12.9 (2.8)	13 (3.1)
	Number of Flowers on Day 110						
Vermicompost	0.7 (0.5)	1.9 (1.5)***	1.6 (1.0)***	2.8 (1.0)***	2.9 (1.6)***	5.5 (1.7)**	4.9 (2.1)***
Dairy sludge	0.5 (0.5)	8.9 (2.1)	10.3 (1.7)	8 (3.9)	11.9 (4.1)	11.6 (2.8)	12.1 (3.3)
	Diameter of Flowers on Day 78 (cm)						
Vermicompost	3 (1.8)	5.1 (0.6)	6.3 (0.5)	7.2 (0.3)	7 (0.4)*	7.1 (0.3)	7.3 (0.4)
Dairy sludge	2 (2.1)	5.4 (1.8)	6.4 (0.4)	7.6 (0.4)	7.7 (0.3)	7.4 (0.3)	7.3 (0.5)
	Diameter of Flowers on Day 110 (cm)						
Vermicompost	1.4 (1.9)	7.6 (1.8)***	7.4 (2.4)***	7.3 (0.6)**	7.2 (0.6)	7.5 (0.4)	7.4 (0.5)
Dairy sludge	2.2 (1.4)	5 (0.4)	4.6 (0.4)	5.9 (0.8)	6.4 (0.4)	6.7 (0.3)	7 (0.5)
	Wet Shoot Weight (g)						
Vermicompost	2 (1)	16 (10)***	25 (6)***	47 (9)***	57 (13)***	77 (12)***	89 (10)***
Dairy sludge	1 (1)	66 (18)	81 (14)	116 (34)	161 (22)	176 (21)	180 (24)

* = P<0.05, ** = P<0.01, *** = P<0.001 (Values in parenthesis are SD of 10 results)

On day 78, there was no significant (P>0.05) difference in the number and diameters of flowers between the dairy sludge and vermicompost treatments. However on days 101 and 110, the marigolds grown in dairy sludge had significantly (P<0.001) more flowers than the vermicompost treatments.

However, on day 110 overall the dairy sludge treatments had significantly (P<0.001) larger diameters compared to the vermicompost treatments. At the end of the trial on day 110, the wet weights of the shoots (stems and flowers) were significantly (P<0.001) heavier for the dairy sludge than the vermicompost treatments and controls. The control had significantly (P<0.001) less flowers than the dairy sludge and vermicompost treatments on days 78, 101 and 110. It also had significantly (P<0.001) shorter flower diameters after 78 and 110 days of growth.

Observations

- At the end of the trial there was extensive moss growth on all the vermicompost treatment pots (Plate 41) compared to the dairy sludge pots (Plate 40).

Plate 40. Moss on dairy sludge pots **Plate 41.** Moss on vermicompost pots

Timing of flowering
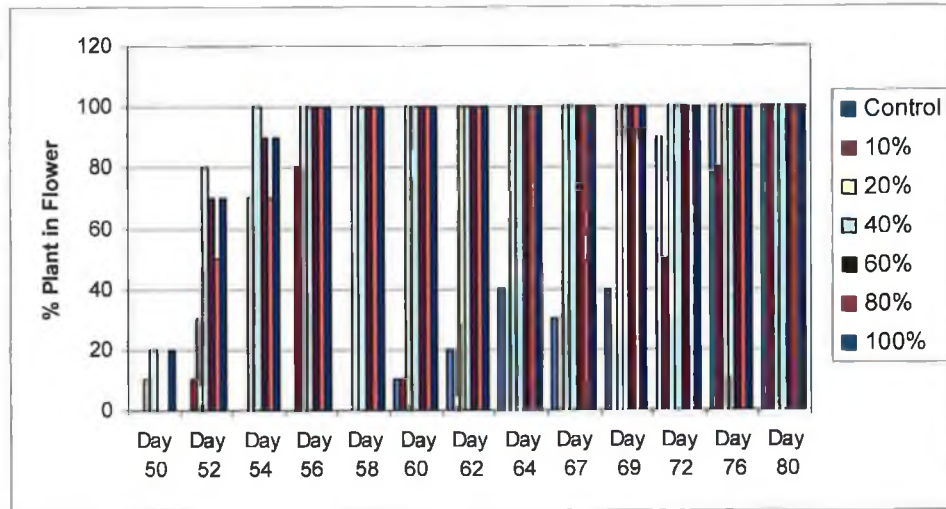
Figure 18. Flower initiation vermicompost
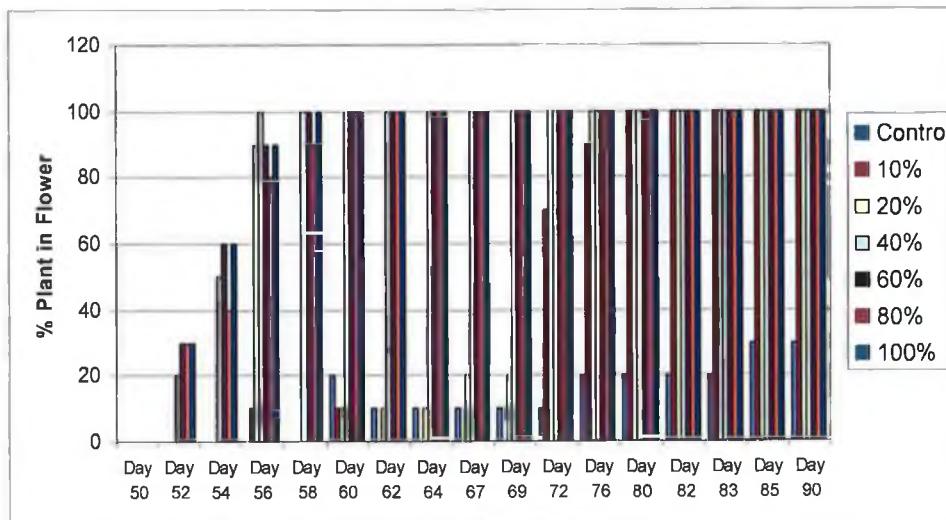
Figure 19. Flower initiation dairy sludge

Figures 18 and 19 showed that marigolds grown in vermicompost flowered earlier compared to the dairy sludge treatments. On day 80, the dairy sludge and vermicompost treatments were all in flower. The plants in the dairy sludge treatments control had not all come into flower by day 90. The control and 10% vermicompost were the last plant to come into flower. The results here show some correlation with Vikram (1988) who found that there was earlier flowering with *Vinca rosea* (Poinsettia) when a medium or soil was mixed with vermicompost.

104

Photographs show Pots (L to R) 0%, 10%, 20%, 40%, 60%, 80% & 100%

Plate 42. Marigolds grown in dairy sludge day 101

Plate 43. Marigolds grown in vermicompost day 101

105

4.9   Barley Results
4.9.1   Seed germinated

Table 47. Number of barley seeds germinated (week 2)

Dairy Sludge	0%	10%	20%	40%	60%	80%	100%
Mean	5 (1.7)	6 (1.2)	4 (1.2)	5 (1.7)	5 (3.0)	5 (2.5)	6 (0.6)
Vermicompost	0%	10%	20%	40%	60%	80%	100%
Mean	4 (0.6)	6 (3.5)	6 (2.1)	4 (1.5)	7 (1.2)	3 (0.6)	2 (1.5)

Values in parenthesis are SD of 3 values

The germination rate of the barley seed after two weeks was low at approximately 25% (Table 47) and there was considerable variation between treatments.

4.9.2   Grain quality at harvest

Table 48. % Crude protein in barley grains

	Control	10%	20%	40%	60%	80%	100%
Vermicompost	13 (2)	9 (1)	10 (2)	9 (1)	10 (0)	9 (1)	10 (0)
Dairy sludge		20 (2)	20 (3)	23 (2)	20 (3)	24 (2)	26 (1)

Values in parenthesis are SD of 3 values

The crude protein in the grain from the dairy sludge treatments was significantly ($P<0.001$) higher than that for the vermicompost treatment and control.

4.9.3   Yield of barley at harvest

The number of plants and number of heads per pot for the vermicompost and dairy sludge treatment are shown in (Appendix F, Tables F3 & F4).

Table 49. Number of heads per plant

Concentrations	0%	10%	20%	40%	60%	80%	100%
Vermicompost	1 (0)	3 (1.3)	5 (4.5)	7 (4.7)	5 (4.2)	11 (4.1)	18 (0.8)
Dairy Sludge	1(0.3)	5(0.6)	13(1.6)	15(1.7)	17(0.5)	13(2.4)	7 (2.2)

Values in parenthesis are SD of 3 values

The number of heads produced per plant varied considerably (Table 49). The number of head per plant increased with increasing concentration of vermicompost. This trend was not as apparent for the dairy sludge.

106

Table 50. Barley harvest results

	Concentration						
	Control	**10%**	**20%**	**40%**	**60%**	**80%**	**100%**
Weight per Head (g)							
Vermicompost	0.1 (0.0)	0.4 (0.1)	0.6 (0.1)	0.7 (0.1)**	0.6 (0.1)	0.7 (0.2)**	0.8 (0.3)**
Dairy sludge	0.1 (0.0)	0.5 (0.0)	0.5 (0.1)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.3 (0.1)
Grain Weight per Head (g)							
Vermicompost	0.1 (0.0)	0.7 (0.1)	0.8 (0.2)**	0.8 (0.1)**	0.7 (0.1)*	0.8 (0.2)**	0.8 (0.3)**
Dairy sludge	0.1 (0.0)	0.5 (0.1)	0.5 (0.1)	0.5 (0.2)	0.4 (0.0)	0.4 (0.1)	0.4 (0.1)
50 Grain Weight (g)							
Vermicompost	1.4 (0.5)	2.4 (0.2)***	2.5 (0.2)***	2.8 (0.2)***	2.6 (0.1)***	2.8 (0.1)***	2.8 (0.2)***
Dairy sludge	1.4 (0.5)	1.5 (0.1)	1.6 (0.2)	1.6 (0.3)	1.8 (0.1)	1.6 (0.1)	1.3 (0.2)
Grain Weight per Plant (g)							
Vermicompost	0 (0.0)	2.5 (1.0)	3.9 (0.7)	5.7 (2.1)	3.8 (0.7)*	7.7 (0.4)	14 (3.9)***
Dairy sludge	0.1 (0.0)	2.7 (1.0)	6.7 (3.4)	6.9 (2.8)	7.2 (2.0)	7 (1.7)	2.7 (0.1)
Weight of Head per Plant (g)							
Vermicompost	0.1 (0.0)	1.5 (0.5)	3 (0.6)*	5 (1.9)	3.4 (0.5)*	7.5 (0.4)	13.6 (3.8)***
Dairy sludge	0.1 (0.1)	2.4 (0.9)	6.6 (3.7)	6.6 (3.0)	7.3 (2.7)	5.2 (2.0)	2.2 (0.3)

*= P<0.05, ** = P<0.01, *** = P<0.001 (Values in parenthesis are SD of 3 results)

The vermicompost had significantly heavier (P<0.001) weight per head, grain weight per head, and 50 grain weight (Table 52), compared to the dairy sludge treatments. Generally the control was significantly lighter than the dairy sludge and vermicompost treatments.

The vermicompost and dairy sludge treatments did not yield significantly different weight of head per plant (P>0.05) and grain weight per plant (P>0.05) (Table 50). Both parameters showed similar trends for the dairy sludge and vermicompost over the range of concentrations. The dairy sludge weights decreased at the higher concentrations of 80 and 100%, while the opposite occurred with the vermicompost.

Observations

- It was noticed that barley leaves showed signs of withering and dying. Five plants in each pot were assessed with the degree of wilting using a photograph key.
- Greenflies were on the barley and were subsequently sprayed with an organic spray 'Natural Pest Control' on (29/7/04) and (24/8/04).
- At the end of the trial, the vermicompost plants were all tall and able to support themselves (Plate 49). The vermicompost plants had a lot less foliage than the dairy sludge plant. The dairy sludge plants had to be supported with bamboo canes (Plate 48). When the canes were removed the plants fell over.

It was noticed that barley leaves showed signs of a possible nutrient deficiency or disease. A photograph key of the four main stages of the effect was used. Plate 43 showed little sign and the degree of severity increased to Plate 47. This key was used to assess how many plants had this condition.

Plates 44- 47.   Photograph Key For Condition Stage A, B, C & D.

Plate 44. Stage A

Plate 45. Stage B

Plate 46. Stage C

Plate 47. Stage D

Plants of barley were assessed for this condition with the photograph key. It was noticed that the worst stage of the condition (stage D) was more predominant in the barley grown in dairy sludge (Figure 20) than vermicompost (Figure 21). It had been noticed that vermicompost had disease resistant properties (Edwards and Arancon, 2004). The conditions found on barley leaves might have been caused by greenhouse condition and might not exist in field trials. Further investigation into possible disease resistant properties of the vermicompost in barley is warranted.
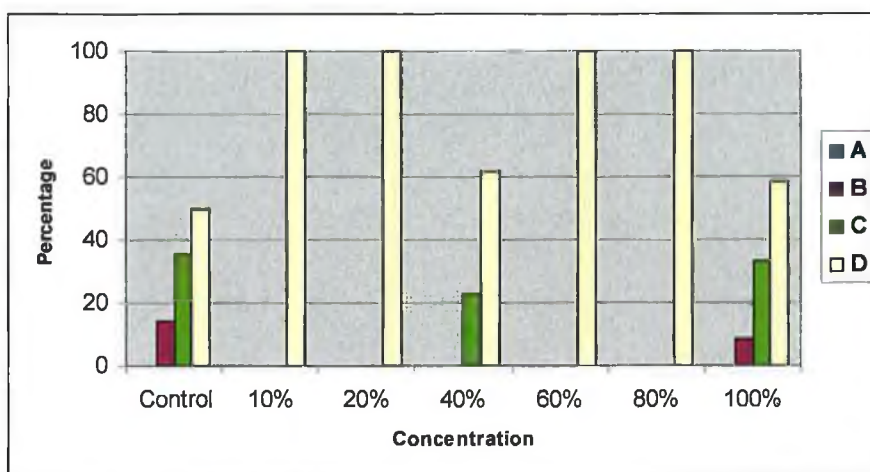
Figure 20. Percentage of the stage of the condition affecting barley grown in dairy sludge
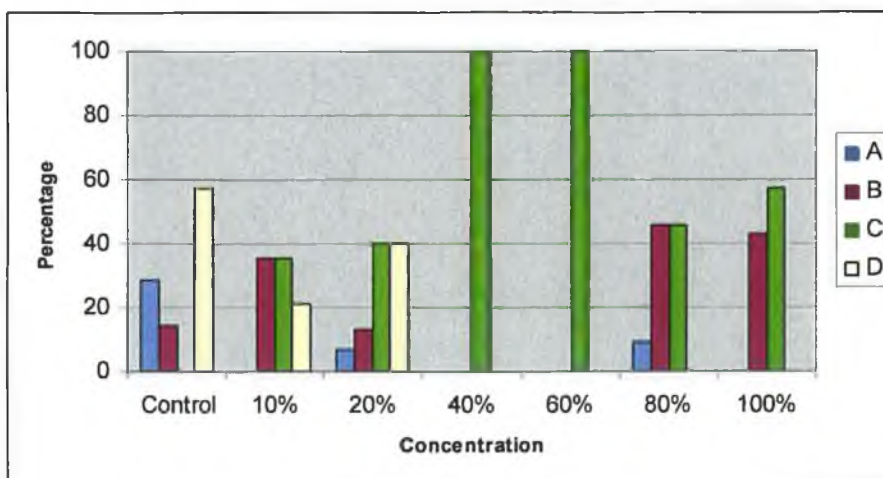
Figure 21. Percentage of the stage of the condition affecting barley grown in vermicompost

Another barley trial was fed fertiliser. Similar browning to figures 20 and 21 was obtained, and this ruled out that it was a nutrient problem. Edwards and Arancon (2004) stated that when vermicompost consisted of a low percentage (10 to 30%) of a medium it suppressed

diseases *Phthium* and *Rhizoctonia*. In field trials vermicompost suppressed *Verticillium* wilt on strawberries, Phomopsis and powdery mildew (*Sphaerotheca fulginae*) on grapes.

Photograph show Pots (L to R) 0%, 10%, 20%, 40%, 60%, 80% & 100%

Plate 48. Barley grown in dairy sludge at harvest

Plate 49. Barley grown in vermicompost at harvest

4.10 Discussion

A series of trials using, ryegrass, radishes, marigolds and barley were conducted in a polytunnel to compare the effects of dairy sludge and vermicomposted dairy sludge on plant growth and yield.

Ryegrass

The grass grown in dairy sludge was generally taller than the grass grown in the vermicompost. The control had the shortest grass. Overall, when the four harvest yields were combined, the dairy sludge treatments had significantly heaviest grass wet and dry weight yield ($P<0.001$) than the vermicompost and control treatments.

At the first two harvests, the dairy sludge treatments produced the heaviest dry weight yields (Table 42) and wet weight yields (Table 41). At the third harvest, the dry weight yields were similar for both the dairy sludge and vermicompost treatments at most of the concentrations (Table 42). However, overall at the third harvest dairy sludge had the heaviest dry weight (Table 42) and wet weight (Table 41) yields. At the final harvest (week 16) there was no significant difference ($P>0.05$) in the wet weight yield between the vermicompost and dairy sludge treatments. However the vermicompost treatments had produced the heavier dry weight yields than the dairy sludge treatments. At all the harvests both the dairy sludge and vermicompost treatments were heavier than the controls.

The dry weight yield in the vermicompost treatments increased linearly with increased concentrations of vermicompost in the peat media, on weeks, 8, 12 and 16. The possible improved grass growth due to the vermicompost could be related to the high nitrate levels. The nitrate could act as a slow release fertiliser supplying the plant with nutrient needed, causing the grass to grow better then the control (Atiyeh, 2000). Grass grown in vermicompost had a more predictable growth and yield compared to the dairy sludge. The vermicompost had lower yields than the dairy sludge. However it had more predictable growth which might be more useful to farmers.

The grass grown in the dairy sludge grew faster and had heavier yields at the beginning (weeks 4 and 8). This was probably due to the level of phosphate in the dairy sludge. After

week 8, the phosphate in the sludge was possibly used by the grass and thus the grass growth slowed down in subsequent weeks 12 and 16. The vermicompost was able to provide consistent growth over the 16 week period.

The higher yield of grass, due to dairy sludge in this pot trial, was also found in other field trial studies. The application of dairy sludge on field trial on soil (Humic Cambisol) in Spain increased the yield of ryegrass by 65% compared to the control (López-Mosquera *et al.* 2002*b*). In another field trial by López-Mosquera *et al.* (2002*a*), it was determined, that in the short term, the dairy sludge was as effective as mineral fertiliser in providing nutrients required for grass growth.

The grass yield peaked at the second harvest at week 8 and decreased gradually afterwards in harvest 3 and 4. The decline was most likely due to removal of nutrients by the plants. Another possible reason for the decline was possibly due to the time of the year. During weeks 8-16 (August to October) the days were shorter and colder. The lower levels of light restricted the growth of the grass (Culleton *et al.* 1992).

When plants were unfertilised, increased plant growth was correlated with increased concentrations of vermicompost (Atiyeh *et al.* 2001). At the higher concentrations of vermicompost, it was found that, plant growth decreased at the higher concentrations. This was due to possible high soluble salt content, heavy metal toxicity, poor aeration and or plant phytoxicity in the vermicompost (Atiyeh *et al.* 2000*a*). The grass results with the vermicomposted dairy sludge did not correlate with Atieyh *et al.* (2000*a*). The grass dry yield did not decline at the higher percentages of vermicompost.

Radish

Overall the radishes grown in the vermicompost medium had better growth than those grown in the dairy sludge medium. At the end of the trial the heaviest radish root wet and dry weights obtained was when the vermicompost constituted a 60% proportion of the total volume of the medium mixture (Table 45). The weight of the roots (wet and dry) increased in weight linearly up to 60% concentration of vermicompost substituted with peat media. At the higher concentrations of 80% and 100% of vermicompost, the weight decreased. Atiyeh *et al.* (2000*a*) noticed in a tomato trial the decline in plant growth at higher

112

concentration of vermicompost. The decline in plant growth was attributed to possible high soluble salt content, heavy metal toxicity, poor aeration and or plant phytoxicity in the vermicompost (Atiyeh *et al.* 2000*a*; Arancon *et al.* 2004). The growth and yield of the tomatoes at the higher concentrations of vermicompost declined above 60% vermicompost and 40% Metro Mix 360 soil-less media.

At harvest, the radishes grown in untreated dairy sludge were slightly disfigured (e.g. Plate 29) compared to the radishes grown in the vermicomposted sludge which were regular in shape (Plate 30).

The dairy sludge had the similar trend in yield, but at lower weights. The wet weight and dry weight (Table 45) of the root increased progressively to 60% concentration and declined at the higher concentrations. The decline in dairy sludge root yield could be possibly due to reasons similar to vermicompost decline in root yield.

Overall the radishes in dairy sludge vermicompost preformed better than the control.

Discussion Marigold

Overall the marigolds grew better in the dairy sludge medium compared to those grown in the vermicompost medium. On Day 78 the dairy sludge and vermicompost treatments had similar numbers of flowers and diameters (Table 46). However the dairy sludge treatments had significantly ($P < 0.001$) higher number of flowers on days 101 and 110 and largest diameters on day 110 compared to the vermicompost treatments (Table 46). The marigolds grown in 10% dairy sludge had the largest diameters. This was possibly because the vermicompost flowered earlier than the dairy sludge treatments and were in stages of dying off. Perhaps the dairy sludge medium provided the right amounts of nutrients compared to the vermicompost.

The dairy sludge treatments had heavier wet shoot weights than the vermicompost treatments and control at the end of the trial (Table 46).

The results showed that there was a general trend in improved plant performance, when peat is substituted with dairy sludge or vermicompost. The dairy sludge and vermicompost

113

had more flowers with larger diameters than the controls. This showed that the dairy sludge and vermicomposted sludge are good peat replacement medium for the horticultural industry.

The only advantage of the vermicompost treatments (Figure 18) compared to the dairy sludge treatments (Figure 19) was accelerated flowering. This could free greenhouse space in a commercial environment and increase overall production.

Discussion Barley

Generally the yield parameters for the vermicompost were higher then those for the dairy sludge. Increased barley yields with vermicompost were also found in field trials in Belarus. The yield of barley (Gonar) increased by 8.7dt/ha compared to the control barley plot (Tsyganov *et al.* 2004). Overall, the vermicompost produced a significantly (P<0.001) heavier yield then the dairy sludge for the yield parameters weight per head, grain weight per head and 50 grain weight. For the yield parameters, weight of head per plant and grain weight per plant, there was no significant difference (P>0.05) between the vermicompost and dairy sludge. Generally the control had the lowest yields compared to the dairy sludge and vermicompost treatments.

Grain protein level is a key criterion in barley used in the malting process. Good malting barley requires low protein of 9-12% (Hector *et al.* 1996). Barley with a high protein is used in animal feed and gets a lower payment (Mahon, 2001). The Guinness Group- Diageo refuses to accept cereal crops from producers that use sewage sludge as a fertiliser or soil amendment in Scotland (Mahon, 2001). The situation involving the use of dairy sludge on barley crops in Ireland will have to be investigated. The grains from the vermicompost plant were around 9% protein and were suitable for the malting process (Table 50) and would achieve a higher premium. However, the grains from the dairy sludge plants were only suitable for use in animal feeds and would get a lower premium. The crude protein is based on the total nitrogen content in the barley grain. The vermicompost had much higher nitrate content than the dairy sludge. In theory the barley grown in vermicompost should have contained higher crude protein content, but this was not the result obtained. It is possible that nitrate leached out of the vermicompost, which resulted in lower crude protein content in the barley grown in vermicompost.

114

Vermicompost had properties that provided sturdy tillers and no excessive growth (Plate 51). The dairy sludge had excessive foliage (Plate 50) but had weaker tillers that had to be supported with bamboo canes. Witter (1978) showed the application of earthworms casts before sowing of a wheat culture inhibit tillering. A similar result was observed in this trial with vermicompost. The vermicompost has less tillering compared to the dairy sludge (Plates 51 & 50).

4.11   Conclusions

- Vermicompost provided the best plant growth for radishes and barley. The radishes grown in vermicompost had the heaviest radish root yields and the barley grown in vermicompost had the highest grain yields and best protein content for malting barley.

- Dairy sludge provided the best plant growth for ryegrass and marigolds, because overall grass grown in dairy sludge had the heaviest wet and dry weight yields. However during the trial the growth of the grass grown in the vermicompost was predictable (because the yields increased with increasing concentrations) and may be more suited for use as a fertiliser. The marigolds grown in dairy sludge had the highest number of flowers with largest diameters after 101 and 110 days of growth and heavier wet shoot weight.

4.12   Mechanisms by Which Dairy Sludge and Vermicomposted Dairy Sludge Influence Plant Growth

4.12.1 Introduction

In the previous plant trials using vermicompost and dairy sludge fed with water, the vermicompost and dairy sludge generally provided better plant growth than the control (peat media). To investigate if vermicompost and dairy sludge had other properties other than nutrients that provided better plant growth than the control (peat medium). Nutrient limitations were eliminated by fertilising the plants. This was investigated using marigolds and barley grown in vermicompost and dairy sludge. The results from this experiment (4.12) should be interpreted with caution. This experiment needs to be repeated again with a range of concentrations of the fertiliser.

4.12.2 Aims

- The aim was to investigate if, when fertilised, if any of the vermicompost and dairy sludge treatments resulted in improved growth compared to the control.
- To record and compare the growth and flowering characteristics of marigolds grown for 110 days in vermicompost and peat medium.
- To record and compare the growth and flowering characteristics of marigolds grown for 110 days in dairy sludge and peat medium.
- To compare the barley yields of the vermicompost and control treatment.
- To compare the barley yields of the dairy sludge and control treatment.

4.12.3 Materials and Methods

The dairy sludge, vermicomposted dairy sludge and peat medium were obtained by the same way as the previous trials. The materials and methods were adapted from the work by Atiyeh *et al.* (2002*b*).

4.12.4 Design of Barley and Marigold trials

The design of these trials is the same as the barley and marigolds trials in section 4.4. The trials were conducted at the same time from June to September 2004. The only difference was that these trials were fed fertiliser. Both the vermicompost and dairy sludge treatments were fertilised three times a week with 15-5-15 +7Ca0 +3Mg0 + TE Peters Excel plant nutrient solution. The plants were fed tap water when required. Peters Excel is a water soluble fertiliser that is recommended for continuous liquid feed programs of plants and contains 15% Total N, 5% P, 15% K, 7% Ca, 3.2 Mg, 0.02% B, 0.010% Cu, 0.12% Fe, 0.05% Mn, 0.010% Mo, 0.030% Zn. These trials were in a randomised block design within the previous trials.

4.12.5 Statistical analysis

The data was analysed statically by one-way ANOVA in a general linear model using SPSS software. The vermicompost or dairy sludge concentrations were then compared to the control, using LSD. Significance was defined as $P<0.001$, 0.01 and 0.05. All of the replicates of each parameter were not significantly different.

4.12.6 Results

Barley Results grown in vermicompost

Table 51. Harvest yields vermicompost

Control	10%	20%	40%	60%	80%	100%
50 Grain Weight (g)						
2 (0.3)	2 (0.3)	3 (0.2)*	3 (0.1)	3 (0.3)*	2 (0.4)	3 (0.3)
Grain Weight per Plant (g)						
4 (2)	9 (2)	11 (8)	9 (4)	10 (6)	7 (2)	8 (2)
Weight per Head (g)						
0.5 (0.1)	0.5 (0.1)	0.7 (0.0)**	0.6 (0.0)	0.7(0.1)**	0.5 (0.2)	0.5 (0.1)
Weight per Head per Plant (g)						
0.4	7 (1)	10 (11)	11 (10)	8 (2)	11 (8)	7 (6)
Grain Weight per Head (g)						
0 (0.1)	1 (0.1)	1 (0.1)*	1 (0.0)	1 (0.1)*	1 (0.2)	0 (0.1)

$* = P<0.05, ** = P<0.01, *** = P<0.001$

Values in parenthesis are SD of 3 results

All results presented are at 9% moisture content. Overall there was no significant difference ($P>0.05$) in weight per head per plant and grain weight per plant between the vermicompost concentrations and control. The 20% and 60% vermicompost concentration had significantly ($P<0.05$) heavier grain weight per head, weight per head and 50 grain weight (Table 53) than the control.

Plate 50. Barley grown in vermicompost at harvest (L to R) 0% control, 10%, 20%, 40%, 60%, 80% and 100% vermicompost

117

Barley Results grown in dairy sludge

Table 52. Harvest Yields Dairy Sludge

Control	10%	20%	40%	60%	80%	100%
50 Grain Weight (g)						
2 (0.1)	2 (0.2)	2 (0.2)	2 (0.1)**	2 (0.2)**	2 (0.1)**	2 (0.3)**
Grain Weight per Plant (g)						
4 (0.4)	8 (2)*	8 (1)*	7 (1)*	7 (1)*	4 (2)	4 (1)
Weight per Head (g)						
0.5 (0.0)	0.6 (0.1)	0.5 (0.0)	0.4 (0.1)	0.5 (0.0)	0.4 (0.0)	0.4 (0.1)
Weight per Head per Plant (g)						
0.4 (0.2)	7 (6)*	7 (4)*	10 (6)*	6 (5)*	9 (1)*	2 (1)*
Grain Weight per Head (g)						
0.5 (0.1)	0.6 (0.2)	0.5 (0.0)	0.6 (0.2)	0.4 (0.0)	0.5 (0.1)	0.4 (0.0)

*= P<0.05, ** = P<0.01, *** = P<0.001
Values in parenthesis are SD of 3 results

All results presented are at 9% moisture content. Overall there was no significant difference (P>0.05) in the grain weight per head between the dairy sludge concentrations and control. The 80% and 100% dairy sludge concentration had significantly (P<0.05) lighter weight per head than the control. There was no difference between the control and 10%, 20%, 40% and 60% dairy sludge. The 40%, 60%, 80% and 100% dairy sludge concentrations had significantly (P<0.01) lighter 50 grain weight than the control. Overall the dairy sludge concentrations had significant (P<0.05) heavier weight per head per plant (Table 52) than the control. The 80% and 100% had not significantly different grain weight per plant than the control.

Plate 51. Barley grown in dairy sludge at harvest (L to R) 0% control, 10%, 20%, 40%, 60%, 80% and 100% dairy sludge.

118

Marigolds results grown in vermicompost

Table 53. Growth of Marigolds on Days 78, 101 and 110.

Control	10%	20%	40%	60%	80%	100%
Number of Flowers Day 78						
1 (1)	1 (1)	2 (0)**	3 (1)***	3 (1)***	3 (1)***	3 (1)***
Number of Flowers Day 101						
2 (1)	5 (2)***	4 (2)*	5 (1)***	6 (1)***	7 (2)***	6 (2)***
Number of Flowers Day 110						
3 (1)	6 (0)***	5 (1)**	6 (1)***	6 (2)***	7 (2)***	7 (1)***
Diameter of Flowers Day 78						
5 (1)	6 (1)***	6 (1)***	7 (0)***	7 (0)***	7 (0)***	6 (1)***
Diameter of Flowers Day 110						
6 (1)	7 (0)***	7 (1)***	7 (1)***	7 (1)***	7 (1)***	7 (1)***
Wet Shoot Weight (g)						
2 (1)	16 (10)***	25 (7)***	47 (9)***	57 (13)***	77 (12)***	89 (10)***

* = P<0.05, ** = P<0.01, *** = P<0.001
Values in parenthesis are SD of 10 results

The vermicompost had significantly (P<0.001) higher number of flowers compared to the control on days 78, 101 and 110. All of the vermicompost concentrations had significantly (P<0.001) larger diameters of flowers compared to the control on days 78 and 110. All of the vermicompost concentrations had significantly (P<0.001) heavier fresh shoot weights (Table 53) compared to the control.

Plate 52. Marigolds grown in vermicompost on day 101, (L to R) 0% control, 10%, 20%, 40%, 60%, 80% and 100% vermicompost.

119

Marigolds Results grown in dairy sludge

Table 54. Growth of Marigolds on Days 78, 101 and 110.

Control	10%	20%	40%	60%	80%	100%
Number of Flowers Day 78						
1 (0)	1 (0)	3 (1)***	4 (2)***	4 (1)***	3 (1)***	3 (1)***
Number of Flowers Day 101						
3 (1)	6 (1)***	7 (2)***	10 (3)***	9 (2)***	7 (3)***	8 (2)***
Number of Flowers Day 110						
4 (1)	11 (2)***	8 (2)***	9 (3)***	9 (3)***	8 (3)***	9 (3)***
Diameter of Flowers Day 78						
3 (3)	6 (1)***	7 (1)***	7 (0)***	7 (0)***	5 (0)***	7 (1)***
Diameter of Flowers Day 110						
7 (1)	7 (1)**	7 (1)*	7 (1)**	7 (0)*	7 (0)	7 (1)**
Wet Shoot Weight (g)						
1 (1)	66(17)***	81(14)***	116 (34)***	161(22)***	176(21)***	180(24)***

$* = P<0.05, ** = P<0.01, *** = P<0.001$
Values in parenthesis are standard deviation of 10 results

All of the dairy sludge concentrations had significantly ($P<0.001$) higher number of flowers compared to the control on days 78, 101 and 110. All of the dairy sludge concentrations had significantly ($P<0.001$) larger diameters of flowers compared to the control on days 78 and 110. All of the dairy sludge concentrations had significantly ($P<0.001$) heavier fresh shoot weights (Table 54) compared to the control.

Plate 53. Marigolds grown in dairy sludge on day 101, (L to R) 0% control, 10%, 20%, 40%, 60%, 80% and 100% dairy sludge

4.12.7 Discussion

To investigate if vermicompost and dairy sludge had other properties other than nutrients that provided better plant growth than the control (peat medium). Nutrient limitations were eliminated by fertilising the plants. The growth and yield of marigolds and barley grown in dairy sludge and vermicompost were compared to the control (peat/perlite media). The results from this experiment (4.12) should be interpreted with caution. This experiment needs to be repeated again with a range of concentrations of the fertiliser.

Discussion of Barley grown in vermicompost

A balanced fertiliser of 15-5-15 was selected. A rate of 0.5g/L was used and provided 75ppm N. Reflecting on this experiment, the concentration of fertiliser used should have been proved that it provided the optimal amount of nutrients using a range of concentrations.

The trend was that 10%, 40%, 80% and 100% vermicompost had similar results to the control and, in general, the vermicompost did not have properties other than nutrients that provided superior plant growth. However 20% and 60% had higher yields than the control. This would indicate that at these two concentrations, the vermicompost had properties other than nutrient related, such as improved physical structure, presence of beneficial microbial activity, plant growth regulators, and/or humic acids (Arancon *et al.* 2004*a*) that provided better barley growth and yield.

Generally the results showed that there were some similarities between the control and vermicompost concentrations. This would indicate that, in general, the vermicompost did not have properties other than nutrients that provided superior plant growth.

Discussion of Barley Results grown in dairy sludge

A balanced fertiliser of 15-5-15 was selected. A rate of 0.5g/L was used and provided 75ppm N. Reflecting on this experiment, the concentration of fertiliser used should have been proved that it provided the optimal amount of nutrients using a range of concentrations. In general, the trend was that dairy sludge had similar results to the control

or had results lower than the control. This indicated that the improved plant growth provided by dairy sludge in section 4.0 was not related to the nutrients in the dairy sludge.

Discussion of Marigolds grown in vermicompost

A balanced fertiliser of 15-5-15 was selected. A rate of 0.5g/L was used and provided 75ppm N. The manufacturer of the fertiliser recommends that bedding plants be fed 50-150ppm N of this fertiliser. Reflecting on this experiment, the concentration of fertiliser used should have been proved that it provided the optimal amount of nutrients using a range of concentrations.

Overall the vermicompost provided better plant growth than the control (peat) medium. The vermicompost concentrations generated more flowers with larger diameters on days 78, 101 and 110. On day 110 the all the vermicompost concentrations had heavier wet shoot weights than the control.

Even with nutrients supplied to the vermicompost, it acted better than the control, indicating that vermicompost had properties other than nutrient related that provided superior growth e.g. such as improved physical structure, presence of beneficial microbial activity, plant growth regulators, and/or humic acids (Arancon *et al.* 2004*a*).

When the marigold plants were supplied with all the nutrients required, the substitution of peat medium with small proportion (10%) of vermicompost, increased the plant growth significantly (Table 53). The vermicompost used in this experiment has potential as a component of peat medium for horticultural use. It could be used to maximise the production of marigolds in a polytunnel when substituted at a low concentration (10% by volume) into a peat medium. The use of vermicompost would be economically attractive since acceletered growth of bedding plants is required and would make better use of polytunnel space in the horticultural industry.

Atiyeh *et al.* 2002*b* grew marigolds (Queen Sophia) in pig manure vermicompost and a soil-less medium (Metro Mix 360). Atiyeh *et al.* (2002*b*) had similar findings that when the vermicompost was supplied with nutrients, it provided better plant growth than the control. After 121 days of growth Atiyeh *et al.* (2002*b*) the vermicompost provided a range of 16-23

122

flowers across the concentrations, compared to this experiment after 110 days of growth the range was 4-9 flowers. The results from this experiment are not directly comparable to Atiyeh *et al.* (2002*b*), but are an indication that vermicomposted pig manure provided more flowers that vermicomposted dairy sludge.

Discussion of Marigolds grown in dairy sludge

A balanced fertiliser of 15-5-15 was selected. A rate of 0.5g/L was used and provided 75ppm N. The manufacturer of the fertiliser recommends that bedding plants be fed 50-150ppm N of this fertiliser. Reflecting on this experiment, the concentration of fertiliser used should have been proved that it provided the optimal amount of nutrients using a range of concentrations.

The dairy sludge concentrations generated more flowers with larger diameters on days 78, 101 and 110. On day 110 all the dairy sludge concentrations had heavier wet shoot weights than the control.

Overall the dairy sludge provided better plant growth than the control (peat) medium. This indicated that either the fertiliser concentration was not high enough or that the dairy sludge had possibly other properties other than nutrient related. These properties provided better plant growth such as improved physical structure, water holding capacity, aeration, particle size or microbial activity.

4.12.8 Conclusions
- With the vermicompost, the marigolds indicated that vermicompost had properties other than nutrient related that provided superior growth e.g. such as improved physical structure, presence of beneficial microbial activity, plant growth regulators, and/or humic acids
- The general trend with barley grown in vermicompost and dairy sludge was the better growth was related to nutrients.
- The marigolds grown in dairy sludge had better growth. This was possibly due to other properties other than nutrient related that provided better plant growth such as improved physical structure, water holding capacity, aeration, particle size or microbial activity.

4.13 Radishes grown in sterilised vermicompost and vermicompost

4.13.1 Introduction

The method in this research trial was the same as Buckerfield *et al.* (1999), who sterilised vermicompost from animal and plant wastes. Buckerfield found the radishes grown in sterilised vermicompost had reduced growth compared to non-sterilised vermicompost. The sterilised vermicompost plant growth was similar to radishes grown in sand (control). Buckerfield stated that vermicompost is superior was due to 'biological component'.

4.13.2 Aims

- To compare the growth and yield radishes grown in 60% vermicompost and 60% sterilised vermicompost.

4.13.3 Materials and methods

Radish seeds (Scarlet Globe) were germinated and grown in trays, containing 60% vermicompost and/or sterilised vermicompost (by volume) with 40% peat/perlite media. The Vermicompost was sterilised by autoclaving at 120^0C at 105KPa 60 minutes (Buckerfield *et. al.* 1999)

The tray consisted of 40 inverted pyramid cells. After sowing, all trays were placed in a room 15-20°C until germinated and then were moved to the polytunnel. The number of seeds germinated each day was counted. 10 radish seedlings were removed at random from the tray and transplanted into pots (10cm diameter). These were filled with same proportion of vermicompost/sterlised vermicompost that it has been germinated in.

30 days later plants were removed and measured for length of roots and tops of plants per potting mixture. The wet weight of tops and bottom was determined. Plants were oven dried at 60°C for 5 days to determine top and bottom dry weights.

4.13.4 Results

Table 55. Water soluble chloride, nitrate, phosphate & sulphate content of mediums

Media	Chloride (mg/kg)	Nitrate mg/kg)	Phosphate (mg/kg)	Sulphate (mg/kg)
Vermicompost	**495** (212)	**39,030** (2242)	**0** (0)	**4,769** (2543)
Sterilised Vermicompost	**872** (229)	**43,880** (4868)	**0** (0)	**6,497** (881)

Values in parentheses are standard deviation of mean of 7 samples

Table 56. Exchangeable potassium, calcium and magnesium content of the mediums

Media	Potassium (mg/kg)	Calcium (mg/kg)	Magnesium (mg/kg)
Vermicompost	**8,973** (3459)	**5,178** (1057)	**365** (60)
Sterilised Vermicompost	**6,812** (4018)	**5,672** (714)	**398** (49)

Values in parentheses are standard deviation of the mean of 3 samples

There was no significant difference $(P>0.05)$ in the nutrient content of the sterilised vermicompost and non-sterilised vermicompost.
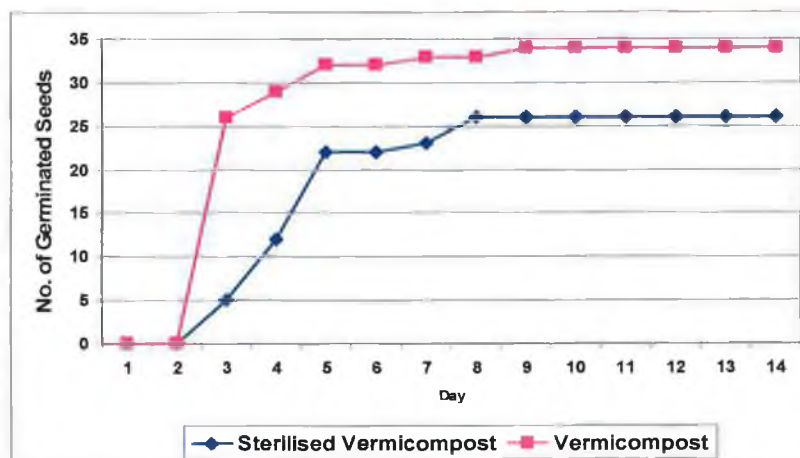
Figure 22. Germination of seeds over 14 days

Table 57. Yield of radishes at harvest

	Sterilised	Not-Sterilised
Number of Leaves	**8** (1)*	**7** (1)
Length of Bulb (cm)	**12** (3)	**17** (5)*
Length of Tops (cm)	**15** (1)*	**10** (1)
Top Wet Weight (g)	**7** (2)*	**3** (1)
Top Dry Weight (g)	**0.51** (0.1)*	**0.27** (0.0)
Bulb Wet Weight (g)	**12** (7)	**7** (4)
Bulb Dry weight (g)	**0.62** (0.3)	**0.47** (0.2)

* = **P<0.05**

125

4.13.5 Discussion

After six weeks of growth the radishes were harvested. The radishes grown in sterilised vermicompost had significantly (P<0.05) more leaves, longer tops, heavier top wet and dry weights compared to the radishes grown in vermicompost. The radishes grown in vermicompost had significantly (P<0.05) longer bulbs (Table 57).

The main yield parameter in assessing radishes is the weight of the bulb. There was no significant (P>0.05) difference between bulb wet and dry weights. Because the radish yield (bulb weight) was not between vermicompost and sterilised vermicompost, it was determined that vermicompost improved radish growth property and was not related to microbial activity within the vermicompost. The research with the vermicomposted dairy sludge did not correlate with Buckerfield *et al.* (1999). This indicated that the vermicomposted sludge did not provide improved plant growth because of a biological component. This finding has been correlated with more recent research by Atiyeh (2000). He used a different method of sterilising vermicompost, but found that sterilised vermicompost had similar plant growth to non-sterilised vermicompost. Improved plant growth maybe is due to other factors such as humic acids or plant growth factors Atiyeh (2000).

4.13.6 Conclusions

- The improved radish growth property was not related to microbial activity within the vermicompost.

Plate 54. Radishes at harvest grown in 60% vermicompost

Plate 55. Radishes at harvest grown in 60% sterilised vermicompost

4.14 Leaching of nitrate from vermicompost

4.14.1 Introduction

After vermicomposting there is a high level of nitrate in the vermicompost. The vermicompost used in plants (section 4.1-4.12) had 33,000mg/kg of nitrate. Because the vermicompost contained a high level of nitrate, it was wondered how much might leachate out of it. Nitrate leaching is a major environmental issue for agriculture (Ceccon *et al.* 1996). It is a concern of this study if vermicomposted dairy sludge leaches out high level of nitrate, it restricts the use of vermicompost.

4.14.2 Aim:

- To determine the amount of nitrate leachated out of vermicomposted dairy sludge.

4.14.3 Materials and Methods

Vermicomposted sludge similar to that used in plant trials was used. It was mixed with the peat/perlite growth medium to give the following concentrations (by volume) 0% (control), 25%, 50%, 75% and 100% (three replicates). For the first five days the grass was kept in the laboratory until the grass had germinated. During this time the seeds were sprayed with a atomiser. When germinated, the pots were moved into the polytunnel, where each pot had a leachate collection system as shown in Figure 23. Throughout the growth trial the temperature in the polytunnel was recorded using a maximum/minimum thermometer and the temperature ranged from 7°C and 39°C. The trial was conducted from May to June 2005. Each pot was watered daily with 300ml of water and the leachate collected daily to prevent denifrification or assimilation by bacteria and algae, and frozen (Anon., 1992). The volume of leachate was noted daily and 60ml aliquots of mixed replicates samples were removed daily. Like McInerney and Bolger (2000) samples were analysed within two weeks for nitrate. Composites samples were made from days 0 to 10 and days 11-20.

Grass

Plant Pot

Wire mesh

Funnel with pipe
attached on end, going
into bottle

Bottle for collecting
leachate
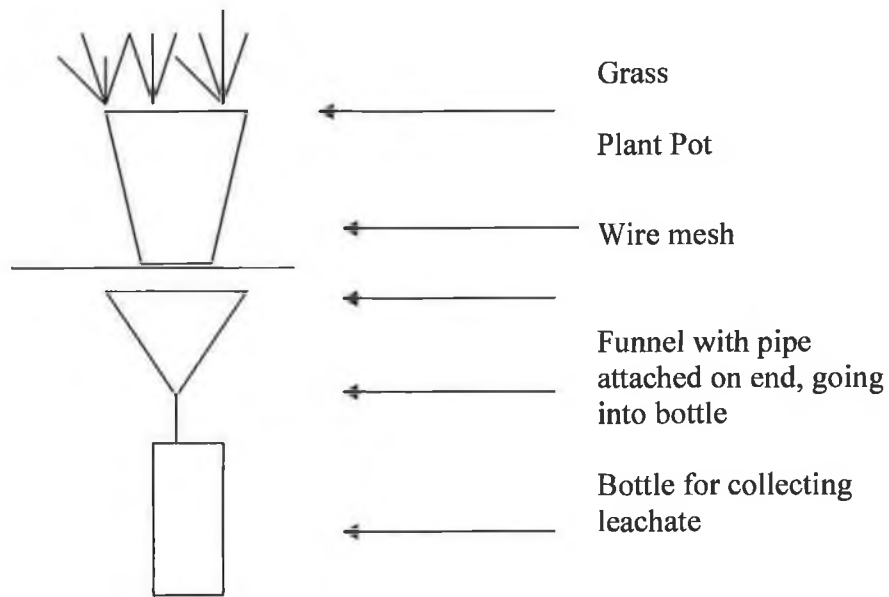
Figure 23. Design of leachate collection system

Plate 56. Overview of trial

4.14.4   Results
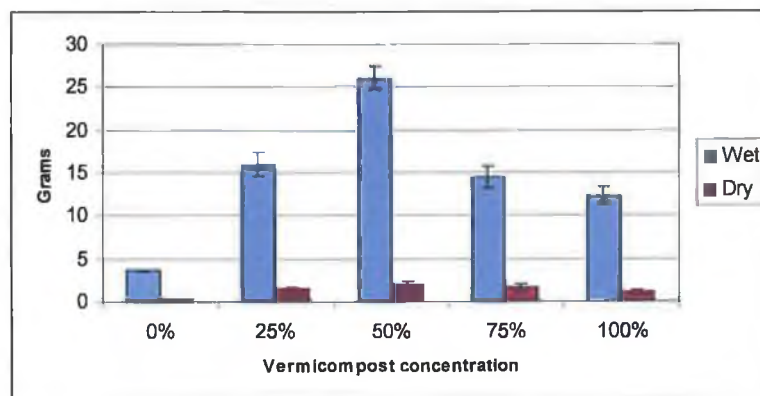
Figure 24. Wet and dry weight of grass yields at harvest

128

The grass grown in 50% vermicompost had the significantly highest (P<0.05) wet and dry weight yields.

Table 58. Nitrate concentration in grass

Vermicompost (%)	mg/l D.W. Nitrate
100%	983
75%	873
50%	250
25%	19
0%	Insufficent sample size

With increasing concentrations of vermicompost, the level of nitrate increased in the grass.

Table 59. Nitrate concentration (mg/L) in leachate from days 0-10 and Days 11-20.

Vermicompost (%)	mg/l Nitrate	
	Days 0-10	Days 11-20
100	2,318 (93)	696 (0)
75	1,455 (0)	585 (12)
50	3,092 (0)	650 (0)
25	2,331 (23)	293 (29)
0	17 (1)	5 (0.3)
Tap Water	1 (0.01)	1 (0.1)

From days 0-10 there was a higher level of nitrate that leached out compared to days 11-20.

Table 60. Nitrate concentration in the vermicompost at the start and end of the trial

Vermicompost (%)	Start (mg/kg)	End (mg/kg)	% Loss of nitrate
100	46,878	20,409	57
75	41,902	1,851	57
50	25,597	1,358	95
25	7,907	283	96
0	187	67	64

4.14.5 Discussion

The vermicompost used in this experiment was left over from trial 3.2. The level of nitrate was 46,878 mg/kg. There were still some earthworms in the vermicompost. In the plant trials (section 4.1-4.12) the amount of nitrate in the vermicompost was 33,000 mg/kg. The 50% vermicompost provided significantly heaviest wet and dry weight grass yield. When plants were unfertilised, increased plant growth was correlated with increased concentrations of vermicompost (Atiyeh *et al.* 2001). At the higher concentrations of

129

vermicompost, it was found that, with vermicompost, plant growth decreased at the higher concentrations. This was due to possible high soluble salt content, heavy metal toxicity, poor aeration and or plant phytoxicity in the vermicompost (Atiyeh *et al.* 2000). The grass results with the vermicomposted dairy sludge did correlate with Atieyh *et al.* (2000). The grass dry yield did decline at the higher percentages of vermicompost. The grass uptake (Table 58) in the 100% vermicompost of nitrate was 983 mg/l which was below the toxic limit of 1500mg/l. Nitrate content in grass above this limit is lethal to animals (Gillingham *et al.* 1969). The amount of nitrate that leached out of the vermicompost diluted with peat/perlite media at different concentrations is shown in Table 59. High level of nitrate leached out of the 50% concentration probably because of increased surface area of vermicompost mixed with the peat/perlite media. The highest amount of nitrate leached out from days 0-10.

If the vermicompost was used in the field it is possible the nitrate would leach out and possibly enter surface or groundwater. This may present a risk to human health, if this water is used for abstraction of drinking water. It could also contribute to eutrophication of freshwater ecosystems (Wilkins, 1996). However nitrate may not leach out at a high level in the field, because the pots were watered heavily in this trial. This is why a field trial is warranted. In Table 60 the amount of nitrate in the vermicompost at the start and end was analysed. It showed that at the lower concentrations (50% & 25%) most of the nitrate was lost due to leaching and plant uptake. This loss could be attributed to greater space/surface area of vermicompost in the pot where water had contact with it. In the 100% and 75%, the vermicompost had a 'cheese cake' appearance and the water flowed down the sides near the pot edge, thus the water had less contact with the vermicompost.

The limit of nitrate permitted in groundwater is 50mg/L (Nitrate Directive 91/676/EEC). The results in this survey would probably exceed this limit in field conditions. If a field grass trial using vermicomposted sludge was conducted, there would be a possible risk of contamination of groundwater by nitrate leaching out of the vermicompost.

4.14.6 Conclusion
- High level of nitrate leached out of vermicomposted dairy sludge.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.0   Conclusions

This study set out to determine if earthworms would survive, grow and digest dairy sludge, and with the digested sludge, if plants would grow in it. Returning to the aims posed at the beginning of this study it is now possible to state that:

(1) This study has shown that earthworms grew and reproduced in dairy sludge. *D. veneta* and *E. fetida* dominating the polyculture. However, if the vermicomposting system maintained a sustainable earthworm population, this was less than inconclusive. The trial period was too short to determine if it was a sustainable system.

(2) The vermicomposting process was improved by reducing the levels of ammonia. There was a pattern with high ammonia levels in fresh dairy sludge and earthworms dying. This was overcome by manually aerating the sludge to volatilise the ammonia or by addition of zeolite; which absorbed the ammonia.

(3) Chemical, biological and physical analysis was conducted on dairy sludge and vermicomposted dairy sludge. There was no leachate generated during vermicomposting.

(4) Vermicompost was generated and has potential uses in the agriculture and horticultural industries.

(5) Plant growth trials with ryegrass, radishes, marigolds and barley grown in dairy sludge and vermicompost were conducted.

(6) It is possible to vermicompost dairy sludge. However, determination that over a continual period of vermicomposting that the earthworm population was sustainable (i.e. extra earthworms not added as time progresses) was not determined. Thus determinating that this system is sustainable was not confirmed. The high level of nitrate that leached out of the vermicompost also questions that the process is not sustainable because of potential pollution of water sources with nitrate.

(7) In relation to the working document on sludge 3rd draft, vermicomposting dairy sludge complies with it.

Other conclusions;

- After vermicomposting, the amount of the plant available nutrients nitrate, calcium and sulphate had increased. Phosphate was not detectable after vermicomposting.

- Due to the increased nutrient content in the dairy sludge from the vermicomposting bed compared to its parent material (fresh dairy sludge), it was determined that it was vermicompost.

- Nitrate levels are the best indicator as to when the dairy sludge is changed into vermicompost. This parameter will also give an overall indication on the general performance of the vermicomposting process.

- The nitrate and phosphate levels could be used as indicators of the extent of vermicomposting.

- Dairy sludge alone can be changed into vermicompost without the addition of any other material such as a carbon source.

- The metal content of dairy sludge and vermicomposted dairy sludge was below limits in the draft Sewage Sludge Directive.

- Generally the pathogen content in the dairy sludge was below the draft Sludge Directive limits for *Salmonella* and *E. coli*. However, on one occasion *E. coli* was above the limit.

- In plant trials comparing dairy sludge and vermicomposted dairy sludge, the vermicompost provided better growth and yield of radishes. The barley grown in vermicompost had higher grain yields and quality. However the dairy sludge provided heavier grass yields. The result is not conclusive because the vermicompost provided lower grass yields but had more predictable growth. Which might be more useful to farmers. Marigolds grown in dairy sludge had more flowers with larger diameters than the marigolds grown in vermicompost.

- Vermicompost and dairy sludge had properties other than nutrient related that provided superior marigold growth.

- Nutrients provided the improved growth of barley in vermicompost and dairy sludge.

- Improved radish growth property was not related to microbial activity within the vermicompost.
- The high level of nitrate in the vermicompost resulted in a high level of nitrate leaching, which can cause environmental pollution of groundwater and water courses.
- Polyelectrolyte is toxic to earthworms above normal dosage.
- There was a small reduction in sludge weight after vermicomposting.

This study has gone some ways towards enhancing our understanding of vermicomposting dairy sludge. It will serve as a base for future studies on this subject.

Recommendations

This research has thrown up many questions in need of further investigation. It is recommended that further research be undertaken in the following areas:

- Conduct a trial over all seasons in an insulated polytunnel. This would be a better environment to conduct vermicomposting. The advantages to an insulated polytunnel are (1) the use of lights to prevent earthworms migrating out of the bed (2) heating the beds by heating the air (3) maintaining the desired environmental conditions.
- Conduct a large scale trial to determine how long vermicomposting takes and if the earthworm population is sustainable.
- Ammonia levels in the sludge need to be reduced. A bed in the design of a continuous flow reactor would mean the bed is more exposed more to air, preventing ammonia build up in the bottom.
- Measures to prevent rats getting into vermicomposting bed should be taken.
- Investigate changing the carbon to nitrogen ratio to increase the rate of vermicomposting.
- Field crop trial to investigate leaching of nitrate using lysimeters.
- Investigate the use of zeolite to reduced nitrate leaching.
- Investigate markets for vermicompost, where nitrate leaching is not issue.

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APPENDICES

APPENDIX A
SPIKE RECOVERIES 3.4

TABLE A1. Spike Recovery of Nutrient Analysis in Section 3.4

Percentage Recovery		
Potassium	Calcium	Magnesium
98	95	104

TABLE A2. Spike Recovery of Anion Analysis in Section 3.4

	% Recovery			
	Chloride	Nitrate	Phosphate	Sulphate
Week 0	90	90	113	95
Week 0	89	94	91	82
week 3	93	92	102	92
week 6	110	143	90	93
week 9	75	85	108	89
week 14	89	121	97	96
week 14	108	111	89	100

APPENDIX B
SPIKE RECOVERIES 4.0

Table B1. Spike Recovery of Anion

	% Recovery			
	Chloride	Nitrate	Phosphate	Sulphate
Dairy Sludge	90	90	113	95
Dairy Sludge	89	94	91	82
Vermicompost	78	119	96	87
Vermicompost	81	64	110	88
Vermicompost	71	82	81	78
Peat	70	94	89	92
Peat	84	91	87	88

Table B2. Spike Recovery of Calcium, Potassium and Magnesium

	Percentage Recovery		
	Calcium	Potassium	Magnesium
Dairy Sludge	106	100	98
Vermicompost	76	96	105
Peat	100	100	104

APPENDIX C

SPIKE RECOVERIES 3.3

Table C. Spike Recovery of Nutrient Analysis in Determination of Vermicomposted Dairy Sludge by Nutrient Characteristics

	% Recovery of spikes			
	Potassium	**Magnesium**	**Calcium**	**CEC**
Samples	5ppm	5ppm	5ppm	1ppm
Fresh Dairy Sludge A	100	104	95	90
Fresh Dairy Sludge B	100	104	95	90
Fresh Dairy Sludge C	100	104	95	90
Vermicomposted Dairy Sludge A	100	104	95	90
Vermicomposted Dairy Sludge B	100	104	95	90
Vermicomposted Dairy Sludge C	100	104	95	90
Horse Manure Vermicompost	100	104	95	90
Horse Manure Vermicompost	100	104	95	90
Horse Manure Vermicompost	100	104	95	90
Samples	**Chloride**	**Sulphate**	**Nitrate**	**Phosphate**
Fresh Sludge 5ppm	84	166	80	22
Fresh Sludge 5ppm	91	183	80	22
Fresh Sludge 1ppm	68	184	68	33
Fresh Sludge 1ppm	101	169	60	26
Vermicompost A 5ppm	71	-36	-317	33
Horse manure Vermicompost	72	159	80	19

APPENDIX D

SPIKE RECOVERIES 4.13

Table D1. Recovery of spikes

	% Recovery			
	Chloride	Nitrate	Phosphate	Sulphate
Peat	85	92	87	89
Vermicompost	80	126	125	184
Sterilised Vermicompost	99	89	112	105

APPENDIX E
SPIKE RECOVERIES 4.14

Table E1. Recovery of Spikes in Nitrate Analysis

	% Recovery of spike
Vermicompost start of trial	123
Vermicompost end of trial	81
Leachate days 0 - 10	101
Leachate days 11-20	93

APPENDIX F
Radish, Marigold and Barley results

RADISH TRIAL DAY 16 (SECTION 4.7)

After sixteen days growth there was no significant difference (P>0.05) between the dairy sludge and vermicompost treatments for the lengths of tops and wet weights of plants grown in. However, the radishes grown in dairy sludge had significantly (P<0.01) longer roots.

Table F1. Radish trials Day 16

	Control	10%	20%	40%	60%	80%	100%
	Length of Root (cm)						
Vermicompost	5 (3)	5 (1)	5 (2)**	6 (3)	6 (4)**	5 (2)	8 (3)
Dairy sludge	7 (3)	5 (2)	7 (3)	6 (3)	9 (4)	6 (3)	6 (3)
	Length of Tops (cm)						
Vermicompost	5 (5)	7 (7)	8 (8)	8 (8)*	7 (7)	5 (5)	5 (5)
Dairy sludge	6 (6)	7 (7)	7 (7)	6 (6)	7 (7)	6 (6)	6 (6)
	Wet Weight (mg)						
Vermicompost	238 (102)	353 (123)***	432 (93)	499 (162)	419 (229)	448 (200)**	481 (189)**
Dairy sludge	288 (77)	527 (134)	496 (113)	473 (154)	372 (153)	310 (101)	362 (143)

* = P<0.05, ** = P<0.01, *** = P<0.001

MARIGOLDS PLANT GROWTH DAY 30 (SECTION 4.8)

On day 30 the vermicompost treatments had significantly (P<0.001) more leaves, heavier shoot and root wet weights compared to the dairy sludge treatments. The dairy sludge treatment had significantly (P<0.001) taller plant height compared to the vermicompost treatments.

Table F2. Marigold Trial Day 30

	Control	**10%**	**20%**	**40%**	**60%**	**80%**	**100%**
Plant Height Day 30 (cm)							
Dairy sludge	3 (0.3)	4 (0.2)	4 (0.4)	4 (0.4)***	4 (0.4)**	4 (0.7)	4 (0.4)**
Vermicompost	3 (0.4)	4 (0.3)	4 (0.3)	3 (0.4)	3 (0.3)	3 (0.4)	3 (0.2)
Total Leaf Number Day 30							
Dairy sludge	4 (0)	6 (0)	6 (0)***	8 (0)	7 (1)*	7 (1)	7 (1)
Vermicompost	5 (1)	6 (0)	7 (1)	8 (1)	8 (1)	7 (1)	8 (0)
Shoot Weight Day 30 (g)							
Dairy sludge	0.1 (0.02)	0.8 (0.1)	1.1 (0.2)	1.4 (0.3)***	1.4 (0.2)***	0.3 (0.2)***	0.8 (0.1)***
Vermicompost	0.2 (0.04)	0.7 (0.1)	1 (0.1)	2 (0.2)	1.8 (0.3)	1.2 (0.4)	1.5 (0.2)
Root Weight Day 30 (g)							
Dairy sludge	0.1 (0.1)	0.8 (0.2)	0.8 (0.2)	1.1 (0.4)***	1.1 (0.4)	0.3 (0.2)	0.7 (0.3)
Vermicompost	0.2 (0.1)	0.9 (0.1)	0.9 (0.2)	1.5 (0.3)	1.3 (0.4)	0.6 (0.3)	1 (0.3)

* = P<0.05, ** = P<0.01, *** = P<0.001

BARLEY TRIAL SECTION 4.9

Table F3. Number of plants per pot

Vermicompost	**0%**	**10%**	**20%**	**40%**	**60%**	**80%**	**100%**
Rep 1	5	3	6	6	7	3	3
Rep 2	5	8	3	3	7	4	2
Rep 3	4	4	4	4	5	5	2
Average	5	5	4	4	6	4	2
Dairy Sludge	**0%**	**10%**	**20%**	**40%**	**60%**	**80%**	**100%**
Rep 1	5	7	4	6	2	6	6
Rep 2	7	5	3	4	7	5	4
Rep 3	4	7	5	2	5	5	6
Average	5	6	4	4	5	5	5

Table F4. Total Number of barley heads per pot

Vermicompost	0%	10%	20%	40%	60%	80%	100%
Rep 1	2	12	32	32	38	43	51
Rep 2	4	20	22	26	37	34	32
Rep 3	4	15	13	25	28	44	42
Average	3	16	22	28	34	40	42
Dairy Sludge	0%	10%	20%	40%	60%	80%	100%
Rep 1	7	27	53	70	39	46	33
Rep 2	6	34	58	87	140	90	26
Rep 3	4	34	39	24	53	64	48
Average	6	32	50	60	77	67	36

APPENDIX G
CHAPTER THREE

Polytunnel

The polytunnel had an arch-shaped roof with two gable ends and measured 8.5m long, 10 wide and 4m high (Plate 1). The frame consisted of tubular aluminium and was covered with polyethylene. The polytunnel was located on a concrete base in a farmyard that was positioned so that it was not affected by shade from any farm buildings. The entrance consisted of two sliding doors (1 m x 2.3m). The polytunnel had air vents near the apex at each end.

Plate. Polyethylene tunnel at Barroe House Farmyard

Plate. Anti-crawl on edge of bed

Sampling

In order to monitor the progress of the earthworms, six weeks after the introduction to the sludge, a sampling programme was initiated. In order to sample the earthworm population, concentric cores were used. Cores with a large diameter might damage the bed and take longer to sort. It was decided to use a small cores size of 11cm for the first sampling occasion.

Plate. Concentric cores of 11cm, 16cm & 31.5cm diameters

Method of Earthworm sampling

The materials in the cores were emptied out. The earthworms and cocoons were separated out by hand sorting. Then they were examined for clitellum development and weighed.

The worms were weighed without voiding them. It has been reported that the gut content would be approximately 10% of live weight. Corrections for the gut content were not applied to any of the data in this study (Neuhauser *et al.* 1980). The live weight was determined after hand sorting and removal of all extraneous material (Ndegwa and Thompson, 2000).

Earthworm Length

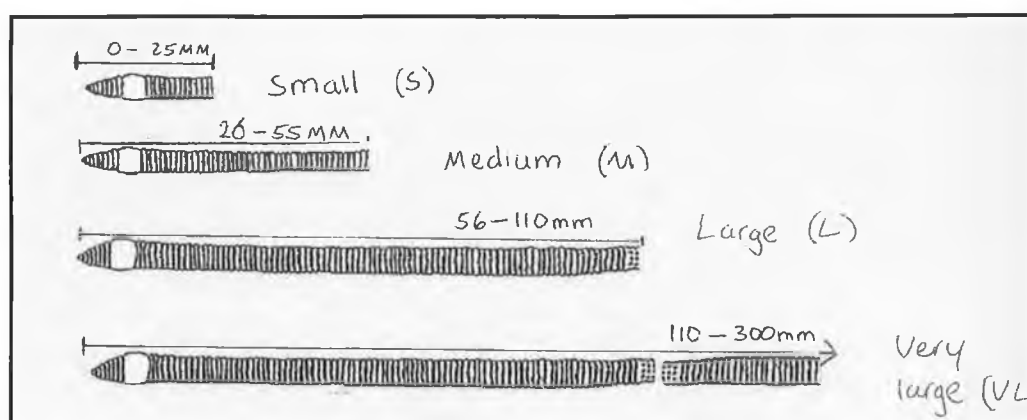Earthworm length was measured by placing earthworms upon the scale shown in figure 7.

Figure. Earthworm length scale

Species Identification

Earthworm species were identified using a dichotomous key by Sims and Gerards (1999)

Methods of sludge parameters

pH and Electrical Conductivity

Determination of pH was done using an 'Orion' pH meter which was calibrated using a buffer solution of pH 7 and pH 4. A 5g sample of sludge was suspended in 45mls of de-ionised water (1/10 w/v) (Atiyeh *et al.* 2000c) in a beaker using a mechanical shaker at 230rpm for 30 minutes. This is a slight modification of the procedure adopted by Erhart and Burian (1997) who used 0.01M $CaCl_2$ solution was used instead of de-ionised water. The pH was measured immediately after suspension.

Electrical conductivity was measured using the same sample (Atiyeh *et al*. 2001) that was used for pH determination. An ORION electrical conductivity meter and probe was used having been calibrated in a 0.01M and 0.001M solution of potassium chloride.

Moisture Content

Crucibles were placed into the muffle furnace for 1 hour at 550°C and cooled in a dessicator. Approximately 10g sludge was placed into a weighed crucible and placed in an oven at 105°C until constant weight was obtained. The final weight was recorded and moisture content calculated (APHA, 1989).

$$\% \text{ Moisture content} = \frac{(A-B) \times 100}{C-B}$$

A= Weight of dried residue and crucible. B= Crucible. C= Weight of wet sample and crucible

Percentage Volatile Solids

Using the same crucibles and samples for moisture analaysis, it was continued on with percentage volatile solids analysis. The samples were placed in a muffle furnace at 550°C for 1 hour, and cooled in a dessicator. The samples were reweighed and loss of weight was calculated % volatile solids (APHA, 1989).

$$\% \text{ Volatile solids} = \frac{(A-D) \times 100}{A-B}$$

A= Weight of dried residue and crucible. B= Crucible. C= Weight of wet sample and crucible. D= weight of residue and crucible after ignition

Ammonia Selective Electrode Method

An 'Orion' ammonia selective electrode (model 95-12) with a hydrophobic gas permeable membrane was used to calculate ammonia. Dissolved ammonia (NH_3 and NH_4^+) is converted to NH_3 by raising the pH to above 11 with a strong base. NH_3 diffuses through the membrane and changes the internal solution pH that is sensed by a pH

electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion selective electrode that serves as a reference electrode (APHA, 1989).

Ultrapure water from a 'Millipore 2' analytical grade water purification system (Millipore, Badford, MA) was used in making all reagents and dilution of samples. All glassware was washed with 5% nitric acid wash and rinsed three times with ultrapure water. A stock ammonium chloride solution was prepared by dissolving 3.819 anhydrous NH_4Cl (dried at 100°C) in ultra pure water and diluted to 1000ml. A series of standards of 1000, 100, 10, 1 and 0.1mg NH_3-N/l was prepared by making dilutions of the stock solution with water. 100ml of each standard was placed in 150ml beaker. The electrode was immersed in the lowest concentration standard and mixed with a magnetic stirrer. The speed of the stirrer was slow, to reduce possible loss of ammonia from the solution. A sufficient amount of $10N$ NaOH solution (usually 1ml) was added to raise the pH above 11. The same stirring rate and temperature was maintained throughout calibration and testing. The electrode was kept in the solution until a stable millivolt reading was obtained. The above procedure was repeated from the lowest to highest concentration standards. Sludge samples were prepared by adding 50g wet sludge into a one litre graduated cylinder and made up the mark with ultra-pure water. The cylinder was then shaken for 30 seconds and the suspension was filter through a 'whatman No.1' filter paper. Standards and samples with $\leq$ 1mg NH_3-N/l, took about 2-3 minutes for millivolt reading to stabilise.

The results were graphed semilogarithmic. The ammonia concentration was plotted in milligrams NH_3-N per litre on the log axis vs. potential in millivolts in the linear axis.

$$\text{mg } NH_3\text{-N/l} = A \times B \times \frac{100 + D}{100 + C}$$

A= Dilution factor
B= Concentration of NH_3-N/l, mg/l, from calibration curve
C= Volume of $10N$ NaOH added to calibration standards, Ml, and
D= Volume of $10N$ NaOH added to sample, ml.

A sub-sample was measured for moisture content and the results were expressed on dry weight basis.

Biological Oxygen Demand (BOD

The dilution water that was used for BOD analysis comprised distilled water which was aerated with a fish pump at 20°C overnight. While the standard BOD method is designed for liquids, in this study a weighed amount of solid sludge was re-suspended into distilled water and made up to one litre in a volumetric glass cyclinder.

Samples of sludge suspensions were placed in BOD glass stopped bottles to the point overflow, and incubated at 20 ± 1°C for five days in the dark. Using a 'Syland' dissolved oxygen meter and probe dissolved oxygen (DO) was measured initially and after incubation. The BOD_5 was calculated from the difference in the initial and final DO. Each BOD determination had to meet an requirement of depletion of at least 2.0mg/l DO and at least 1.0 mg/l residual DO. If the dilution water blank showed more than 0.2 mg/L DO depletion after five days, the results were not used (APHA, 1989).

BOD calculation:

$$BOD_5, mg/kg = \frac{DO_0 - DO_5 \times 1000}{Wet\ sludge\ (g)}$$

$$BOD_5, mg/kg\ Dry\ solids = \frac{BOD_5,\ mg/kg \times 100}{\%\ dry\ solids\quad 1}$$

DO_0 = DO (mg/l) of diluted sample immediately after preparation.
DO_5 = DO (mg/l) of diluted sample after 5 day incubation at 20°C.

Chemical Oxygen Demand (COD)

While the standard COD method is designed for liquids, in this study a weighed amount of solid sludge was re-suspended into distilled water and made up to one litre in a volumetric glass cylinder. The COD 'Hach' method was used in this study (Anon. 1992), using 2ml of samples of sludge suspension in COD reagents. The prepared COD vials (0-15,000 range) were inverted gently to mix the content and placed in into a preheated

reactor for 2 hours at 150°C The reactor was turned off after two hours and allowed to cool for 20 minutes. The vials were then removed and allowed to cool to room temperature. A blank COD vial containing 2ml distilled water was also used. COD results were obtained by reading on a 'HACH DR 2000' spectrophotometer at 620nm which was zeroed using a blank vial.

COD calculation:
COD mg/kg   = COD mg/L*2000

COD mg/kg D.S.   = COD mg/kg
                          % dry solid of sludge

Total kjeldahl nitrogen (TKN)

The method used for determining TKN in the sludge was based on a method by (Bremner, 1965). In this method, 1 gram of air dried sludge (<2mm) was placed in a dry macro-Kjeldahl flask with two kjeldhal catalyst tablets and 25ml of redistilled water used to wash down the walls of the flask. 30ml of concentrated analar grade H_2SO_4 was then added dropwise to the flask in a fumehood. The flask was cautiously heated in the digestion block and when the water was removed and frothing ceased, heat was increased until such time as the digest was clear. The contents were then boiled for 5 hours. After completion of the digestion, the flask was allowed to cool and 50ml of redistilled water was added to the flask. The flask was gently swirled and the cold solution made up to 250ml in a volumetric flask, with redistilled water. The distillation procedure involved adding 10ml of 2% boric acid to a 50ml Erlenmeyer flask and covering with parafilm. The buchi distillation unit was preheated. Then, 25ml of digested sample was pippetted into a clean digestion flask and the walls washed down with 25ml of redistilled water. The digestion flask was attached to a preheated buchi distillation unit and a 50ml Erlenmeyer flask containing 10ml of 2% with boric acid indicator solution was placed under the digestion outlet with the tube resting in the 2% boric acid indicator solution. Fifteen ml of 32% (NaOH) was added to the digested sample and distilled for 5 minutes. Following distillation, the Erlenmeyer flask was removed and the contents titrated with

0.02N HCl until there was colour change from green to wine red. Blanks containing redistilled water were digested and distilled in the same manner as above.

Percentage N in the sample was calculated using the formula;

$$\% \, N = xml \, HCl \quad \frac{0.28 \times 250 \times 100}{1000 \times 25 \times W}$$

W = Weight of sludge (g)

Total Organic Carbon (TOC)

Total carbon consists of inorganic carbon and organic carbon. Inorganic carbon is mostly present as carbonates (e.g. calcite and dolomite). Organic carbon is derived from the decomposition of animal and plants e.g. leaves, branches. The method used for determining TOC is based on the loss on ignition method where the temperature is maintained below 440°C to avoid the destruction of any inorganic carbonates. The destruction of carbonates could lead to an increase sample weight loss and overestimation of the organic matter content (Schumacher, 2002).

The organic carbon (OC) is calculated by dividing the organic matter content by the Van Bemmelen factor of 1.724. This factor is based on the assumption that the organic matter contains 58% organic carbon (Nelson and Sommers, 1982)

10g of predried was placed into a preweighed crucible. The sample was then combusted in a muffle furnace at 430°C overnight (15 hours). The sample was cooled in dessicator and then reweighed.

$$\% \, organic \, matter = \frac{(B-A) \times 100}{B}$$

A= dry weight of sludge before combustion
B= dry weight of sludge after combustion

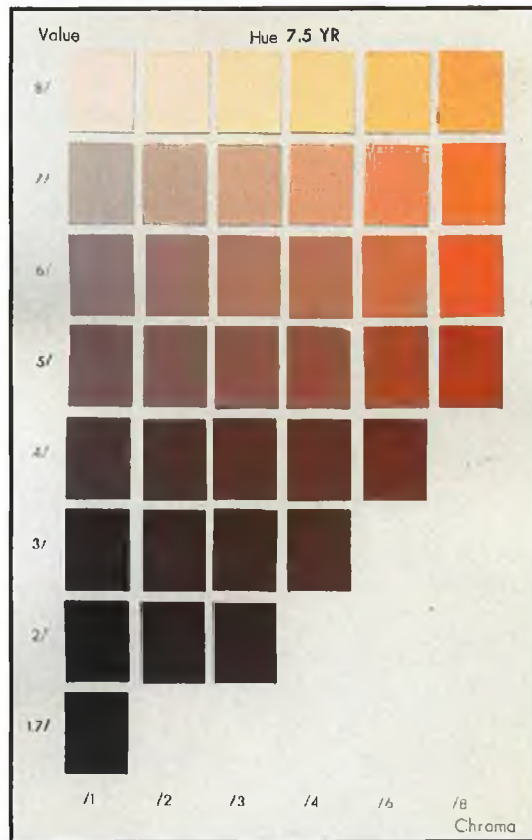% Organic carbon = % organic matter / 1.724

Soil Colour Chart

Figure. Soil Colour Chart (Anon., 1970)

Temperature

The temperature was recorded by three methods

(a) minimum and maximum air thermometer

(b) four temperature probes and datalogger from June to October (Weeks 14 to 31) and

(c) spot readings of the temperature (MT 100 KC Temperature probe) of the bed was recorded in a grid pattern at three different heights in the bed (the top of the bed had temperature readings taken at 7.5cm form the surface, the readings for the middle of the bed were taken 15cm from the surface and the readings for the bottom of the bed were taken 22 cm from the surface) on dates in March, April, May and June. A Grant 1200 series Squirrel datalogger and four temperature probes were obtained in week 14, three probes recorded the temperature of the bed and one recorded the air temperature.
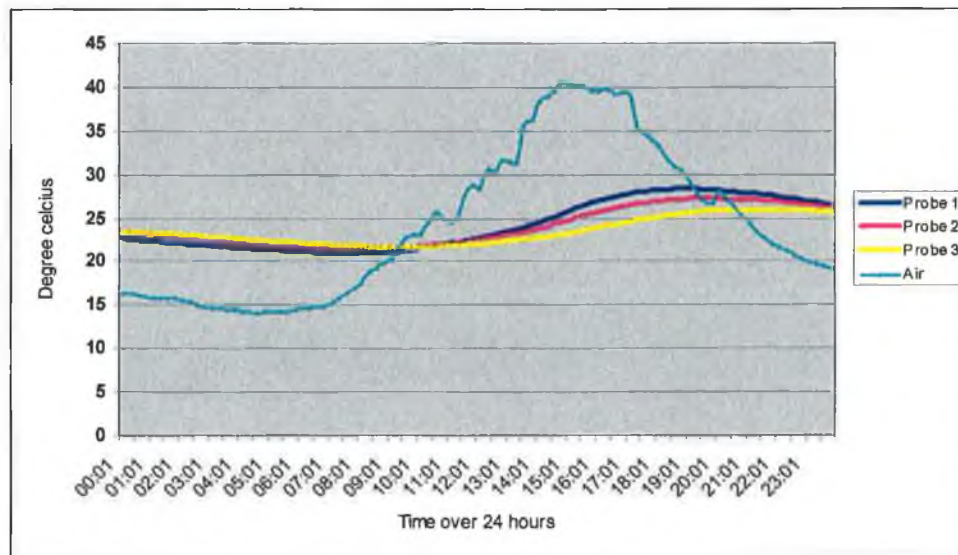
Figure. Temperature over a 24 hours period on 28th June 2003

The Figure above showed that while there was considerable variation in air temperature over 24 hour period 14-40°C, the variation in the temperature of the sludge was considerably less (21-28°C).

Observations Trial 3.2

- After the first 2/3 days approximately 90 worms migrated out of the bed and died. After the first week approximately 100 worms were dead on surface of the bed.

- On the 7th March, green algae were observed on surface of the beds, except where direct sunlight could not reach surface. There is a pattern with the growth of algae and exposure to sunshine.

- On the 28th March, approximately 30 dead earthworms were on the surface of the bed. Usually there were no dead earthworms, except after the initial few days at start of trial.

- On the 1st May, there were thousands of black mites visible on the surface of the bed. Melon skins were placed on the surface to attract the mites to the melon skins. This had limited effect due to the number of mites present. After 1 / 2 weeks the mites were no longer present.

- On the 19th May, it appeared the potworms (enchytræids) had fragmented much on the surface of the bed and had consumed the green algae on the surface.

- On the 26th May, there were a lot of potworms visible on surface of the bed and 7 dead earthworms were found.
- On the 30th May- there was a 'squeshing noise' from the vermicomposting bed. Around the same time the potworms came to the surface of the bed.
- Near the end of the trial there was a large number of springtails and woodlice in the vermicomposting.
- When taking the cores, the height of black colour sludge on the bottom of the bed was lower near the edge of bed. This was possibly due to more air, compared to cores taken in the centre of bed.

Rat infestation
- There was a rat infestation in the bed during the winter and some of the earthworms were eaten. Prevention measures should be taken on large scale trials.

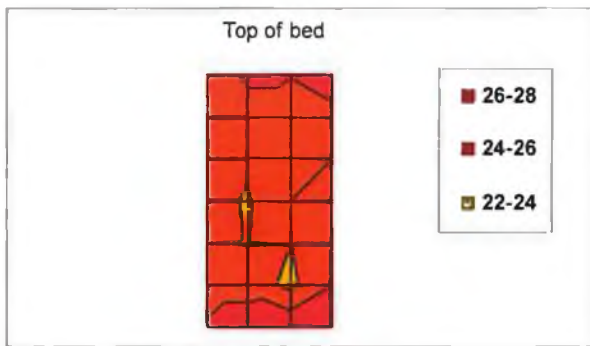**Plate.** Rat holes in vermicomposting bed (red circles are location of rat holes)
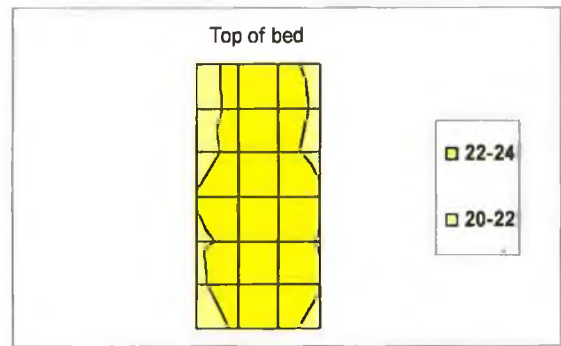

Figure . 9.30pm Top temps (30/5/03)
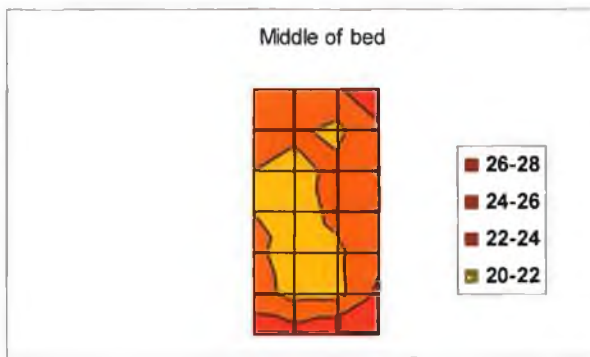

Figure. 9.30am Top temps (1/6/03)

Figure. 9.30pm Middle temps (30/5/03)
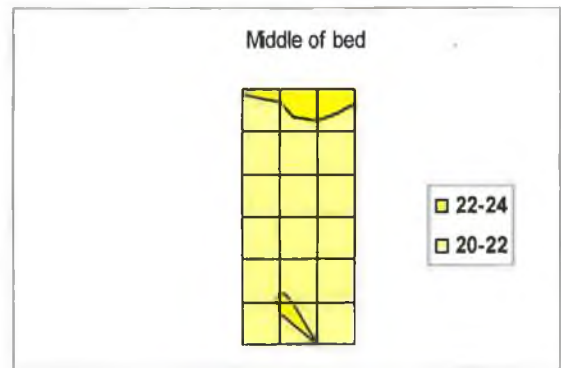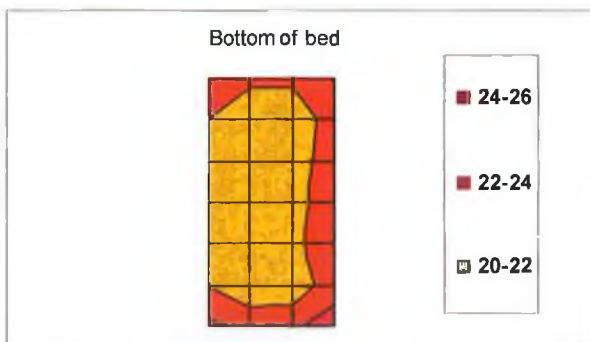

Figure. 9.30am Middle temps (1/6/03)

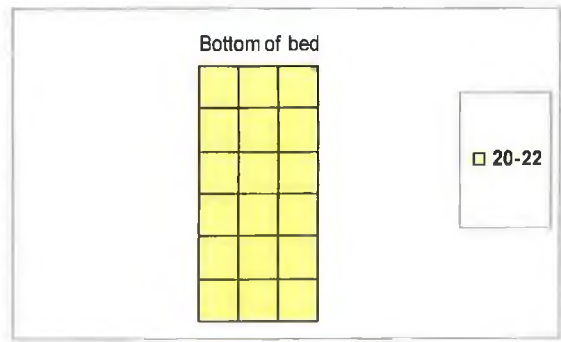**Figure.** 9.30pm Bottom temps (30/5/03)

Figure. 9.30am Bottom temps (1/6/03)

The six figures above were taken at 9.30pm on 30/5/03 as an example of temperatures on a warm day. The air temperature was 23°C. The top of the bed had temperature readings taken at 7.5cm form the surface. The readings for the middle of the bed were taken 15cm from the surface and the readings for the bottom of the bed were taken 22 cm from the

surface. Figures 25-27 were taken at 9.30am on 1/6/03. The 29th and 30th were hot days. At 9.30pm on 30/6/03 there were some earthworms massed together at the bottom of the vermicomposting bed underneath the plywood. There were 10 dead earthworms on the surface of the bed. The top of the bed was the warmest because of direct sunlight. The middle and bottom part of the bed were warmest at the edges and coolest in the centre. On hot days the earthworms try to move to a cooler temperature.

APPENDIX H
Extraction of Earthworms from the vermicomposting bed

The extracting of the earthworms from the vermicompost was investigated by (1) vibration (2) electrical stimulation (3) migration method. The migration method was the most successful in extracting the earthworms from the vermicompost. The methods are described below.

Vibration method

To investigate if earthworms were stimulated to the surface of the bed by vibrations. A series of 10 metal rods were inserted at random into the windrow bed. The rods were then hit with another metal rod to make them vibrate. This method did not encourage any earthworms to the surface of the bed.

Electrical stimulation (Octet method)

The electrical Octet method is the least destructive sampling method used for earthworm population sampling in field conditions. It works by inserting a series of metal rods into the ground in a circle, which are then attached to a large ring on the surface of the ground. The ring is then attached to a control box (DEKA 4000) which sends a series of electrical currents into each of the metal rods (Plate H2). The electrical current stimulates the worms to the surface of the bed, where they can be picked off. Based on knowledge to date, no evidence was found as to whether this method was ever used before to extract earthworms from a windrow bed of vermicomposted dairy sludge. The method was therefore tested on the windrow bed, but unfortunately a very low number of earthworms came to the surface.

Migration method

A migration technique used by Masciandaro *et al.,* (2000), was used to successfully extract the majority of earthworms out of the vermicompost. Earthworms may have the ability to sense fresh food. A belt of fresh aerated sludge was placed around the edge of the vermicompost. Over a period of a week the earthworm migrated into the fresh sludge (Plate H1). The remaining sludge contained small number of earthworms and cocoons, which were removed by manually sorting through the vermicompost. This was very time consuming.
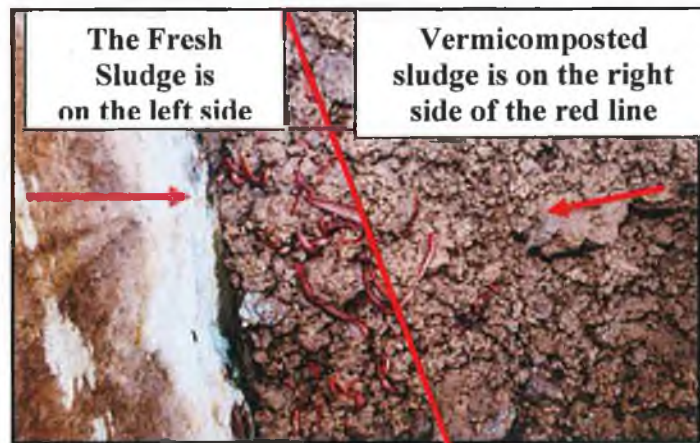

Plate H1. Earthworms concentrated in the fresh aerated sludge

Plate H2. Octet ring on the vermicomposting bed

APPENDIX I
Methods of Nutrient Analysis

Methods

Exchangeable Cations (Calcium, Magnesium and Potassium)

Exchangeable cations were determined using the Ammonium Acetate extraction method (Thomas, 1982). Approximately 10g of air-dried sludge (<2mm sieved) was weighed and transferred into 100ml conical flask. 40ml of 1N ammonium acetate (CH_3COONH_4) was added and covered with parafilm. The flask and contents were swirled and let stand. After one hour the contents were pre-filtered through a Buchner funnel fitted with No. 40 Whatman filter paper before being filtered through a 0.45μm filter. The extracts were then transferred into 100ml volumetric flasks and made up to volume by rinsing the suction flask with 1N ammonium acetate.

Calcium and Magnesium were then determined with a Perkin Elmer 2380 Atomic Absorption Spectrophotometer using an air/acetylene flame. Calcium was determined at wavelength 422.7nm and a calibration curve prepared using standards in the range 0-10 mg/l Ca. Magnesium was determined at wavelength 285.2nm using standards in the range 0-2 mg/l Mg.

The interference of phosphate in magnesium was overcome by the addition of lanthanum (APHA, 1989). Phosphate, sulphate and aluminium interfere in the analysis of calcium, were overcome with the addition of lanthanum solution (US EPA, 1983).

Lanthanum is an example of a releasing agent which preferentially combines with interferent in this case the phosphate ion. This leaves the calcium free to produce a absorption signal. It is common practice to add lanthanum in the form of 10% lanthanum chloride solution.

Potassium was determined using a 'Corning 400' flame photometer. The photometer calibrated using the standards prepared and once calibrated was periodically checked using the 0 and 20ppm standards (0 and 20ppm gave emission readings of 0 and 100 respectively; if they had drifted they were reset). A calibration curve was constructed of emission versus potassium concentration and determined the concentration of exchangeable potassium in the sludge samples. The results were expressed as mg/kg.

Cation Exchange Capacity

Cation exchange capacity was determined using the method described by Chapman (1965). The method involved placing 4g of air dried sludge (<2mm sieved) in a 50ml centrifuge tube, 33mls of 1N (CH_3COONH_4) solution was added. This tube was then mechanically shaken for 5 minutes and centrifuged until the supernatant was clear. The liquid was decanted and the procedure repeated three times. The samples were then washed three times with, 33mls aliquots of 99% isopropyl alcohol. Using the shaking and centrifuging procedure employed previously on the sample, absorbed Na was replaced by means of 33ml aliquots of 1N (CH_3COONH_4) reagent. Each decant was placed in a 100ml volumetric flask and was made up to the mark with CH_3COONH_4 reagent. Sodium was determined using a corning 400 flame photometer with a range of prepared standards. Results were expressed in milliequivalent per 100g of air dried sludge.

Water Soluble Anions

Water soluble inorganic ions of nitrate, phosphate, chloride and sulphate (NO_3^-, Cl^-, PO_4^{3-} and SO_4^{2-}) were determined using 'Dionex 100 Ion Chromatograph'. Samples were injected into a stream of carbonate-bicarbonate eluent and passed through a series of ion exchangers (APHA, 1989).

<u>Method for preparing sludge and Extraction Method</u>

An extraction method described by Chaoui *et al.* (2003) was used. Samples were oven dried at 30°C. The samples were grinded in a food blender. The samples were sieved with a 2mm aperture size sieve. A 5g sample was added to 50ml of extractant (ultra pure water) in conical flask. The conical flask was placed in a reciprocal shaker for 15 min at

200 oscillations min^{-1}. The solution was filtered using a 'Whatman No. 1' filter paper. After which it was filtered again with a smaller 'Pall' 0.45μm filter paper to remove fine particles.

All standards and buffer were made up fresh on day of use with ultra pure water. All chemicals were analar grade. A buffer was prepared by dissolving 0.3816g sodium carbonate (Na_2CO_3) and 0.2856g sodium bicarbonate ($NaHCO_3$) in 2 litres of water. The standards were oven dried for 1 hour at 104°C. A solution of mixed standards (nitrate, chloride, phosphate and sulphate) was prepared by weighting out 0.163g of KNO_3, 0.16482g NaCl, 0.144g KH_2PO_4 and 0.2567g Mg $SO_4.7H_2O$ and made up to 1litre using ultra-pure water. Standards of 5, 10 15 & 20ppm were prepared from the stock solution

Procedure

0.7ml of standards and samples were injected into the ion chromatograph and the peak heights and retention times were recorded on the strip chart recorder. After the last peak (sulphate) had disappeared and the conductivity signal had returned to the base line, another sample was injected. Samples were injected until good reproducibility in results was obtained. The concentration of each anion was calculated from a calibration curve. (APHA, 1989).The results of the blank were subtracted from sample results. The results obtained in mg/l were converted to mg/kg dry weight.

Samples Interferences

Samples were spiked with known standards to check the performance of the ion chromatograph and not all of percentage recoveries were within the accepted recovery range. This may be due to sludge matrix inferences from either (1) transition metals (Cd, Cu, Mn or Zinc) inference forming complexes (2) Aluminium contamination resulting in low phosphate recovery (3) Unexpected recoveries were possible due to the presence of nitrifying/denitrifying microbes rather than any chromatographic resolution problems. Initial analysis of dairy sludge samples by ion chromatography resulted in unacceptable spike recoveries. Acceptable spike recovery is between 80-120% for anions in all matrices when using the AS14 column with the exception of nitrite and nitrate in domestic wastewater sample. However, the unexpected recoveries were possibly due to

sludge matrix inferences from either (1) transition metals (Cd, Cu, Mn or Zinc) inference forming complexes (2) Aluminium contamination resulting in low phosphate recovery (3) Unexpected recoveries were possible due to the presence of nitrifying/denitrifying microbes rather than any chromatographic resolution problems. (Anon, 2001).

Sample Preparation – Matrix Elimination

It is often essential that some sample pre-treatment be done prior to analysis with ion chromatography. This may be filtration or a more complex time consuming chemical matrix elimination step. Complex samples, such as wastewaters and solid leachates, usually need further clean –up of the sample in order to eliminate matrix inferences. Solid phase extraction (SPE) cartridges were used to remove inferences which can affect ion chromatographic analysis (Jackson, 2000). 'Dionex OnGuard® II H' cartridges were used in this study. The cartridge was activated by injecting 10mls of ultrapure water through it. The first 3mls of a sample passed through the cartridge was discarded. One cartridge was used per sample.

Cleaning of the Column

Metal contamination of the column was removed by cleaning the column with 100mM citric acid on a regular basis. This procedure involved reversing the order of the guard column and analytical column in the eluent flow path. The flow rate was reduced to 1ml/minute. The column was rinsed for 15 minutes with ultrapure water before pumping the 100Mm citric acid solution for 60 minutes. The column was futher rinsed with ultrapure water for 15 minutes. The eluent was then pumped over the column for 30 minutes. The guard column was then replaced into the original position.

APPENDIX J
Phytotoxicity Method

The phytotoxicity bioassay is a slight modification of the method described by Zucconi *et al.* (1981). Water extracts (1:5) from substrates were incubated at $25^{\circ}C$ in the dark for 24 hours with cress seeds *Lepidium sativum*.

A sample of 5g of sludge with 25ml of distilled water was placed into a centrifuge tube, covered with parafilm. It was placed in a mechanical shaker for one hour. Then it was centrifuged at 5000rpm for 10 minutes. In a petri dish lined with filter paper, ten cress seeds were placed. There were five replicates for each extract. 1ml of extract or 1ml distilled water (control) was placed on the filter paper in each petri dish. The petri dishes containing the seeds and extracts were incubated at $25°C$ in the dark for 24 hours.

After 24 hours, the germinated seeds (G) were counted and radicle length was measure (L). The germination index was calculated according to the formula GI= (G/GO) x (L/LO) x 100. GO was germination percentage and LO was the radicle growth of the control.

APPENDIX K
Metal and Pathogen Methods

Metals

Metal analysis was carried out by Dr. Noel Casey and Dr. Ted McGowan of Plasmatech Ltd, Institute of Technology, Sligo.

Sludge Samples

The metal analysis was conducted on three separate composite samples obtained from the windrow bed (section 3.2) in the polytunnel on 12th May 2004 after 64 weeks of vermicomposting. Because of the loss of the control windrow bed due to earthworm migration into it, fresh sludge was used. Composite fresh dairy sludge samples were obtained from the pile of sludge at the belt press at Glanbia, Kilkenny on three separate occasions on 21st November and 12th December 2003 and 19th April 2004.

Method

All metals were analysed by Inductively Coupled Plasma Mass Spectrometer (ICPMS), except Iron was analysed by Flame Atomic Absorption Spectrometer (FAAS).

Metal Sample Collection and Preparation

5% nitric acid wash was placed in the plastic sampling containers for one week. The content were emptied and washed with ultrapure water. The plastic sampling trowel was soaked in acid wash for one week, washed with ultrapure water and air dried.

Sample Preparation

Samples were prepared for analysis by placing 1kg of sludge into a pre acid washed glass tray in a carbolite oven at 100°C for 24 hours. After this sub-samples were placed into pre-acid washed porcelain crucibles and placed in a muffle furnace at 200°C for 8 hours. Samples were removed and placed in a dessicator. The samples were then crushed in a pre-acid washed pester and mortar, sieved through a 2mm sieve and stored in pre-acid washed air dried plastic containers.

Method
Digestion Procedure

(1) 0.3g of sample was weighed into a pre-acid washed glass test tubes. (2) 5ml of Romil HNO_3 SPA was added to each test tube, and placed into a heating water in a glass test tube at approximately 90°C on a hot plate situated in a fume hood. (3) 1ml of nitric acid was added to each test tube after approximately 1 hour. (4) 1ml of hydrogen peroxide was then slowly added the each test tube. (5) The digested samples were allowed to cool and were filtered through cellulose membrane filters, using a Millipore filtration apparatus. The apparatus was rinsed with ultra pure water into 50ml volumetric flasks, before being made up to the volume. (6) Three method blanks were carried through the same digestion procedure.

ICP-MS Instrumentation

ICP-MS measurements were made using a VG Elemental PlasmaQuad II Inductively Coupled Plasma Mass Spectrometer. The original PQ1 ICP-MS instrument was upgraded with a high performance ion sampling interface. A microconcentric nebuliser MCN-100 (CETAC Technologies, USA) was used throughout. Optimisation of the instrument was performed using a multielement solution of 100µg l^{-1} Be, Sc, Co, Y, In, Bi.

Reagents and Materials

All reagents were certified high purity trace metal analysis grade or were purified in-house before use. Ultrapure water was used throughout. Multielement standard solution (SPEX CertiPrep, USA) was used to prepare calibration and standard solutions. Single element (Be, Sc, Co, Y, In, Bi) certified standards (SPEX CertiPrep, USA) were used for the preparation of internal standard and spike solutions in 2% nitric acid. This was prepared by dilution of concentrated Nitric acid *(Romil-SpA, UK)*. Ultrapure water from a Millipore 2 analytical grade water purification system (Millipore, Badford, MA), Cellulose nitrate whatman filter paper 0.4μm, Millipore filtration apparatus, Inductively Coupled Plasma Mass Spectrometer (ICPMS) VG Elemental PlasmaQuad II, Flame Atomic Absorption Spectrophotometer (FAAS) Perkin Elmer 560, Carbolite Oven, , Acid washed test tubes, Beaker, Heating plate, Volumetric Flasks (50mls), Acid wash (5% Nitric acid), Romil HNO_3 super purity acid (SPA) 69%, Romil H_2O_2 super purity acid (SPA) 30%.

Pathogens

Fresh dairy sludge samples were analysed by Bord na Móna Horticulture Ltd. for pathogens *Salmonella, Escherichia coli.*

Methods

Table. Methods for pathogen analysis

Microorganisms	Method
Salmonella	TP 11 based on ISO6579:20
E. coli	TP 6 based on ISO 7251

Samples

Sludge samples were collected on 16th December 2004, 27th July and 10th August 2005 from the sludge pile at the belt press at Glanbia. The samples were stored in sterilised bags.

APPENDIX L
Consumption Trials Section 3.8

Plate. 1000g Earthworms dead

Plate. 100g Earthworms- alive

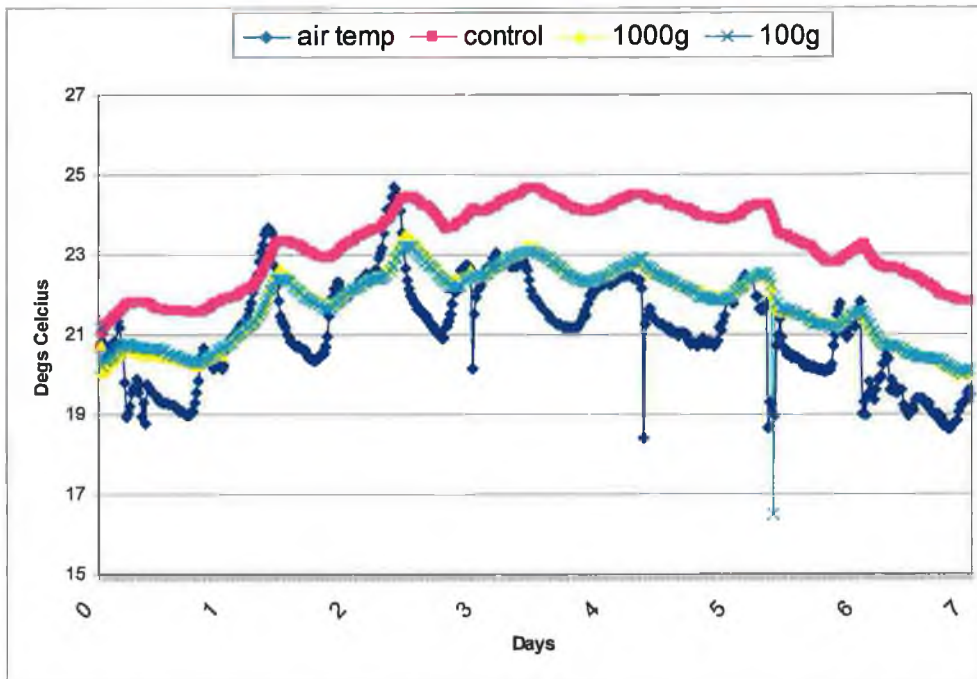**Plate.** 1000g Dead earthworms emptied into a tray

Figure. The temperature in the control sludge, sludge with earthworms (100g and 1000g) and air temperature during the trial.

APPENDIX M   SLUDGE SURVEY

Irish Dairy Wastewater Sludge Survey

Facility Name: _____

1. Do you have a wastewater treatment plant (WWTP) on site for your facility?

   _____

2. If yes, please state type of WWTP (primary, secondary, tertiary).

   _____

3. How many tonnes of dairy wastewater sludge were generated during the period 1ST January to 31st December 2003 at your facility?

   _____

4. What was the dry solid content of the dairy sludge?

   _____

5. During the same period what was the method of disposal?
   A. Landspreading
   B. Landfill
   C. Composting
   D. Lime Stabilsation   _____
   E. Other (please specify)

6. Have you ever carried out trials/research with alternative disposal methods? If yes, please specify.

   _____

Thank You for your Cooperation

Please return to Percy Foster by post with the enclosed envelope, or by fax at 071-9146802

If you would like a copy of the survey results please include your Email address: _____

8th August 2004

RE: Irish Dairy Wastewater Survey

Dear sir/madam,

I'm a M.Sc. research student in I.T. Sligo. My research is assessing vermicomposting as a method to treat dairy wastewater sludge in Ireland. The research is in association with Glanbia, Kilkenny (Paula Neilan), and is funded by the Department of Agriculture and Food under FIRM (Food Institutional Research Measure) and the Environmental Protection Agency ERTDI (Environmental Research Technological Development and Innovation) fund.

As part of my research I would like to get a 'recent picture' of the dairy wastewater generation and method of disposal in Ireland. In order to this I am conducting a survey of dairy processing facilities. If you would like to participate in the survey there is copy enclosed with this letter. The survey will only take a few short minutes to fill in.

No one company will be mentioned in the results, as the results will be expressed in charts, e.g. total amount of sludge generated, percentage of sludge in Ireland landspread, etc. If you would like to get a copy of the survey results please include your email at the bottom of the survey.

By carrying out the survey I hope to get an accurate idea of the dairy wastewater sludge generation in Ireland, this can only be achieved by a high response rate to the survey. I hope you will take part.

Thank you for your cooperation.

Kind Regards

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