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Evaluation of vermicompost from composts for agricultural and horticultural uses

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EVALUATION OF VERMICOMPOST FROM COMPOSTS FOR AGRICULTURAL AND HORTICULTURAL USES

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SUMMARY

The EU landfill directive has imposed a challenging set of targets for the UK to reduce the amount of waste sent to landfill. This has resulted in an increased realisation that wastes can be recycled and reprocessed into valuable products. One such area that is undergoing significant development is the composting of biodegradable waste products. The primary or secondary treatment of wastes by earthworms (vermicomposting) has been proposed as a mechanism to enhance the commercial value of composts. The commercialization of these technologies, however, requires the development of stable markets and consumer confidence in the end products. Currently, in the scientific literature, there are several reports that vermicomposts enhance plant growth; however the mechanism for this enhancement is poorly understood. The first experimental chapter of this thesis presents data from an experiment into the in-vessel co-composting of Green Wastes (GW), Green waste with Paper Pulp (GW/PP), and Green waste with Biosolids (GW/SS) using Ecopod® composting process. It aimed to determine whether compost chemistry and end-use was affected by feedstock quality. Consequently, three feedstock were made by mixing green waste with paper pulp or biosolids (paper I). Vermicomposts were subsequently produced from the three Ecopod® composts. In three separate plant growth trials the presence of vermicompost significantly affected plant growth. However, not all plant species responded in the positive manner previously reported (paper II). In cereal growth, substituting inorganic fertiliser with vermicompost did not decrease yield as long as some inorganic fertiliser was present in the feeding regime. This is true for wheat and maize (paper III, appendix 2). Similarly silage grass responded much better to applications of vermicompost than to conventional composts applied at the same rate (appendix 2). Tomatoes grown in commercial growth media substituted with vermicompost did not respond in the same way as reported in previous studies; no significant yield increases were observed. Few studies report on the effect of growing medium/ fertilising regime on vitamin content of foods. With increasing interest in organic food production systems in particular, it is becoming increasingly important that we understand the effects that growing conditions have on the nutritional properties of foods. In this case there was no effect of growing medium on ascorbic acid (vitamin C) content of tomatoes (paper IV). The final chapter (paper V) was a collaborative work with A.P. Williams and investigates the effect of earthworm digestion on the survival and proliferation of *E. coli* O157 in composts and soil. Litter dwelling earthworms (e.g. *Dendrobaena veneta*) significantly aided the lateral movement of *E. coli* O157 within compost. Our results imply that whilst long-term persistence of *E. coli* O157 in soil and compost may be unaffected by the presence of earthworms, digestion from worms may aid proliferation of the pathogen during initial stages of soil or compost contamination. In summary, this thesis shows that feedstock can be used to manipulate compost product quality. After vermicomposting the plant growth response is often species specific. Our failure to replicate US studies suggests that vermicompost production methods and process management may also affect end product quality. This will hinder commercialisation of the technology. Significant further work is required to identify the method by which plant growth enhancement is facilitated by vermicomposts and to what extent this is specific to a particular vermicompost production method.

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Table of Contents

EVALUATION OF VERMICOMPOST FROM COMPOSTS FOR AGRICULTURAL AND HORTICULTURAL USES i

ACKNOWLEDGEMENTS. iii

List of Figures xiv

List of Tables xviii

List of Abbreviations xx

CHAPTER 1: INTRODUCTION 1

1.1 General introduction and the need for research 1

1.2 Plan of thesis 2

1.3 Aims and Objectives 4

CHAPTER 2: LITERATURE REVIEW 6

2.1 INTRODUCTION 7

2.1.1 The Composting Process 9

2.2 THERMOPHILIC COMPOSTING 10

2.2.1 Experimental Equipment 10

2.2.2 Microbiology 11

2.2.3 Pathogen Reduction 15

2.2.3.1 Sources 16

2.2.3.2 Human pathogens 16

2.2.3.3 Animal Pathogens 18

2.2.4 Chemical and Physical Changes 20
2.2.4.1 Nitrogen 21
2.2.4.2 Phosphate 23
2.2.4.3 Pollutants.....24
2.2.4.3.1 Heavy metals.....24
2.2.4.3.2 Organic Pollutants.....25
2.3 VERMICOMPOSTING.....26
2.3.1 Effect of ingestion on organic matter.....27
2.3.2 Microbiological.....27
2.3.2.1 Pathogen reduction.....28
2.3.3 Chemical and Physical changes 29
2.3.3.1 Nitrogen 30
2.3.3.2 Phosphorus.....32
2.3.4 Pollutants.....33
2.3.4.1 Heavy Metals 33
2.3.4.2 Organic Pollutants.....34
2.4.1 Soil 35
2.4.1.1 Physical 35
2.4.1.2 Chemical and Biological.....36
2.4.2 Plant growth 39
2.4.3 Plant health.....42
2.5 SUMMARY 44
2.6 REFERENCES: 45

CHAPTER 3: BACKGROUND MATERIAL AND EXPERIMENTAL METHODS.....65

3.1 ARTICLE 1. IN-VESSEL CO-COMPOSTING OF GREEN WASTE WITH BIOSOLIDS AND PAPER WASTE 66

3.1.1 Experimental Design.....66

3.1.2 Sampling strategy.....67

3.1.3 The EcoPOD® Composting System.....67

3.2 ARTICLE II. RESPONSE OF COMMON POT GROWN FLOWER SPECIES TO PLANT GROWTH MEDIA SUBSTITUTED WITH VERMICOMPOST.....70

3.2.1 Vermicompost production 70

3.2.2 Choosing Flower Species.....71

3.3 ARTICLE III YIELD RESPONSES OF WHEAT (Triticum aestivum) TO VERMICOMPOST APPLICATION 73

3.4 ARTICLE IV. YIELD AND VITAMIN CONTENT OF TOMATOES (Lycopersicon esculentum) GROWN IN VERMICOMPSTED MANURES.....74

3.4.1 Identifying marketable fruits.....74

3.5 ARTICLE V. EARTHWORMS AS VECTORS OF ESCHERICHIA COLI O157:H7 IN SOIL AND VERMICOMPOST.....76

3.5.1 Keeping earthworms in experimental beds.....76

3.6 EXPERIMENTAL METHODS.....78

3.6.1 Moisture Content 78

3.6.2 Ph and Electrical Conductivity (EC).....78

3.6.3 Extractions 78

3.6.5 Total Dissolved Organic and Inorganic Carbon 79

3.6.5.1 Total Dissolved Carbon:.....79

3.6.5.2 Inorganic Carbon (IC) 79

3.6.5.3 Total Organic Carbon.....80

3.6.6 Total Dissolved Nitrogen (Tdn), Inorganic Nitrogen (Din) and Dissolved Organic Nitrogen (Don).....80

3.6.6.1 Total Nitrogen (TN) 80

3.6.6.2 Total Inorganic Nitrogen (TIN).....81

3.6.6.3 UV-Visible Spectroscopy.....81

3.6.6.4 Nitrate NO_3^-.....81

Automated method 81

Manual method.....82

3.6.6.5 Ammonium NH_4^+ 83

Automated method 83

Manual method.....84

Dissolved Organic Nitrogen 85

3.6.7 Phosphate 85

3.6.8 Potassium, Sodium and Calcium 86

3.6.9 Statistical Analyses 87

3.6.9.1 One way Analysis of Variance (ANOVA).....88

3.6.9.2 Two way ANOVA.....88

3.6.9.3 Experimental Design 89

3.6.10 REFERENCES 90

CHAPTER 4: In-vessel Co-composting of Green Waste with Biosolid and Paper Waste 92

4.1 ABSTRACT 94

4.2 INTRODUCTION 94

4.3 MATERIALS AND METHODS 4.3.1 Composting Process 98

4.3.2 Feedstocks 99

4.3.3 Sampling Strategy 101

4.3.4 Chemical Analysis 101

4.3.5 Statistical Analysis 102

4.4 RESULTS 103

4.4.1 Visual Observations 103

4.4.2 Spatial Variability During Active Composting 104

4.4.3 Soluble Inorganic N 109

4.4.4 Compost Maturation 110

4.4.5 Leachate Production During Maturation 111

4.4.6 Spatial and Temporal Dynamics of Other Nutrients and Metals 113

4.5 DISCUSSION 117

4.5.1 Success of Mixed Waste Composting 117

4.5.2 Nitrogen Dynamics 118

4.5.3 Covering Maturing Composts 119

4.5.4 Potentially Toxic Elements (PTEs).....120

4.6 CONCLUSIONS.....121

4.7 REFERENCES 122

CHAPTER 5: Responses of Common Pot Grown Flower Species to Commercial Plant Growth Media Substituted with Vermicomposts.

.....127

5.1 ABSTRACT.....129

5.2 INTRODUCTION 130

5.3 MATERIALS AND METHODS.....133

5.3.1 Composts.....133

5.3.2 Growing media.....133

5.3.3 Germination experiment 134

5.3.4 Plant growth experiment.....135

5.3.5 Compost analyses.....135

5.3.6 Statistical analysis.....136

5.4 RESULTS 137

5.4.1 Germination 137

5.4.2 Plant Growth 138

5.4.3 Flowering and Seed Production.....141

5.5 DISCUSSION.....142

5.6 CONCLUSION.....145

5.7 REFERENCES 145

CHAPTER 6: Yield Responses of Wheat (Triticum aestivum) to Vermicompost Applications. 151

6.1 ABSTRACT 153

6.2 INTRODUCTION 153

6.3 MATERIALS and METHODS 156

6.3.1 Site and Vermicompost Production 156

6.3.2 Glasshouse Experiments 157

6.3.3 Winter Wheat Field Experiment 158

6.3.4 Chemical Analyses 160

6.3.5 Cost benefit analysis 160

6.3.6 Statistical Analysis 161

6.4 RESULTS 161

6.4.1 Climate 161

6.4.2 Glasshouse Lysimeter Experiments 162

6.4.3 Wheat Yield in Glasshouse Lysimeters 165

6.4.4 Field Experiments 167

6.4.5 Wheat Yield in Field Experiments 168

6.5 DISCUSSION 174

6.6 CONCLUSION 177

6.7 REFERENCES 178

CHAPTER 7: Yield and Vitamin Content of Tomatoes (Lycopersicon esculentum) Grown in Vermicomposted Wastes 186

7.1 ABSTRACT 188

7.2 INTRODUCTION 189

7.3 MATERIAL AND METHODS 191

7.3.1 Growing media 191

7.3.2 Germination experiment 191

7.3.3 Plant growth experiment 192

7.3.4 Compost Analysis 192

7.3.5 Vitamin analysis 193

7.3.6. Statistical analysis 196

7.4. RESULTS 196

7.4.1 Germination and plant growth 196

7.4.2 Fruit yield and quality 197

7.5 DISCUSSION 200

7.6 CONCLUSIONS 205

7.7 REFERENCES 206

CHAPTER 8: Earthworms as Vectors of Escherichia coli O157:H7 in Soil and Vermicomposts 211

8.1 DECLARATION 212

8.2 ABSTRACT 214

8.3 INTRODUCTION 215

8.4 MATERIALS AND METHODS.....217

8.4.3 Preparation of boxes and cores.....220

8.4.4 Preparation of E. coli O157:H7 inoculum.....221

8.4.5 Preparation and application of spiked manure.....221

8.4.6 Harvests.....222

8.4.7 Determination of E. coli O157:H7 numbers on and within earthworm tissue 224

8.4.8 Total microbial activity.....225

8.4.9 Data analysis .....:.....225

8.5 RESULTS 226

8.5.2 Survival and lateral movement of E. coli O157:H7.....227

8.5.2.2 Compost.....227

8.5.3 Survival and vertical movement of E. coli O157:H7 in soil.....230

8.5.4 E. coli O157:H7 numbers on and within earthworm tissue.....233

8.5.5 Total microbial activity.....234

8.6 DISCUSSION.....236

8.7 REFERENCES 240

CHAPTER 9.....249

GENERAL DISCUSSION 249

9.1 Discussion of Results.....249

9.2 Further work.....254

APPENDICES.....259

APPENDIX I 260

I.I COMPOSTING in UK WASTE MANAGEMENT POLICY 260

I.I.I Commercial Composting Methods 261

I.I.I.I Windrow 262

I.I.I.II Static Pile 262

I.I.I.III In-Vessel 263

I.I.II Vermicomposting methods 264

I.I.II.I Feeding rates and stocking densities 265

I.II COMPOST APPLICATIONS and USES 265

I.II.I Other uses 266

I.III ENVIRONMENTAL BENEFITS 266

I.IV CURRENT PRACTICES and FUTURE TARGETS. 268

I.IV.I Current state of waste management 268

I.V MATERIALS, OPERATORS and SITES 270

I.V.I Sources 270

I.V.II Collection methods 271

I.V.III Sites 271

I.VI PROCESS TYPES IN USE IN THE UK 272

I.VII RECENT TRENDS 275

I.VIII FUTURE TARGETS 278

I.VIII.II Assuring end product quality 280

Appendix 2 285

List of Figures

Figure 2.1	Schematic of the composting process	10
Figure 2.2	Basic processes of the composting process	13
Figure 2.3	Diagramatic representation of the role of composting in N cycling	22
Figure 2.4	Detail of upper digestive tract of earthworms	27
Figure 2.5	Changes to concentrations of total nitrogen of vermicomposts and composts	31
Figure 2.6a	CO_2 evolution rates during incubation at 10 °C of unamended soil soil amended with anaerobically stabilized sludge and compost	37
Figure 2.6b	Inorganic N during incubation at ambient temperature c unamended soil, soil and anaerobically digested sludge, and compost.	37
Figure 2.7	Yield and size of tomato fruits produced in a standard commercial potting medium substituted with different concentrations of pig manure vermicomposts.	40
Figure 2.8	Shoot weight and height of marigold seedlings grown in Metro-Mi 360 substituted with different concentrations of pig manu vermicompost	41
Figure 2.9	Pest suppression by vermicompost	44
Figure 3.1	Experimental design of EcoPOD	66
Figure 3.2	Filling the CT5 with green waste	69
Figure 3.3	Experimental EcoPOD at Henfaes research centre	69
Figure 3.4	Covered, maturing green waste	70
Figure 3.5A	Experimental worm bed	71
Figure 3.5B	Hand-sorting earthworms from vermicompost	71
Figure 3.6	Sunflowers (*Helianthus annua)*	72
Figure 3.7	Cosmos (*Cosmos bipinnatus)*	72
Figure 3.8	Californian Poppy (*eschscholzia californica)*	73
Figure 3.9	Wheat growth trials at Henfaes Research Station (Spring 2005 showing vermicompost application plots. Darkest plots are 30t ha equivalent of vermicompost application.	74

Figure 3.10	Common physiological fruit disorders of tomato, A. Blossom End Rot; B. Cat Facing; C. Cracking www.uga.edu/vegetabl/tomato.html).	75
Figure 3.11	A. Experimental vermicomposting beds showing feeding strip of manure and several *D. veneta* earthworms. B. Opened soil core prior to sampling, earthworms were fed from the top of the core (top of the picture).	77
Figure 3.12	Identifying outliers in final *Helianthus* length using Box Plots. Outlier is highlighted by red circle	88
Figure 3.13	Temperature gradients within greenhouse	89
Figure 4.1	Temperature profiles during the in-vessel composting of different waste mixtures.	105
Figure 4.2	pH profiles during the in-vessel composting of different wast mixtures	106
Figure 4.3	Changes in NO_3^- and NH_4^+ during the in-vessel composting of different waste mixtures	110
Figure 4.4	NO_3^- in leachate from maturing compost piles derived from different waste mixtures	113
Figure 4.5	Available Zn concentration in the composts derived from different waste mixtures during the active and maturation composting phase	115
Figure 4.6	Changes in total heavy metals concentrations (Zn, Cu and Pb) in composts derived from different waste mixtures during the active and maturation composting phases.	116
Figure 5.1	Percentage germination of *Helianthus annuus, Cosmos bipinnatus* and *Eschscholzia californica* grown in commercial peat based potting compost substituted with vermicomposted green waste.	137

Figure 5.2	Plant growth rates measured at 31 days after transplanting, and 83 days after transplanting into commercial peat based compos substituted with green waste derived vermicompost for *Helianthu annuus* and *Cosmos bipinnatus.*	139
Figure 5.3	Final shoot lengths and above ground biomass of *Helianthus annuus, Cosmos bipinnatus* and *Eschscholzia californica* grown in commercial potting compost substituted with green waste derived vermicompost	140
Figure 5.4	Flowering responses of *Cosmos bipinnatus* and *Eschscholzia californica* to commercial peat based potting composts substituted with green waste derived vermicompost	142
Figure 6.1A	Height and chlorophyll pigment development of field grown wheat	164
Figure 6.1B	Height and chlorophyll pigment development of glasshouse grown Wheat	164
Figure 6.2	Yield (Mg ha^{-1}) of glasshouse grown wheat	165
Figure 6.3	Regression analysis of glasshouse grown winter wheat properties	167
Figure 6.4	Yield (Mg ha^{-1}) of field grown winter wheat	169
Figure 6.5	Regression analysis of field grown winter wheat properties	171
Figure 7.1	Germination of tomato seeds grown in a commercial growth medium substituted with four different vermicomposts at rates	196
Figure 7.2	Mean fruit yield of tomatoes grown in a commercial peat-based growth medium substituted with four different vermicomposts a rates	197
Figure 7.3	Percentage of marketable tomato fruit after growth in a commercial peat-based growth medium substituted with four differer vermicomposts	198
Figure 7.4	Ascorbic acid content of tomatoes grown in a commercial peat-based growth medium substituted with vermicompost	199
Figure 8.1	Experimental design of vermicomposting and soil boxes.	222

Figure 8.2	Experimental design of soil cores.	223
Figure 8.3	Lateral movement of *E. coli* O157:H7 by the earthworm *D. veneta* in actively vermicomposting cattle manure	229
Figure 8.4	Vertical movement of *E. coli* O157:H7 by the earthworm *L. terrestris* in soil	232
Figure 8.5	Distribution of *E. coli* O157:H7 in the earthworm *D. veneta* after feeding on contaminated cattle manure	233
Figure 8.6	Mineralization of ^{14}C-Glucose in soil, compost, manure, *L. terrestris* earthworm-digested soil, and *D. veneta* earthworm-digested compost	235
Figure 8.7	Mean survival of *E. coli* O157:H7 over 21 d in soil cores and in compost boxes	236
Figure I	Schematic of the overall composting process	262
Figure II	Static pile composting	263
Figure III	In-vessel composting process	264
Figure IV	Distribution of composted products 2003/2004	266
Figure V	Greenhouse gas fluxes from composting and AD of putrescibl wastes	267
Figure VI	Distribution of waste produced per capita in the UK	269
Figure VII	UK waste composted per capita	269
Figure VIII	Percentage of centralised composting schemes by authority type	273
Figure IX	The increase in composting sites in the UK	275
Figure X	Growth in material composted in the UK	276
Figure XI	Municipal waste management in England: 1996/97 to 2000/01	277
Figure XII	Household waste and recycling in England: 1983/84 – 2000/01	277
Figure XIII	Grassland biomass response to vermicompost application.	285
Figure XIV	Yield of Maize (*Zea mais*) in response to vermicompost applicatior	286
Figure XV	Radish root weight in response to compost and vermicompost application.	288

List of Tables

Table 2.1	Comparison of the heavy metal content in the four main fractions of biowaste	25
Table 2.2	Changes in sludge properties before and after vermidigestion	30
Table 2.3	Changes in the amounts of organic and inorganic P in municipal waste subjected to windrow composting and vermicomposting.	32
Table 2.4	Comparison of metal concentrations found in un-digested and vermidigested sewage	34
Table 2.5	Effects of compost application on selected soil chemical and biological properties in long term field experiment	38
Table 4.1	Characteristics of the raw feedstock mixes used in the composting Trials	100
Table 4.2	Chemical properties of the covered and uncovered waste-derived composts at the end of their maturation phase	108
Table 5.1	Chemical properties of the conventional peat-based compost (Humax®), the green waste derived vermicompost and the sand	134
Table 5.2	Plant growth responses of Eschscholzia to a commercial peat based compost (Humax®) substituted with different amounts of vermicomposted green waste	141
Table 6.1	Chemical properties of green waste/ biosolids derived vermicompost, lysimeters and field soil	157
Table 6.2	Monthly averages of temperature, rainfall and sunlight hours with comparison of long-term means for the field site at Henfaes Research Station, Gwynedd, UK	162
Table 6.3a	Summary budget of fixed costs, variable costs, gross crop value and relative net economic benefit of vermicompost application excluding fertilizer application to winter wheat	172
Table 6.3b	Summary budget of fixed costs, variable costs, gross crop value and relative net economic benefit of vermicompost application including fertilizer application to winter wheat	172
Table 7.1	Chemical properties of the commercial peat-based growth medium	194

and four vermicomposts prior to planting

Table 8.1 Chemical and microbiological properties of soil, compost and manure 218

Table 8.2 Statistical comparisons of *E. coli* O157:H7 numbers (log_{10} CFU g^{-1}) in earthworm-digested compost in comparison with control 230

Table 8.3 Statistical comparisons of *E. coli* O157:H7 numbers (log_{10} CFU g^{-1}) in earthworm digested soil in comparison with control 231

Table 8.4 Variability of microbial mineralization of ^{14}C-Glucose 235

Table I Management of BMW in selected EU countries 260

Table II Compostable waste collected through different schemes for household wastes 270

Table III Composting site types, 1999 272

Table IV Type of composting process, all site types 274

List of Abbreviations

ABPO	Animal Byproducts Order
BS	Biosolid
BSE	Bovine Spongiform Encephalopathy (Mad Cow Disease)
DEFRA	Dept. of the Environment, Food and Rural Affairs (UK)
DIN	Dissolved Inorganic Nitrogen
DON	Dissolved Organic Nitrogen
EA	Environment Agency (UK)
EPA	Environmental Protection Agency (USA)
EU	European Union
FMDV	Foot and Mouth Disease Virus
GW	Green Waste
GW/BS	Green Waste and Biosolid
GW/PP	Green Waste and Paper Pulp
IC	Inorganic Carbon
NAW	National Assembly for Wales
PP	Paper Pulp
PTE	Potentially Toxic Elements
TC	Total Carbon
TDN	Total Dissolved Nitrogen
TN	Total Nitrogen
TSE	Transmittable Spongiform Encephalopathic Organisms
UK	United Kingdom
USA	United States ofAmerica

CHAPTER 1: INTRODUCTION

1.1 GENERAL INTRODUCTION AND THE NEED FOR RESEARCH

The UK has traditionally relied on landfill as the primary method of waste disposal. However, recent introduction of EU legislation (EC/31/1999) sets challenging targets for reducing the amount of waste the UK sends to landfill. Composting of biodegradable waste is increasingly recognized as playing a key role in attaining these targets. Although the Composting Association has shown that the number of composting facilities in the UK has risen by nearly 50% since 2002, the 1.97 million tonnes of garden waste composted in 2003/2004 represents only 20% of the estimated total of UK household garden waste. At present, the composting industry is focused on the recycling of green waste, however, as yet there is almost no composting of the estimated 6 million tonnes of kitchen waste and this remains an untapped resource (Slater et al., 2005; Spencer 2005).

The basic characteristics of the thermophilic composting processes are well understood. It is known to be an aerobic process mediated by a suite of microorganisms, the community dynamics of which change with increasing and decreasing temperature. Plant nutrient dynamics, in particular losses of nitrogen (N) is of concern. N is primarily lost during the thermophilic stage when high temperatures and somewhat alkaline conditions encourage the proliferation of ammonifying microorganisms and NH_3 volatilization. There is a need to identify strategies for minimising N losses either by manipulating the feedstock or the composting conditions.

Much less is understood regarding the processes involved in the breakdown of organic materials by earthworms (vermicomposting). Until recently this has been a process adopted, on a small scale, by many householders to recycle organic wastes. In the near future, it is likely to become a secondary process for adding value to conventional composts; moreover, increasing volumes of vermicompost originating from the earthworm breeding process are being sold to amateur gardeners and professional horticulturalists. Much of the research is industry driven and concentrates on the development of end uses in order to consolidate an emerging market.

Vermicompost induced plant growth and yield enhancement have been reported by scientists in the USA. It is often used as a marketing tool but sometimes misreported outside of the scientific community. However, in order to maintain consumer confidence and allow industry expansion, there is a need to further our understanding of plant growth responses to vermicompost and to do this in a rigorous scientific manner. In a similar vein, only one study to date reports on pathogen reduction in vermicomposts. There is an urgent need to elaborate on this and report on more specific human pathogenic species.

1.2 PLAN OF THESIS

This research was funded by the company Organic Resource Management Ltd. (ORM). The company is active in two key areas namely, selling commercial composting equipment namely a static pile, forced aeration in-vessel system Ecopod® and providing an advice and consultancy service for those setting up Ecopod® composting sites. ORM have also developed a network of earthworm breeders. The

company acts as a hub for these breeders, providing advice and support and marketing earthworms on the breeder's behalf.

The remainder of this thesis is divided into eight chapters; it starts with a literature review of the current position of compost and vermicompost research. This illustrates current understanding of the composting and vermicomposting process; the response of soil to compost amendment and to plant growth responses both in small pot scale and larger field scale environments.

The thesis is presented as 5 separate scientific papers, some repetition of introductory material occurs but unavoidable when preparing a thesis of this type.

Chapter 3 introduces some background material that is not included in the five research papers

Chapter 4 describes an investigation into producing composts of differing nutrient status by manipulating initial feedstock proportions. Three compost types were produced; low N status green waste and paper pulp (GW/PP), high N status green waste and biosolid (GW/SS) and medium N status green waste (GW). Compost nutrient changes were monitored during active composting and a comparison of compost management options during maturation was made.

These composts were subjected to earthworm ingestion, and chapter 5 reports on growth responses of three flower species to vermicompost addition.

Chapter 6 reports on responses of wheat (*Triticum aestivum*) to applications of vermicomposted GW/SS both in greenhouse and field scale trials. The potential for substitution of inorganic with vermicompost was investigated.

The public are increasingly concerned about large scale food production. Consumption of organically produced food is increasingly popular. Previous US studies have reported enhanced plant growth and yield of tomatoes when grown in growing media containing low percentages of vermicomposts. Chapter 7 was designed to study the effect of UK produced vermicomposts on tomatoes. In addition, we also studied tomato vitamin C content in response to changes in growth media.

The final experiment reported here is a collaborative study which reports on the persistence and dispersal of *E. coli* O157:H7 by earthworms in soil and vermicompost.

Chapter 9 includes a general discussion of results from previous experimental chapters. Conclusions are drawn and areas of further work identified. Appendices include a review of the role of composting in UK waste management policy; a more in depth explanation of common experimental methods adopted in the experimental chapters. Finally, appendix 2 consists of a presentation of a selection of results that are not included in the experimental chapters.

1.3 AIMS AND OBJECTIVES

The aims and objectives of this research were agreed with the funding company prior to the commencement of the study period. These were:

- To assess the potential of customising compost nutrient content to satisfy a range of uses and to identify any potential spatial variability that might occur in the Ecopod composting system (Chapter 4).

- Identify plant growth responses of a broad range of agricultural and horticultural crops to waste derived vermicomposts (Chapter 5, 6, 7).
- Determine to what extent inorganic fertiliser can be replaced by vermicomposts without reducing final yield (Chapter 6).
- Confirm that reported plant growth responses to US produced vermicomposts is attributable to similar products produced by different methods in the UK (Chapter 7)
- Examine the potential for human pathogen dispersal during the vermicomposting process (Chapter 8).

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CHAPTER 2 LITERATURE REVIEW

2.1 INTRODUCTION

In the UK today, waste management has an unrivalled political profile (Read, 2001), with increasing pressure on waste management bodies to reduce the volume of wastes sent to landfill sites from both public and legal domains. New legislation in the form of the European Landfill directive (1999/31/EC, 1999), and a commitment to reduce greenhouse gas emissions is now forcing waste management bodies throughout Europe to consider alternative methods of managing biodegradable wastes.

Each person in the UK generates about 0.42 tonnes of waste p.a., with an estimated growth of 3% p.a., this quantity is expected to double in 20 years (Read, 2001). In order to achieve landfill reduction targets set by the EU landfill directive*, large volumes of waste will need to be diverted from landfill to recycling or composting for treatment or recovery in other ways. "Assuming 60% of municipal waste is biodegradable, the UK needs to divert at least: 3.2 million tonnes of biodegradable municipal waste each year to meet the first target; 7.5 million tonnes of biodegradable municipal waste each year to meet the second target; and 10.1 million tonnes of biodegradable municipal waste each year to meet the third target", (Letsrecycle undated). In addition alternative routes need to be cost effective and the end product marketable in order to be a realistic proposition for both local authorities and commercial waste management companies alike.

* EU landfill directive targets: reduce waste sent to landfill to 75% of total waste produced in 1995 by 2010, 50% of total waste produced in 1995 by 2013 and 35% of total waste produced in 1995 by 2020

Composting can be an important element of sustainable waste management for the UK and will have a significant role to play in meeting the UK's landfill directive obligations, (Slater and Frederickson, 2001). The Composting Association survey and compile an annual report on "The State of Composting in the UK" (Slater et al, 2005). In summary, for the period 2003/4, a total of 2Mt of wastes were composted and 87% of this was in centralised sites; although significant growth in on-farm composting was recorded in the same period. 95% of centralised sites are operated by waste management companies, processing 2000 – 5000 tonnes per year, and dedicated composting companies, processing 5000 – 10,000 tonnes annually. Increasingly common in rural areas are small on-farm sites, 85% of which are run by agricultural operators, these process on average < 1000 tonnes. Since food waste composting is tightly regulated; at the time of writing, no licensed sites exist in the UK, although there are several engaged in the licensing process; the industry is largely dependent on green waste composting. However, the industry is in its infancy and it will be some time before its presence will influence the data presented in reports such as this. For the UK to meet its EU Landfill directive targets, this sector of the industry needs rapid development. At present, regulatory requirements are hampering this growth and unless this is addressed it is probable that the UK will fail to meet its Landfill Directive obligations.

Agriculture is the largest and fastest growing market sector for composted products (40%) followed by landscape restoration and landfill engineering, (24%). The remainder is sold to professional and amateur gardeners, landscaping and grounds maintenance.

Digestion by earthworms (vermicomposting) is increasingly seen as a low cost method of treating organic wastes; although at present, it is conducted on a much

smaller scale than thermophilic methods. Most vermicompost that is marketed in the UK today is a by-product of earthworm breeding. In comparison to conventional composts it is marketed at the high value, amateur and professional horticulture sector.

An in depth review of the development and adoption of composting as a waste management strategy in the UK is included in Appendix 1.

2.1.1 The Composting Process.

Composting is a biological, aerobic process that uses naturally occurring micro-organisms to convert biodegradable organic matter into a humus like product (Imbeah, 1998). Its primary use in waste management is as an alternative to landfilling, producing a valuable product that can be reused as a slow release fertiliser on agricultural, horticultural land and amenity sites. The emphasis on aerobic composting of organic wastes has led to an increasing interest in understanding the microbial processes that result in the conversion of wastes into organic manure rich in humic substances and plant nutrients (Sharma et al., 1999). The microbial process changes many of the fundamental properties of the wastes and many studies have reported on these changes and the general processes are well understood. In contrast, studies into the changes occurring during the vermicomposting of wastes has only recently begun with several speculative reports on overall processes and the fate of waste contaminants.

2.2 THERMOPHILIC COMPOSTING

2.2.1 Experimental Equipment

The study of thermophilic composting is done in one of three ways

- Using composts from large scale compost producers
- Obtaining raw feed stocks and composting outdoors using windrow or static pile techniques
- Smaller, laboratory scale bioreactors.

Laboratory scale bioreactors are most often used to gain a deeper insight into the composting processes they allow manipulation of critical parameters. More importantly with food waste compost they allow the study of human and other commercially important pathogens although some researchers have studied pathogen reduction in windrow systems (Garcia-Siera et al., 2001). Arguably, using commercially produced composts gives a more accurate representation of composts in general use than those produced under optimum conditions. Many bench scale bioreactors are described in the literature (Ashbolt and Line, 1982; Sikora et al., 1983; Clarc et al., 1977; Mote and Griffis, 1979; Miller et al., 1990; Nakasaki et al., 1990; Deschamps et al., 1979; Schultze, 1960; Hogan et al., 1989, Jeris and Regan, 1973; Magalhaes et al., 1993; Smårs et al.,2001 Körner et al., 2003), and a further discussion on designs and processes is given in Smärs et al., (2001)

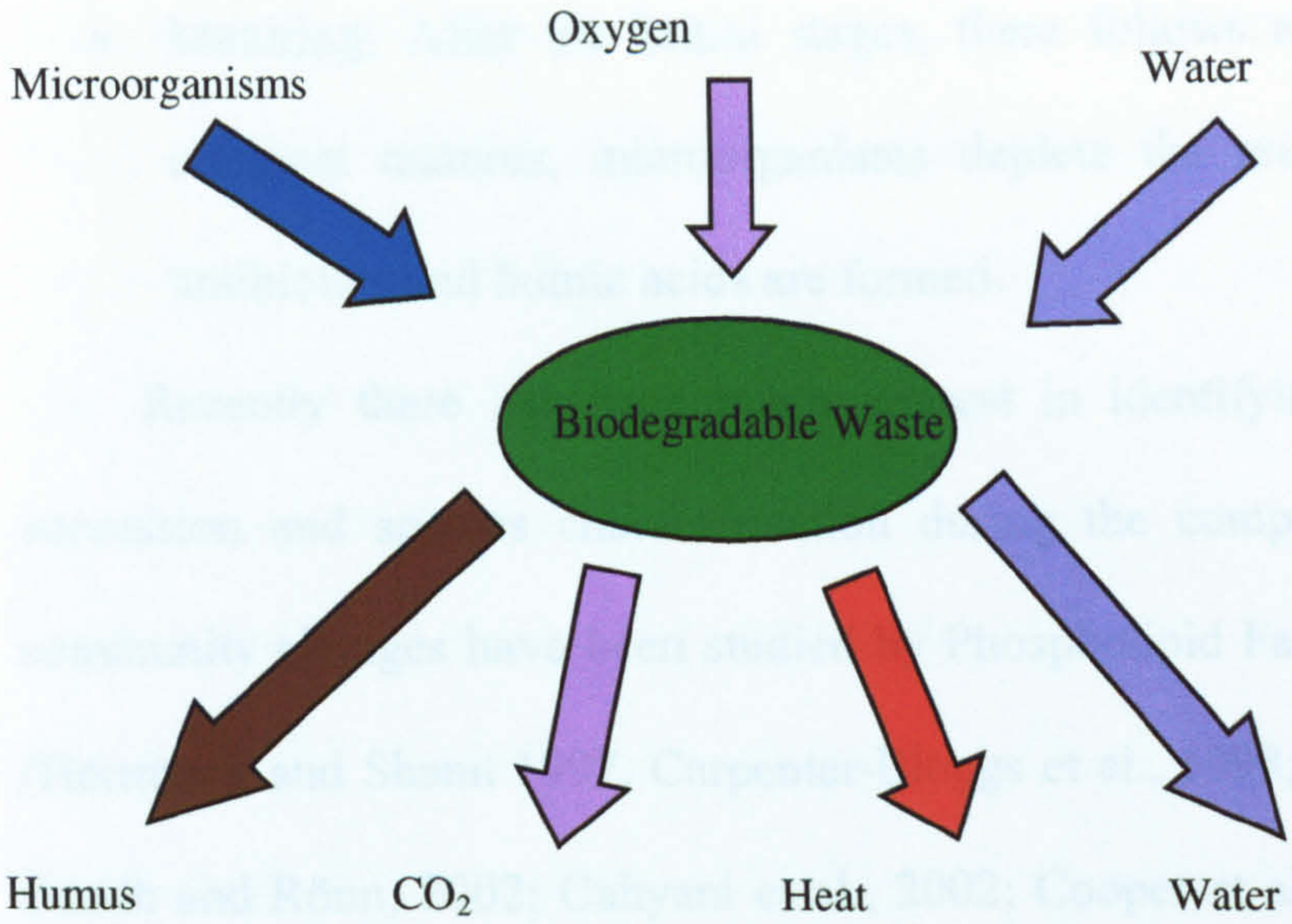


Figure 2.1 Schematic of the composting process

2.2.2 Microbiology

The composting process is complex but the main characteristics are well understood. Gray and Biddlestone (1981) identified four main categories;

- Mesophilic: Microbiological breakdown commences and heat is generated; temperatures increase; organic acids are produced and pH falls.
- Thermophilic: Thermophilic organisms, primarily actinomycetes and spore forming bacteria take over the breakdown process. High temperatures neutralise pathogenic organisms and weed contaminants. Ammonia is liberated from proteins and pH rises.
- Cooling: The temperature falls as the reaction rate drops, Mesophilic organisms reinvade, polymers breakdown.

- Maturing: After the initial stages, there follows a long period where the compost matures, microorganisms deplete the readily available nutrients, antibiotics and humic acids are formed.

Recently there has been much interest in identifying microbial community succession and species characterisation during the composting process. Bacterial community changes have been studied by Phospholipid Fatty Acid (PLFA) analysis (Herrmann and Shann 1997, Carpenter-Bloggs et al., 1998; Klamer and Bååth 1998; Sundh and Rönn, 2002; Cahyani et al., 2002; Cooper et al., 2002; Lei and Van der Gheynst, 2000). Highest microbial diversity is found in the initial mesophilic stage. Communities are dominated by fungi and gram-negative bacteria in the initial mesophilic stage. These are replaced by gram-positive bacteria and actinomycetes in the thermophilic stage; (Cahyani et al., 2002; Hermann and Shann, 1997). Sundh and Rönn (2002) also found actinomycetes fatty acid after the initial thermophilic stage. Actinomycetes persist into the curing stage alongside re-colonisation by gram negative and eukariotic bacteria (Cahyani et al., 2002). Hermann and Shann, (1997) reported reappearance of fungal PLFA during the curing stage. Full characterisation of microbiological species has been undertaken using terminal restriction fragment length polymorphisms (T-RFLP) of PCR-amplified 16s rRNA genes (Michel et al., 2002; Tiquia and Michel et al., 2002).

Considerable amounts of carbon and nitrogen are lost from compost in gaseous form. Most carbon is lost as CO_2 losses but CH_4 production can account for 1.9 g kg^{-1} of organic C loss (Fukumoto et al., 2003). For the majority of composting environments, CO_2 generation and oxygen consumption peaks at periods of high temperature during early stages of the composting process, (Robertson, 2002). In windrow composting turning induces a temporary drop in temperature, this results in

a decrease in CO_2 production and oxygen consumption (Hao et al., 2004). All composting matrices develop small anaerobic microsites and the emission of very low levels of CH_4, H_2S and N_2O is regularly measured (e.g. Robertson, 2002; Beck-Friis et al., 2001; Hao et al, 2004).

Quantity and speciation of gaseous emissions from compost environments is largely determined by physical and chemical properties of the matrix (Sommer and Moller, 2000). Elevated moisture content and high proportions of dense materials such as wet sludges, manures and food waste decreases the degree to which oxygen diffuses through the compost mass allowing anaerobic conditions to develop and inhibiting the development of higher temperature. The development of anaerobic sites is responsible for reductive environments encouraging production and emissions of gases such as CH_4, H_2S and N_2O by microorganisms. In poorly managed composts that develop significant anaerobic areas, the emission of larger volumes of CH_4 H_2S and N_2O can be a major environmental hazard and levels of 5g CH_4 m^2 h^{-1}, 0.5 mg N_2O m^2 h^{-1} have been measured (Beck-Friis et al., 2000; Hobson et al., 2005). It seems that compost production method also influences gaseous emissions and that composting with large windrows encourages the development of anaerobic sites and CH_4 and N_2O emission (Fukumoto et al., 2003). Little work exists on the production and emission of H_2S from the composting process although initial studies report levels of 3 parts per million (ppm) during the thermophilic sage of aerobic compost. Higher levels (> 50 ppm) have been recovered from composts that have been managed to mimic anaerobic conditions (Robertson 2002). Much of the work on gaseous emissions of the composting process is conducted using bench-scale composting bioreactors to impose a temperature regime on the degrading matrix. The imposition of a temperature regime on a degrading mass has been shown to adversely

influence results (Sundh and Rönn, 2002). Similarly, microbial biomass shows no increase in externally heated composts while increasing 3-4 fold in self-heating composts, (Rönn, 1999). A discussion of NH_3 loss and its implications for final compost nitrogen content is included in section 2.2.4.1. Compost research typically uses a wide range of feedstock and often incorporates some degree of co-composting of different wastes. This makes identifying common biological chemical and physical characteristics of the composting process very difficult since such properties are often dependent on initial feed stock characteristics.

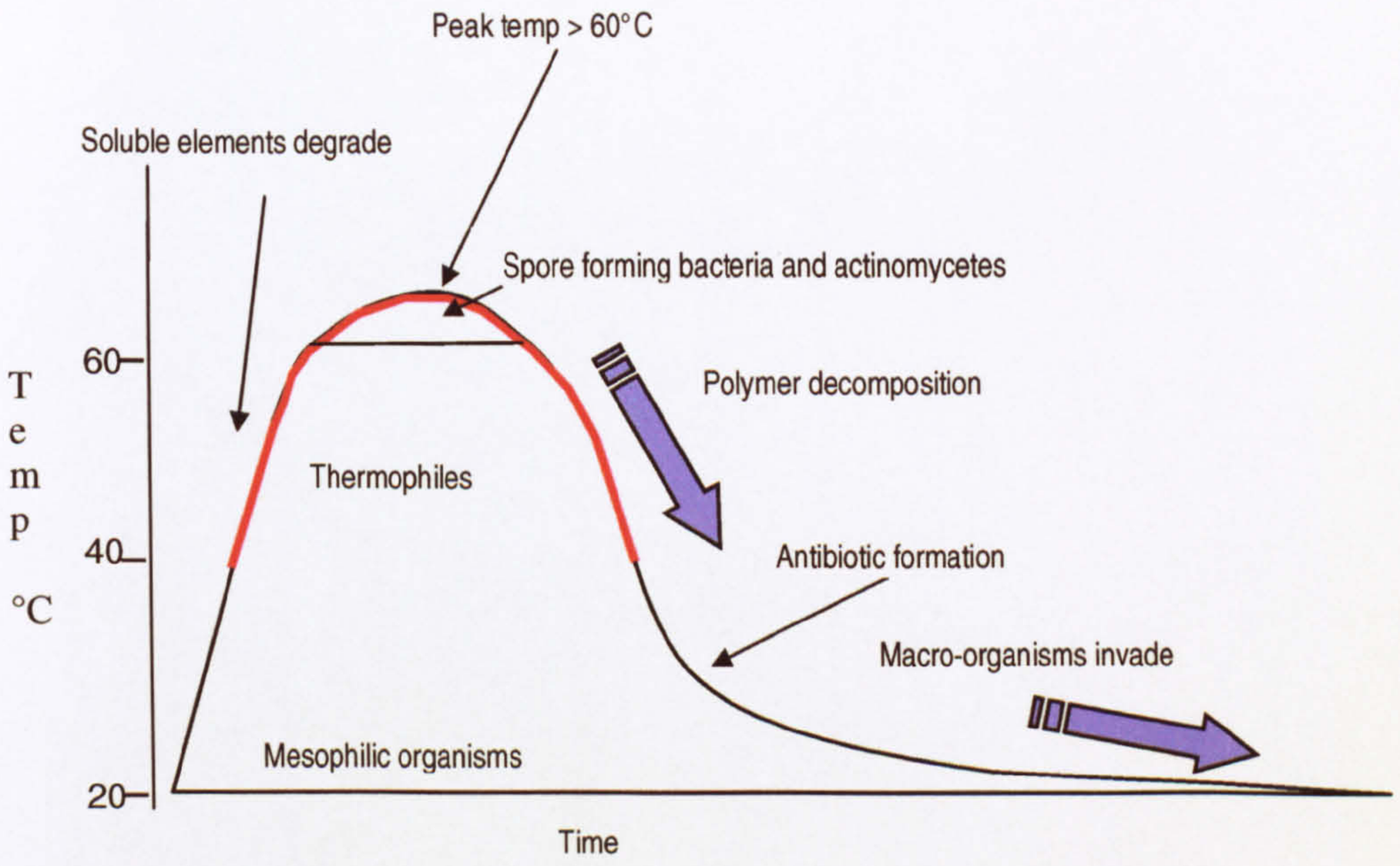


Figure 2.2 Basic processes of the composting process

2.2.3 Pathogen Reduction

High temperatures during the composting process are known to kill most pathogens that are of concern. Green wastes are not thought to be of particular concern; however, since food wastes and in particular, meat in kitchen wastes are potential reservoirs of serious human and animal diseases, the EU and individual member states have introduced strict requirements on the management of the composting process where feed stocks contain kitchen/ catering wastes.

The Animal By-products Order, 1999/ 2003 (ABPO) (EU 2003) was introduced in order to ensure the removal of human and animal pathogens during the management of wastes containing meat or other products of animal origin. Prior to 1 July 2003, the Animal By-products Order 1999 banned the use of composted wastes that contained meat, or of composted catering waste, which originated from premises in which meat or products of animal origin was handled, on land even when it was treated. This was due to a requirement in the legislation to ban access to treated and untreated wastes by livestock including wild birds, (DEFRA 2003). The new ABPO ((EC) No. 1774/2002) permits the composting of low risk catering wastes and animal by-products under strict conditions. Domestic householders are exempt from these conditions, they may compost their own kitchen scraps provided that they do not keep ruminants, pigs or poultry on the premises. On premises where poultry are kept, composting of kitchen waste is only allowed in enclosed containers; where pigs or pet ruminants are kept, composting of kitchen scraps is not allowed. It is questionable whether this part of the legislation is enforceable; in addition it is unlikely that compost produced in domestic households attains temperatures to kill potential pathogens that might be present. Although it is recommended that meat should not be

composted as it attracts vermin, some compost bin manufacturers advertise their product as vermin proof and suitable for composting meat scraps.

2.2.3.1 Sources

Pathogen sources in MSW have been identified as, (Gale 2002):

Uncooked meat in kitchen and catering wastes. Primarily from domestic kitchens,

- Pet food
- Dog and cat faeces (cat litter)
- Dead pets
- Nappies
- Poultry

2.2.3.2 Human pathogens

The sanitization effect of thermophilic composting of sewage sludges and manure feed stocks is well documented and since the processes are similar, it is considered to be a good model for the behaviour of human pathogens in food waste composting. The primary human pathogens that are known to proliferate in food wastes are *Salmonella* spp, *Shigella* spp, *Staphylococcus* spp, *Listeria,* and *Escherichia coli.*

The composting process is known to successfully eliminate *Salmonella* spp, *E. coli* and other enteric viruses (Watanabe et al., 1997; Hassen et al., 2001; Lung et al., 2001; Garcia-Siera et al., 2001; Ranalli et al., 2001; Turner, 2002; Sidhu et al., 2001). Composting has also been reported as being more effective than anaerobic and aerobic digestion at eliminating human pathogens (Ponugoti et al,. 1997). However,

improperly managed composts can encourage the proliferation and dispersal of potentially pathogenic organisms (Hassen et al., 2001; Beffa et al.,1996; Millner, 1995).

Droffner and Brinton, (1996) has reported concerns regarding the behaviour of *Listeria* spp during the composting process. They found that false negative results were obtained using the standard Listeria Selective Medium (UVM-1) suggesting that heat stressed *Listeria* organisms were unable to survive in this but were recorded when using other media. Some researchers have reported slight re-infection of composts by human pathogens during the cooling and maturation stage, although there is some evidence that indigenous microflora suppresses this re-infection but this effect diminishes with time (Hassen et al., 2001; Sidhu et al., 2001). Tiquia et al., (1998) has questioned the efficacy of windrows in eliminating faecal streptococci. In particular, the cooler areas at the outer edges of the windrows can potentially reduce the sanitisation properties of the process. However, in most well managed windrow compost sites regular mixing ensures that virtually all material is subjected to temperatures above 55°C. Stenbro-Olsen et al., (1995); Joshua et al., (1998) and Deportes et al., (1998) have reported significant reductions of faecal coliforms in MSW composts. Similar concerns regarding the edge cooling effects in "in-vessel" systems have also been expressed, (Gale, 2002), and legislation now requires composting kitchen/ catering wastes be carried out in enclosed systems and maintained at a minimum of 60°C for 2 days.

Although composting is an aerobic process, anaerobic microenvironments exist. This allows the development of anaerobic microbial communities within the compost mass. In recent investigations, Böhnel and Lube (2000) detected *Clostridium. botulinum* in more than 50% of sampled composts in Germany. It is thought that the

spores of *Clostridia* may survive adverse conditions (Mitscherlich and Marth 1984) and that the sanitisation of substrates that occurs during composting might not destroy all *Clostridia* spores, (Böhnel et al., 2002). Determination of *C. botulinum* is difficult and few scientific groups are known to work with them and according to De Groot and Steenhof, (1997) this probably results in the underestimation of botulism in European countries. However, spore levels predicted in composts are no higher than reported in some soils, (Gale, 2002).

Workers on composting sites are potentially exposed to a range of pathogenic organisms. Of particular concern, is the volume of potentially pathogenic *Aspergillus* spores that workers on composting sites are exposed. In a recent study of source separated kitchen wastes in Finland, concentrations of endotoxins in the cabin of the wheel loader exceeded all recommended limits, particularly of some thermotolerant *Aspergillus* spp, (Hassen et al., 2001, Koivula et al., 2000; Beffa et al., 1996). The adverse health effects resulting from exposure to such organisms far outweighs likelihood of disease transfer resulting from incomplete sanitisation of the composting matrix.

2.2.3.3 Animal Pathogens

Since the major proportion of food waste composts will be used in the agricultural sector, the fate of animal pathogens is also of concern. To date there has been no study into the fate of Transmittable Spongiform Encephalopathic (TSE) organisms in the composting process, however, it is thought that composting will have no effect on BSE agents, (Gale 2002), nor will there be any subsequent decay in soil (Gale and Stansfield, 2001). The restrictions placed on composting catering

wastes by the Animal By-Products Order (EU 2003) should mean that no infected cattle carcasses should enter the composting process within the EU. This diversion of potentially infected material from the composting process results in a lower likelihood of re-infection of cattle grazing on land where composted kitchen waste is applied than where sewage sludge is applied (Gale and Stansfield, 2001). The position of Ovine TSE, i.e. Scrapie poses a more complex problem; the BSE agent is present in the inedible part of cattle whereas the Scrapie agent is found in muscle tissue of sheep and will enter the composting process. Cooking kills most Scrapie agents but questions exist regarding its behaviour in soil. Brown and Gajdusek (1991) noted a three year decline in Scrapie infectivity in soil but were unsure whether thus was due to decay or adsorption to soil particles.

The recent outbreak of Foot and Mouth virus (FMDV) in the UK and its potential spread by food waste is also of concern. The virus is known to be present in milk before external symptoms of the disease are seen but is believed to be denatured when exposed to temperatures above:

- 80 – 100°C for 2-3 min
- 70°C for 25 minutes (MacDarmid, 1991)

Turner et al., (2000) found that FMDV was deactivated at temperatures above 55°C. In an earlier study, however Turner and Burton, (1997) found that FMDV was inactivated in pig slurry at 50°C if maintained at pH 8 for 48 hours.

Composting is known to deactivate several other animal viruses:

- Classical Swine Fever is inactivated at temperatures above 55°C, (Turner et al., 2000).
- African Swine Fever undergoes rapid initial destruction at 56°C, (Plowright and Parker, 1967).
- Swine Vesicular Disease is reduced to below detectable levels above 50°C at alkaline pH, (Turner and Williams, 1999), or at 40°C when maintained for 48 hours, (Turner and Burton, 1997)
- Aujeszky's disease at 40°C for 5 hours, (Turner and Burton, 1997).
- Avian Influenza Virus, Newcastle Disease and Infectious Bursal Disease, (Senne et al., 1994)

2.2.4 Chemical and Physical Changes

Micro-organisms play a key role in converting wastes into composts. Major components of green waste and MSW are cellulose and lignin. Since these are known to be resistant to microbial breakdown, they can slow the composting process considerably. Singh and Sharma (2001) found that inoculating lignocellulosic wastes with a microbial combination of known lignin and cellulose degraders (*Pleurotus sajor-caju, Trichoderma harzianum, Aspergillus niger* and *Azobacter chroococcum*) reduced the cellulose, hemicellulose and lignin content and composting times significantly. In addition, N,P,K content in the final product was shown to be enhanced. Senesi and Brunetti (1996) reported that in comparison with soil; the humic and fulvic fractions in composts generally have a high degree of molecular heterogeneity and a lower degree of aromatic compounds.

2.2.4.1 Nitrogen

Nitrogen plays an important part both in the composting process and in the subsequent value of the finished compost as a soil additive. Its fixation, transformation and recycling by microorganisms is of great interest in environmental sciences. Current concerns include gaseous emissions of nitrous oxides and their contribution to atmospheric changes and climate change, and their high concentrations in ground and surface waters in relation to drinking water quality and eutrophication.

There is general agreement about N dynamics during the composting process, (Tiquia, 2002; Körner and Stegman, 2002). Most N is in an organic form and is only slowly converted to inorganic N pools. Nitrification is limited by temperatures above 30°C and concentrations are low during the thermophilic stage. Nitrification is only seen during the cooler maturation stage as the populations of nitrifying bacteria re-infect the composting matrix. Denitrification occurs during the initial stage of the composting process and populations of denitrifying bacteria decline during the composting process. In feed stocks rich in kitchen wastes, up to 50% of the initial N was lost as gaseous NH_3. Korner et al. (2003) in their study of N dynamics in a mixture of kitchen wastes and green wastes have reported that between 40 – 70% of N is ammonified. It was hypothesised that these high levels of gaseous N loss was as a result of differences in biochemical components; (i.e. cellulose, hemicellulose and lignin in green waste and starch, carbohydrates and proteins in kitchen wastes). Since food waste contains high quantities of water, it is more likely that increased moisture content influenced oxygen diffusion and it was the development of anaerobic conditions leading to increased ammonification that led to the high losses reported.

N immobilisation has to occur since microbial proteins are formed during the composting process in order to maintain the microbial population. However, since it can only be detected by measuring an increase in the organic N pool, it can only be visualised when the immobilisation rate is higher than ammonification (Körner and Stegman, 2002). In their study, Körner and Stegman, (2002), conclude that immobilisation of the inorganic fraction is due to chemical-physical processes and not by the microbial biomass i.e., it was bound up with the humic - lignin complex. Parkinson et.al. (2004) suggest that there is a close link between nutrient losses and turning frequency with increased NH_4- N losses with each turn. A similar pattern was seen in leachate content with increased NO_3- N losses in leachate collected from frequently turned composts compared with static compost piles. Nitrogen dynamics and its place in larger scale N cycling is summarised in figure 2.3

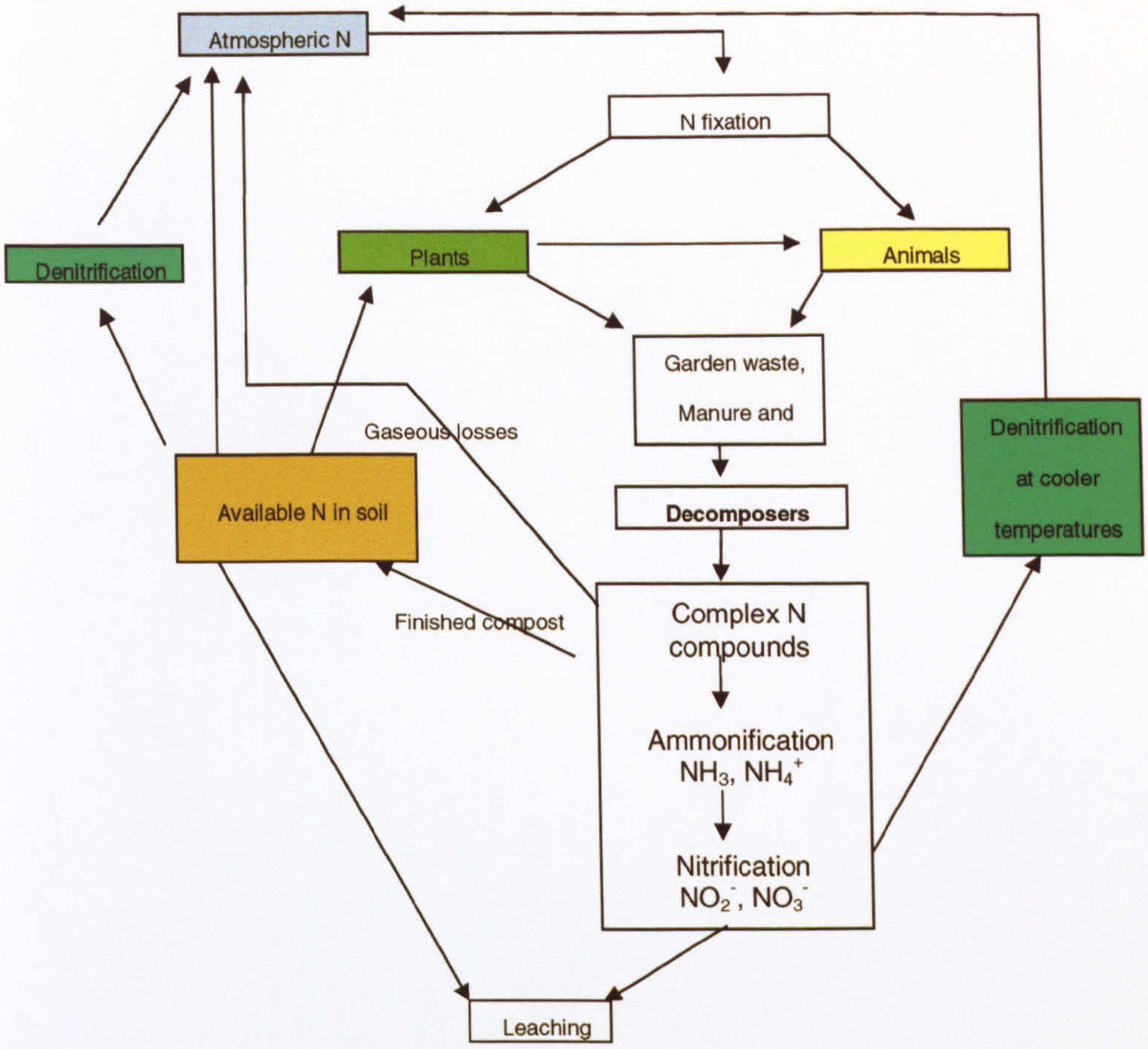


Figure 2.3 Composting and the Nitrogen cycle

2.2.4.2 Phosphate

Most research is focused on changes in P availability in soil after compost application and its effect on plant growth and yield (Mkhabela, and Warman, 2005; Speir et al., 2004) whereas studies into P dynamics during active composting are scarce. Moreover, results from the few that exist are inconclusive and suggest that change in P speciation is dependent on feedstock (Traoré et al. 1999; Felton et al., 2004). A common conclusion is that total P concentrations increase; however, studies into levels of plant available P were inconsistent. Parkinson et al., (2004) found that leachate originating from composting of animal manures contain in excess of

40mg P l^{-1} throughout the composting process and increased with increased compost turning. This is contradicted by Felton et al. (2004) who report higher concentrations of water soluble P in compost that had lowest turning frequency.

2.2.4.3 Pollutants

2.2.4.3.1 Heavy metals

The composted organic fraction of municipal solid waste can be reused for soil conditioning. However, heavy metal concentrations are known to increase during the composting process even when initial pre-treatment concentrations are similar to background levels (Table 2.1). Frequent application of composts to soil systems may lead to the accumulation of heavy metals in soils. Concentrations of Cu, Pb and Zn are shown to increase significantly during the composting process. Intense microbial activity results in significant gaseous losses of C as CO_2, reducing total volume considerably (Veeken and Hamelers, 2002). There is some evidence that heavy metal bioavailability decreases during the composting of process (Amir et al., 2005); the degree to which metals become bound to organic fraction is likely to be metal dependent and more work is required to further elucidate with the likely impact of long term application. The heavy metal content of biowaste-composts frequently exceeds the legal standards, and thus raises a conflict between two governmental policies: the recycling of solid waste on the one hand, and the protection of natural ecosystems and public health on the other (Veeken and Hamelers 2002).

Table 2.1 Comparison of the heavy metal content in the four main fractions of biowaste (in italics) and the natural background content of the original biowaste constituents, (Veeken and Hamelers, 2002).

Fraction	Heavy metal content (mg kg^{-1} DM)			
	Cd	Cu	Pb	Zn
Organic fraction >1 mm	*0.24±0.10*	*10±1*	*30±3*	*87±10*
Indoor organic waste	0.3±0.4	7±8	1±4	45±35
Outdoor organic waste	0.4±0.2	8±5	5±7	60±100
Organic fraction 0.05–1 mm	*0.61±0.28*	*28±7*	*94±17*	*196±49*
Humus layer	2±1	20±9	100±64	150±80
Inorganic fraction 0.05–0.5 mm	*0.05±0.01*	*3.1±1.5*	*26±10*	*24±3*
Soil sand	0.2±0.3	12±8	15±11	35±42
Organo-mineral fraction <0.05 mm	*1.0±0.5*	*63±26*	*157±30*	*338±58*
Humus layer	2±1	20±9	100±64	150±110
Soil loess	0.2	25±34	20±5	30±12
Soil clay	0.4	30±25	55±17	70±42

2.2.4.3.2 Organic Pollutants

In addition to metal enrichment of the soil, other organic pollutants that accumulate in treated sewage sludges are known to accumulate in soils. Secondary composting of contaminated sludges results in further volume reduction and potential concentration of contaminants. A number of organic pollutants, such as hydrophobic, organic contaminants and surfactants, some of which have hormone mimicking properties, are known to accumulate in organic wastes (During and Gath 2002,).

Compost feedstocks also contain significant levels and variety of polynuclear aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) (McGowin et al., 2001; Rogers, 1996; Moreda et al., 1998). Increased microbial activity in soil increases the degradation rates for most pollutants, phthalic acid esther pesticides such as organophosphates and carbamate pesticides are rarely seen in the end product,

whereas organochlorines such as chlordane can persist through the composting process and is measured in some mature composts, (Amir et al., 2005; Lee et al., 2003; Büyüksönmez et al., 1999). Concentrations of PAHs and PCBs are known to persist or increase in composts to such an extent that they can be released back into the environment and significant contamination of finished composts has been reported (Lazzari et al., 1999; Büyüksönmez et al., 2000; McGowin et al., 2001; Moeller and Reehu, 2003). Fricke et al. (1996) concluded that concentrations of lower chlorinated polychlorinated dibenzo-p dioxin and dibenzo furans (PCDD, PCDF), decrease during the composting process with furans being more susceptible to degradation; but concentrations of hepta- and octa - PCDD can increase; however, since these are not considered to be particularly toxic, it is not thought to increase the overall toxicity of the compost. The nature of the organic compound, specific composting conditions and procedures, the microbial communities present, and the duration of composting affect the extent and the mechanism of degradation (Büyüksönmez 1999). Of recent concern in the USA is the persistence of chlordane. Lee et al. (2003) concluded that some composts contribute to anthropogenic cycling of POPs through the biosphere.

2.3 VERMICOMPOSTING

Charles Darwin was the first person to document the ability of earthworms to breakdown a range of organic wastes (Darwin, 1881). However, it is only recently with increasing awareness of the environmental effect of landfilling wastes that scientists have investigated the potential of using earthworms to process organic wastes.

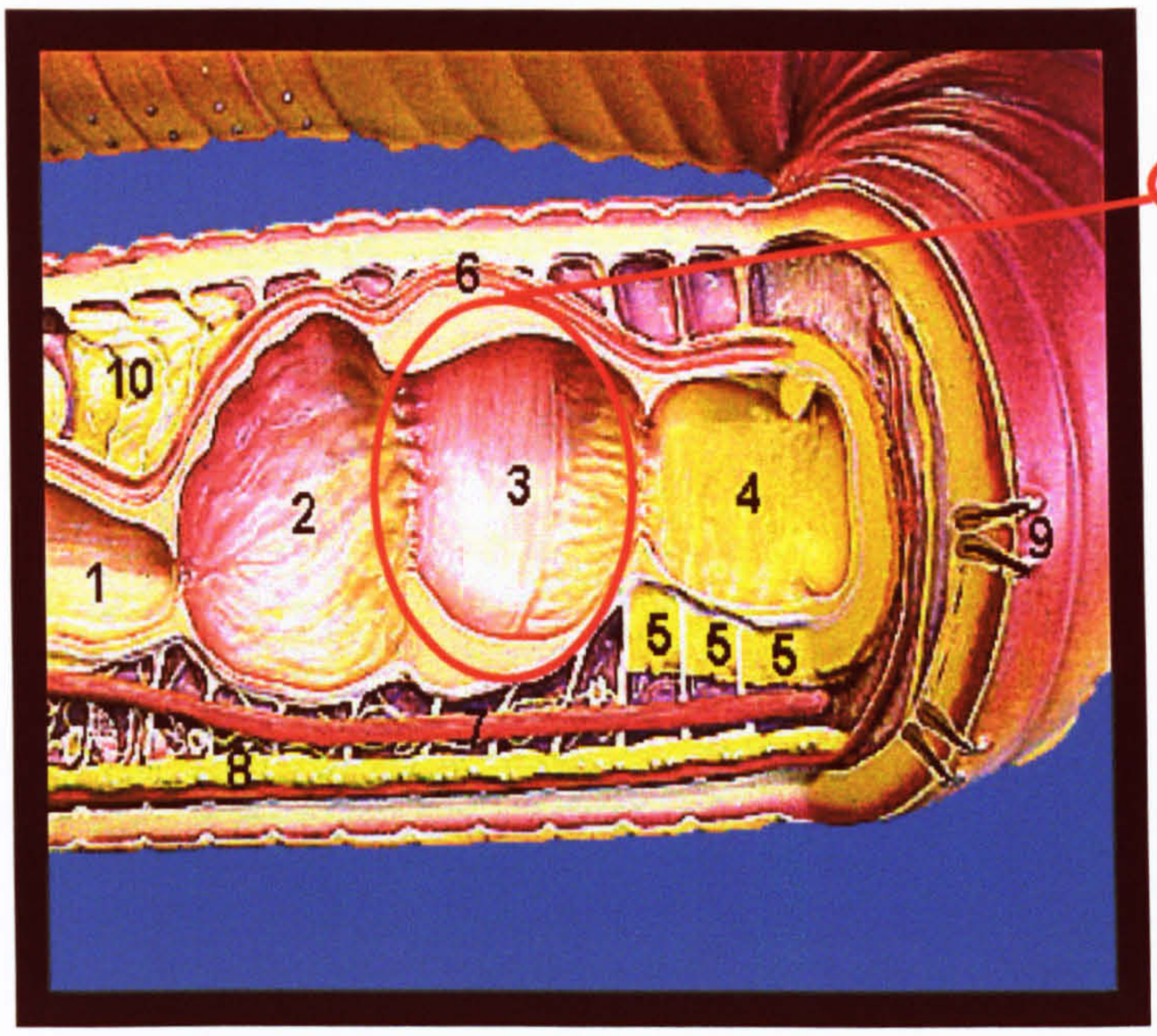
2.3.1 Effect of ingestion on organic matter.

Vermicomposting in its basic form it is a low-cost method of treating organic wastes (Hand et al., 1988) by exploiting the ability of some earthworms to fragment the organic matter in a grinding gizzard (Fig 2.4). They consume and excrete organic matter rapidly; living off the micro-organisms in these materials. These casts contain significant quantities of polysaccharides which stimulate microbial activity resulting in rapid stabilisation of the feed stock.

2.3.2 Microbiological

The casts excreted by earthworms are more microbially active than the source material consumed, thus increasing the speed of stabilisation (Edwards and Bolhen 1996; Vinceslas-Akpa and Loquet, 1996). Several studies have shown that vermicomposting wastes accelerated the stabilisation of sewage sludge wastes three times faster than non-ingested sludge (Hartenstein 1978; Neuhauser et al., 1988; Frederickson et al., 1997; Singh and Sharma 2002). Some worms however, require pre-composted waste. For example Singh and Sharma (2002) found that pre-composting wheat straw with known lignocellulose degrading micro organisms followed by vermicomposting produced a compost rich in N and P and could potentially reduce composting times by up to 40 days.

PLFA profiles show that the most common microorganisms present in vermicomposts are *Pseudomonas* (27%), *Bacillus* (37%), *Aeromonas* and *Vibrio*. There is some evidence to suggest that microbial communities in vermicomposts reflect its source with some common rumen microorganisms found in cow manure vermicomposts (Verkhotseva et al., 2002).



1. Oesophagus
2. Crop
3. Gizzard
4. Intestine
5. Chloragogue cells
6. Dorsal blood vessel
7. Ventral blood vessel
8. Ventral nerve cord
9. Setae
10. Seminal vesicles

Figure 2.4 Detail of upper digestive system of earthworms (Gillis and Garo, undated)

2.3.2.1 Pathogen reduction

Since worms will not tolerate temperatures in excess of 35°C the process must be maintained at temperatures below this. The resulting product will not meet UK Environment Agency (EA) rules on pathogen reduction and some form of pre-composting is required in order that the product meets national guidelines. Researchers in the USA however, have reported pathogen reduction sufficient to meet EA guidelines during the vermicomposting of biosolids (Eastman, 1999; Dominguez et al., 1997). Work by Ndegwa and Thompson (2001) concluded that when studying vermicomposting of biosolids, in order for the final product to comply with the US Environmental Protection Agency (EPA) standards then a combination of composting followed by vermicomposting was the only method that resulted in satisfactory

pathogen reduction. In addition, Smith (2001) has noted the presence of *Listeria* spp and *Legionella monocytogenes* in the liquor produced in household vermicomposting units.

Most vermicompost retailers target the high value end of compost market, i.e. amateur gardeners and horticulturalists that use the compost for growing fruit and vegetable. Given that some of this produce will be eaten raw, the potential exists for human infection from consuming food grown on such contaminated wastes. More recently pre-harvest contamination of vegetables with *E. coli* O157:H7-infected compost is known to be responsible for enterohemorrhagic food poisoning outbreaks (Islam et al., 2005). There is a significant need for further work on vermicomposting and pathogen reduction. Little is known of human pathogen fate but no workers have reported on animal pathogen removal. This lack of information will retard the adoption of vermicomposting as a waste management method given the current strict requirements currently imposed on thermophilic composting methods.

2.3.3 Chemical and Physical changes

Several researchers have reported on the physical and chemical changes that occur as a result of ingestion and excretion by earthworms. The grinding action in the gizzard increases the surface area of the material (Shi-wei and Fu-zhen 1991) as well as increasing its cation exchange capacity (CEC), decreasing its pH and increasing aeration status (Hartenstein and Hartenstein, 1981; Bernal et al., 1996); (Table 2.2).

Table 2.2 Changes in sludge properties before and after vermidigestion (adapted from Hartenstein and Hartenstein 1981)

	Sludge	Castings
pH	6.84	6.2
Eh (mV)	182.5	397.25
CEC	19.55	22.1

2.3.3.1 Nitrogen

Studies into the changes in nitrogen content of vermicomposted substrates do not give consistent results; however, these studies have been conducted on a range of feedstock presumably with variable initial N content. Vinceslas-Akpa and Loquet (1997) concluded that vermicomposting of lignocellulosic maple wastes resulted in a weight loss of 35% in the first seven months of the vermicomposting process. In addition the C:N ratio decreased from 62:1 to 27:1 in vermicomposted samples primarily due to C decrease "and a higher proportion of total N content in the vermicompost" (Fig 2.5).

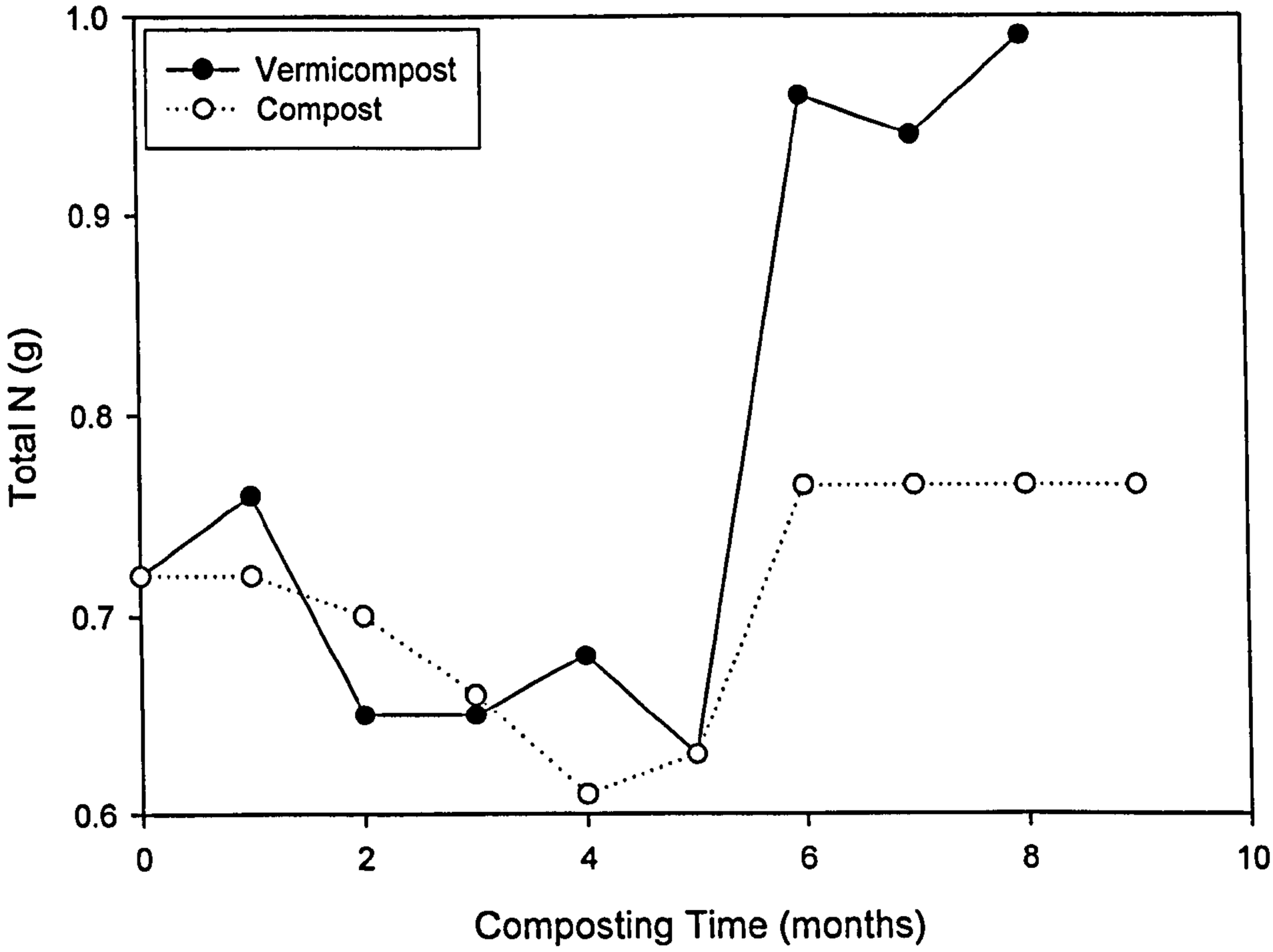


Figure 2.5 Changes to total nitrogen contents of vermicomposts and composts, (Vinceslas-Akpa and Loquet M. 1997)

Similar initial changes in C:N have been reported by Bernal et al., (1996); however this was considered being due solely to a decrease in carbon content rather than an any increase in nitrogen. In contrast, Singh and Sharma (2002) reported a decrease in nitrogen content during vermicomposting of pre-composted wheat straw which may have been due to ammonification, NH_3 volatilisation, and denitrification (Martins and Dewes, 1992; Bernal et al., 1996). Since Singh and Sharma's study only lasted 30 days it is possible that what they are reporting is the initial decrease in N content seen by Vinceslas-Akpa and Loquet, (Fig 2.5) although Benitez et al., (1999) reported a loss of 31% of total N during an 18 week vermicomposting trial of sewage sludges.

2.3.3.2 Phosphorus

Changes in P speciation from organic to inorganic forms have been documented (Ghosh et al., 1999). Wastes treated with earthworms showed a faster reduction in organic P content than those without and several workers report increases in concentrations of total P (Singh and Sharma, 2002; Kaushik and Garg, 2004; Ghosh et al., 1999). The amount of total inorganic P (Pi) increased with incubation period; those wastes treated with earthworms showed the highest rates of mineralisation, however, changes in total Pi did not follow the same trend as mineralisation of organic P possibly due to variations in growth and multiplication of worms in different waste streams, (Table 2.3).

Table 2.3 Changes in the amounts of organic and inorganic P in municipal waste subjected to windrow composting and vermicomposting. (adapted from Ghosh et al., 1999)

	Weeks incubation			
	Composted		Vermicomposted	
	1	7	1	7
Municipal Waste				
Organic P (mg kg^{-1})	2962	2466	2358	1086
Inorganic P (mg kg^{-1})	2193	1619	135	1464
Easily extractable P (mg kg^{-1})	369	497	410	1389

Studies into concentrations of plant available P are contradictory and seem to be feedstock specific. Bhattacharya and Chattopadhyay (2002) and Ghosh et al. (1999) report increases in plant available P when vermicomposting fly ash and manure. They hypothesise that phosphate solubilizing bacteria increased in

vermicomposted waste thus transforming insoluble P into plant available forms; however, it has been suggested that earthworms stimulate microbial metabolism and cause an immobilisation of free PO_4^{-3} into worm and microbial tissue (Benitez et al., 1999). Benitez et al. (1999) reported such reductions in water extracted PO_4^{-3} over 18 week period of their study from an initial concentration of 1302 mg kg^{-1} to 0 mg kg^{-1} by the 6th week.

2.3.4 Pollutants

2.3.4.1 Heavy Metals

Earthworms from base metal mining areas are known to accumulate, excrete and change available metal concentrations (Gish and Christensen, 1973; Van Hook 1974; Ireland, 1975 a; b; Helmke et al., 1979). Increases in concentrations of heavy metals in vermicomposted sludges has also been reported (Table 2.4) but this could be as a result of increased mineralisation rates in vermidigesting processes and that given sufficient time, similar concentrations would be seen in thermophillic composting processes (Hartenstein and Hartenstein, 1981).

Table 2.4 Comparison of metal concentrations found in un-digested and vermidigested sewage sludges after 10 days (adapted from Hartenstein and Hartenstein, 1981).

Element	Sludge (mg kg^{-1})	Castings (mg kg^{-1})	Change (%)
Fe	13300	13630	+10.0
Mn	339	377	+11.2
Cu	431	448	+3.9
Zn	1780	1890	+8.4
Al	8833	9480	+7.3
Cd	8.5	9.6	+12.9
Pb	170	200	+17.6

2.3.4.2 Organic Pollutants

To date, only one study reports on pollutant degradation by vermicompost (Forouzangohar et al, 2005). This concentrates on the use of vermicompost as a remediation strategy on contaminated soil and not on degradation during the vermicomposting process. The extent to which utilizing vermidigestion as a primary or secondary treatment might affect organic pollutant concentrations in contaminated composts has yet to be determined.

2.4. PROPERTIES OF COMPOSTS AND VERMICOMPOSTS, THEIR EFFECT ON SOIL AND PLANT GROWTH.

2.4.1 Soil

Composted soil amendments have consistently been shown to change soil physical and chemical characteristics consistent with improved soil fertility. However, whereas physical characteristics improve over the long term; chemical changes can appear in the first few weeks after application but some changes are only seen after several years of regular compost application . Overall effects are moderate with only N and P availability being significantly influenced by compost application. No comprehensive studies have targeted the effects of vermicompost on soil properties. It is thought that these products will predominantly be used in smaller scale "pot" type situations

2.4.1.1 Physical

Soil organic matter has been identified as one of the key components of structural stability, and regular addition of composted materials may increase overall organic matter content thus increasing soil stability (Albiach et al., 2001; Debosz et al., 2002) However, due to the variable nature of residues, results from studies into aggregate stability and organic matter components from organic residues are inconclusive. Pagliai et al. (1981) reported slight increases in soil stability following application of sewage sludge applied at 50 t ha^{-1} and composted MSW applied at 150 t ha^{-1}. Guidi et al. (1988) found no changes in soil stability following application of 300kg N ha^{-1} as compost from sewage sludge and MSW. It was suggested that

variability is due to climatic differences but could also result from differences in soil characteristics prior to compost application.

In situ vermicomposting of biological sludges in laboratory studies designed for optimum worm activity increases soil polysaccharide concentration which act as aggregating cements, subsequently increasing the formation of small soil pores (<500μm), (Masciandaro et al., 2000) and therefore enhancing water retention, oxygen diffusion and nutrient availability. Additions of both organic and inorganic soil amendments improve soil porosity (Marinari et al., 2000), inorganic fertilisers increase the number of rounded pores to a greater degree than organic amendments, whereas organic additives increase the frequency of large, irregular and long pores (Marinari et al., 2000).

2.4.1.2 Chemical and Biological

Changes in soil organic matter composition after the application of composts show the ongoing decay of the easily degraded fraction, primarily polysaccharides and microbial biomass. The more recalcitrant components such as lignin remained unchanged after 18 months (Liefeld et al., 2002). Compost addition to soil resulted in increases in polysaccharide concentration and a decrease in reactive olefinic compounds, however levels are restored to normal when humification of composts is complete (Giggliotti et al., 1997).

Biomass carbon levels are stimulated briefly with compost addition and the evolution of CO_2 shows a pronounced but short lived peak, (Fig 2.6a). A peak in levels of inorganic N is seen two months after application followed by subsequent depletion during the following autumn by soil microbial population, (Fig 2.6b),

(Debozs et al., 2002). In longer-term trials (three years) by the same author, the addition of compost increased extractable P, total N, biomass C and soil respiration, (Table 2.5).

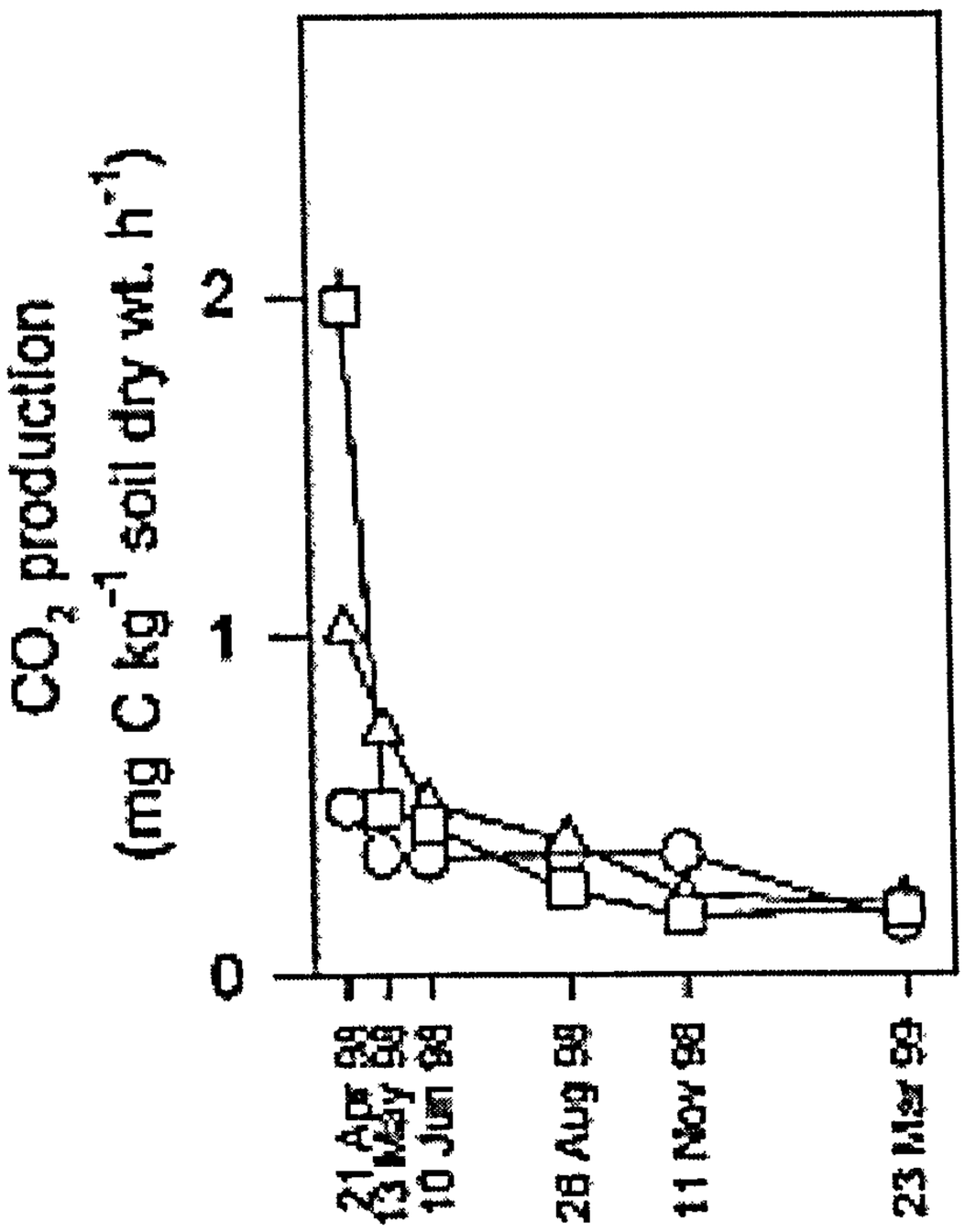


Figure 2.6a CO_2 evolution rates during incubation at 10 °C of unamended soil (O), soil amended with anaerobically stabilized sludge(△) and compost (□).Vertical bars represent standard deviations. (Debosz et al., 2002)

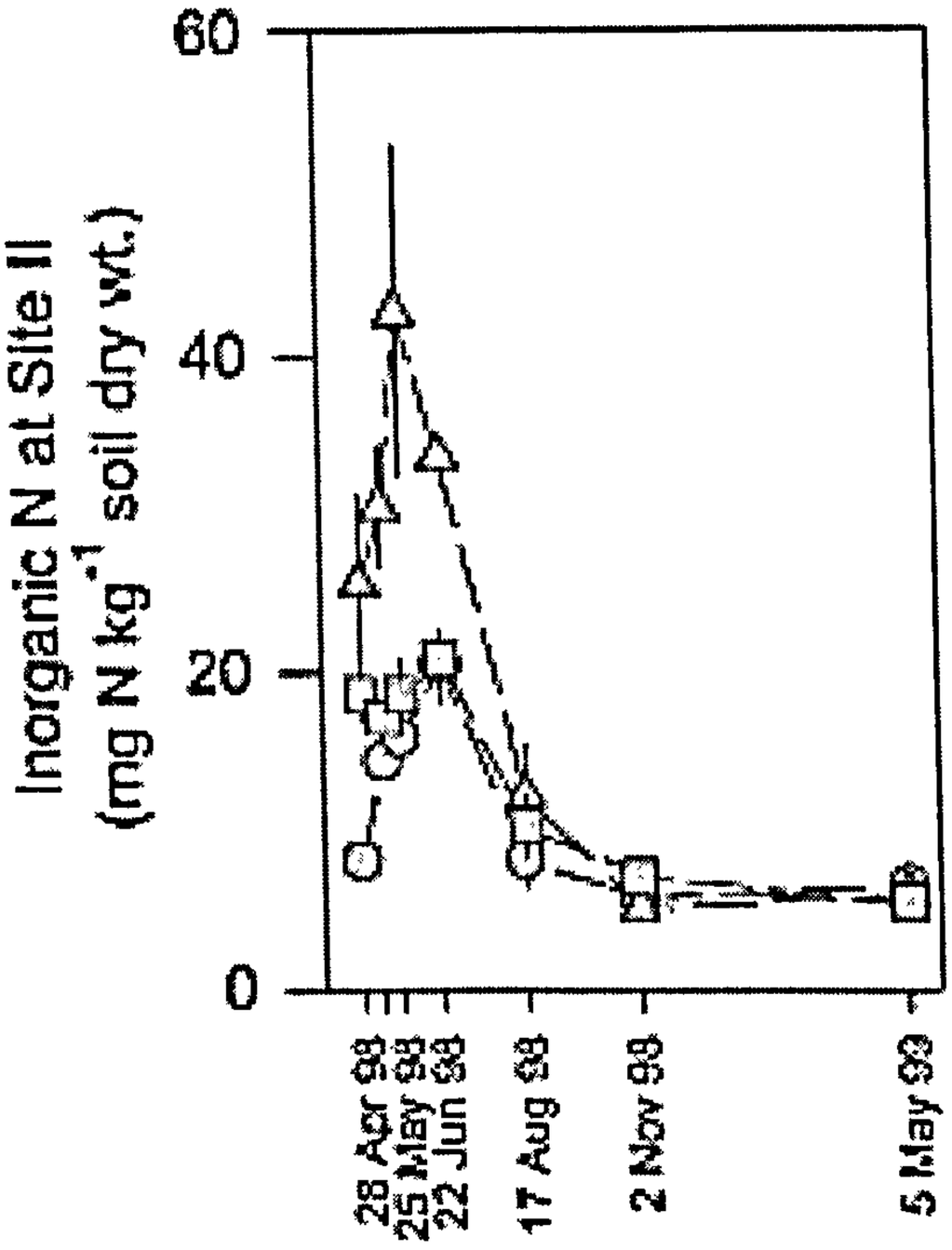


Figure 2.6b Inorganic N during incubation at ambient temperature of unamended soil (O), soil and anaerobically digested sludge (△), and compost (□). Vertical bars represent standard deviations. (Debosz et al., 2002)

Table 2.5 Effects of compost application on selected soil chemical and biological properties in long term field experiment on sandy loam soil, (adapted from Debosz et al.,2002).

	No amendment	Compost amendment
Extractable P (mg P kg^{-1} dry wt. soil)	32.6	41.4
Total N (mg N kg^{-1} dry wt. soil d^{-1})	2.6	4.4
Biomass C (mg C kg^{-1} dry wt. soil)	282.1	329.9
Soil respiration (CO_2 as mg C kg^{-1} dry wt. soil h^{-1})	0.1	0.2

Albiach et al., (2000) have reported increases in soil microbial biomass and enzymatic activities following the application of several organic amendements. 24t $ha^{-1}y^{-1}$ of MSW compost increased biomass and enzyme activity significantly whereas soils amended with the manufacturer's recommended rate of 2.4t $ha^{-1}y^{-1}$ of vermicompost remained unchanged. Vermicompost is expensive to purchase and it was suggested by the authors that this influences the manufacturers recommended application rates. So little scientific work reports on the use of vermicompost on land that it is difficult to see how the manufacturers could conclude that such small amounts (2.4t ha^{-1} y^{-1}), was sufficient to instigate any changes in soil properties.

Compost application usually increases soil pH, (Bulluck et al., 2002, Leifeld et al., 2002; Zheljazkov and Warman 2003). In one instance Leifeld reports increases in pH from 4.5 to 6.5 in a compost amended dystric cambisol. Increases in soil EC are also reported, (Zheljazkov and Warman, 2003) and CEC (Bernal et al., 1996). These increases are logical since most composts have higher pH, EC and CEC than most soil. Summarising the effect of composts on metal concentrations is difficult. It

usually reported that adding compost to metal contaminated soil reduces the bioavailability of the contaminant (Nachtegaal et al., 2005; Garrido et al., 2005). However, the process is influenced by physico-chemical characteristics of soil, compost and metal and the decrease in bioavailable concentrations is not always observed. Some reports conclude that concentrations of some metals, (Ca, K, Mg, Mn P, Na Pb and B) increase on application of organic soil amendments (Bulluck III, et al., 2002), this is not surprising since most composts will contain elevated concentrations of plant macro and micro nutrients. The increased level of Pb in this case is surprising. Applications of composts containing elevated concentrations of Pb may initiate this response but in this case the composts applied originated from farmyard. This does not explain the increased level of Pb observed in this study. The same studies report no change in HNO_3 extractable Cd, Co, Cr, Mo, Ni, Se. (Bulluck III, et al., 2002; Zheljazkov and Warman, 2003).

2.4.2 Plant growth

Vermicomposts have consistently been shown to increase plant growth rates in pot and field trials, whether used as a soil additive or in soilless media (Fig 2.8) (Atiyeh et al., 2000, 2001, 2002). In recent studies Atiyeh et al., (2002) found that applying 50-500 mg/ kg of vermicompost derived humic acids to tomatoes and cucumbers increased growth rates significantly whereas a significant decrease was reported in application rates of 500-1000mg/ kg and suggests that it is the hormone-like action of humic acids that is responsible for the increase in growth rates observed. Substitution of 20 – 40% of commercial growth medium (Metro-Mix 360) with vermicomposts has been shown to increase plant growth and yield (Fig 2.7 and 2.8), (Atiyeh et al., 2002a; Atiyeh et al., 2000; Arancon et al., 2003a,b, 2004a, b,

2005b; Acevedo and Pire, 2004;). Canellas et al., (2002) have reported enhanced root growth and increase in lateral root emergence sites in maize plants exposed to humic acids derived from vermicomposted cattle manure. In addition, Arancon et al. (2003b) have extracted several plant growth hormones from vermicomposts. Canellas et al., (2002) have confirmed their presence within the humic structure.

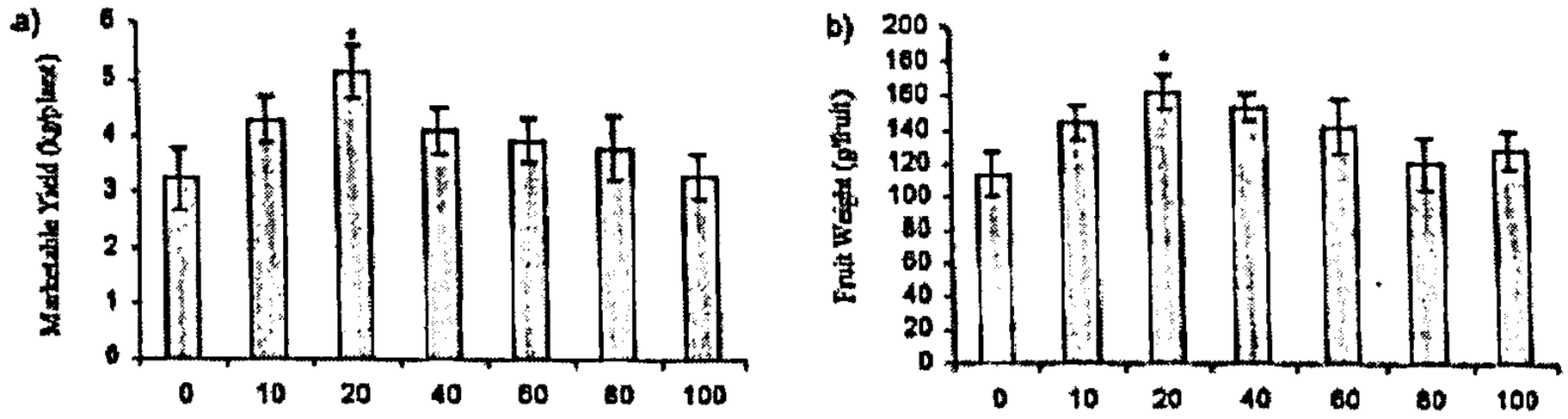


Figure 2.7 Yield and weight of tomato fruits produced in a standard commercial potting medium (Metro-Mix 360) substituted with different concentrations of pig manure vermicomposts. Columns marked * are significantly different from Metro-Mix 360 control (0% vermicompost) (Atiyeh et al., 2000)

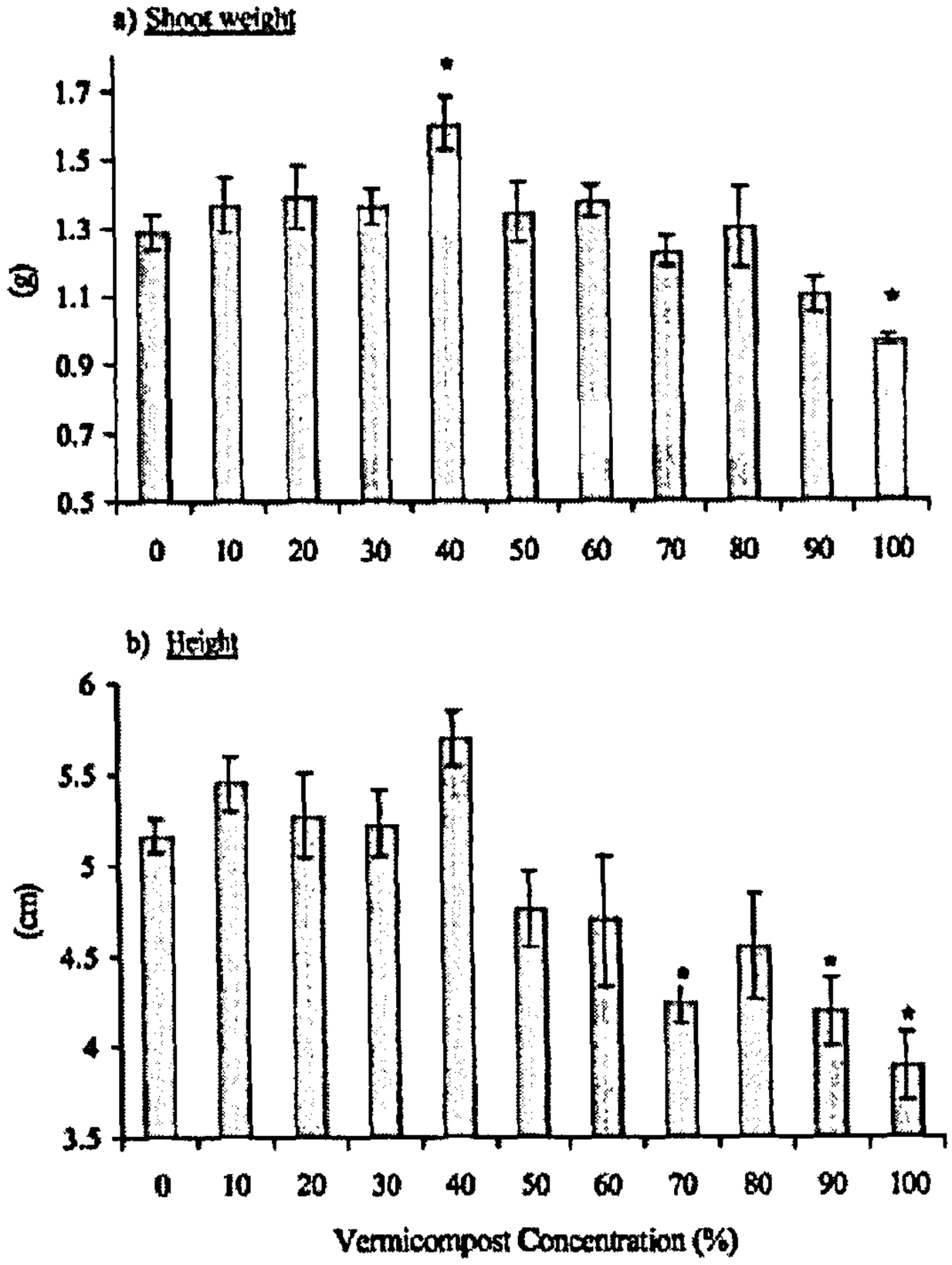


Figure 2.8 Shoot weight and height of marigold seedlings grown in Metro-Mix 360 substituted with different concentrations of pig manure vermicompost, 28 days after seeding (Atiyeh et al., 2002)

Most studies have reported beneficial effects of vermicompost on germination, plant growth and yield with substitutions of 20 to 40% of vermicompost into a commercial growth medium. The potential for yield enhancement is not unique to vermicompost; Maynard et al., (1993, 1995) also demonstrated increases in tomato yield when grown in municipal solid waste derived compost. However, not all plant growth experiments have produced such positive results (e.g. Gardener, 2004; Cavender et al., 2003), and it may be that plant responses to vermicompost are more species specific than previously reported. There is also some evidence that vermicomposts enhance rates of mycorrhizal inoculation (Cavender et al., 2003). This enhanced extra radical mycorrhizal network development coupled with root growth

stimulation may increase the plants' ability to exploit added inorganic nutrients thus maintaining or enhancing yield.

Conventional composts and inorganic composts decreases trophic groups of soil arthropods whereas vermicompost application results in increase of the same groups, although the mechanisms responsible for this change are not understood, (Gunadi et al., 2002).

2.4.3 Plant health

In addition to increased plant growth, the suppression of plant diseases by composts and vermicomposts is widely reported. Composted MSW is less effective than conventional pesticides at suppressing a broad range of plant pathogens, however in contrast to chemical pesticides, composts enhance plant-beneficial soil microorganisms (Pascual et al., 2002). In addition, composts are known to suppress a range of turf grass pathogens (Boulter et al., 2002 a, b, c.; Nelson and Boehm, 2002); Fusarium wilt of Flax (Serra-Wittling et al., 1996); *Fusarium oxysporum* in sweet basil (Reuveni et al., 2002); soil borne pathogens in cereal production, namely *Gaeumannomyces, Phoma and Plasmodiophora* (Tilston et al., 2002).

Two mechanisms of pathogen control have been described, (Hoitink et. al., 1996 Amir and Alabouvette, 1993; Toyota and Kimura, 1993; Toyota et al., 1995);

- General suppression of *Pythium* and *Phytophthora* spp. The high microbial activity prevents germination of the spores.
- Specific antagonistic suppression of *Rhizoctonia solani* and *Sclerotium rolfsii* by *Trichoderma* spp and *Peniciliium* spp respectively

The suppressive effect of the compost and vermicomposts appears to be dose dependent and increased with an increase compost application rates and

vermicomposts contains twice as many antagonistic bacterial as peat (Szczeck, 1999). There is broad agreement that it is microbial antagonism that is responsible for the Fusarium suppression observed, (Serra-Wittling et al., 1996; Toyota et al., 1995) however, suppressing agent of other pathogens is not known.

Pot experiments with pathogenic fungi (*Phytophthora nicotianae* var. *nicotianae, Fusarium oxysporum* f. sp. *lycopersici, Plasmodiophora brassicae* and nematodes, *Heterodera schachtii* and *Meloidogyne hapla* on tomato and cabbage revealed a suppressive effect of commercial earthworm compost toward the pathogenic fungi but not toward the parasitic nematodes (Szczech et al.,1993).

Subsequent comparative studies of vermicomposted sewage sludge and animal manures have shown that sewage sludge derived composts do not protect tomatoes from *Phytophthora nicotianae* and significantly reduces their growth rate whereas manure derived compost reduced infection and increased growth rates. The sewage sludge compost inhibited the growth of *P. nicotianae* and the growth of plants, (Szczech and Smolinska, 2001).

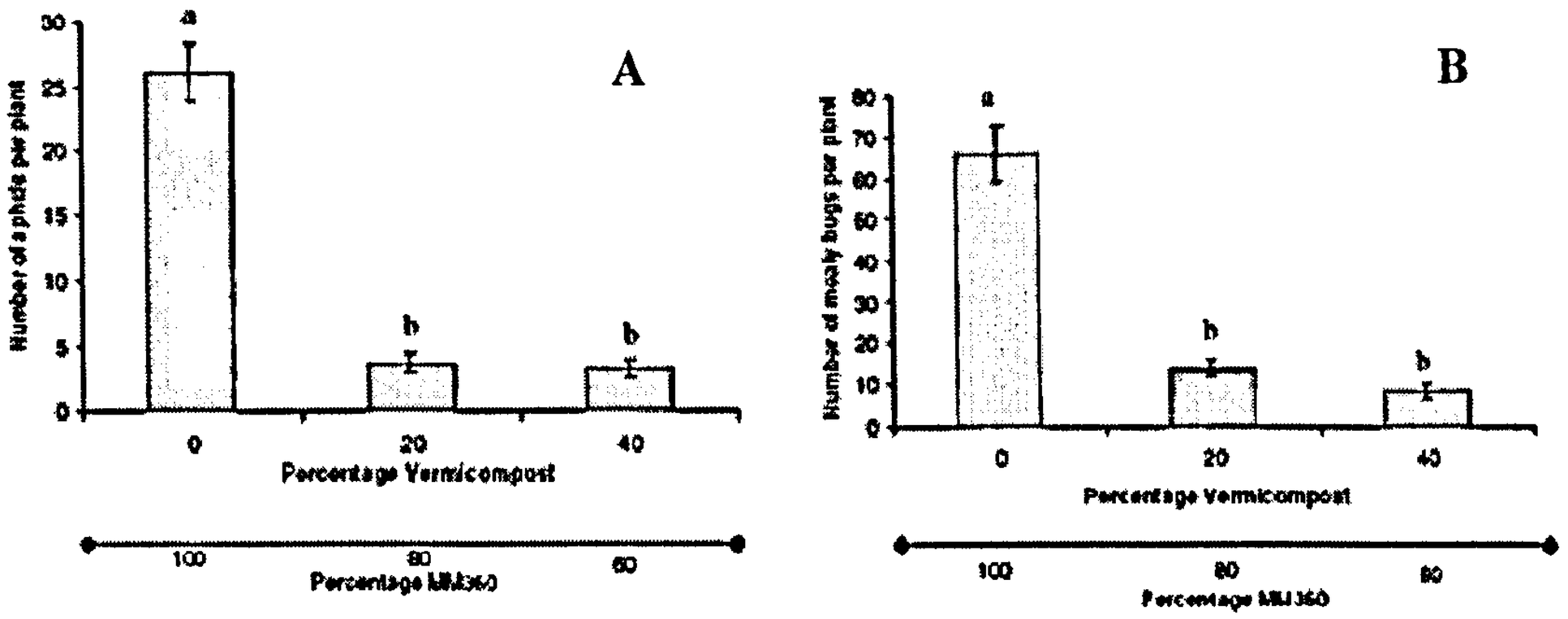


Figure 2.9 Pest suppression by vermicomposts.

A) Aphid suppression in peppers planted in soil-less medium (MM360) substituted with vermicompost presented as number of aphids in pepper plant. **B)** Mealy bug suppression in peppers planted in soil-less medium (MM360) substituted with vermicompost presented as number of mealy bugs per pepper plant. Columns followed by the same letter do not differ significantly at $P \leq 0.05$. Adapted from Arancon et al., (2005a).

Only one study reports on the ability of vermicompost to reduce infestations of common insect pests such as aphids (*Myzus persicae* Sulz.), mealy buds (*Pseudococcus* spp.) and cabbage white caterpillars (*Pieris brassicae* L.) in greenhouse experiments. In all plants studied in this case, infestation was significantly reduced by the presence of 20%-40% v/v vermicompost in the growing medium (Arancon et al., 2005a), (Fig 2.9a,b).

2.5 SUMMARY

The UK is facing major challenges in order to achieve our EU landfill directive targets and composting is rapidly becoming a key method by which we are reducing the volume of waste we send to landfill. Many of the very basic physical and chemical processes of thermophilic composting are well understood. Current research is targeted at more complex processes of nutrient losses, heavy meatal speciation

during active composting and after application onto contaminated soils and worker health and safety implications resulting from emission of bioaerosols (fungal spores and bacteria) and gaseous losses.

In complete contrast, vermicomposting as a primary and secondary treatment of food wastes has received little attention. The understanding of their plant growth potential is being studied by a few researchers but needs further work to develop a broader picture. Sanitation properties have also been studied but this also requires further work for this to become more widely accepted. In addition, if vermicomposting is to be adopted on a broader scale it becomes likely that these composts will be applied to agricultural land although the most lucrative markets will be in the amateur gardening and horticulture sectors. Although the physical, chemical and microbiological changes that occur in soil following application of vermicomposts has received some attention, neither their potential for growth and yield enhancing properties or optimum application rates have been determined.

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CHAPTER 3

BACKGROUND MATERIAL AND EXPERIMENTAL METHODS

Included in this chapter are further details of specific methods, and experimental considerations that presenting a thesis as a series of papers does not allow. Also included are further details of analytical materials and methods which are common to each of the experiments outlined in this thesis.

3.1 *ARTICLE 1.* IN-VESSEL CO-COMPOSTING OF GREEN WASTE WITH BIOSOLIDS AND PAPER WASTE

3.1.1 Experimental Design

When studying several different feed stock as in this study, for both process management and statistical rigour it would be desirable to do this in replicated, separate EcoPOD®'s; however, restrictions, both financial and regulatory meant that we were restricted to one EcoPOD®. It therefore has to be assumed that aeration regimes adopted here could not be fully adapted to a particular feedstock and were managed so as to ensure that the wettest mixture composted fully. It was also for this reason that the Green waste/ Biosolid mixture was located nearest the fan in order to maximise aeration, the EcoPOD® was therefore set up as in fig 3.4 with buffering strips in between each feedstock and at each end to minimise edge effects.

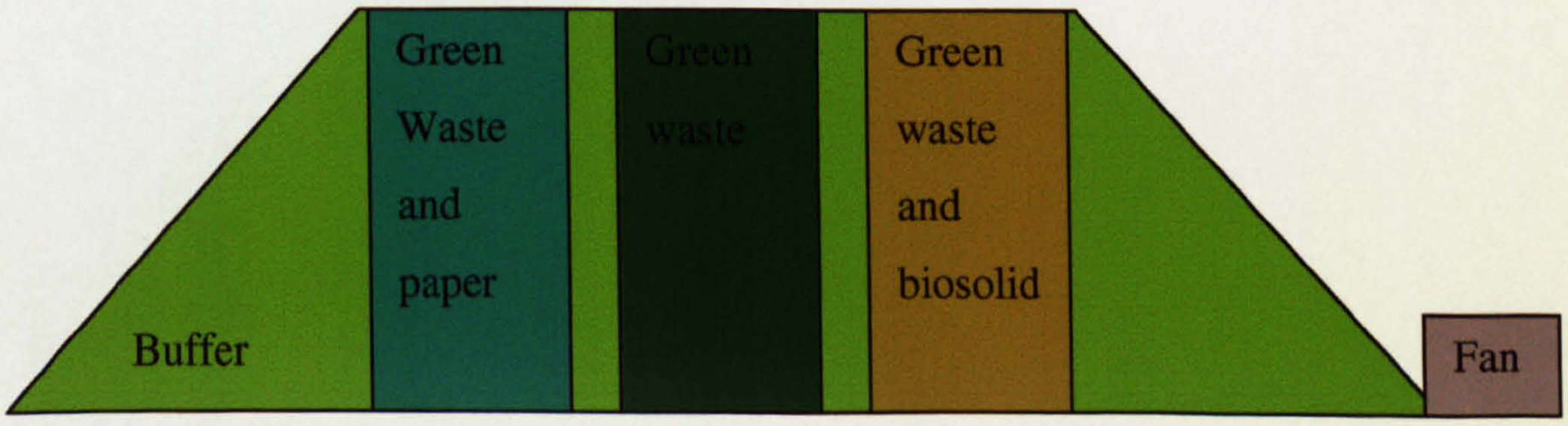

Figure 3.1 Experimantal design of EcoPOD®.

3.1.2 Sampling strategy

Although the feed stocks were well mixed, heterogeneity on a smaller scale is inevitable. In order to produce a representative sample, the Composting Association recommend pooling sub samples before analysis; however, in an experimental setup such as this it is preferable to report the variability, therefore the samples were not pooled. EN 12579:1999 recommends a minimum of 12 samples for growing media. In this experiment we considered each feedstock type as a separate unit and as such considered 12 samples from each feedstock type to be sufficient to give a representative sample. Although sampling from the same location might suggest the use of a Repeated Measures ANOVA. This statistical test assumes that the factor being measured will be identical at each sampling time, this statistical method attributes more of the variance to the between groups part of the ANOVA thus increasing the power of the test. However, since one of the objectives was to measure variability, it was decided that this was not desirable and we decided to use a conventional one way ANOVA for statistical analysis of these results

3.1.3 The EcoPOD® Composting System

This is a low-cost, forced aeration, in-vessel composting system. It is essentially a plastic tube that is filled with compostable material using either a CT 5® or CT 10® depending on the bag diameter. The CT 5® used in this experiment is in essence a hopper that incorporated a hydraulic ram that pushes the material into the EcoPOD®, simultaneously inserting a perforated plastic pipe in the base. This pipe is attached to a timed fan which allows aeration of the system (Fig 3.2). One complete CT 5® EcoPOD® can hold 75 tonnes of composting material. The experimental EcoPOD® in this study contained 30 tonnes of material and was approximately 30 m long, (Fig

3.3). When composting is complete, the bag is opened and the compost removed, stored and allowed to mature. The plastic is either recycled or landfilled but cannot be reused for composting. In this study the plastic was used as a cover for the maturing composts, (Fig 3.4).

Figure 3.2 Filling the CT5® with green waste

Figure 3.3 Experimental EcoPOD® at Henfaes Research Centre

Figure 3.4 Covered, maturing greenwaste compost.

3.2 *ARTICLE II.* RESPONSE OF COMMON POT GROWN FLOWER SPECIES TO PLANT GROWTH MEDIA SUBSTITUTED WITH VERMICOMPOST.

3.2.1 Vermicompost production

To produce vermicompost for future experiments, six worm beds were set up. Since earthworms are less active during the winter months when this was set up and in order to preserve nutrient content, these experimental worm beds were located in a heated outbuilding (Fig 3.5). Each bed was covered in black, gas permeable membrane and the room maintained at an average temperature of 20°C. Each wormbed initially contained 5 kg m^{-2} of the earthworm *Dendrobaena veneta.* These were fed semi-mature compost in thin layers as required (2 weekly). Moisture, pH and

EC were monitored weekly and adjusted if required. After approximately two months and every 6 weeks thereafter, the beds required emptying, this was done by hand-sorting half of the worm bed, separating and removing the vermicompost from the earthworms. The earthworms were weighed and returned to the worm bed on each occasion. The vermicompost was retained for growth trials. On average, the earthworms processed 1.3 kg m^{-2} d^{-1}. Although some breeding and cocoons were observed, this was insufficient to maintain original population numbers and earthworm numbers declined throughout the vermicomposting period. When sufficient vermicompost had been produced, the remaining earthworms were transferred to an outdoor bed. Vermicomposted green waste was used for studying flower responses (Chapter 5) and vermicomposted green waste and biosolid for studying wheat yield responses (Chapter 6).

Figure 3.5 A Experimental worm bed. B Hand-sorting earthworms from vermicompost

3.2.2 Choosing Flower Species

The main objective of this experiment was to study growth responses of flower species that a) represented wide range nutrient requirements, b) developed single and multiple flowers, c) produced seeds in a form that was easy to measure and, d) commonly grown in the UK and were likely to perform well in greenhouse conditions.

It was decided to use Sunflowers (fig 3.6), Cosmos (fig 3.7) and Californian poppy (fig 3.8)

- Easy to grow
- Large nutrient demanding plant
- Single flower
- Good seed producer

Figure 3.6 Sunflowers (*Helianthus annua)*

- Easy to grow
- Grows in low nutrient growth media
- Multiple flowers
- Good seed producer

Figure 3.7 Cosmos (*Cosmos bipinnatus)*

- Easy to grow
- Grows in medium nutrient growth media
- Multiple flowers
- Good seed producer

Figure 3.8 Californian Poppy (*Eschscholzia californica).*

3.3 *ARTICLE III* YIELD RESPONSES OF WHEAT (*Triticum aestivum*) TO VERMICOMPOST APPLICATION

The fields at Henfaes Research Station have, in the past, been used for a wide range of growth trials and it is likely that at some point the fields used for this experiment had received various fertilizer treatments. However, for several years prior to this experiment the fields had been used as grazing for the farms sheep flock. For this experiment we assumed that no previous inorganic N treatments were likely to influence our results. The experimental area was planted as four drill strips, subsequently blocked and treatments applied (fig 3.9).

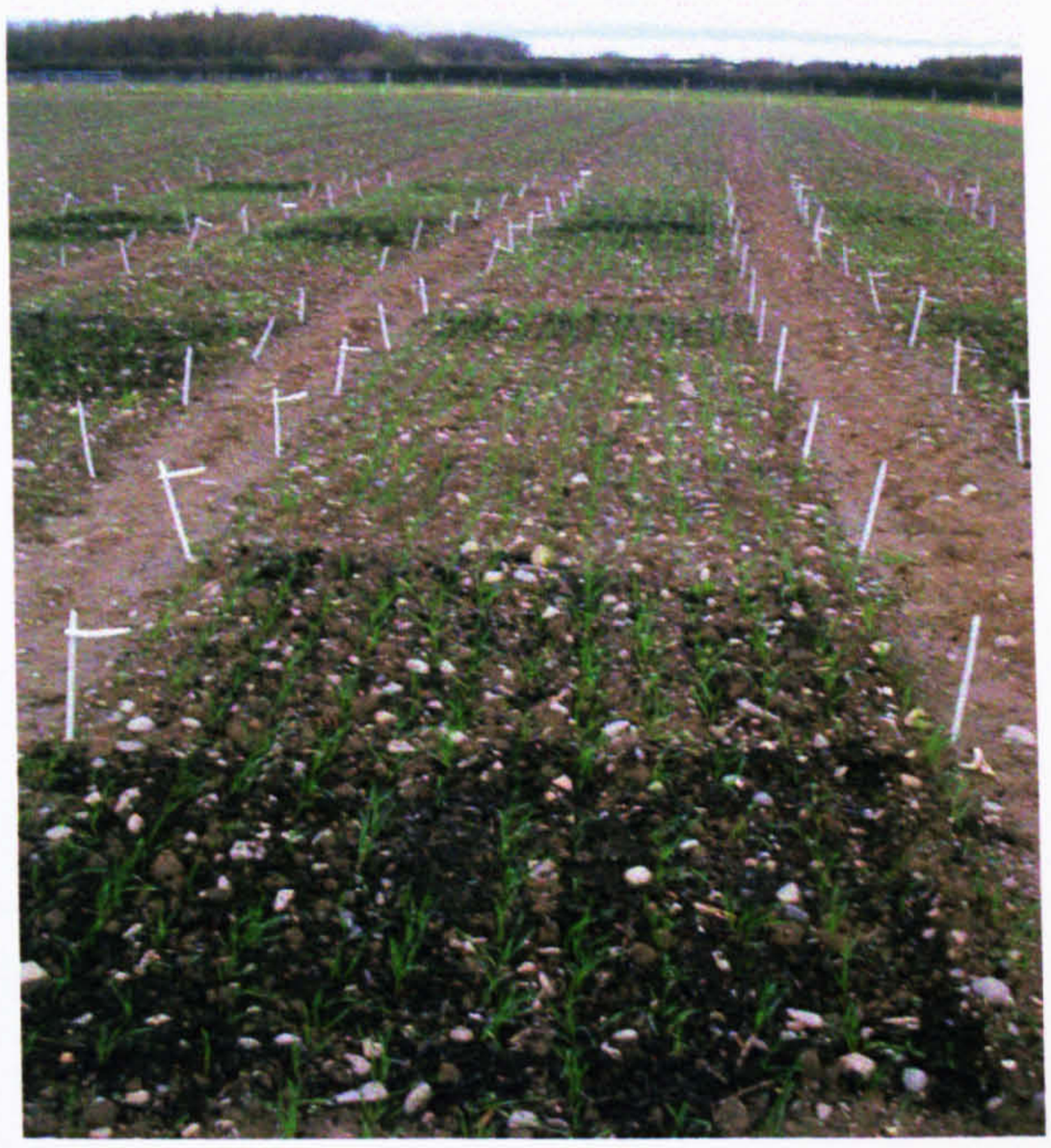

Figure 3.9 Wheat growth trials at Henfaes Research Station (Spring 2005), showing vermicompost application plots. Darkest plots are 30t ha^{-1} equivalent of vermicompost application.

3.4 *ARTICLE IV.* YIELD AND VITAMIN CONTENT OF TOMATOES *(Lycopersicon esculentum)* GROWN IN VERMICOMPSTED MANURES

3.4.1 Identifying marketable fruits

Providing supermarkets with fruit that is of acceptable quality often results in high levels of rejected fruits. For this reason, the production of a high proportion of marketable fruits from a crop is of primary importance. As well as measuring total tomato yield, we decided that it was equally important to determine the effect of growth medium on the production of marketable fruits. However no definitive guidelines exist on determining what is a marketable fruit, we therefore decided on a strategy for determining marketable fruit. All fruits had to be completely free of skin blemishes and disease free. No fruits displaying any of the physiological conditions described below were accepted, neither were any fruit below 4cm diameter.

In a large growth trial such as this it was inevitable that some proportion of the fruits harvested will be unmarketable. Of primary concern were a range of physiological disorders that are common in tomatoes. It became obvious at the beginning of the fruit development stage that some of the first fruits were developing a physiological condition known as Blossom End Rot (fig 3.10A), this is a common response to calcium deficiency usually induced by water stress. Similarly a few fruits became misshapen, a disorder known as "cat facing" (fig 3.10C), this is primarily a response to cold. Toward the end of cropping period, some fruits developed radial cracks (fig 3.10C). This is a response to swings in moisture content and although great care was taken to minimise this several fruits became severely cracked. One plant developed verticillium wilt at the very end of the cropping period; this did not affect our results.

Fig 3.10 Common physiological fruit disorders of tomato, A. Blossom End Rot; B. Cat Facing; C. Cracking (www.uga.edu/vegetabl/tomato.html).

3.5 *ARTICLE V.* EARTHWORMS AS VECTORS OF *ESCHERICHIA COLI* O157:H7 IN SOIL AND VERMICOMPOST

3.5.1 Keeping earthworms in experimental beds

Maintaining a healthy and active earthworm population in small indoor beds is never easy. Earthworms are very sensitive to external factors such as moisture content, pH and electrical conductivity of the bedding material and these factors are sometimes overlooked in an experimental environment. Since this experiment was conducted indoors we were particularly concerned that the bedding material would dry out and moisture content was monitored and adjusted regularly in compost boxes (fig 3.11A). It was also for this reason that we chose a mixture of compost and paper pulp as the initial bedding material for the compost part of the experiment thus giving the grey appearance in fig 3.11A. Since we did not want to perpetuate any vertical movement of *E. coli* in the soil cores by watering from above, the moisture content was maintained by standing the cores in water in a large basin and the water level replenished as required. The lowest portion of the soil did become waterlogged and developed a slight gleyed appearance (fig 3.11B). In both experiments earthworms were fed with cattle manure as required.

Fig 3.11A. Experimental vermicomposting beds showing feeding strip of manure and several *D. veneta* earthworms. B. Opened soil core prior to sampling, earthworms were fed from the top of the core (top of the picture).

3.6 EXPERIMENTAL METHODS

3.6.1 Moisture Content

Approximately 10 g of compost or soil was weighed out into a ceramic crucible and placed in an oven at 105°C for 24 hours. Moisture content was calculated as the weight difference before and after weighing and expressed as a percentage of dry weight. Some slight loss of volatile organic compounds may occur at this temperature, however, at the beginning of analytical work, an oven set at lower temperature was unavailable. The same method was then adopted throughout the remainder of the analytical work.

3.6.2 Ph and Electrical Conductivity (EC)

Equal volumes of compost and distilled water (1:1 v/v) were mixed thoroughly and allowed to equilibrate for 30 min. The pH and EC was determined from the same compost/ water suspension using a pH (Orion 410A pH meter), or EC electrode (Jenway 4010 meter) according to the method of Smith and Doran (1996).

3.6.3 Extractions

All composts/ soils were extracted with 1 M KCl (1:5 w/v compost-to-KCl ratio) for 1 h on a reciprocating shaker at 250 rev min^{-1}. The extracts were then centrifuged for 10 min at 10000 g, the supernatant filtered through Whatman 40 filter paper and the samples stored in polypropylene bottles at -20°C to await analysis. These extracts were used for all analyses with the exception of K, Na and Ca. For K, Na and Ca analyses, the samples were extracted with 0.5 M CH_3COOH (1:5 w/v compost-to-CH_3COOH ratio) as described above.

3.6.4 Total Carbon and Nitrogen

Total C and N were measured by combustion analysis on oven-dried composts using a LECO CHN 2000 analyser. Weighed samples are combusted in a furnace at 930°C under a constant flow of oxygen. The resulting CO_2 is then passed through an infrared cell and its concentration measured. NO_x gases are passed through a copper catalyst where it reduced them to N_2. This is measured by thermal conductivity once the gases have been scrubbed of CO_2 and H_2O. C and N are given as percentages of total weight and C-to-N ratio (C:N) calculated by division.

3.6.5 Total Dissolved Organic and Inorganic Carbon

Dissolved organic C (DOC) and Inorganic C (IC) were measured using a Shimadzu TOCV-TN analyzer (Shimadzu Corp., Kyoto, Japan) as described in Jones et al. (2002).

3.6.5.1 Total Dissolved Carbon:

The sample is burned in a combustion tube at 720°C to form CO_2, this is cooled and dehydrated, passed through a halogen scrubber and analysed by a Non-Dispersive Infra Red gas analyser, (NDIR).

3.6.5.2 Inorganic Carbon (IC)

IC analysis involves acidifying the sample to a pH of less than 3 with HCl, this converts all carbonates to CO_2. Both the CO_2 and the dissolved CO_2 are volatilised by sparging with air and measured by NDIR.

3.6.5.3 Total Organic Carbon

Two methods can be used for determining Dissolved Organic Carbon (DOC),

1. TC- IC = TOC. This includes errors associated with both TC and IC analyses.
2. Non-purgeable organic carbon (NPOC). This involves acidifying and sparging the sample to eliminate the IC component, the TC remaining is referred to as NPOC to distinguish between the two methods. NPOC is generally used when a sample contains more IC than TOC.

The first method was used throughout this study although both methods are in essence the same and give similar results except when there is a significant amount of purgeable organic component present in the TOC. The relationship between TOC concentration and peak area is determined using a TOC standard (potassium hydrogen phthalate).

3.6.6 Total Dissolved Nitrogen (Tdn), Inorganic Nitrogen (Din) and Dissolved Organic Nitrogen (Don)

Total soluble N (TSN) were determined using a Shimadzu TOCV-TN analyzer (Shimadzu Corp., Kyoto, Japan).

3.6.6.1 Total Nitrogen (TN)

The sample is introduced into a combustion tube at 720°C. The nitrogen in the sample is decomposed to NO. The carrier gas (N_2) containing the NO is cooled and dehumidified and enters the chemiluminescence gas analyser. The gas analyser detects the TN concentration.

3.6.6.2 Total Inorganic Nitrogen (TIN)

Measuring concentrations of inorganic nitrogen species in compost extractions requires the addition of several reactants to the extract to produce a coloured chromophore. Measuring the intensity of colour development enables the determination of concentration. This is done by UV-Visible Spectroscopy

3.6.6.3 UV-Visible Spectroscopy

Ultraviolet-Visible spectroscopy measures the transmission of light through a substance. When light is absorbed, the radiant power of the light is decreased. The light passes through a monochromator in order to select one wavelength; this light with a known radiant power strikes the sample and the decrease in radiant power is measured. Beer's law tells us that absorption is proportional to concentration of absorbing molecules. Lambert's law says that the fraction of absorbed light is independent of the intensity of the radiation. Combining Beer's and Lambert's laws enables a calculation of concentration from absorbance.

3.6.6.4 Nitrate NO_3^-

Automated method

All analysis in chapter 3 was performed on a Skalar San++ segmented flow autoanalyser (Skalar Inc., Norcorss, GA). The reaction is based on the reduction of NO_3^- to NO_2^- by passing the sample through an activated Cadmium/ Copper column. The resulting NO_2^- is reacted with a primary aromatic amine (sulphanilamide) then coupled with α-napthyl-ethylenediamine dihydrochloride to form a coloured azo dye. There is a potential for interference from Fe, Cu and other metals that can give negative NO_3^- results.

Reagents

1. *Ammonium Chloride Buffer*: 50 g NH_4Cl in 1 litre distilled water, pH adjusted to 8.2 with ammonia solution, add 1 ml Brij

2. *Colour Reagent*:

   150 ml o-phosphoric acid in 700 ml distilled water, add 10 g sulphanilamide $C_6H_8N_2O_2S$ and 0.5 g α-napthyl-ethylenediamine dihydrochloride $C_{12}H_{16}Cl_2N_2$ and make up to 1 litre.

Standards

3.034 g $NaNO_3$ in 500 ml distilled water gives a 1000 ppm stock and was diluted to the following concentrations for calibration: 0, 2, 4, 6, 8, and 10 mg NO_3^- - N l^{-1}

Manual method

In the remainder of the thesis, the same reaction is used but the analysis was done manually using a microplate reader using the hydrazine, *N*-1-napthylethylenediamine assay, (Downes 1978). This method is sensitive between 0 – 2.5 mg NO_3^- - N l^{-1} and most samples required dilution to bring them within the calibration range.

Reagents

1. Catalyst Solution. 0.0354 g A.R. $CuSO_4.5H_20$ plus 0.9 g $ZnSO_4.7H_2O$ in 1 litre of distilled water.

2. 1 M NaOH (0.8 g in 20 ml of distilled water)

3. Hydrazine sulphate solution (0.0342 g in 20 ml of distilled water)

4. N-1-napthylethylenediamine dihydrochloride (NED; 0.1g in 100 ml of distilled water)

Method

Into each well of a microplate:

1. Add 114 µl sample or standard
2. Add 20 µl catalyst, mix
3. Add 20 µl NaOH, mix
4. Add 20 µl hydrazine, mix
5. Incubate for 7.5 min at 33 °C
6. Add 60 µl sulphanilamide
7. Mix for 4.5 minutes
8. Add 20 µl NED
9. Mix for 4.5 minutes
10. Read at 540 nm

3.6.6.5 Ammonium NH_4^+

Automated method

In chapter 4 all NH_4^+ analyses were done using a Skalar San++ segmented flow autoanalyser using the Berthelot or Indophenol Blue reaction to give the coloured indophenol blue dye, the intensity of which can then be measured by absorbance at 667 nm. This reaction is not specific to NH_4^+ since a variety of organic compounds having a free amino group are known to develop the same blue colour (Mulvaney, 1996).

Reagents

1. *Buffer*: 33 g Potassium Sodium Tartate ($C_4H_4O_6KNa_3.2H_2O$) and 24 g sodium citrate ($C_6H_8O_7Na_3\ 2H_2O$) in 1 litre distilled water, adjust pH to 5.2 with HCl, add 1 ml Brij
2. *Sodium Salicilate:* Add 25 g Sodium Hydroxide (NaOH) and 80 g Sodium Salicilate ($C_7H_5NaO_3$) to 1 litre distilled water (stable for 1 week)
3. *Sodium Nitroprusside:* 1 g Sodium Nitroprusside, ($Na_2[Fe(CN)_5NO].2H_2O$), in 1 litre distilled water, (stable for 1 week in dark bottle)
4. *Sodium Dichloroisocyanurate:* 2 g Sodium Dichloroisocyanurate ($C_3H_4Cl_2N_3NaO_5$) in 1 litre of distilled water, (stable for 1 week)

Standard

0.19095 g Ammonium Chloride (NH_4 Cl) in 500ml gives a 100 mg N l^{-1} stock solution and were made up into the following concentrations for calibration 0, 2, 4, 6, 8, and 10 mg NH_4^+ - N l^{-1}

Manual method

In the remainder of the thesis, the same reaction is used but the analysis was done manually using a microplate reader (Mulvaney 1996).

Reagents

1. *Sodium Salicilate- Sodium Nitroprusside :* Dissolve 0.78 g Sodium Salicilate ($C_7H_5NaO_3$) and 0.0125 g Sodium Nitroprusside, ($Na_2[Fe(CN)_5NO].2H_2O$) in 10 ml distilled water .
2. *Buffered Hypochlorite Reagent:* Dissolve 0.296 g Sodium Hydroxide (NaOH), 0.996 g Sodium Monohydrogen Phosphate Heptahydrate

($Na_2HPO_4.7H_2O$)in approx 8 ml distilled water, add 1 ml Sodium Hypochlorite (NaOCl). Adjust pH to 13 with NaOH and make up to 10 ml with distilled water.

3. Ethylenediaminetertaacetic acid (Na_2EDTA). Dissolve 0.6 g of Na_2EDTA in 10 ml distilled water.

The same 100 mg $NH_4Cl - N\ l^{-1}$ standard stock solution was used for both methods.

Method

Into each 0.3 ml microplate well:

1. Add 25 µl of sample or standard
2. Add 15 µl EDTA, mix
3. Add 60 µl Na salicylate – nitroprusside reagent, mix
4. Add 134 µl distilled water, mix
5. Add 30 µl of hypochlorite reagent, mix
6. Put in an incubator at 37 °C for 30 min (or at room temperature, 20 – 25 °C for 2 hours)
7. Read at an absorbance value of 667 nm

Dissolved Organic Nitrogen

Dissolved Organic Nitrogen is calculated by TDN – TIN = DON

3.6.7 Phosphate

Phosphate was determined colourimetrically using the method of Murphy and Riley (1962). Under acidic conditions, phosphate reacts with ammonium molybdate to

form an ammonium phosphomolybdate complex which is then reduced by ascorbic acid to produce molybdenum blue. The absorbance can be measured at 820 nm.

$$PO_4^{3-} + 12(NH_4)2MoO_4 + 24H^+ \rightarrow (NH_4)3PO_4.12MoO_3 + 21NH_4^+ + 12H_2O$$

Reagents

1. 0.42% ammonium molybdate in 1 N H_2SO_4
2. 10% ascorbic acid in distilled water

Method

1. Pipette 80 µl standard or sample into a well of a microplate.
2. Add 30 µl of Ascorbic acid.
3. 180 µl ammonium molybdate reagent.
4. leave for 30 minutes for the colour to develop and read on a microplate reader at 820 nm.

3.6.8 Potassium, Sodium and Calcium

K, Na and Ca were measured using a Sherwood 410 flame photometer (Sherwood Scientific, Cambridge, UK). CH_3COOH extracts were always diluted with distilled water for K analysis. Compost is invariably rich in K and a 10 fold dilution is always necessary. In composts that are particularly high in K, a dilution of 100 fold was sometimes required in order to bring the concentration to within the calibration range. Na and Ca were analysed in the same 10 fold dilution to bring the samples to within the calibration range. Flame photometry exploits the ability of hot atoms to emit light when their electrons have been promoted to excited states, in this case by a flame. Comparing

emission of the unknown with a standard calibration curve enables a determination of concentration to be made.

3.6.9 Statistical Analyses

All statistical analyses were conducted using SPSS (v 11.5), (SPSS Inc. Headquarters, 233S.Wacker Drive, Chicago, Illinois 60606 USA).

All statistical methods used in this thesis assume that the data is normally distributed around the mean. In all cases, data was tested for normality by using the Kolmogorov-Smirnov test for normality, (Pallant 2001). Where data was found not to be normally distributed, the data set was transformed. If data presented is a transformed data set, the method of transformation is quoted separately in each chapter. Outliers were identified using SPSS "Normality plots with test" command (Figure 3.12). Outliers are defined by SPSS as values that are 1.5 box lengths from the edge of the box; each box length is the interquartile range for that particular variable. These were further analysed by a Q test and if confirmed as outliers they were removed from the data set before continuing with the analysis. This was only necessary for a small number of values in greenhouse flower trials and not for any other study in this thesis.

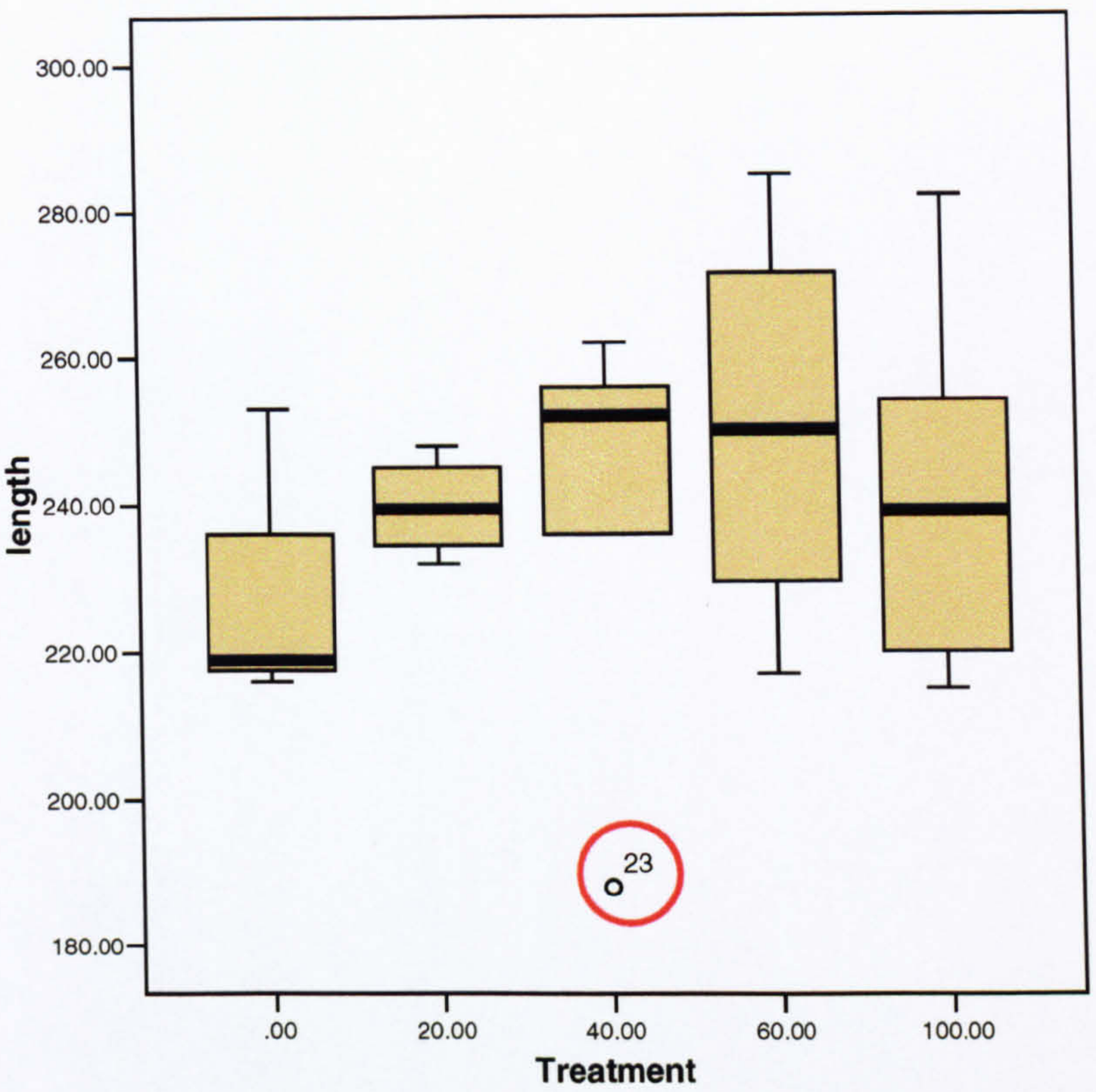

Figure 3.12 Identifying outliers in final *Helianthus* length using Box Plots. Outlier is highlighted by red circle

3.6.9.1 One way Analysis of Variance (ANOVA)

All results in chapter 4 were analysed using a One Way Analysis of Variance (ANOVA) with Post Hoc Tukey test to identify significantly different treatments. Significance is defined as $p \leq 0.05$ unless otherwise stated.

3.6.9.2 Two way ANOVA

Two Way ANOVA allows an analysis of two individual factors and the joint effect of both these factors. The greenhouse used for both lysimeter experiments and all pot experiments in this thesis is known to have two distinct temperature gradients (Figure 3.13). It becomes necessary to test for the effect of temperature on the experimental result as well as the effect of treatment. This is done by adopting a

Randomised Block Design for treatment allocation, and analysing the results by Two Way ANOVA to test for the effect of the secondary variable, i.e. temperature in this case. In addition it enables us to test for the more ambiguous interaction effect of both temperature and treatment. Where an interaction effect is determined as statistically significant, then no definitive conclusion about the effect of either treatment or temperature can be made.

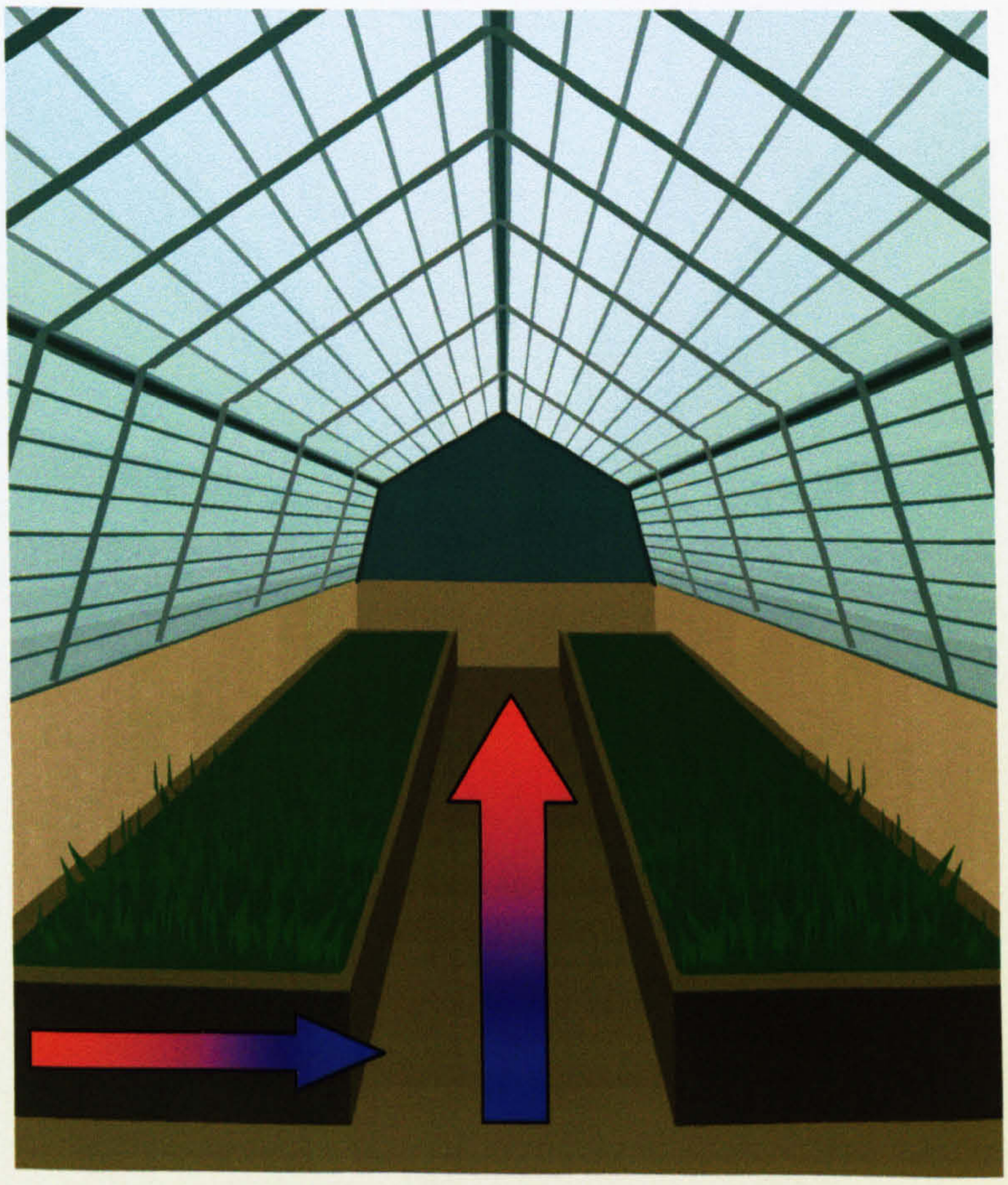

Figure 3.13 Temperature gradients within greenhouse

3.6.9.3 Experimental Design

A fully randomised design runs the risk of having all the replicates of one treatment together in one temperature regime. In circumstances such as the greenhouse experiment it is necessary to overcome this by blocking the experimental design and

assigning treatments randomly within each block. Therefore it is guaranteed that there is an equal number of treatment replicates within each temperature block. This enables us to test for the effect of the second variable as treatment on the result. In this thesis, none of the growth studies were large enough to require an analysis of both temperature gradients shown in figure 3.13 simultaneously.

Similar patterns can be seen in field experiments although the temperature variable is usually replaced by other factors such as aspect, soil drainage etc. The principle for experimental design and statistical analysis is the same randomised block design with Multiple Factor ANOVA. Both field scale experiments in this thesis were designed using a Randomised Block Design and analysed by Two Way ANOVA with block and treatment, (vermicompost/ fertiliser application rates), as dependent variable and the measurable variable (i.e. yield, flower number etc) as the independent variable. Significant differences were identified using either a Post Hoc Tukey Test or Least Significant Differences Test, (LSD) with significance defined as $p \leq 0.05$ unless otherwise stated.

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CHAPTER 4

Article I

Roberts P., Edwards-Jones G., Jones D.L. In-vessel Co-composting of Green Waste with Biosolids and Paper Waste.

Submitted to *Compost Science and Utilization*

In-vessel Co-composting of Green Waste with Biosolids and Paper Waste

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Abbreviations: DON, dissolved organic nitrogen; GW, green waste; PP, paper processing waste; BS, biosolids.

CHAPTER 5

Article II

Roberts, Edwards, Edwards-Jones, Jones Responses of common pot grown flower species to commercial plant growth media substituted with vermicomposts.

Submitted to *Bioresource Technology* January 2005.

Responses of common pot grown flower species to commercial plant growth media substituted with vermicomposts.

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CHAPTER 6

Article III

Roberts, Edwards-Jones, Jones: Wheat *(Triticum aestivum)* to Vermicompost Applications. Accepted for publication in *Compost Science and Utilization*

Yield Responses of Wheat *(Triticum aestivum)* to Vermicompost Applications.

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CHAPTER 7

Article IV

P.Roberts, D.L.Jones, G.Edwards-Jones. Yield and Vitamin Content of Tomatoes (*Lycopersicon esculentum*) Grown in Vermicomposted Wastes

Submitted to *Journal of the Science of Food and Agriculture*

Yield and Vitamin Content of Tomatoes (*Lycopersicon esculentum*) Grown in Vermicomposted Wastes

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CHAPTER 8

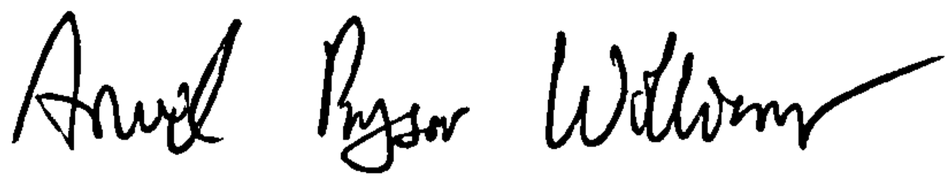
Article V

A.P. Williams, P. Roberts, L.M. Avery, K. Killham, D.L. Jones. Earthworms as vectors of *Escherichia coli* O157:H7 in soil and vermicomposts

Published in FEMS Microbial Ecology

8.1 DECLARATION

This was a collaborative piece of research between Arwel Prysor Williams and Paula Roberts. We declare that we both participated equally in the experimentation, data analysis and manuscript preparation.

Arwel Prysor Williams 

Paula Roberts 

Earthworms as vectors of *Escherichia coli* O157:H7 in soil and vermicomposts

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CHAPTER 9

GENERAL DISCUSSION

9.1 Discussion of Results

This thesis has attempted to achieve two objectives.

1. Further the current understanding of nutrient dynamics within the thermophilic composting process in particular when adopting Ecopod® system of composting. Evaluate the potential for producing composts of a particular nutrient loading by manipulating the initial feedstock, either by diluting with paper pulp or biosolids.

2. Extend understanding of plant growth responses of manure and compost derived vermicomposts.

Spatial variability within the EcoPOD® composting vessel was minimal ensuring the production of a uniform compost end product. We were successful in manipulating the final compost nutrient content to target different end-uses by selecting appropriate waste feedstocks (*paper I*). We also showed that despite the often negative public perception of the use of biosolids and industrial wastes for land application co-composted, sanitized wastes is likely to pose little environmental risk assuming that they are spread to land in an environmentally compliant manner.

Prior to this thesis most vermicompost research originated from Ohio State University, USA and focused on the plant growth enhancing properties of vermicomposts. These previous studies have shown that plant responses to vermicompost addition have always caused a positive stimulation of growth with one exception (Cavender et al., 2003). In the US, vermicomposting is an indoor process using the earthworm *E. foetida.* In the UK at present, most vermicompost is produced as a by product of the earthworm *D. veneta* breeding process. The major part of the breeding process, which produces the vermicompost, is conducted outdoors with only the final fattening of adult worms being done in indoor heated environments. The objectives of papers *II – IV* were to determine whether the same growth enhancing properties of *E. foetida* produced vermicomposts was also true of those produced by *D. veneta.*

From results of an initial study into the growth of radish, (*Raphanus sativus* var. Scarlet globe L.) (results, appendix 2) it was clear that the enhancement response was species specific. Radish dry weight was significantly decreased by vermicompost addition at most percentage substitution rates. It was decided to expand into common garden flowers to explore the species specificity of the growth enhancing response. Flower species were chosen to represent three broad classes:

A. Sunflower, *Helianthus annuus*, a large, single stemmed, mycorrhizal, nutrient demanding plant with rapid stem elongation that should respond to the presence of plant growth regulators in early growth stages.

B. Aster, *Cosmos bipinnatus.* A medium sized, multi stemmed, mycorrhizal, low nutrient demanding plant

C. Californian poppy, *Eschscholzia californica*. A small plant that develops a bulbous tap root. Is unclear whether this plant develops mycorrhizal associations, at best it is only develops weak associations.

These plants were all grown in various proportions of vermicompost produced from composted material from paper *I*. We confirmed from that study that indeed, plant response to vermicompost addition was species specific.

Paper *III* investigates plant responses on a much larger scale. Whilst there is some work investigating the role of vermicomposts in field scale production of some salad vegetables namely tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*), no work reports on growth responses of arable crops. We were interested in determining the potential that vermicompost might enhance nutrient uptake by wheat, thus enabling farmers to reduce inorganic nutrient input without compromising yield. Our results confirmed our hypothesis that when vermicompost is mixed with small amounts of inorganic fertilizer, wheat yield is not compromised; however, vermicompost addition on its own did result in significant reduction in yield when compared to NPK additions. Similar results were also observed in an identical greenhouse experiment with maize (result appendix 2).

Having succeeded in inducing a plant growth response from our own vermicomposts produced on an experimental scale from pre-composted material; we wanted to confirm the results of Atiyeh et al. (2000) using commercially available, manure-derived, vermicompost that originates from the UK earthworm breeding process; Paper *IV* aimed to do that. In a small preliminary study on tomato growth the previous summer, using our own vermicompost, we observed similar results to Atiyeh

et al. (2000). Consequently, we were expecting to see a similar yield response using commercial UK vermicompost. This did not happen. Not only was plant response to vermicompost addition dependent on plant species it also seemed to depend on individual compost type.

It has been hypothesised that the presence of plant growth regulators (PGR) within vermicompost are major factors which influence plant growth responses to vermicomposts. PGRs have been isolated from vermicomposts and soils supporting high earthworm densities and shown to stimulate root elongation, lateral root emergence (indole acetic acid; Canellas et al., 2002; cytokinins and auxins; Krishnamoorthy and Vajranabhaiah, 1986).

PGRs and plant growth promoting rhizobacteria (PGPR) are ubiquitous in soils and other organic soil amendments (Arshad and Frankenburger, 1998), yet rarely have they been shown to actually cause a yield enhancement under commercial conditions; moreover high numbers of IAA producing PGPRs have been isolated from conventional growing media and shown to actively suppress tomato, lettuce and beet seedling growth (de Brito Alvarez et al., 1995; Gamliel et al., 1993; Loper and Schroth, 1986). It is expected that PGRs are rapidly degraded in soil, subjected to leaching or adsorbed on to the solid phase (Arshad and Frankenburger, 1998), and that the concentrations are insufficient in some composts to initiate a response.

Cavender et al. (2003) reported an enhancement of mycorrhizal associations in plants grown with vermicompost. The potential exists that this root growth stimulation coupled with enhanced extra radical mycorrhizal network development may increase the plants' ability to exploit available nutrients.

Without detailed information on mycorrhizas and PGPRs it will remain difficult to formulate effective quality standards that may be used by the vermicompost industry to ensure product quality and maintain consumer confidence. Given the claims surrounding the plant growth promoting potential of vermicomposts, more work is required to elucidate the factors that govern plant growth enhancement of some vermicomposts.

Several thousand tones of vermicompost are being sold in the UK at present. Considering that most of this will eventually be used to grow salad vegetables that are often eaten raw sanitization of compost is essential. This coupled with increased amounts of vermicompost produced from cattle manure, it became clear that a study into the potential spread of the human pathogen *E. coli* O157 by earthworms in vermicompost and soil was needed. Paper *V* presents the results of such a study. We concluded that the direction of *E. coli* O157 movement by earthworms was dependent on earthworm species. There was an initial increase in pathogen numbers where earthworms were present followed by a sharp decline to levels similar to substrates not containing earthworms. Cattle are asymptomatic carriers when infected with *E. coli* O157 and as such are rarely tested for *E. coli* O157 infection. In the light of this, there is little a worm breeder/ vermicompost producer can do to reduce the risk of *E. coli* O157. Although the likelihood of human infection resulting from consuming food grown on *E. coli* O157 contaminated compost is very low it has been documented (Islam et al., 2005). It is necessary for vermicompost producers to be aware of the potential that *E. coli* O157 may be present in their product.

9.2 Further work

We now know that plants respond to vermicomposts in different ways. What is unknown is why. Plant growth by similar species is enhanced by some vermicomposts but not by others; similarly vermicomposts induce a positive plant growth response in some plant species but not in others.

9.2.1 Vermicomposts vary significantly in their ability to enhance plant growth. It is likely that some of this variability arises from compost process management and storage conditions. In order to further develop and maintain consumer confidence in vermicomposts, there is a need to identify the processes responsible for this variability. Furthermore, the mechanism of growth promotion needs further work.

9.2.2 Vermicomposts appear more "soil like" than their thermophilic compost counterparts. The ingestion process changes the particle size distribution and increases their moisture holding capacity. It is possible that some of the growth enhancement properties of vermicomposts are directly linked to their physical properties. There is a need to investigate the potential that plant growth responses may be attributed to physical changes in peat based growing media induced by the addition of vermicompost. Physical changes may induce changes in plant nutrient availability. There is also a need to determine to what extent physical changes in the growing medium, changes the bioavailability of plant nutrients, particularly of micronutrients.

9.2.3 The presence of Plant Growth Promoting Rhizobacteria (PGPRs) significantly affects plant growth. No attempt has yet been made to identify numbers and species in either composts or vermicomposts.

9.2.4 Since plant growth enhancement is typically attributed to the presence of Plant Growth Regulator's (PGRs) in vermicompost, a comprehensive study of the presence and concentrations of these chemicals in a wide range of growing media is required. Furthermore, PGRs can be expected to undergo a number of fates in compost (e.g. leaching, biotic and abiotic transformation, sorption etc; Arshad and Frankenburger, 1998). Further work is required to characterize their dynamics in vermicomposts and conventional composts.

9.2.5 The behaviour and lifestyle of *D. veneta* and *E. foetida* are very similar and it is unlikely that earthworm speciation induces different plant growth responses from vermicomposts. However, this factor cannot be discounted and merits further research.

9.2.6 We measured plant growth responses in fresh vermicomposts (paper *II*) but failed to do so in older vermicomposts, (paper *IV*). Some vermicomposts are stored for a considerable time before being sold. The potential exists that a lengthy storage time degrades the product and this needs addressing. However, until the mechanism for growth enhancement is identified, it will be difficult to determine which parameters to measure.

9.2.7 In view of the fact that the non-mycorrhizal plant species studied in this thesis (e.g. Radish) performed less well than those known to develop mycorrhizal

associations, it is necessary to identify the mechanisms that led to this growth suppression.

9.2.8 There is growing interest in the ability of vermicomposts to suppress infestations of pests. However, if vermicomposting of waste is to be seriously considered as an alternative to thermophilic composting, there is a need to develop our understanding of human and animal pathogen responses to earthworm digestion.

9.3 References

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APPENDICES

APPENDIX I

I.I COMPOSTING in UK WASTE MANAGEMENT POLICY.

Composting can be an important element of sustainable waste management for the UK and will have a significant role to play in meeting the UK's landfill directive obligations, (Slater and Frederickson, 2001), however, in comparison to other EU countries, the UK has been slow in adopting alternative waste management strategies such as composting of biodegradable municipal waste (Table I).

Table I Management of BMW in selected EU countries (% of total BMW produced), (adapted from Crowe et al., 2002)

	Landfill	Central composting
Netherlands (1998)	13.1	33.3
France (1998)	40.3	8.9
UK (1998)	86.2	3.0

Composting is a process by which the organic component of the waste is biologically decomposed in controlled aerobic conditions. Micro-organisms oxidise biodegradable wastes to CO_2 and water vapour using atmospheric oxygen as the oxidising agent. The heat produced in the process destroys many human pathogens that would otherwise survive other treatment methods (Pepper et al. 1996). The result is humus like residue that can safely be used as a soil conditioner, for land reclamation or as a growing medium in horticulture.

Many wastes contain considerable amounts of pathogenic organisms, inorganic and organic contaminants such as pesticide residues, aromatic hydrocarbons, and heavy metals (Edwards and Bohlen, 1996). Composting will reduce pathogen levels, encourages degradation of some pesticide residues and aromatic hydrocarbons, but heavy metal concentration increases relative volume (Veeken and Hamelers, 2002). Since 1978 there has been increasing interest in earthworm digestion of metal

contaminated wastes; a process known as vermicomposting. Subsequent research of uncontaminated wastes has highlighted the potential of adopting this process as an alternative to conventional methods. Although it is still a process that is very much in the development stage, there is an increasing acceptance that worms do stabilise organic wastes, rendering them innocuous about 3 times faster than noningested sludge, (Edwards and Bohlen , 1996). However some questions still remain about their ability to remove metals and neutralise pathogenic organisms. Some researchers have suggested that all materials should be composted before worms are applied, (Giggliotti et al. 1997) in order to reduce pathogen levels. Others suggest that "*in situ*" vermicomposting may be as effective in incorporating organic matter into the soil (Masciandaro et al. 2000); however, it is unclear whether either of these methods reduces pathogenic organisms to acceptable levels.

I.I.I Commercial Composting Methods

Many small garden sized bins exist for the composting of small volumes of household waste but their effectiveness in reducing volumes of waste to landfill sites depends on voluntary participation. The waste produced by those households without the space or the inclination to participate in household composting schemes along with putrescible wastes from non household sources will also require treatment, to this end large scale composting systems have been developed. These are generally divided into three categories: Windrow, Static pile and In-vessel; however, the initial stage of removing large debris and recyclable materials is the same for all treatment categories. The general process are outlined in fig I

I.I.I.I Windrow

Wastes are composted in long rows (windrows), the mixture is turned mechanically to expose organic matter to ambient oxygen (Pepper et al. 1996), windrows can be open or contained and covered.

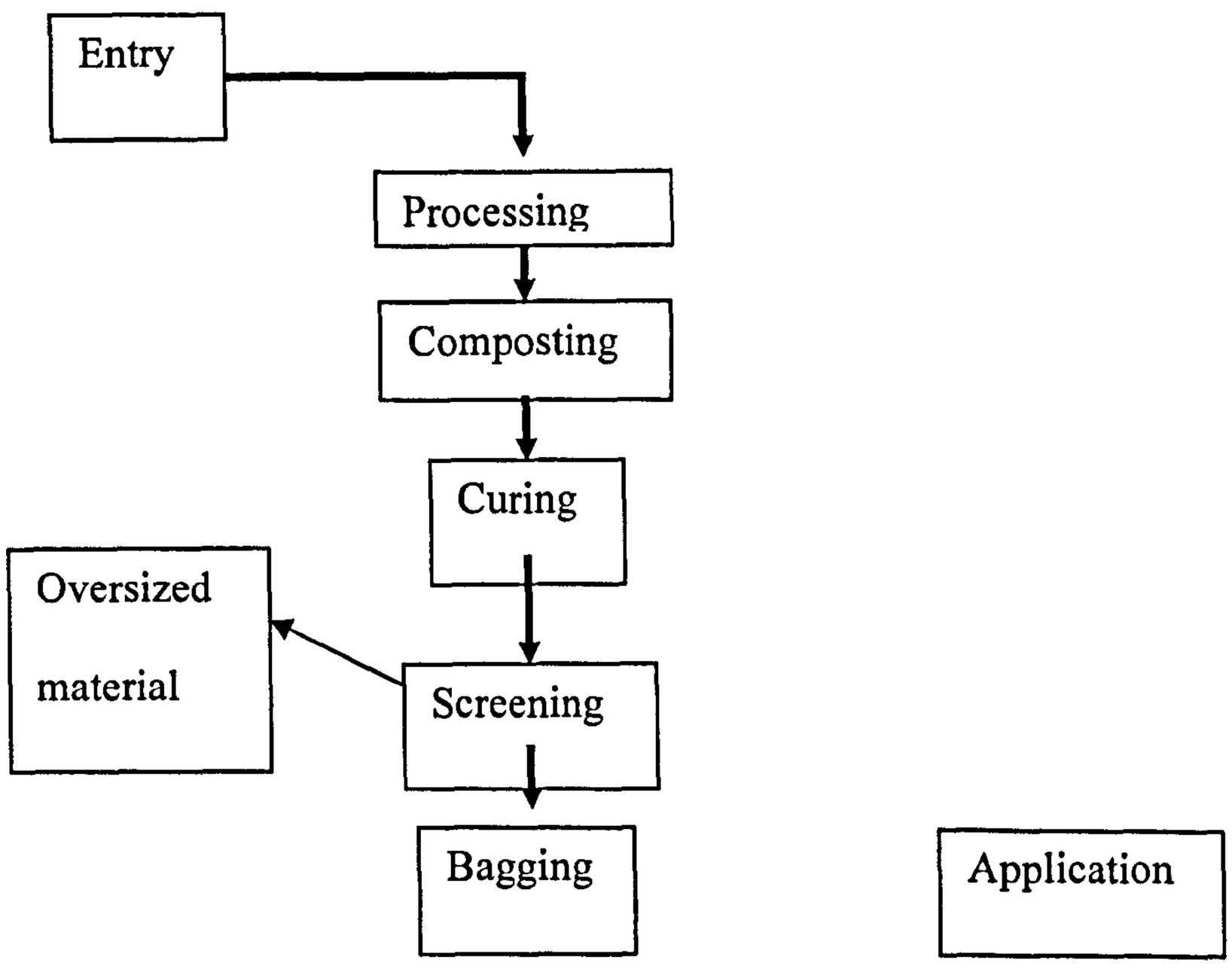

Figure I Schematic of the overall composting process

I.I.I.II Static Pile

Static pile systems are forcefully aerated by perforated pipes installed under the piles to maintain a minimum oxygen level throughout the compost mass.

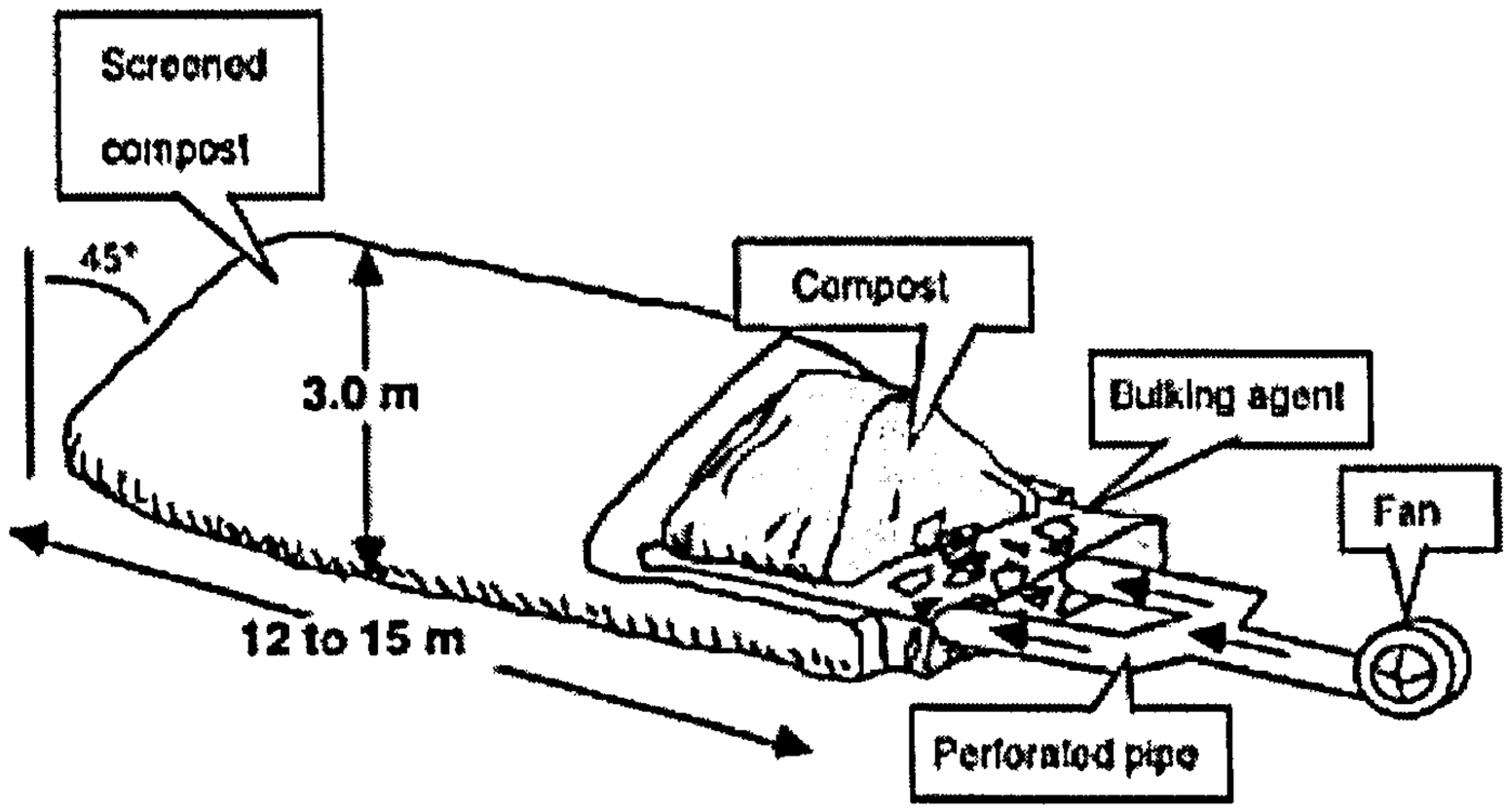

Figure II Static pile composting (Ohio State University undated)

The composting process takes approximately 21 days. It is then dried, screened stored or cured for approximately 30 days and sold or distributed. The large fraction recovered by the screening process and returned to the composting process as a bulking agent (fig II).

I.I.I.III In-Vessel

This process takes place in either fully or partially enclosed systems. Environmental parameters such as oxygen levels, moisture and temperature can be controlled. The size and cost of the reactors will depend on individual requirements. A schematic of the overall process is shown in fig III

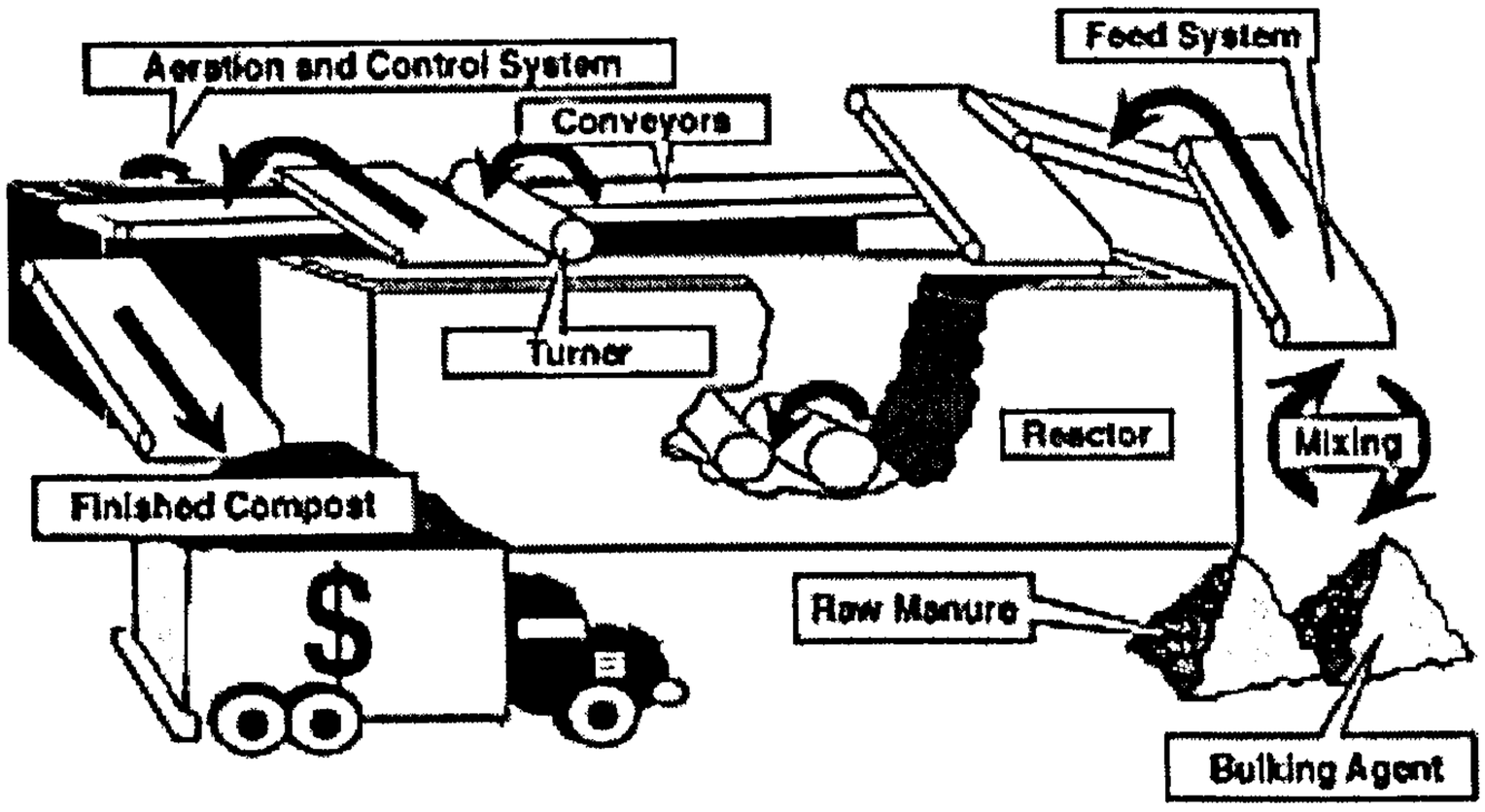

Figure III In-vessel composting process (Ohio State University undated)

I.I.II Vermicomposting methods

Vermicomposting is an "aerobic, bioxidation and stabilisation, non-thermophilic process of organic waste decomposition" (Gunadi et al. 2002). It is a low-cost method of treating organic wastes exploiting the ability of some earthworms to fragment waste residuals in their grinding gizzards (Edwards and Neuhauser, 1988; Edwards and Arancon, 2004; Hand et al., 1988). The digestion process fragments the waste substrate; accelerate rates of decomposition, increases plant available nutrient content (Atyieh et al., 2002; Orozco et al., 1996; Chaoui et al., 2003; Singh and Sharma, 2002). Compared to conventional composts, vermicomposts are richer in microbial diversity, populations and activity, (Gunadi et al., 2002; Subler et al., 1998).

Methods employed when vermicomposting will depend on the feed stock; wet slurry type wastes either need separating or drying to reduce the water content. With other manure type wastes, worms will begin processing cattle solids a few days after collection whereas pig solids may take two weeks. Poultry manures pose a greater problem; the ammonia concentration will have to fall below 0.5mg/ g before earthworms will enter (Edwards and Bohlen, 1996). Industrial wastes are often pre-processed before being exposed to worms; this may involve mixing with a bulking material such as woodchip or paper waste.

Several techniques are available for processing organic wastes with worms:

Lowcost floor beds; Gantry fed beds; containers or boxes; raised gantry fed beds and complete recycling systems. (Edwards and Bohlen 1996). Several epigeic earthworm species are commonly used for vermicomposting are *Eisina fetida* (Tiger worm) *Eudrilus eugieni, Dendrobaena veneta, Perionyx excavatus, Polypheretima elongata.*

I.I.II.I Feeding rates and stocking densities

Ndegwa (2000) have assessed stocking densities and feeding rates using *E. andrei.* Optimum stocking density of 1.60 kg worms m^{-2} and optimum feeding rates of 0.75kg-feed/ kg worm/ day resulting in highest conversion of feed into vermicompost. Other studies have reported that the optimum feeding rate will depend on the feed and its pre-treatment (Wright 1972) and that feeding rates will depend on earthworm species and feed type (Edwards and Bohlen 1996). Neuhauser et al. (1980) reported that ingested volume depended on the total amount of organic matter available, similar results were later obtained by Edwards and Bohlen (1996).

I.II COMPOST APPLICATIONS and USES

The decline in organic matter in agricultural soil as a result of intensive management has stimulated interest in restorative methods. Several organic wastes are currently used as soil amendments and with increasing political pressure to divert the disposal of organic wastes from landfill then it is likely that increasing quantities will find their way onto agricultural land as a method of improving soil quality by reversing the loss of organic matter. Composts are typically used instead of peat as a soil conditioner and growing medium and common uses are given in fig IV

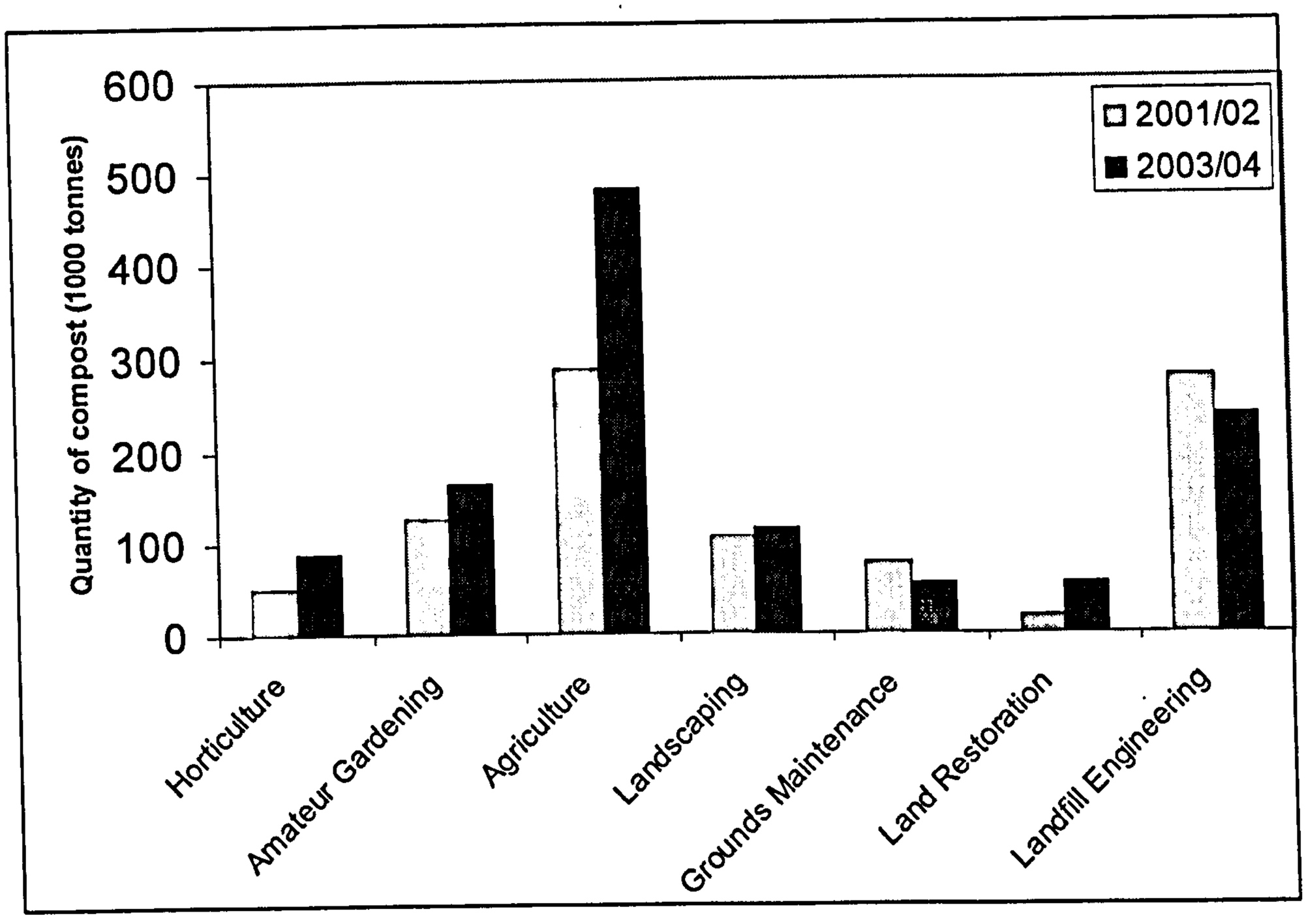

Figure IV Distribution of composted products in the UK 2003/2004 (Slater et al., 2005)

I.II.I Other uses

Compost is a key component of biofilters; these are commonly used for the removal of volatile hydrocarbons, nitrate, ammonia, and other nitrogen based gases from a variety of sources such as landfill leachate, flue gases odours from waste treatment plants (Jokela et al., 2002; Flanagan et al., 2002). Harvesting and subsequent vermicomposting has been shown to be an effective method of managing water-hyacinth *(Echhornia crassipes,* Mart. Solms) for which there is no known conventional control method (Gajalakshmi et al., 2001).

I.III ENVIRONMENTAL BENEFITS

Methane, (CH_4) is a recognised greenhouse gas and its global 'greenhouse' effect is 62 times that of carbon dioxide (Hutchings, 1999). It is a natural product of microbial fermentation and is naturally emitted into the atmosphere from many sources e.g. wetlands; however, recent increases in atmospheric concentrations originates from

anthropogenic sources, (Nebel and Wright, 2000). Landfill sites are recognised as a significant source of methane and emissions from landfills were estimated to have responsible for 28% of total EU emission in 1995, (Hutchings D., 1999) and 10% of annual global contribution (Borjesson, 1999). In a report to the EU, Smith et al., (2001) concluded that one of the most effective method of reducing methane emissions from landfill was by source segregating of MSW and composting (of any type) of putrescible fraction although anaerobic digestion (AD) with combined heat and power (CHP) generation gave the highest net greenhouse gas flux. (fig V). However it must be noted that the net negative greenhouse flux of composting and AD is very small compared to recycling, but together, they offer the lowest greenhouse gas fluxes of all waste management option discussed by Smith et al., (2001).

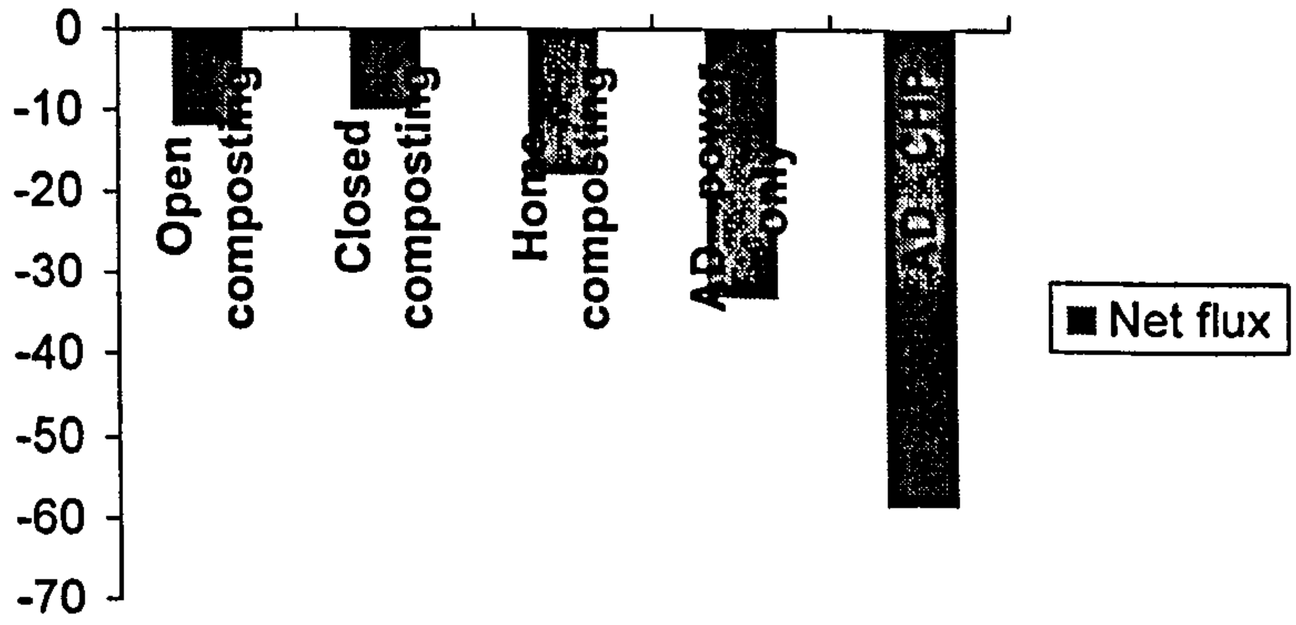

Figure V. Greenhouse gas fluxes from composting and AD of putrescible wastes, assuming average EU electricity replaced. Kg CO_2 eq/ tonne MSW (adapted from Smith et al., 2001).

The contribution that landfill sites make to atmospheric greenhouse gas levels in broadly recognised by all devolved and central governments in the UK and all have set targets for reduction of which increasing composting levels of putrescible wastes plays a key role (NAW 2002).

I.IV CURRENT PRACTICES and FUTURE TARGETS.

I.IV.I Current state of waste management

Overall, waste management employs between 200,000 and 400,000 people in the EU and the trend is toward fewer employees but in higher quality jobs. Currently, England and Wales produces 400 million tonnes of waste every year; of this 106 million tonnes are from commercial, industrial and household sources (DEFRA, 2000). Of the 29.3 million tonnes of municipal wastes collected in England and Wales in 1999/ 2000, 83% is sent to landfill (DEFRA, 2000). The trend for fewer employees per tonne of waste produced may be offset by the growth in waste quantities and increased control and processing of the waste stream (Vernon and George, 2001)

Overall per capita waste generation in the UK 2000 was approximately 0.6 tonnes y^{-1}. N. Ireland produces more waste per capita than any other region (fig VI). England recycles or composts just 12 percent of its household waste, one of the lowest rates in EU but accounts for 92% of the UK total, (Hogg et al., 2002). England and N. Ireland have begun to adopt composting as a waste management strategy, however much more work needs to be done in order to approach European levels. Overall tonnes composted per capita is seen in fig VII.

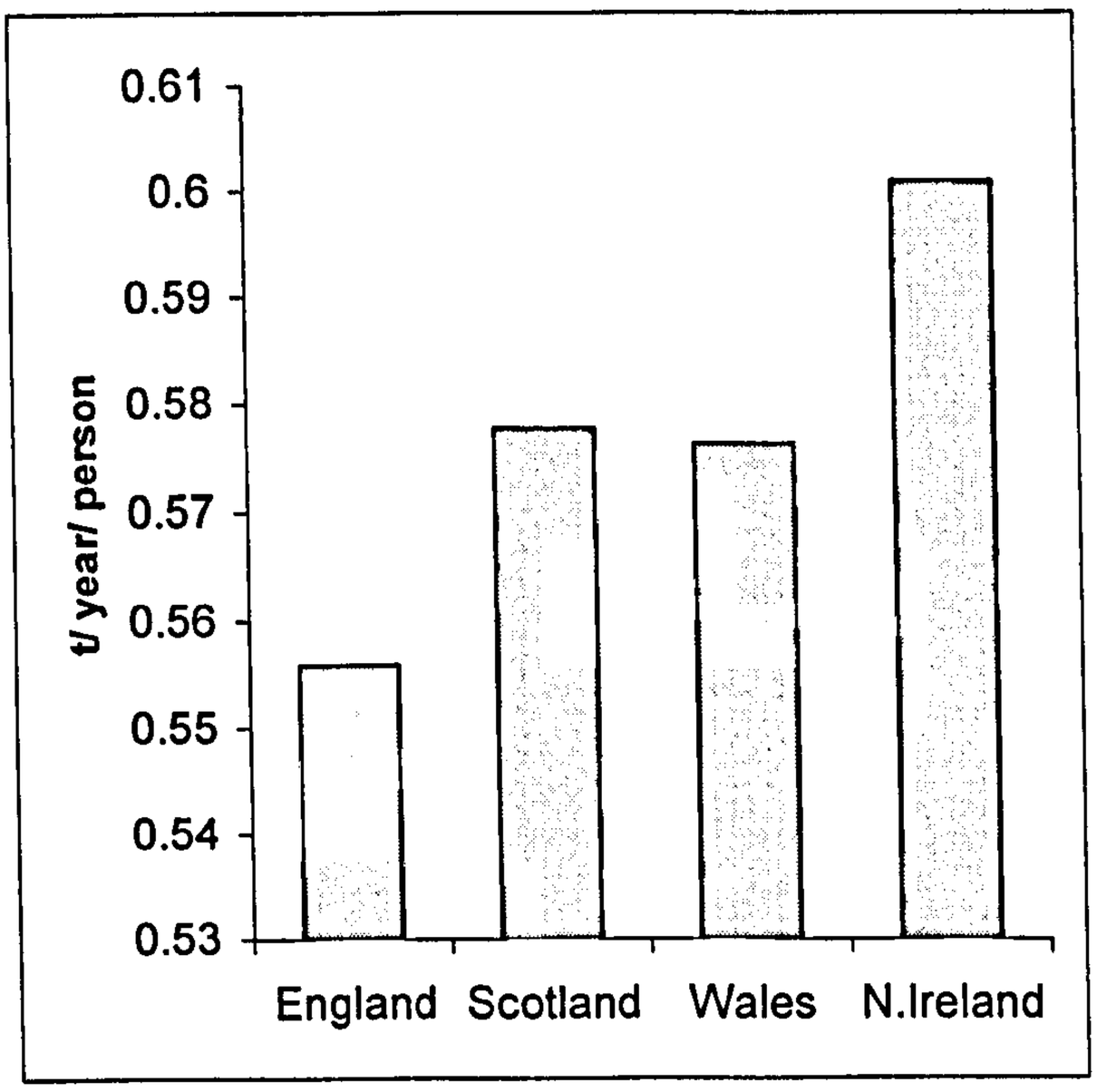

Figure VI Distribution of waste produced per capita in the UK. (adapted from Hogg et al 2002, National Statistics online (2001)

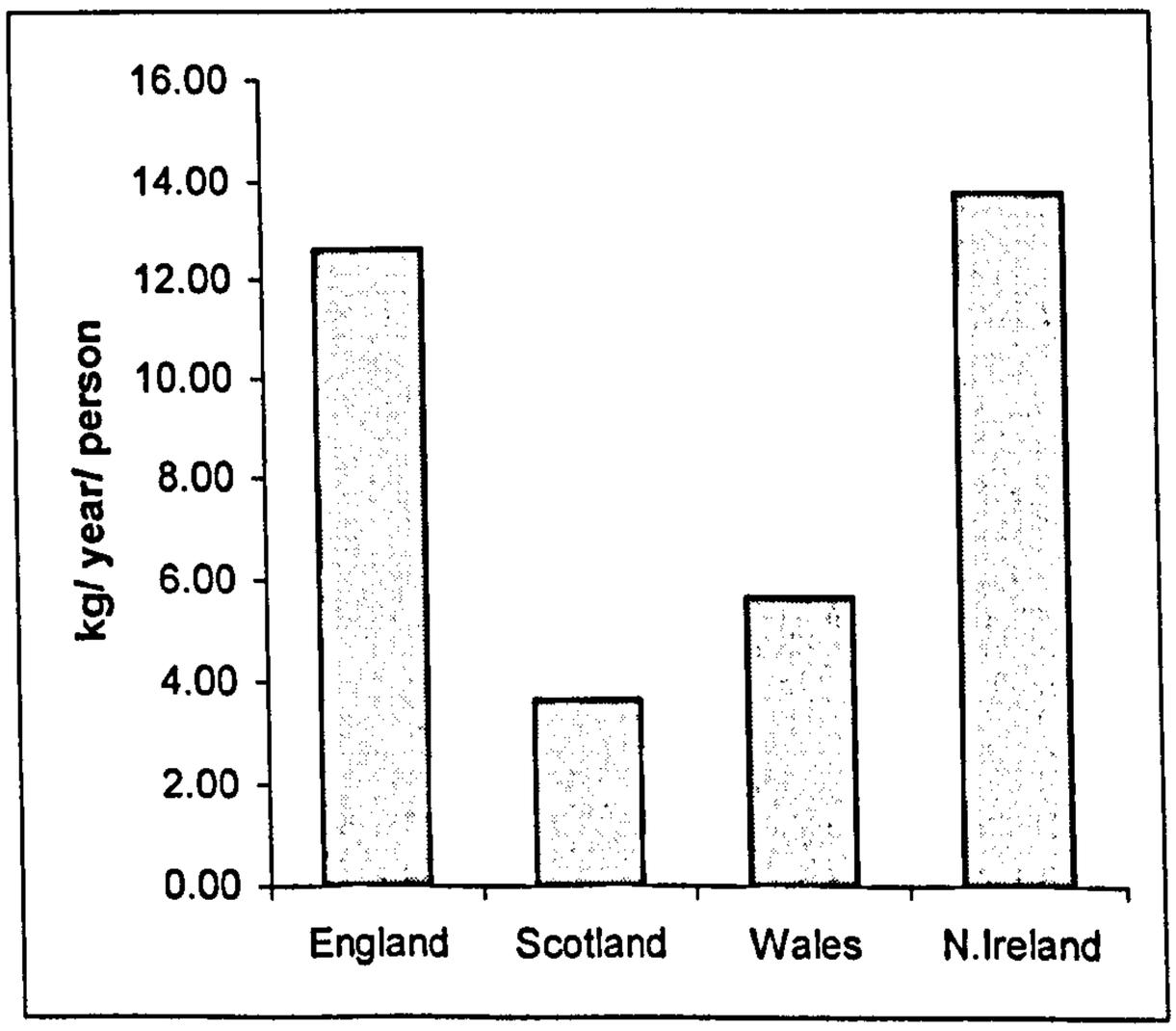

Figure VII UK waste composted per capita (adapted from Hogg et al 2002, National Statistics online (2001)

The government estimated portion of biodegradable wastes found in British MSW (BMSW); (53%) and kitchen and garden wastes (20%) are out of date and widely disputed. Other published figures range from 53% to 61% for BMSW and ranged from 25.7% to 32% for organic wastes (Coggins 1999; Gandy 1993; Robinson and Stentiford 1993; Naseratnam et al., 1997; MEL Research 1994 Slater and Frederickson 2001), garden waste could account for up to 15% of MSW. However, much of the BMSW will consist of paper and cardboard, the BPEO for such wastes is to incinerate combined with energy recovery and that composting efforts should be aimed at the vegetable, food and garden (VFG) wastes, (Slater and Frederickson, 2001).

According to Gale and Stansfield (2002), the total UK household meat consumption is 3.77 Mt y^{-1}, 12% by weight of household refuse consists of cooked meat 1% being raw meat. Assuming a total of 20 million households in the UK and an average of 4kg of putrescible waste per refuse sack, the estimated total of meat discarded is 540,800 t y^{-1} (499,200 t y^{-1} cooked; 41,600 t y^{-1} uncooked). Other studies quoted by Gale and Stansfield (2002) suggest that this is an underestimation and that uncooked meats constitute 3.4% of household refuse. Catering establishments discard very little uncooked meat in their catering waste. Since the 2000 foot and mouth outbreak, there has been considerable concern that the adoption of poor composting practices may result in similar future outbreaks accelerated by the spread of contaminated waste to agricultural land. To this end, the UK government has introduced strict requirement on all food waste composters to ensure adequate sanitisation during active composting and to eliminate recontamination of composted wastes by fresh feed stock (DEFRA 1999).

I.V MATERIALS, OPERATORS and SITES

I.V.I Sources

According to Hogg et al., (2002), in 1999, 74% of the total tonnage of waste materials composted came from municipal sources. Of the 618517 tonnes, 72% was garden wastes from bring sites, 17% was local authority sourced green waste from parks and gardens; kerbside collection accounted for 7.5%, the remainder came from other non-domestic sources.

I.V.II Collection methods

At present, no requirement exists for separate collection of putrescible waste materials in the UK. Many local authorities are faced with recycling and composting targets that can only be met if separate waste collections are initiated. The NAW in their draft waste strategy have specifically stated that "compost"; as far as the waste strategy targets are concerned will only qualify as such, if it is separately collected. (NAW 2001). Collecting methods and quantities are outlined in table II

Table II Compostable waste collected through different schemes for household wastes (DEFRA 2002)

Collection Route	Mt	Percent
Household waste recycling centre	0.93	68%
Kerbside	0.39	29%
Other	0.04	3%

I.V.III Sites

In 1999, 197 sites were identified and divided into three main groups (table III). Of the 833,044 tonnes of materials processed, 765,155 were in centralised sites, 66,401 tonnes in on-farm sites and 1,488 tonnes in community sites. By 2003/ 2004 the number of composting sites had increased to 322, and 1.97 Mt of green waste was processed. For the first time, the on farm site numbers has exceeded centralised site types (Slater et al., 2005). The distribution of existing and proposed centralised composting sites in England is shown in fig VIII. The high level of non respondents to the annual Composting Association survey study make it difficult to make any valued judgements on the adoption and distribution of composting within the study area but if it does accurately represent the current position it is non-metropolitan districts that have developed and established composting as a waste management strategy. It has not been determined whether lack of space or disinterest and inertia is why metropolitan districts lag behind in adopting and implementing such programmes.

Table III Composting site types, 1999 (adapted from Hogg et al., 2002)

	Centralised	On farm	Community
Total	80	65	53
England	67	63	52
Wales	2	1	1
Scotland	4	1	
N. Ireland	5		
Jersey	2		

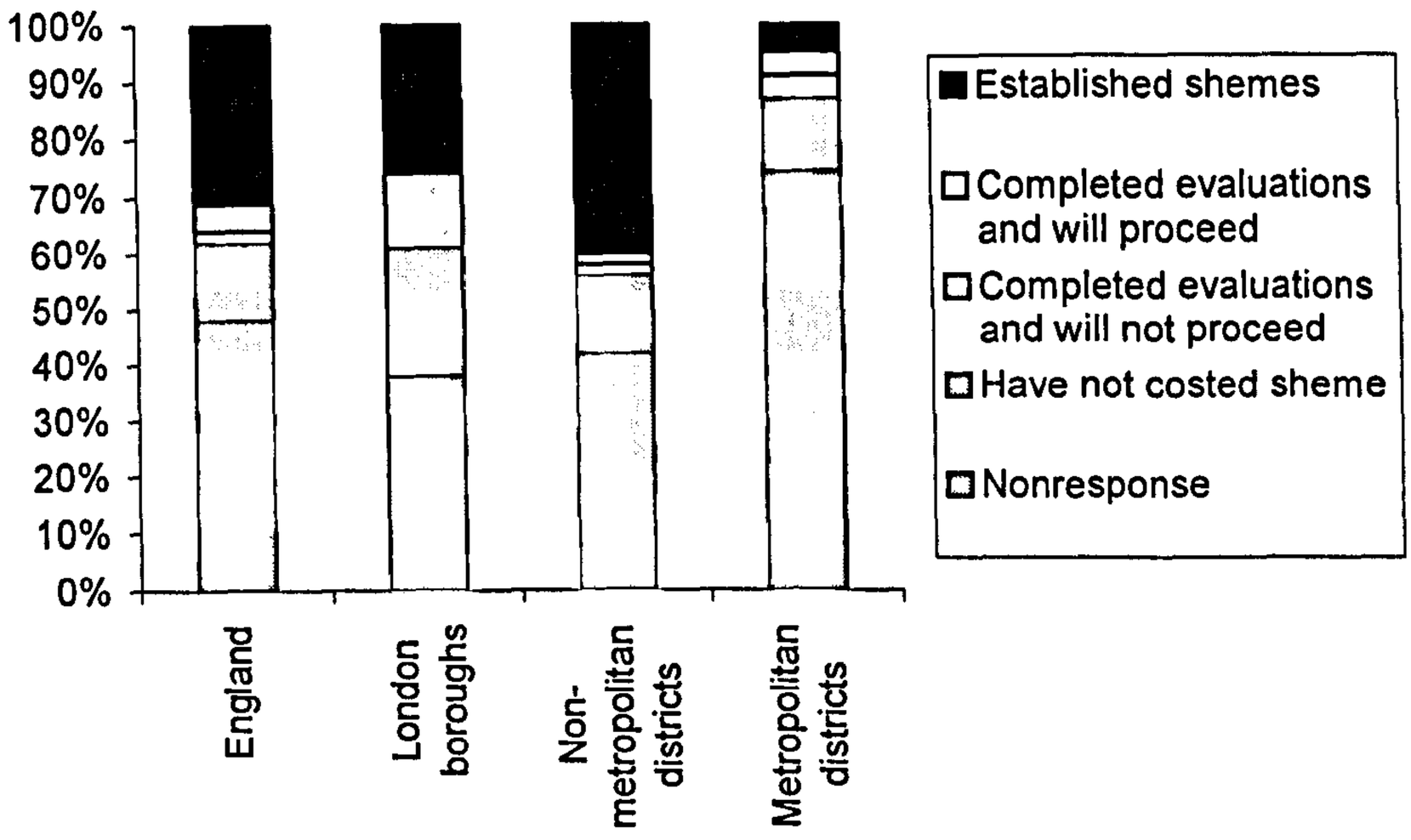

Figure VIII Percentage of centralised composting schemes by authority type; England 2000/ 2001 (DEFRA 2002).

I.VI PROCESS TYPES IN USE IN THE UK

From table IV it can be seen that the recent expansion in composting is based on simple technology of the open-air mechanically turned windrow which accounts for 88% total materials processed.

Since no licensed catering waste composting sites exist in the UK to date, the available data concentrates primarily on green waste and municipal solid wastes (MSW).

Of the 1,663852 tonnes of materials processed, 1,523101 were in centralised sites, 130,402 tonnes in on-farm sites and 10,349 tonnes in other site types with a higher emphasis on on-farm composting in Wales and Scotland. Most composting of green waste is done using simple windrow technology; however, under current regulations this is not suitable for the composting of food wastes and the cost of managing composting premises suitable for treating catering wastes is likely to exclude smaller operators. This is likely to result in development of larger centralised sites in urban areas where higher population densities will attract private business but there is a need to develop approved small-scale units that will attract smaller scale composters in rural areas.

Table IV Type of composting process, all site types, (Hogg et al., 2002)

Process type	Number of sites	Tonnage	Percentage throughput	c
Open-air mechanically turned windrow	121	736,52	88%	
Covered/ contained mechanically turned windrow	5	35,124	4%	
In vessel	7	32,717	4.5%	
Open air static pile with no aeration – centralised	5	15,967	2%	
Open air static pile with no aeration – on-farm	3	630	<1%	
Open air static pile with no aeration – community	23	751	<1%	
Vermicompost	1	250	<1%	
Other centralised – 1 mixed, unknown	2	9,889	1%	
Other – 2 not known, 2 community mixed	30	1,187	<1%	
Total	197	833,04	100%	

I.VII RECENT TRENDS

The growth in the number of composting sites and in tonnage composted is recorded annually by the Composting Association; latest trends are presented in fig IX, X (Slater et al., 2005).

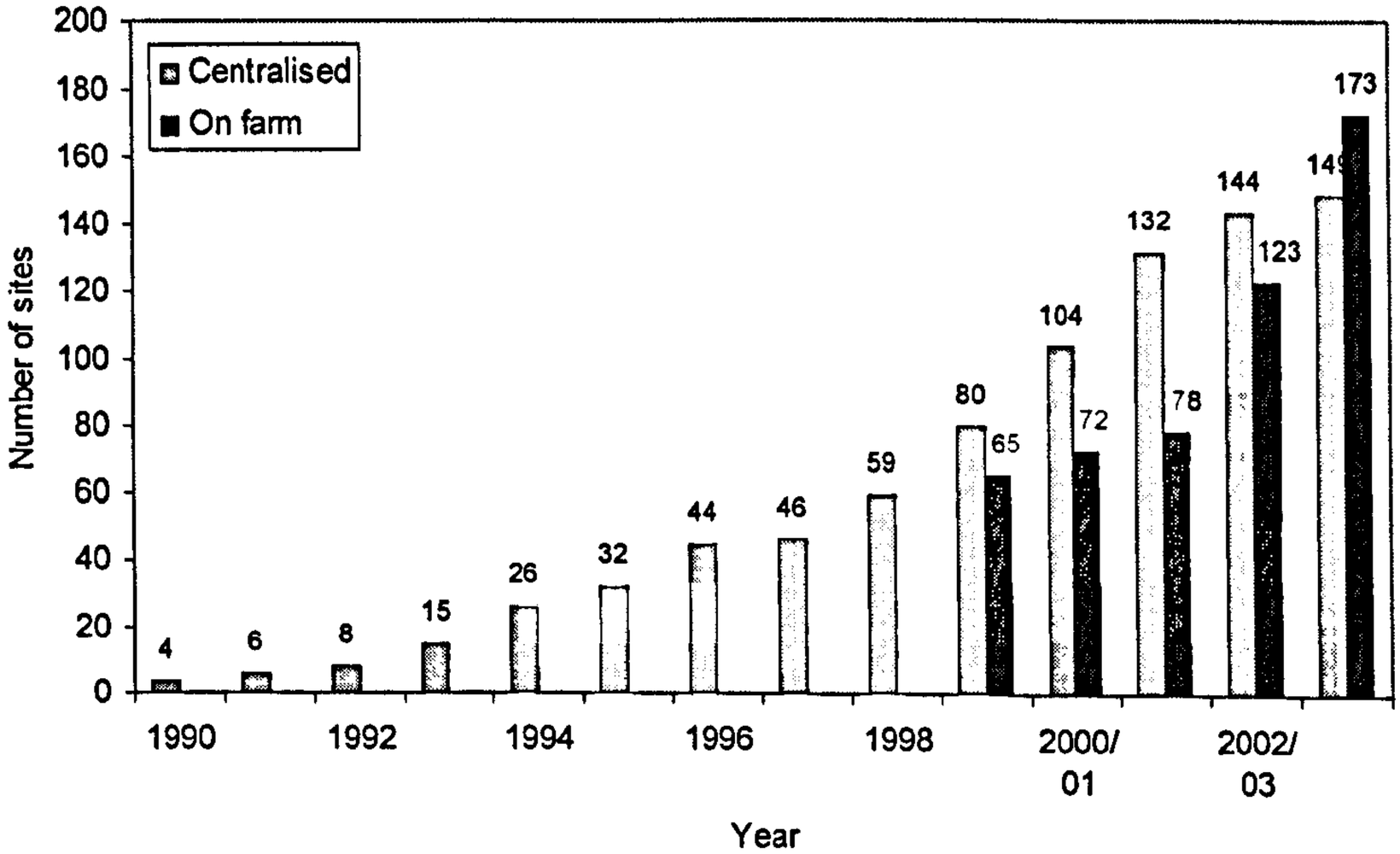

Figure IX The increase in composting sites in the UK (Slater et al., 2005)

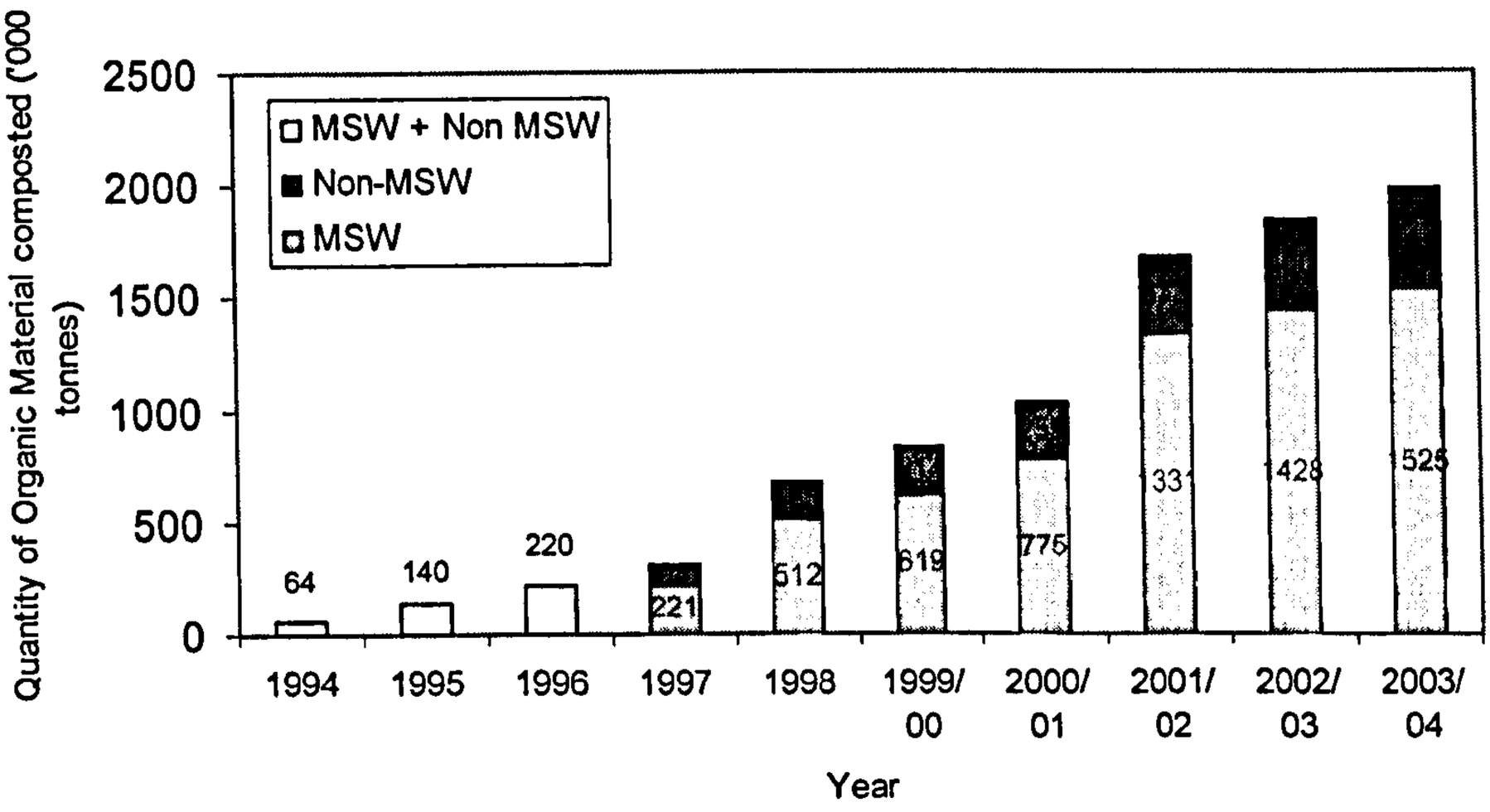

Figure X Growth in material composted in the UK. (Slater et al., 2005)

An increase in recycling and composting levels of municipal wastes (kg/ household/ week) has also been noted by DEFRA (2002) (fig XI). Between 1996 and 2001, the increase in the recycled/ composted portion has compensated for the total increase in waste produced and resulted in only a small decline in overall wastes sent for landfilling.

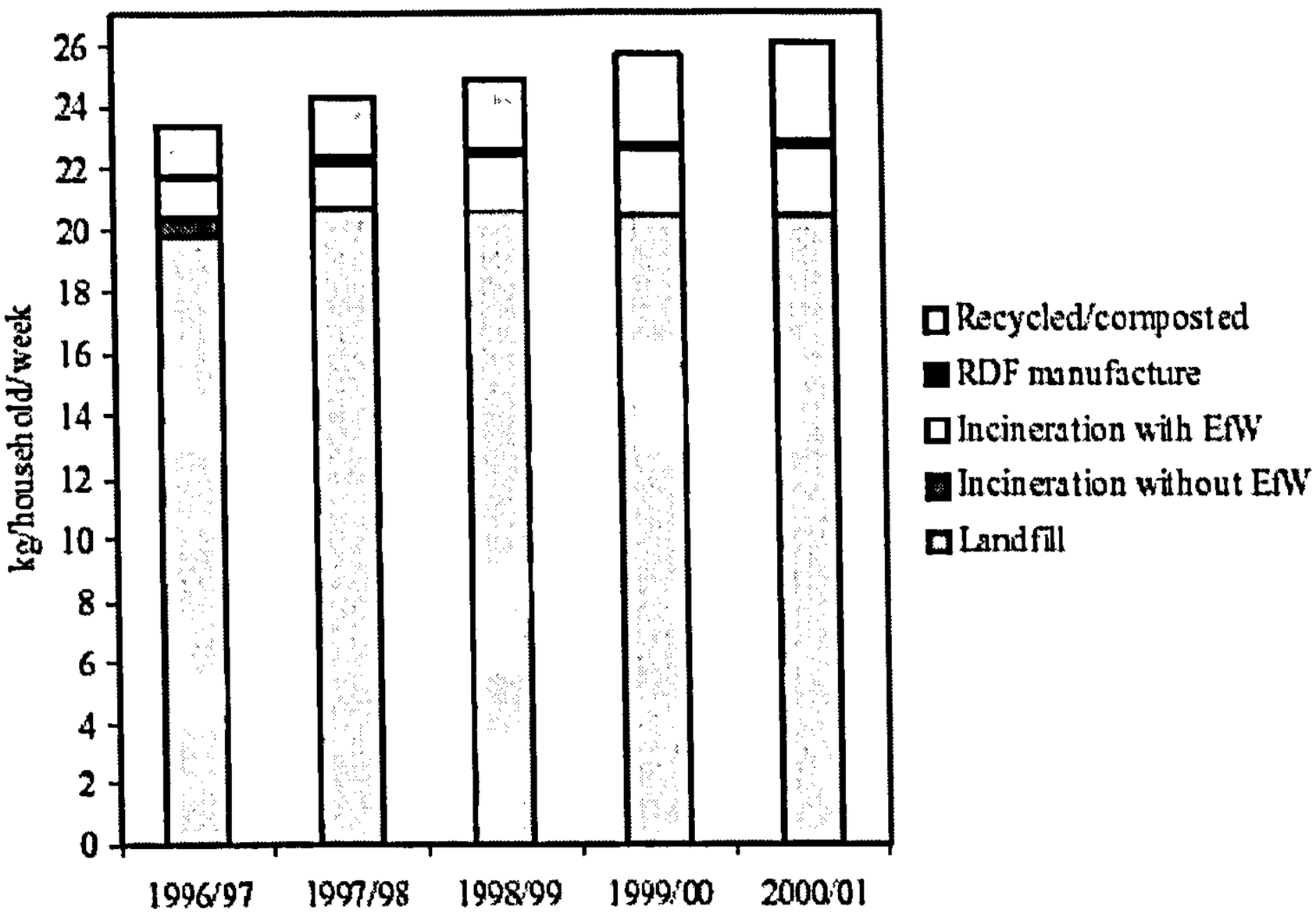

Figure XI Municipal waste management in England: 1996/97 to 2000/01 (DEFRA 2002)

A similar trend was noted in household waste levels, overall tonnage has increased but the increase in the fraction recycled/ composted has largely offset the amount needing alternative treatment. (Fig XII)

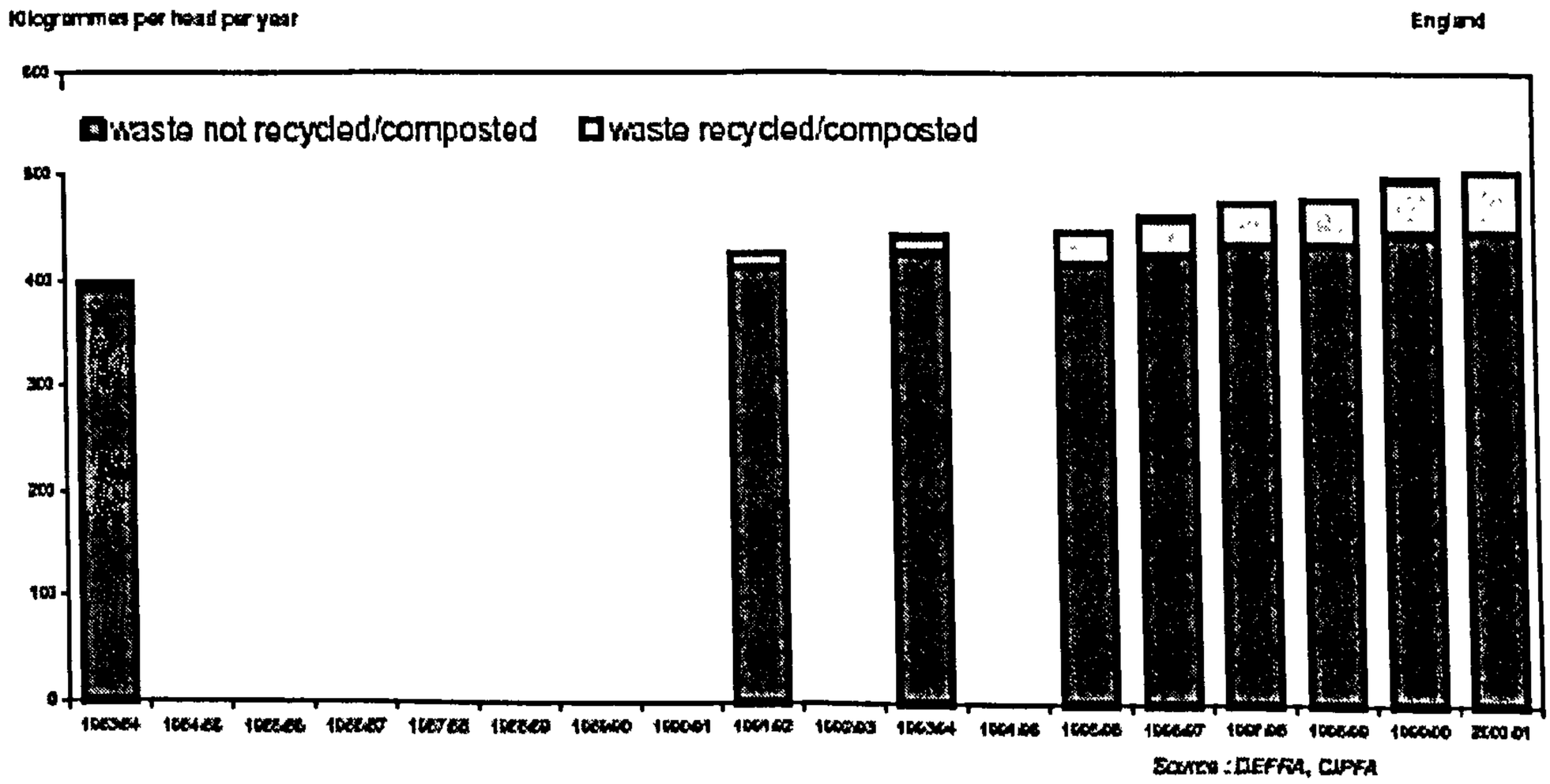

Figure XII Household waste and recycling in England: 1983/84 – 2000/01 (DEFRA 2002)

I.VIII FUTURE TARGETS

In recent years, the political profile of waste management in the UK has increased significantly. This is primarily in response to the European Landfill Directive (EC 1999). This directive places strict limits on the amount of waste that can be disposed of by landfill. For the UK the amount of biodegradable solid waste (BSMW) disposed of in this way must be reduced to:

75% of the amount produced in 1995, by 2010

50% of the amount produced in 1995, by 2013

35% of the amount produced in 1995, by 2020

(Slater and Frederickson,2001)

The directive requires that a minimum of 25% of household wastes be recycled or composted by 2005 rising to 33% by 2015. The National Assembly for Wales (NAW) have set higher targets and intend that 40% of MSW be composted or recycled by 2009/10 with a minimum of 15% source segregated composting. Considering the current low levels of municipal composting in Wales; 2% of the UK total in 2000 (Hogg et al., 2002), this is an ambitious target. Achievement of these targets will require separate collection and composting of kitchen waste by local authorities. The British government has introduced "two major acts, three waste strategy consultation documents, two waste strategies", (Read 2001). However, these strategies failed to recognise the scale of change required to meet their own targets for recycling and recovery (Environment, Transport and Regional Affairs Committee, 1998). This report went on to record its disappointment with waste management in the UK and states that waste management in the UK is still "characterised by inertia, careless administration and *ad hoc* as opposed to science based decisions."

I.VIII.I Maintaining standards, legal regulations

No legal definition of compost exists in the UK. The DETR (1998) definition states that compost is:

"Biodegradable municipal waste which has been aerobically processed to form a stable granular material containing valuable organic matter and plant nutrients which, when applied to land, can improve soil structure, enrich the nutrient content of soil and enhance biological activity."

Whereas the Composting Association defines compost as a "material that has been subjected to controlled, self heating biodegradation under aerobic conditions and stabilised such that it is not attractive to vermin, does not have an obnoxious odour and does not support the regrowth of pathogens and their indicator species. Compost that has been subject to a screening process may be classified in terms of its particle size grade from fine to coarse".

According to Hogg et al. (2002) there is a lack of distinction between those composts that meet the CA standards and those composted materials defined as composts by DETR that do not meet the CA standard. Both define a recognised process but there is still a need to distinguish marketable products from wastes without which, concerns about which material can be applied safely to land will arise. This issue is further complicated by the ambiguity as to whether composts are regarded as wastes under other UK legal regulations (e.g. the Waste management licensing regulation 1994, and the Animal By-product order, 1999), (Hogg et al., 2002).

In response to recent outbreaks of foot and mouth, classical swine fever, BSE scrapie and *E.coli* and *Salmonella* the UK government has proposed and amendment to the Animal By-product order, (1999). The intention is to allow composting of kitchen waste, however, in the proposed measures are adopted it will impose much tighter restrictions on large scale composting methods and materials where meat or food waste that has been on the same premises as meat is present. The composting association maintain that the "prescriptive techniques proposed in the consultation go much further than is seen necessary in mainland Europe and will make the composting of these wastes prohibitively expensive", (Bretton 2002). These requirements will restrict the composting of such wastes to expensive in vessel systems and imposes strict limits on

pathogen numbers. Home composting will not be affected by these restrictions as long as the household does not have a pet pig or ruminant animal.

I.VIII.II Assuring end product quality

The sustainability of this new industry depends on developing high quality products. Moreover, maintaining consumer confidence requires a consistent high standard. Due to the range of wastes that are potentially compostable, the CA has introduced a certification scheme that attempts to guarantee a product of known quality. The CA standards apply to the whole composting process; there is an emphasis on traceability of sources and the composting process; details are required of feedstock sources and pre-preparation Strict monitoring of temperatures within the composting mass is required in order to minimise pathogenic organisms. Recording of the monitoring regime is required as well as identifying actions to be taken is these conditions are not met. Compliance with the CA regime and subsequent CA approval allows the producer to show the CA symbol on their certified products. According to Hogg et al. (2002) the level of uptake of certification to date has been low but recognises that the scheme is new. More recent numbers of compost producers is more encouraging with 42 certified producers. It is a costly procedure, it is estimated that it costs small producers in excess of £2000 pa and larger producers in excess of £3000 pa to register and maintain registration in scheme.

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Appendix 2

Additional Material

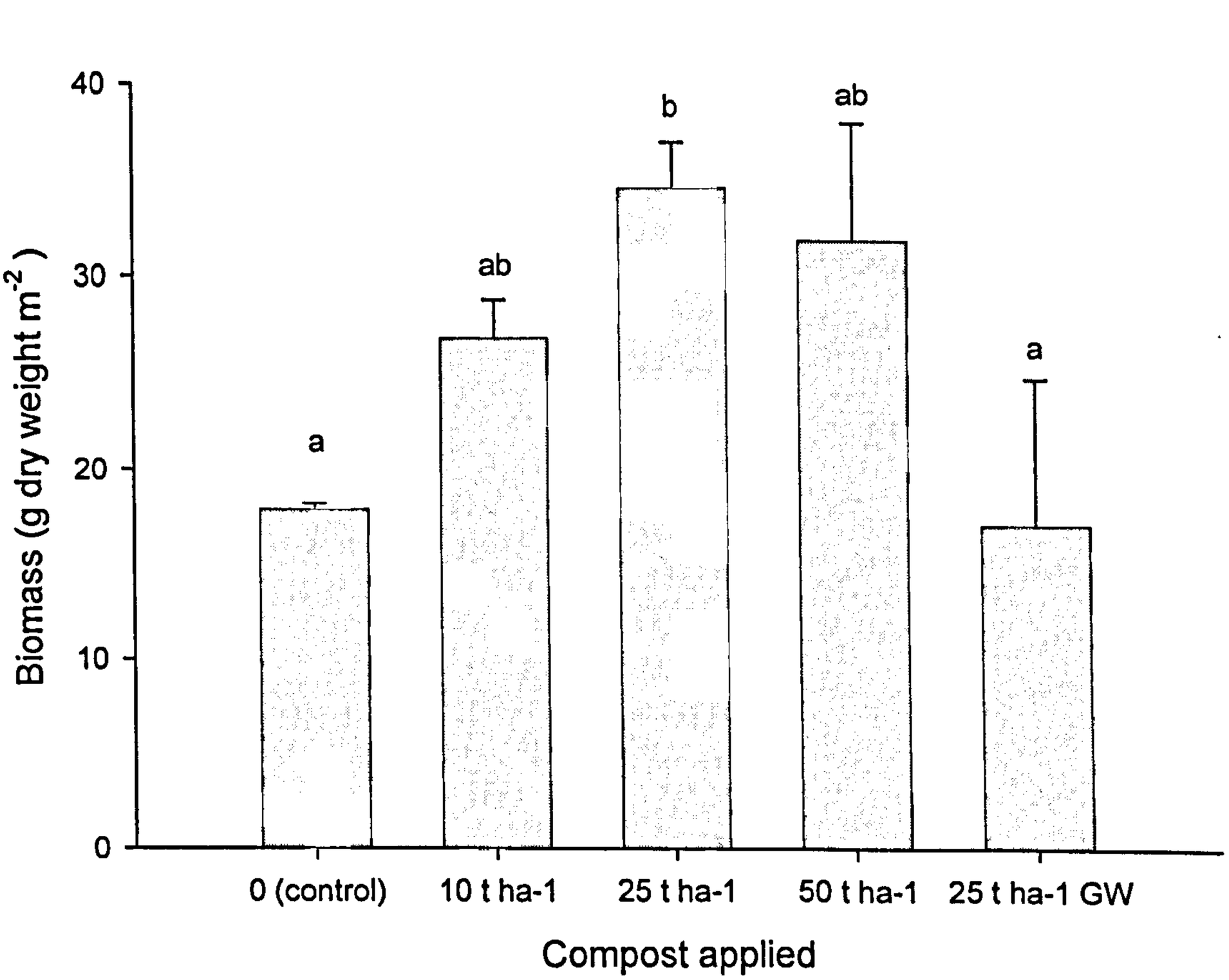


Figure XIII Grassland biomass response to vermicompost application. Where VC - vermicompost, GW – Green Waste. Bars with different letters are significantly different at the $P \leq 0.05$ level. Figures represent mean ± SE, (n = 3).

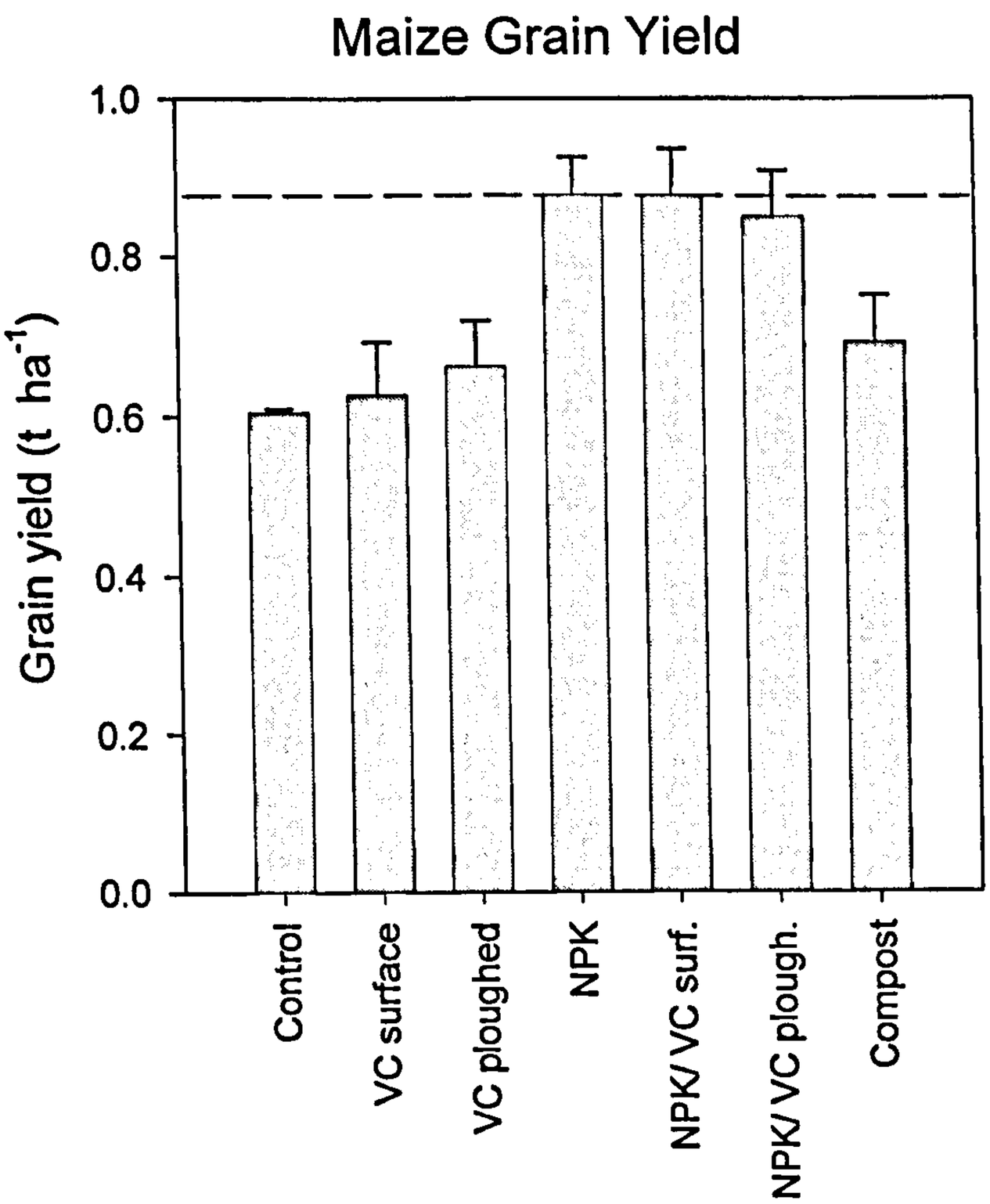
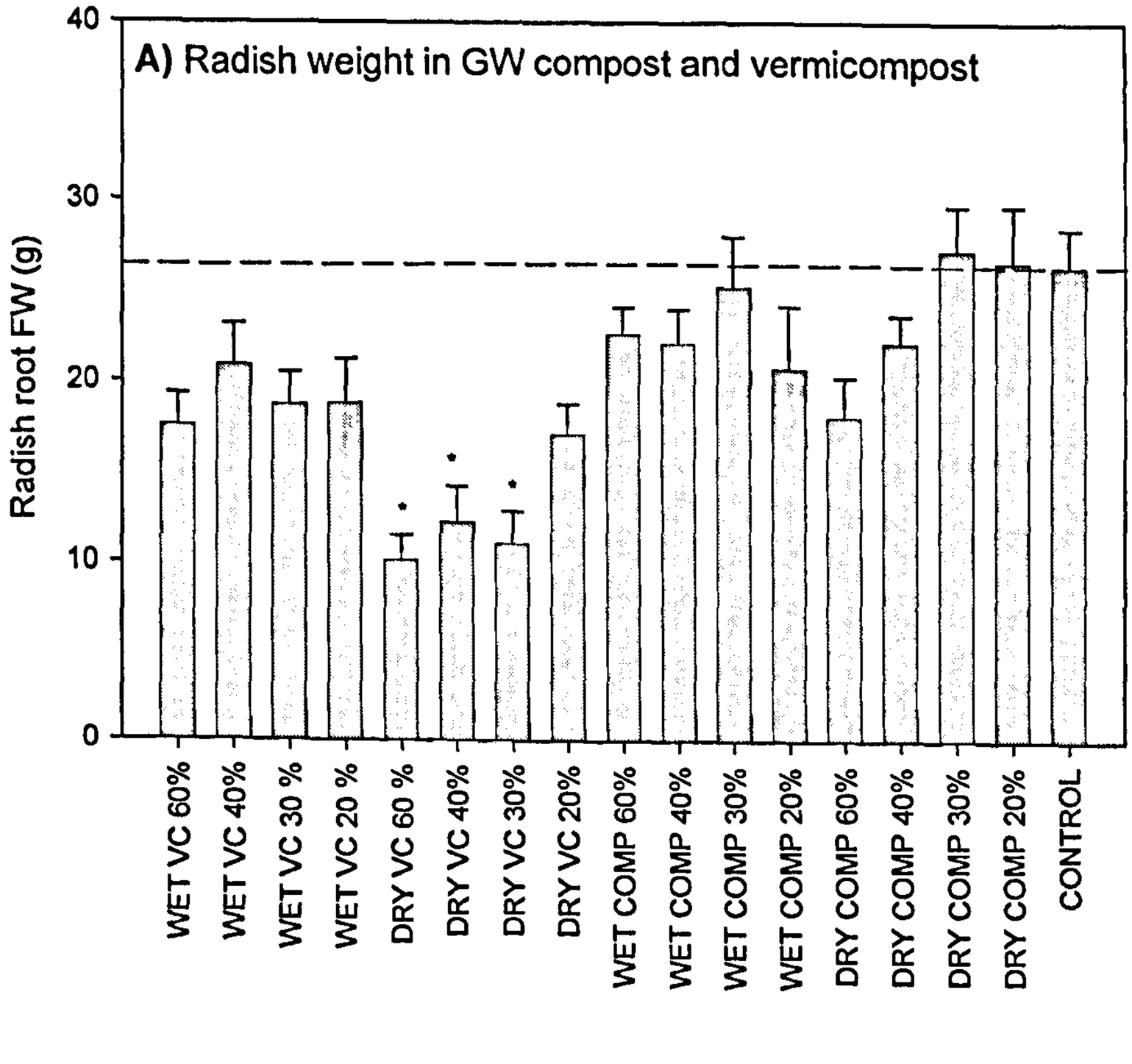
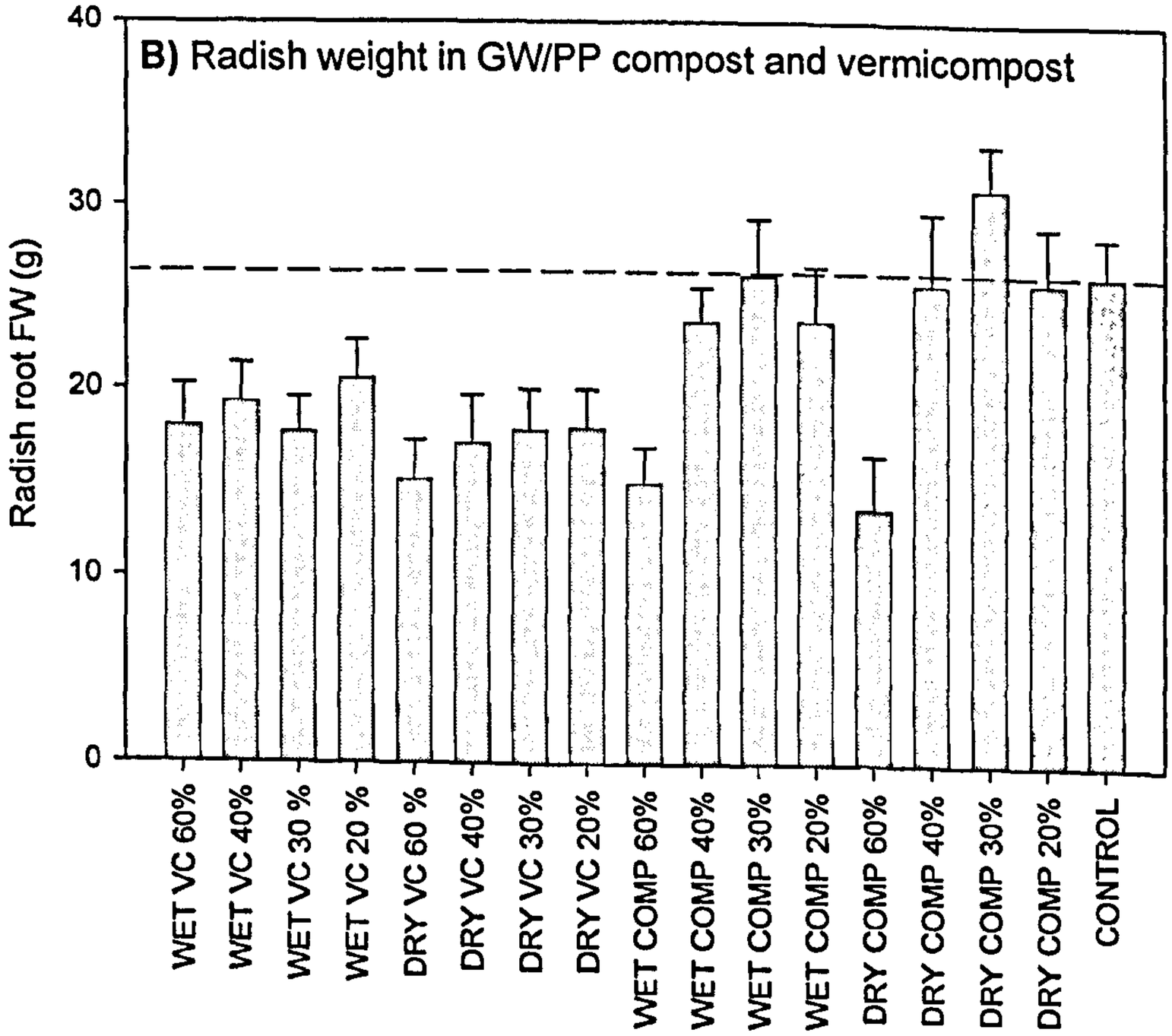


Figure XIV Yield of Maize (*Zea mais*) in response to vermicompost application, where control (no fertilizer amendements); VC surface (25t ha^{-1} vermicompost, surface applied); VC ploughed (25t ha^{-1} vermicompost, ploughed in at seeding); NPK (150 kg ha^{-1} 20:10:10 NPK fertiliser); NPK/ VC surf. (25t ha^{-1} vermicompost plus NPK, surface applied , N content normalised to NPK equivalent); NPK/ VC plough. (25t ha^{-1} vermicompost plus NPK, ploughed in at seeding, N content normalised to NPK equivalent); Compost (25 t ha^{-1} non vermicomposted green waste, surface applied). Asterisk denoted treatments significantly different to the NPK control at the $P \leq 0.05$ level. Figures represent mean ± SE, (n = 3).


A) Radish weight in GW compost and vermicompost
Radish root FW (g)
0
10
20
30
40
WET VC 60%
WET VC 40%
WET VC 30 %
WET VC 20 %
DRY VC 60 %
DRY VC 40%
DRY VC 30%
DRY VC 20%
WET COMP 60%
WET COMP 40%
WET COMP 30%
WET COMP 20%
DRY COMP 60%
DRY COMP 40%
DRY COMP 30%
DRY COMP 20%
CONTROL


B) Radish weight in GW/PP compost and vermicompost
Radish root FW (g)
0
10
20
30
40
WET VC 60%
WET VC 40%
WET VC 30 %
WET VC 20 %
DRY VC 60 %
DRY VC 40%
DRY VC 30%
DRY VC 20%
WET COMP 60%
WET COMP 40%
WET COMP 30%
WET COMP 20%
DRY COMP 60%
DRY COMP 40%
DRY COMP 30%
DRY COMP 20%
CONTROL

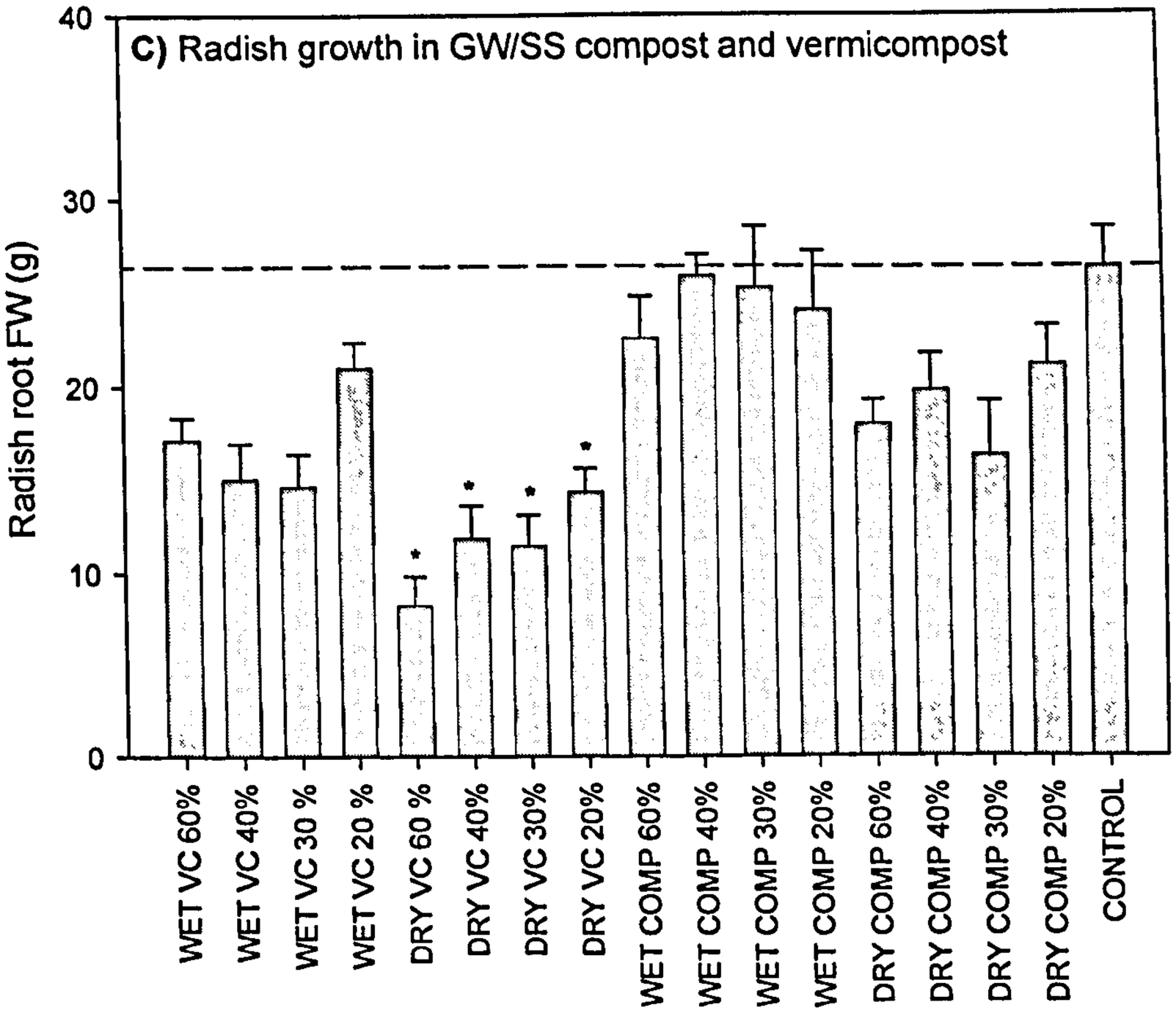


Figure XV Radish root weight in response to compost (comp) and vermicompost (VC) application. Percentage value represents the rate of substitution of peat based growing medium with compost or vermicompost. Plants were grown in three separte wastes, Fig A: GW (green waste only derived compost and vermicompost); Fig. B: GW/PP (green waste and paperpulp derived compost and vermicompost) and Fig. C: GW/SS (green waste and biosolid derived compost and vermicompost). Asterisk denoted treatments significantly different to the peat based growing medium control at the $P \leq 0.05$ level. Figures represent mean ± SE, (n = 5).