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Recycling Solid Waste from the Olive Oil Extraction Process

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Recycling Solid Waste from the Olive Oil Extraction Process

by Assoc. Professor N.G. (Tan) Nair and Dr Julie Markham

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Foreword

The main objective of the project was to recycle solid waste produced from the 2- and 3-phase olive oil mill extraction processes. A composting technique was developed for this purpose that can be applied as an olive mill waste management tool. The technique is characterised by process efficiency and low cost. The process was used for composting olive husk waste collected from 2005, 2006 and 2007 harvests. The study was done over three years at three sites in New South Wales.

The olive industry will benefit from the results of this project by being able to convert liabilities (wastes) to assets (value-added compost). The consequence is management of environmental pollution, and sustainable and responsible farming practices. The amendment of olive grove soil with the olive husk compost will assist in healthy growth of the trees by improving the physical, nutritional and microbial status of the soil.

The key findings of the project are the ability to covert the raw, phytotoxic olive husk waste to non-phytotoxic compost high in organic matter. There was a gradual decrease in the level of phenols in the olive husk during the composting process resulting in a product which is amenable to the growth of plants. A new procedure for extracting phenol was developed successfully in this project for the purpose of assaying phenols in the compost. Using this method, levels of phenol in the compost dropped from 29.68 mg/g at the early stages of the process to 8.9 mg/g at the completion.

Although the majority of Australian olive growers use the 2-phase oil extraction process, the composting procedure developed in this project was found to be applicable to both the 2- and 3-phase olive husk wastes.

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This report is an addition to RIRDC's diverse range of over 1800 research publications and forms part of our New Plant Products Program that facilitates the aims of new industries based on plants or plant products having commercial potential in Australia

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Peter O'Brien
Managing Director

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Executive Summary

What the report is about

The report is about developing an environmentally sustainable system to manage solid waste from the 2- and 3-phase olive oil mill extraction processes. The Australian olive industry has been expanding at the rate of about 9% per annum over recent years. This significant increase in fruit production will result in vast quantities of solid and liquid wastes generated to the detriment of the environment. The industry is therefore faced with the challenge to manage these wastes in order to achieve sustainable production in a clean environment. The research was done over three years at three sites in New South Wales using olive husk waste collected from 2005, 2006 and 2007 harvests. The research work reported here is important because it provides the industry with a tool to recycle processed oil mill waste to improve the health of the crop and the status of the soil.

Who is the report targeted at?

The report is targeted at the Australian olive oil processors and olive growers who will benefit from the results of this project by being able to convert liabilities (wastes) to assets (value-added compost). The consequence is the management of environmental pollution as well as sustainable and responsible farming practices.

Background

The continued rapid expansion in the Australian olive industry can be expected to result in the production of over 62,500 tonnes of solid and over 350 x 10^6 litres of liquid wastes per annum. Olive mill wastes from 2- and 3-phase oil extraction processes are produced in significantly large quantities in short periods of time. The industry is therefore faced with the challenge to manage these wastes in order to achieve sustainable production under a clean environment. The Second RIRDC Plan for the Australian Olive Industry for the five-year period 2003 to 2008 has identified olive processing solid and liquid wastes as a key environmental issue for the industry to address. The olive husk and wastewater produced from oil extraction process contain macromolecules such as polysaccharides, lipids, proteins and a number of monocyclic and polymeric aromatic molecules generally known as phenolic compounds, and hence are highly recalcitrant and toxic to humans, animals and plants (Kistner *et al* 2004). They are important environmental pollutants.

There are currently over one hundred patented techniques covering various aspects of olive waste management (Niaounakis and Halvadakis 2004). These patents were granted over a period of about thirty four years from 1969 to 2003. As far as we know, none of these techniques are universally in use due mainly to their high cost. There is at present a significant shift in the major olive growing countries in the world from the practice of spreading raw, untreated olive husk on farm lands to the use of olive husk that has undergone a bioremediation process. There appears to be a lack of sufficient data on the use of composting as a bioremediation process for olive husk waste management.
The rationales behind the olive solid waste management technology developed in this project are:

- to convert the waste into a value-added product which can be fully utilised for the benefit of the health of olive trees,
- to develop a low cost technology that will enable the olive growers to adopt it on a commercial scale and,
- to implement a farming practice that will overcome social and health implications relating to inherently odorous and biotoxic pollution caused by the olive waste.

Composting is a reliable and natural technique for remediation of toxic olive husk waste. Different composting processes are used in the Mediterranean olive growing countries. As far as we are aware, none of these methods have focused on our objective of humification process in composting, and its consequent impact on soil microbial activity and nutrient cycling beneficial to crop health (Altieri et al. 2002, Raviv 2007). This project aims to develop an environmentally sustainable and a low cost composting technology for bioremediation of the olive husk waste from the 2- and 3-phase olive oil mill extraction processes.

Aims and objectives

The main aim of the project is to develop an environmentally sustainable system to manage solid waste from the 2- and 3-phase olive oil extraction processes. The benefit to the industry is a sustainable waste management system that prevents environmental pollution from irrational disposal of oil mill waste. This project will benefit the community at large by overcoming social and health implications resulting from the inherently odorous and bio-toxic wastes from oil mill wastes.

Key findings

This project has demonstrated that 2- and 3-phase olive husk waste can be successfully converted into compost by using relatively low cost bioremediation technology. It has further shown that the resultant compost is non-phytotoxic and can therefore be recycled as an organic amendment to olive grove soil. The experimental results showed significant reductions in the levels of phenols as a result of composting, and the end product of the composting process supported the growth of plants. A successful composting model was thus developed that can be applied as an olive mill waste management tool. The cost of production of the composting process developed in this project is relatively low. An indicative budget for the production of the 2- and 3-phase olive husk composting process is approximately $50 (fifty dollars) per tonne. The assumptions on which the cost has been based are: olive husk and olive pruning at no cost, chicken manure @ $50.00/Tonne, wheat straw @$7.00/bale (20kg/bale), and microbial mix (eg. Actizyme®) @$30.00/kg. The budget excludes labour cost.

Implications
The main impact of the outcomes of this project on the Australian olive industry is the application of an efficient and economical olive mill solid waste management technology. It is suggested that olive mills adopt the composting process developed in this project either individually or collectively as regional enterprises. The consequence will be the ability to convert liabilities (waste) into assets (value-added compost) thus contributing to sustainable and responsible farming practices.

The composting technology is economical and commercially feasible. An indicative budget for the production of the 2- and 3-phase olive husk composting process is approximately $50 (fifty dollars) per tonne. This cost has been based on the assumption that there is no cost for olive husk and olive pruning and that the ingredients, chicken manure, wheat straw and microbial mix, cost $50/T, $7/bale and $30/kg respectively. Labour cost has been excluded from the budget.

Economic benefits of using compost
Soil amendment using compost has several distinct advantages over the addition of fertilisers to soil. There is significant loss of nutrients from soil after fertilisation due to leaching. Compost retains nutrients in the soil and releases them on demand by the plants due to its high cation exchange capacity. Compost maintains the water-holding capacity of soil, thereby helping the plants to be more drought resistant. Well processed composts can be suppressive to a variety of soil-borne pathogens, including those of olives.

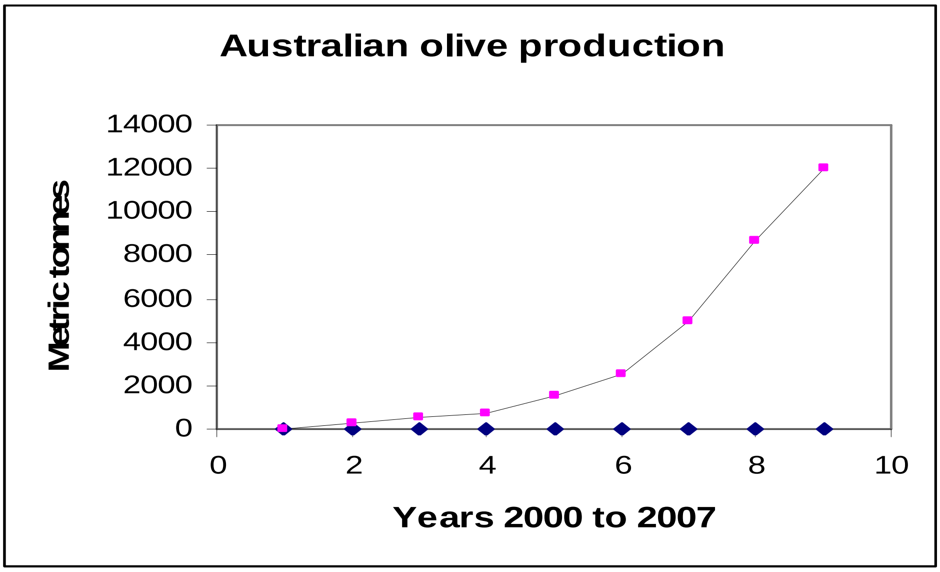
A comparison of the cost of soil amendment with compost and fertilisers indicates that the former is less expensive than the latter. For instance, the cost of fertiliser application to soil involving fertigation and foliar application is approximately $208/hectare. The comparative cost for compost application to soil is approximately $125/hectare.

Far more positive benefits can be gained in terms of economics and sustainability of farming by using compost for amending soil than fertilisers.

Introduction

Australian olive production

The current world production of olives is 2×10^6 metric tonnes per year. The global production of olive oil for 2006-2007 is estimated to be about 2.5×10^6 tonnes, and the Mediterranean countries account for 99% of the total production (Alfano *et al*. 2007). The olive industry in Australia has significantly increased production as the trees planted over the last decade started to mature. The Australian production of olive oil in 2007 was estimated to be 8700 metric tonnes. The local olive industry has been expanding at the rate of about 9% per annum over the recent years (Fig. 1). In contrast to Australia, the average annual olive production growth rate in the Mediterranean and Middle East countries has been 5% over the last fifteen years (Raviv *et al.* 2007).



Source: Australian Olive Association (2007)

Figure 1 – Australian olive production from 2000-2007

The prediction is that in excess of 7.5 million olives will be planted in Australia in the immediate future. Consequently, over 25,000 metric tonnes of oil may be available per annum from Australian producers by 2011 (Miller 2007).

Australian olive waste production

The amount of solid (olive husk) and liquid (olive mill wastewater) waste produced is directly proportional to the production of olive oil. These olive mill wastes are produced in significantly large quantities in short periods of time. In spite of predictions of significant increase in fruit production, the Australian olive industry does not appear to recognise the vast quantities of solid and liquid wastes that will be generated to the detriment of the environment. The continued rapid expansion in the Australian olive industry can be expected to result in the production of over 62,500 tonnes of solid and over 350×10^6 litres of liquid wastes per annum. The industry is therefore faced with the challenge to manage these wastes in order to achieve sustainable production under a clean environment. The Second RIRDC Plan for the Australian Olive Industry for the five-year period 2003 to 2008 has identified olive processing solid and liquid wastes as a key environmental issue for the industry to address.

Australian olive growers use either the 2-phase or the 3-phase process for extraction of olive oil. However, majority of these growers use the 2-phase system which produces more solid but significantly less water waste than the 3-phase process (Table 1).

Table 1 – Comparison of solid and liquid wastes* produced by the 2- and 3-phase olive oil extraction processes

Waste material	2-phase process	3-phase process
Olive husk (%)	82.5	47.8
Waste water (%)	14.5	49.5

* Approximate values Modified from Altieri (2007)

Characteristics of olive mill wastes

The olive husk and wastewater produced from oil extraction processes contain macromolecules such as polysaccharides, lipids, proteins and a number of monocyclic and polymeric aromatic molecules generally known as phenolic compounds (Ehaliotis *et al.* 1999). The levels of phenols in wastewater and olive husk can vary from 1 to 8 g l^{-1}(Di Gioia 2002) and from 2.9 to 3.7mg g^{-1} (Nair *et al.* 2007a, 2007b) respectively. The wastewater is also characterised by dark colour due to chromophoric lignin-related materials with different degrees of polymerisation and a sharp characteristic odour (Sayadi *et* al.1996). Olive husk is also characterised by its phytotoxicity, hydrophobicity, salinity, low pH and polyphenols (Perucci *et al.* 2006). The presence of phenols as well as short and long chain fatty acids is considered to be responsible for the phytotoxicity (Cassa *et al*. 2003, Kistner *et al.* 2004, Isidori *et al.* 2005) and antimicrobial (Fiorentino *et al.* 2003, Isidori *et al.* 2005) nature of these wastes. Well processed composts can be suppressive to a variety of soil-borne pathogens (Raviv *et al.* 2007); however, little is known about the suppressive capacity of compost made from olive solid waste (Pera and Calvert 1989). Recent work has shown that olive waste compost suppressed *Fusarium oxysporum* f. sp. *Melonis* (Raviv *et al.* 2007), and *Verticillim dhaliae* (Alfano *et al.* 2007).

Table 2 – Comparisons of some characteristics of olive waste (olive husk) generated from 2-phase and 3-phase oil extraction processes in Australia and the Mediterranean region

Parameter	Mediterranean 2-phase	Mediterranean 3-phase	Australian 2-phase	Australian 3-phase
Moisture%	54 – 57	45 – 50	52 - 55	42 - 49
Phenols%	2.5 – 2.7	0.35 – 0.37	1.06	0.20
Total nitrogen%	0.43 – 0.48	0.50 – 0.90	1.2 – 1.3	1.2 – 1.4
Total carbon%	25 - 29	29 – 32	50 - 54	49 – 51
C/N ratio	59 - 60	57 – 59	46 - 50	42 – 52

Problems associated with untreated disposal of olive husk and wastewater from olive oil extraction process

Disposal of olive solid and liquid wastes from olive oil mills is already a major environmental issue in several olive growing countries in the world. Spreading the solid waste on farm lands and storing the wastewater in anaerobic ponds causes enormous pollution to the land and air. Inappropriate disposal of olive husk and olive mill wastewater create environmental problems such as odour and ammonia released into the atmosphere and leaching of nitrates and other pollutants into the ground water (Figs 1 and 3 Appendix 2). The work of Sierra *et al.* (2001) has shown that olive mill wastewater infiltration in the soil has caused carbonate dissolution and redistribution and modifications in pH values, electrical conductivity, nutrient contents, phenolic compounds and biological activity of the soil horizons. The olive mill wastes have been, and continue to be, disposed onto farm lands, thus causing the inhibition of numerous microorganisms, a reduction in seed germination and the alteration of several soil characteristics such as porosity and humus concentration (Campanella *et al.* 2004). The introduction of olive solid and liquid waste into soil tends to increase the average diameter of the soil aggregates, bulk density and slows down hydraulic conductivity (Colucci *et al.* (2002). Polyphenols in olive husk are well known to affect nitrification in the soil and have deleterious effect on soil microbial activity. The high C:N ratio and low pH in the olive husk are also known to immobilise nitrogen in the soil (Cardelli *et al.* 1998, Benitez *et al*. 2004).

Common methods of disposal of olive husk and wastewater produced from olive oil extraction process

The common methods used for the disposal of olive solid and liquid wastes from olive oil extraction processes in different olive growing countries in the world are given below:

- uncontrolled spreading of olive husk on farm lands at rates of 100 - 200 m^3 h^{-1} $year^{-1}$
- controlled spreading of olive husk in Italy for 3-phase extraction process at the rate of 80 m^3 h^{-1} $year^{-1}$
- controlled spreading of olive husk in Italy for the classical extraction process at the rate of 50 m^3 h^{-1} $year^{-1}$
- controlled spreading of olive husk in Israel for the 3-phase extraction process at the rate of 40 m^3 h^{-1} $year^{-1}$ every alternate year in the same plot
- irrigation of tree crops with olive oil mill wastewater at the rate of 80 m^3 h^{-1} $year^{-1}$
- irrigation of olive trees with olive liquid waste in Greece at the rate of 1.5 m^3 $tree^{-1}$ $year^{-1}$
- uncontrolled discharge of olive liquid waste in Israel into municipal wastewater treatment plants
- storage of olive liquid waste in open anaerobic ponds
- storage of olive liquid waste using floatation devices
- storage of olive liquid waste using open settling tanks
- limited methods of composting olive solid waste
- biofilters for the treatment of olive liquid waste
- production of biogas (methane) from treatment of olive solid waste.

Cayuela and Sanchez-Monedero (2006) have recently reviewed the situation of olive industry wastes and the methods of treating the problem. The selection of the most suitable or appropriate methods of disposal of olive solid and liquid wastes will depend on the social, agricultural or industrial environment of the olive mill and the olive industry as a whole.

The case for bioremediation of olive solid waste by composting process

There are currently over one hundred patented techniques covering various aspects of olive waste management (Niaounakis and Halvadakis 2004). These patents were granted over a period of about thirty four years from 1969 to 2003. As far as we know, none of these techniques are universally in use. There are two main reasons for this:

- most of the existing technologies are sophisticated and expensive for commercial use
- the technology required for olive waste management needs to be site-specific

Although some workers have shown that it is feasible to spread olive husk in the field, this type of amendment leaves significant amount of non-humified carbon in the soil for a long time (Amirante and Pipitone 2002, Di Giovacchino *et al.* 2002). Such amendments also reduce the rate and degree of humification (Amirante and Pipitone 2002).

There is at present a significant shift in the major olive growing countries in the world from the practice of spreading untreated olive husk in the field to the use of olive husk that has undergone a bioremediation process. Although composting is one such bioremediation process, different composting processes are used in the olive growing countries in the Mediterranean region. As far as we are aware, none of these methods have focused on the importance of the humification process in composting and its consequent impact on soil microbial activity and nutrient cycling. For instance, the technology developed by Altieri and Esposito (2007) in Italy consists of adding appropriate hygroscopic organic waste to olive husk without stones (seeds), packing in net sacks and storing until required for use in the field. Another method of composting being carried out in Israel (Raviv *et al.* 2007) involves the addition of urea to olive husk to bring the C:N ratio to 30 followed by regular turning of the compost mix, and maintenance of compost temperature at different levels and moisture level at 50 – 60%. It appears that the humification process during the composting period was not determined.

The rationales behind the olive solid waste management technology developed in this RIRDC project (UWS-20A) are:

1. to convert the waste into a value-added product which can be fully utilised for the benefit of the health of olive trees. This technology does not create another waste product to deal with, unlike those used in solvent extraction of olive husk waste to maximise oil production, and extraction of biophenols by the chemical and pharmaceutical industries
2. to use a bioremediation process for the olive solid waste where the biological and biochemical properties and activities of naturally occurring microorganisms are harnessed to produce humified compost
3. to use microorganisms in the composting process that will not only accelerate the break down of lignin, cellulose and hemicellulose in the plant materials but also aid in the accumulation of humic ions such as humates, humic acid and fulvic acid
4. to develop a low cost technology that will enable the olive growers to adopt it on a commercial scale
5. to design a system that is suited to the type of infrastructure of the Australian olive industry
6. to develop a process that can be adopted by individual olive presses as well as by composting cooperatives formed by regional groups of olive growers
7. to implement a farming practice that will overcome social and health implications relating to inherently odorous and biotoxic pollution caused by olive waste.

Markers for the determination of waste treatment process efficiency

It is important to identify markers for determining the efficiency of olive waste bioremediation process. Three such markers are levels of phenol, phytotoxicity and humic ions in the final compost product. Some workers believe that phenols are the only compounds affecting phytotoxicity of the olive solid waste (Raviv *et al.* 2007). While different methods of determining phytotoxicity are known, there are no standard published methods of extraction of solvent soluble phenolic compounds in olive solid (olive husk) waste compost. Polyphenols present in both olive oil and in olive husk after extraction of oil exhibit considerable diversity in polarity characteristics ranging from hydrophobic to hydrophilic in nature (Capasso et al, 1994, Owen et al, 2000, Tura and Robards, 2002, Robards, 2003). The methods used for isolating phenols from olive husk ('pomace') waste and soil contaminated by the disposal of oil mill waste vary greatly. A range of extractions, some of which are quite complex, with water (Sierra et al, 2001, Saadi et al 2007), acetone (Julkhen-Tito, 1985), ethanol (Clemente et al, 1997, Blekas et al, 2002, Farag et al, 2003, Bouzid et al, 2005,) or aqueous methanol (Servili et al, 1999, Ryan et al, 2001, Romero et al, 2002), with or without additives to prevent hydrolysis, oxidation and isomerisation of the phenolic compounds have been reported. Additional steps involving aqueous acetone (Guyot et al, 1998, Cardoso et al, 2005) were often employed to ensure extraction of the range of polyphenols present in the sample.

As there are no standard published methods for extraction of solvent soluble phenolic compounds in olive waste compost, this project included the development of a reproducible quantitative method for the extraction of phenolic compounds from olive husk waste and compost prepared from it.

Composting is a bioremediation process, and the process is expected to reduce phytotoxicity of the olive husk waste. The role of phenols in the phytotoxicity is well known; it may be also related to fatty acids (Kistner *et al.*2004). The absence of phytotoxicity is a reliable indicator of mature compost.

The third important marker of an efficient composting process is the level of humic ions in mature compost. The use of an efficient composting technique results in the accumulation of humus like substances from biological and biochemical processes that take place during the process. Humification can be defined as the transformation of non-living microorganisms into humic compounds such as humic acid, fulvic acid and humin. They are the most chemically active compounds in soils with significantly high cation and anion exchange capacities (Tan 2003). Humates have a cation exchange capacity of approximately 400 and therefore have a significantly high storage area for nutrients. The humates also have a high water holding capacity and, this characteristic helps to make crops more drought resistant. Humus increases the capacity of the plant to take up 30-35% more nutrients through the root system.

The main objective of the project is to develop an environmentally sustainable and a low cost system to manage solid waste from the 2- and 3-phase olive oil mill extraction processes.

Objectives

Main objective

The main objective of the project is to develop an environmentally sustainable system to manage solid waste from the 2- and 3-phase olive oil mill extraction processes.

Outcomes of objective

Major outcomes of the objective are the development of an environment management system that will achieve the following:

- Prevent environmental pollution by recycling the olive solid waste that has undergone a bioremediation process
- An economically and operationally effective waste management process
- A waste management process that is capable of converting liabilities into assets
- A waste management system that contributes to sustainable and responsible farming practices

Methodology

Materials

Solid waste (olive husk) from 2- and 3-phase olive oil extraction processes were collected from olive mills at Rylstone Olive press, Rylstone, NSW, Glenlee Olive Grove, Camden, NSW and, Lakelands Olives, Clandulla, NSW during 2005, 2006 and 2007 harvests. These were used in the different composting trials.

Ingredients used in the olive husk composting were wheat straw, chicken manure and olive pruning (leaves and stalks). Actizyme® (Southern Cross Laboratories, Dural, New South Wales) was added to the mix in all the composting trials. Actizyme® contains *Bacillus subtilis* and enzymes such as protease, lipase and cellulase.

The materials used in the phytotoxicity tests were olive husk compost after completion of the composting process, a standard potting mix and seeds of watercress (*Lepidium sativum* L.).

Methods

Physical characteristics of olive husk

The basic profiles of the 2- and 3-phase olive husks were determined by analysis of moisture (%), pH, total carbon (%), total nitrogen (%) and C:N ratio. Standard methods of analysis were used.

Composting process of the 2- and 3-phase olive husks

Seven batches of composting trials were carried out; however, detailed results of three compost trial batches (batches 4, 6 and 7) representing 2- and 3-phase olive husk have been selected for presentation in this report. The compositions of all the seven trial batches are given in Table 3.

Table 3 – Composition of the trial batches used in composting process

Compost trial batch	Kg			
	Olive husk	Wheat straw	Chicken manure	Olive pruning
1[a]	1500	580[d]	500	-
2[a]	2000	400[c]	600	-
3[a]	2100	300[c]	600	-
4[a]	2100	300[d]	600	-
5[a]	2100	300[c]	300	300
6[b]	2000	300[c]	600	-
7[b]	2000	600[d]	400	-

[a] 3-phase olive husk [b] 2-phase olive husk [c] Shredded straw [d] Unshredded straw

The amount of each ingredient in the compost mix was calculated to achieve starting levels of C:N ratio and total nitrogen in the ranges of 25 to 35 and 1.2 to 1.6 respectively. The ingredients were mixed thoroughly with a front end loader, and the moisture content adjusted to 50% saturation. The compost heaps were set up in conical shapes. This geometry allowed efficient aeration and turning of the mix every two days (Fig. 2). Temperatures of the heaps were recorded before each turn at four points in each of the heaps at a depth of 10 cm.

Figure 2 – The lay out of the olive husk trial compost batches. Note the turning operation of the compost heap in the foreground by front end loader, and the steam created in the compost heap by microbial activity.

Samples of wheat straw were taken from 2- and 3-phase olive husk compost at the completion of the composting process, and processed for examination of microbial decomposition using a scanning electron microscope (SEM). The normal processing method for SEM was followed.

Methods for the analysis of phenols in the olive husk composts

Phenol extraction

Prior to phenol extraction, the compost was dried at 55°C for 3 days to constant weight, pulverised using a mortar and pestle, sieved through 2000µm sieve (to get rid of small stones and large pieces of straw) and then stored in air-tight containers. 5g of dried compost was weighed into 250mL flask and methanol:water (80:20 v/v, 30mL) was added. The flasks were placed in a closed shaking incubator (150rpm, 25°C) for 20 hours. The contents of the flasks were centrifuged at 12 000g for 10min at 4°C and the supernatants stored at -20°C. The pellets were resuspended in methanol:water (80:20 v/v, 30mL) and extracted for 48 hours under the same conditions. After centrifugation, the supernatants were collected and the pellets were resuspended in acetone:water (60:40 v/v, 30mL) and incubated for 3 hours. All three supernatants were pooled and stored at -20°C prior to being assayed. The volumes of the combined supernatants were adjusted to 100mL in volumetric flasks with methanol:water (80:20).

Phenol assay

Phenol assays were performed using a modification of the method of Box (1983). Extracts were diluted as required in distilled water and 5 mL samples (4 replicates) were transferred to glass test tubes. 750µl of 20% sodium bicarbonate (Sigma) was added to each tube followed by 250µl Folin-Ciocalteau's phenol reagent (Sigma). The contents of the tubes were mixed by vortexing after each addition. After 60 minutes incubation at room temperature, the tubes were mixed again by vortexing and the absorbance at 750nm was measured. For each assay, a standard curve was constructed. Initially, phenol (0-10mg L^{-1}), caffeic acid (0 - 20mgL^{-1}) and gallic acid (0 - 20mgL^{-1}) were compared as standards (Fig. 3), and caffeic acid was selected as the standard for subsequent experiments. Phenol concentrations were calculated as caffeic acid equivalents.

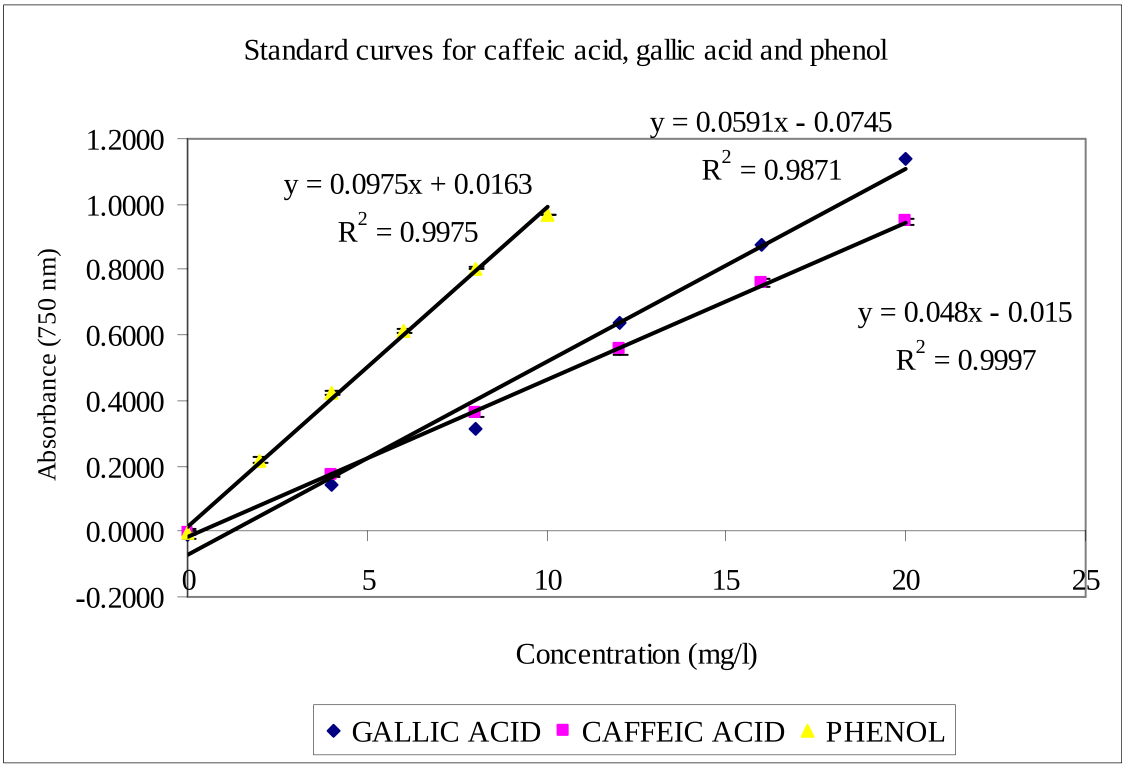


Figure 3 – Standard curves for caffeic acid, gallic acid and phenol

Chemical analysis of the olive husk compost

The chemical analyses (except those of phenols) of the olive husk compost were carried out by the Environment Analysis Laboratory, Lismore, New South Wales. The compost samples were air dried at 65^{0}C prior to crushing (ring mill) to ensure homoegenity. The data are expressed on a dry weight basis. Total nitrogen and total carbon were determined by LECO CNS2000 Analyser and expressed as percentages. pH (1:5 water) was measured according to Rayment and Higgins 4A1 (1992). Conductivity (1:5 water was measured by the methods of Rayment and Higgins 4B1 (1992), and expressed as ds/m (=1mS/cm = 1000µS/cm). The method used for determining humates and humic acid was the same as that used for solid and liquid samples containing 0.5% or more humic acid (Black, 1996). The humic acids were dissolved by treatment with 1N sodium hydroxide and then precipitated with hydrochloric acid. The furnace was added to recheck the sodium hydroxide extraction of the humic acid and to determine the humate. The levels of humic acid and humates are expressed as percentages.

Phytotoxicity analyses of end product of olive husk composting process

Phytotoxicity was determined in the end product of olive husk composting process. Plastic containers were used for testing seed germination in olive husk compost. Fifty seeds of cress (*Lepidium sativum* L.) were placed in the compost, and the surface was covered with a thin layer of vermiculite. The containers were placed in a glass house at 25^{0}C and watered regularly by misting. Percentage seed germination and length of shoots and roots were calculated after seven days. The results were compared with a standard nursery potting mix control.

Statistical analysis

The effect of the variability in the solvent extraction of phenols in the olive husk compost was investigated using the MINITAB 14 (MINITAB Inc., 2003) fully nested ANOVA. The test of significance was considered when α=0.05. The factors selected were the replication of the extraction process, the duplicates in the extraction process and the replication of the Folin-Ciocalteau assay used to measure the phenol level in the compost.

Results

Characterisation of 2- and 3-phase olive husk

There were differences in moisture content, pH, phenol levels, total nitrogen, total carbon and C:N ratio between 2- and 3-phase olive husk (Table 4).

Table 4 – Characteristics of 2- and 3-phase olive husk

Component	2-phase	3-phase
Moisture (%)	52 - 55	42 - 49
pH (1:5)	6.5 – 6.9	6.3 – 7.2
Phenol (%)	1.0	0.20
Total nitrogen (%)	1.2 – 1.3	1.2 – 1.4
Total carbon (%)	50 - 54	49 – 51
C:N ratio	46 - 50	42 – 52

Composting process of 2- and 3-phase olive husk

Table 5 summarises the analysis of individual ingredients used in the different trial batches of compost.

Table 5 – Range in the levels of different components in the ingredients used in the experimental olive husk compost trials

Component	Olive pruning	Wheat straw	Chicken manure
Moisture (%)	42.0 – 45.0	72.0 - 75.0	54.3 – 58.0
pH (1:5)*	7.0 – 7.1	6.0 – 6.2	6.5 – 6.9
Total carbon (%)	26.3 - 27.2	41.0 – 42.2	32.0 – 33.5
Total nitrogen (%)	0.87 – 1.0	0.52 – 0.6	3.2 - 4.6
C:N	30.3 – 32.1	78.0 – 79.0	7 0 – 8.0

* Solid:water ratio of 1:5

Due to the natural variations in the components of the individual ingredients, the quantities of the ingredients were computed to develop compost mixes with the desired starting C:N ratio. The formulae of the compost trial batches used in the composting process are given in Table 3.

Compost temperature profiles during 2- and 3-phase olive husk composting process

The results are presented in Figs. 4 –6. In general, compost temperature followed a normal pattern during the composting process. There was an initial increase in temperature to 63 – 66^{0}C followed by a gradual drop to 18 – 21^{0}C. However, in compost trial batch 6 the compost temperature remained at 34^{0}C on day 29 (Fig.4).

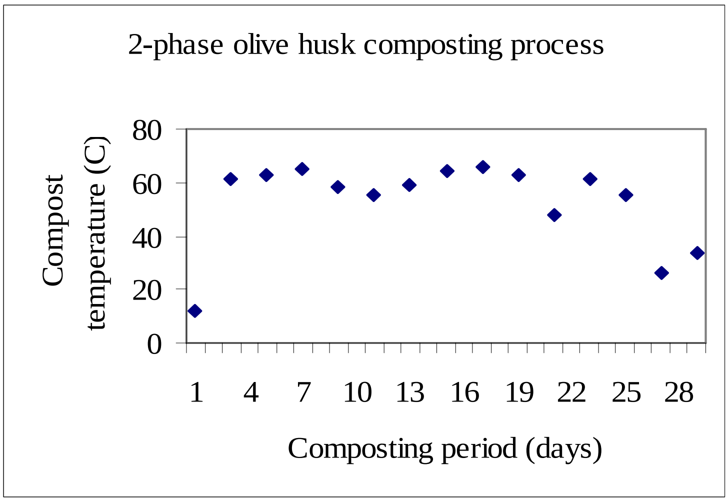


Figure 4 – Temperature (0 C) profile during twenty nine days of 2-phase olive husk composting process
(Compost trial batch 6 – olive husk + wheat straw + chicken manure)

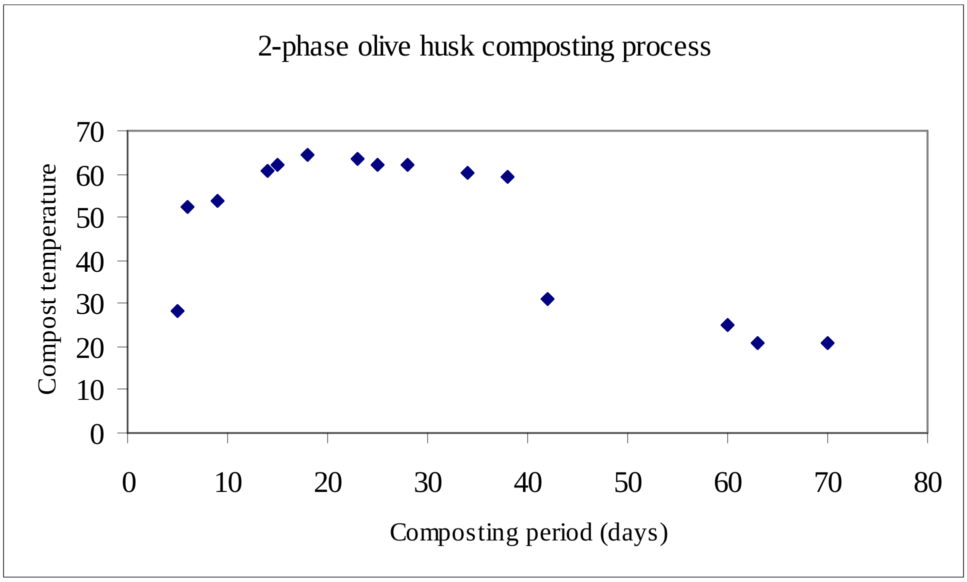


Figure 5 – Temperature (0 C) profile during eighty days of 2-phase olive husk composting process
(Compost trial batch 7– olive husk + wheat straw + chicken manure)

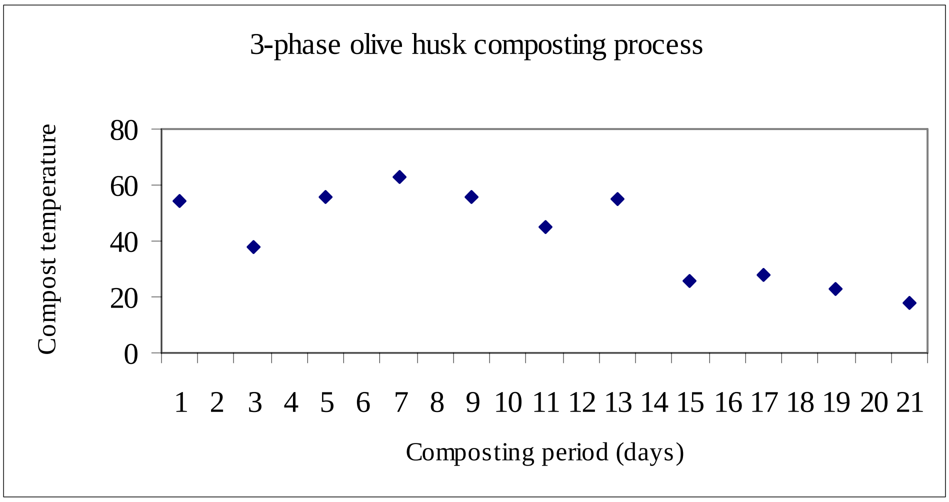


Figure 6 – Temperature (0 C) profile during eighty days of 2-phase olive husk composting process
(Compost trial batch 4– olive husk + wheat straw + chicken manure)

In general, the addition of microorganisms and enzymes to compost in the form of Actizyme® did not influence either the percent total nitrogen or C:N ratio of the olive husk compost at the finish of the composting process (Table 6).

Table 6 – **Effect of supplementing olive husk compost with microorganisms and enzymes** (Actizyme®) on total nitrogen and carbon:nitrogen ratio

Component	Stage of composting	OH+CM	OH+CM+ACT	OH+ST+CM	OH+ST+CM+ACT
%N	Start	1.3	1.2	1.8	-
%N	Final	1.4	1.8	2.0	1.9
C:N	Start	25.3	-	26.2	25.0
C:N	Final	16.8	15.8	15.7	15.0

OH – olive husk, CM – chicken manure, ST – wheat straw, ACT - Actizyme

Profiles of compost pH during 2- and 3-phase olive husk composting process

The results are given in Figs.7 – 9. The shift in pH in the 2-phase olive waste compost (trial batch 6) was from 7.7 at the start to 7.5 at the end of composting. The maximum pH was 8.3 at day 23 (Fig.7). In the case of the 2-phase olive waste compost (trial batch 7) the starting pH was 8.3 reaching a maximum of 9.4 at day 22 and falling to pH 7.7 at day 69 (Fig.8). The pH shift in the 3-phase olive waste compost (trial batch 4) was 7.8 to 7.6 from start to finish (Fig.9).

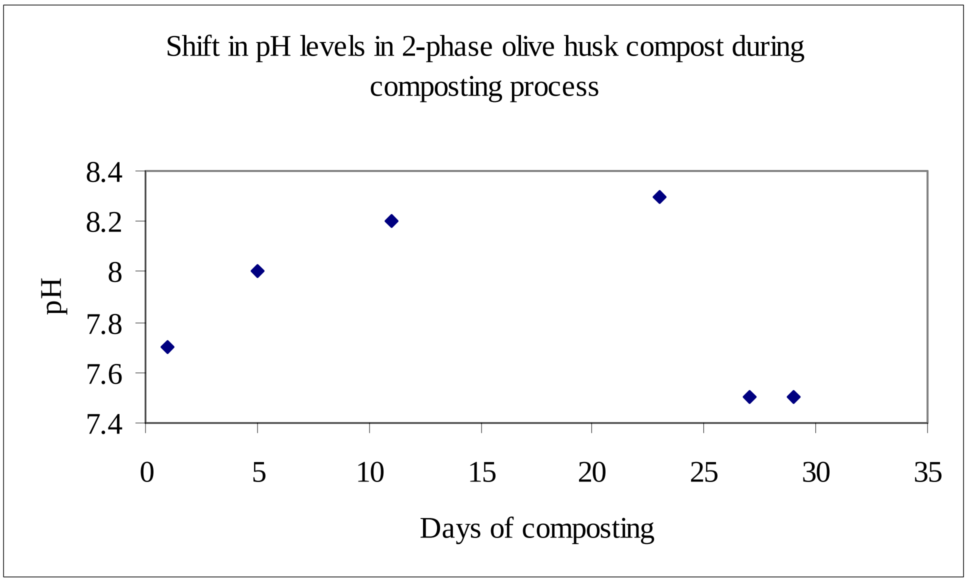


Figure 7 – Shift in pH during 29 days of olive husk composting process
(Compost trial batch 6 – olive husk + wheat straw + chicken manure)

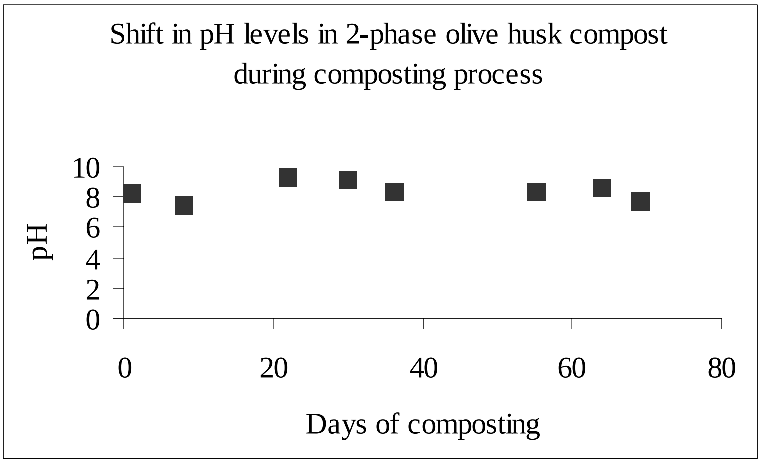


Figure 8 – Shift in pH during 80 days of olive husk composting process
(Compost trial batch 7 – olive husk + wheat straw + chicken manure)

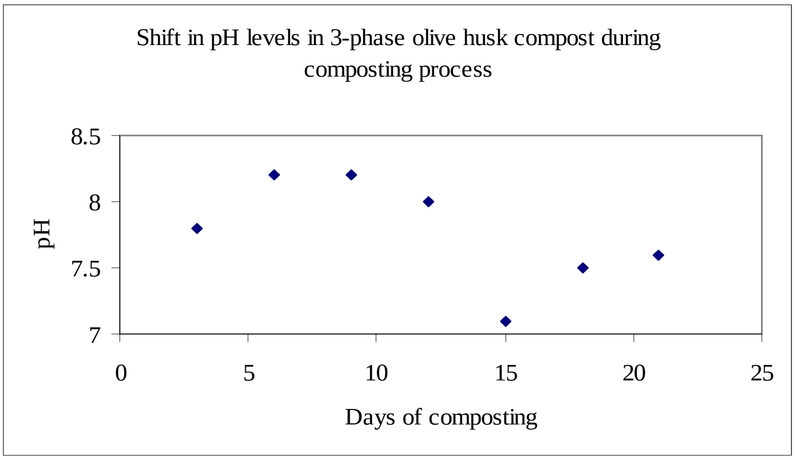


Figure 9 – Shift in pH during 29 days of olive husk composting process
(Compost trial batch 4 – olive husk + wheat straw + chicken manure)

C:N ratio and total nitrogen at the start and finish of 2- and 3-phase olive husk composting process

A typical pattern of changes in total nitrogen, total carbon and C:N ratio during composting 2-phase olive husk compost is given in Table 7. While there was reduction in the C:N ratios in the compost from start to finish of the composting process, the reverse was observed in the levels of total nitrogen and pH.

Table 7 – A typical pattern of the changes in nitrogen, carbon and C:N ratio during composting of 2-phase olive husk

Composting period (days)	Total nitrogen (%)	Total carbon (%)	C:N ratio	pH
1	1.4	39.2	28.0	6.0
11	1.5	34.3	21.3	6.7
30	1.9	27.5	17.0	7.4

All the seven composting processes carried out in the project showed a similar pattern of changes in total nitrogen, total carbon, C:N ratio and pH. The results of compost trial batches 6, and 7 (2-phase olive husk compost), and 4 (3-phase olive husk compost) are shown in Figs. 10 – 15.

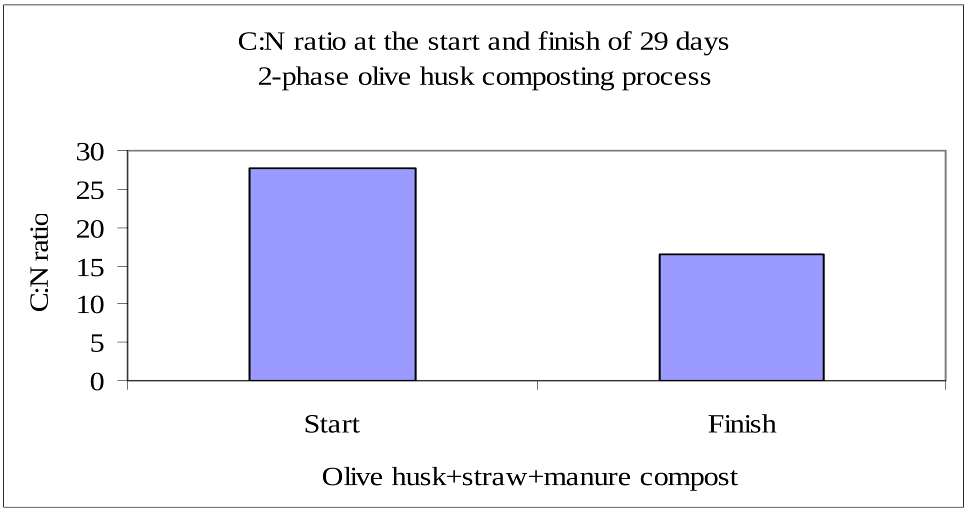


Figure 10 – Compost trial batch 6 C:N ratio

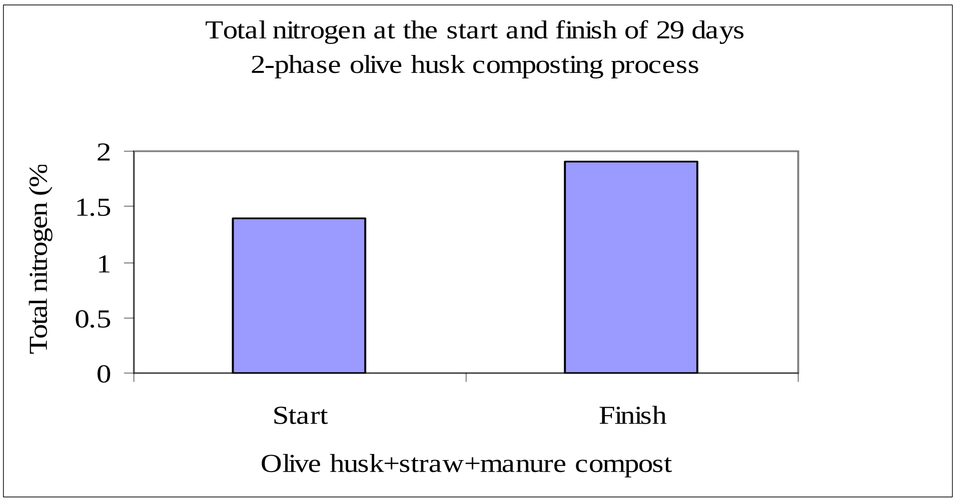


Figure 11 – Compost trial batch 6 total nitrogen %

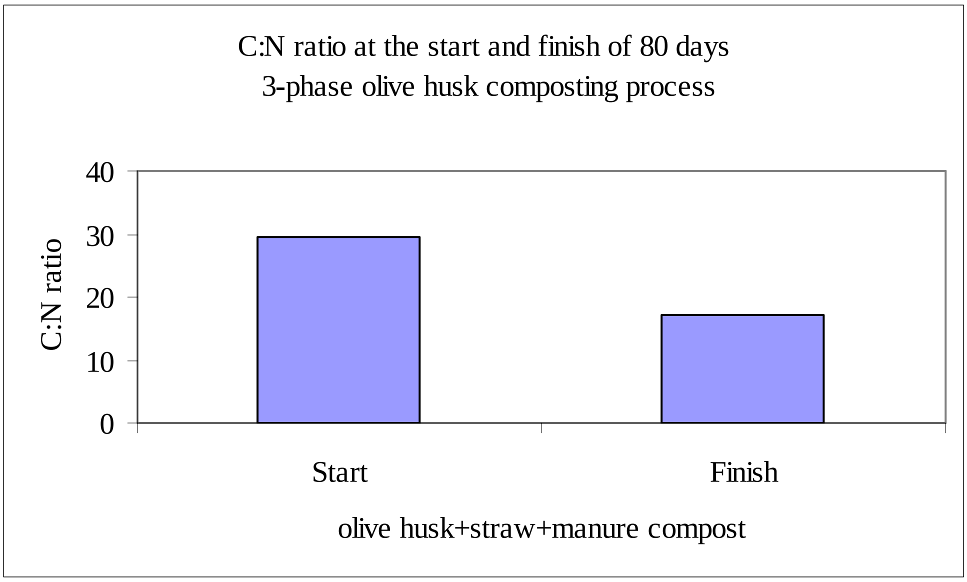


Figure 12 – Compost trial batch 7 C:N ratio

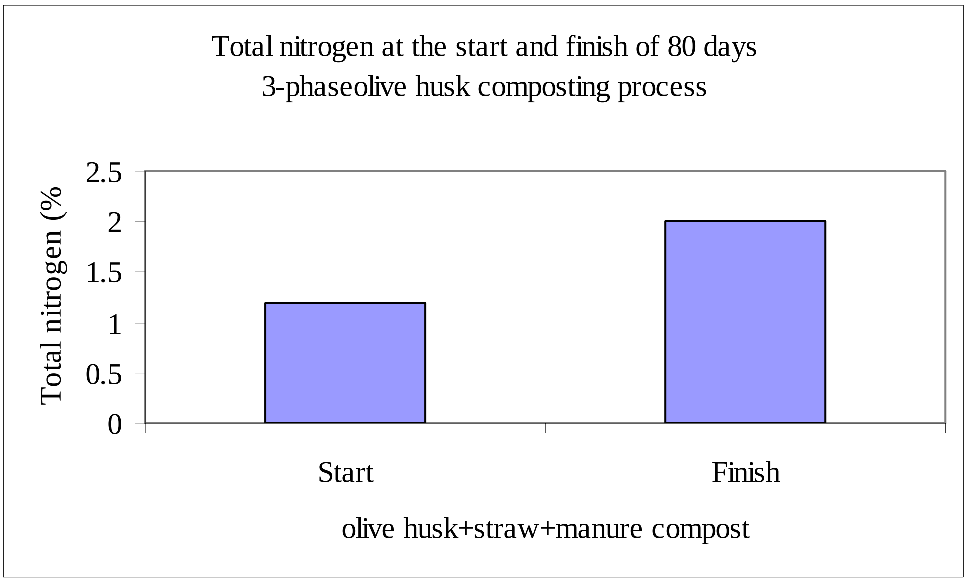


Figure 13 – Compost trial batch 7 total nitrogen %

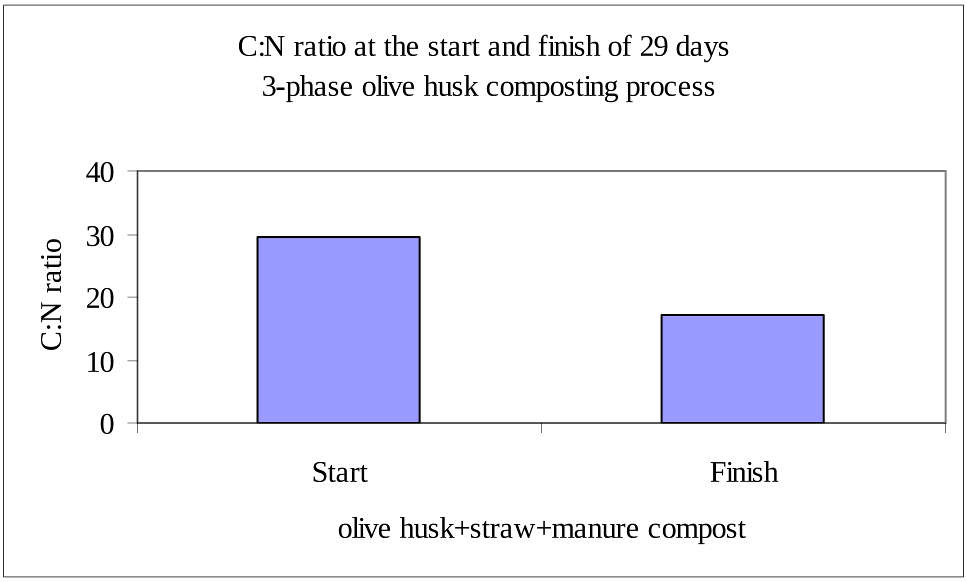


Figure 14 – Compost trial batch 4 C:N ratio

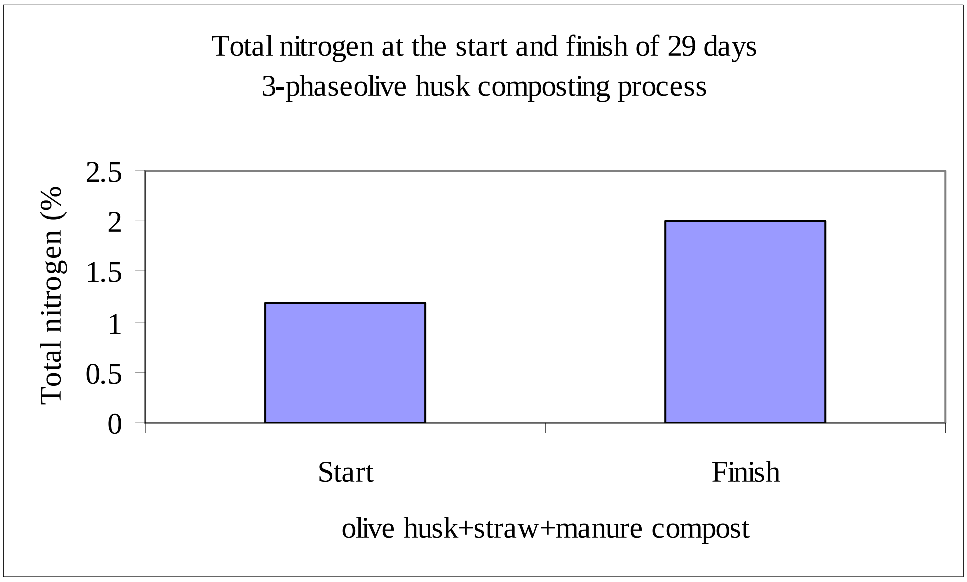


Figure 15 – Compost trial batch 4 total Nitrogen

Humates, humic acid and organic matter at the finish of 2- and 3-phase olive husk composting process

Humates, humic acid and organic matter in the 2- and 3- phase olive husk composts were analysed after the composting processes have been completed. The results are presented in Tables 8 and 9. The percentages of organic matter in compost trial batch 6 at day 29 (end of composting) and in compost trial batch 7 at day 56 (of 70 days composting) were 69.2 and 36.0 respectively (Table 8). Humates were higher in compost of trial batch 7 than in that of trial batch 6. The humic acid contents of both composts were similar.

Table 8 – Organic matter and humic ions in 2-phase olive husk composts[A]

%	Compost trial batch 6[B]	Compost trial batch 7[C]
Organic matter	69.2	36.0
Humates	61.2	70.1
Humic acid	0.60	0.62

[A]Compost ingredients: Olive husk + wheat straw +chicken manure
[B]Composting day 29; [C]Composting day 56

The percentages of organic matter in 2-phase olive husk compost (batch 6) at day 29 was the same as that in 3-phase olive husk compost (batch 4) at day 25 (Table 9) but humates and humic acid were lower.

Table 9 – Organic matter and humic ions in 3-phase olive husk compost*
(compost trial batch 4)

%	Composting day 25
Organic matter	69.2
Humates	51.0
Humic acid	0.51

*Compost ingredients: Olive husk + wheat straw +chicken manure + olive pruning

Phenol levels during composting of 2- and 3-phase olive husk compost

Before monitoring phenol levels in the compost treatments, experiments were performed to develop and validate a method of extracting and measuring total phenols in the compost samples. As noted in the Introduction, there are no standard published methods of extraction of phenolic compounds in olive mill waste, and it has been noted by several workers that extraction protocols affect the determination of phenols in solid and liquid samples (eg review by Obeid et al, 2005). Hence, one of the objectives of this study was to develop a reproducible method to monitor the degradation of solvent extractable, naturally occurring phenolic compounds in compost prepared from solid olive mill waste which contains many compounds of different molecular weight and different structure. No single procedure will extract all with equal efficiency.

The requirements of the protocol were that it optimised extraction of the phenolics present in the waste, that it was suitable for use with a large number of samples, that it minimised the workload and that it was sufficiently sensitive to detect low levels of phenols at the end of the composting procedure. The details of the method that was developed are described in the Methods section of this report and details of its validation are provided in Appendix 1.

All the seven batches of composting carried out in the project showed similar pattern of changes in concentrations of phenols during the process. Phenol levels during composting of 2-phase (compost trial batches 6 and 7) and 3-phase (compost trial batch 5) are given in Figs. 16 – 18.
There was a gradual reduction in the level of phenol from start to finish of the composting process. In 2-phase olive husk compost trial batch 6 the phenol levels dropped from 2.97 mg/g on day 11 to 0.89mg/g on day 44 (Fig. 16).

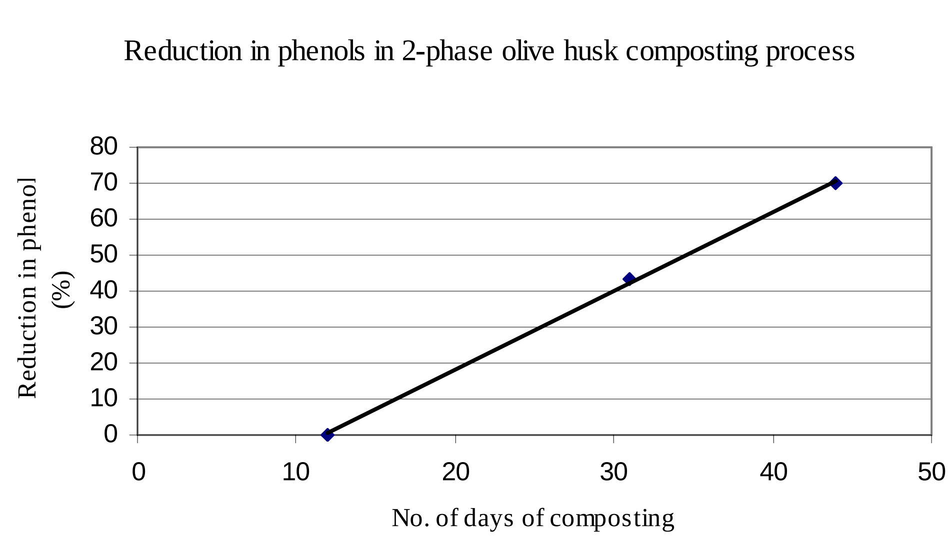


Figure 16 – Reduction in phenols in 2-phase olive husk composting process

Similarly, in 2-phase olive husk compost trial batch 7 the phenol levels dropped from 3.66mg/g on day 1 to 0.99 mg/g on day 76 (Fig. 17).

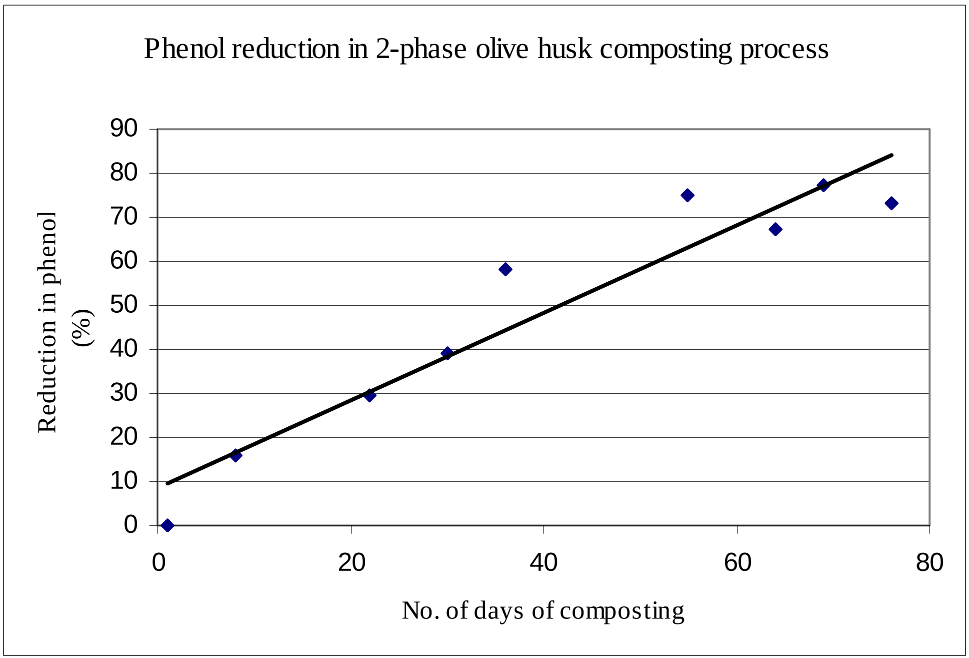


Figure 17 – Phenol reduction in 2-phase olive husk composting process

In 3-phase olive husk compost trial batch 5 the phenol levels in one mix (olive husk + wheat straw + chicken manure) dropped from 3.56 mg/g on day 1 to 1.23 mg/g on day 43, and in another mix (olive husk + wheat straw + chicken manure + olive pruning), the phenol levels dropped from 2.75 mg/g on day 1 to 1.43 mg/g on day 43 (Fig. 18).

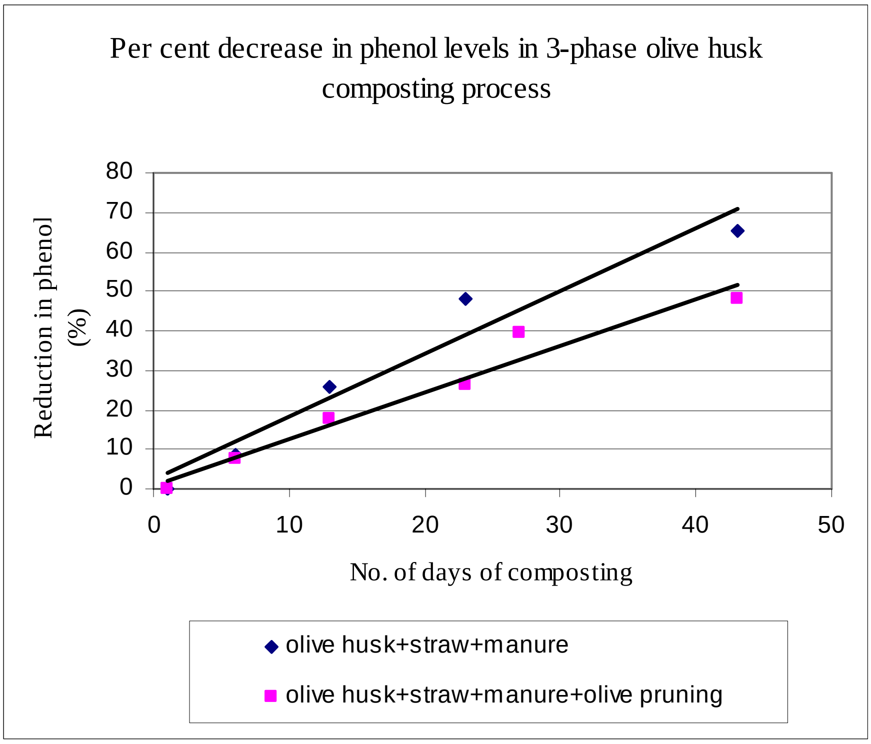


Figure 18 – Per cent decrease in phenol levels in 3-phase olive husk composting process

Phytotoxicity tests of 2- and 3-phase olive husk compost after completion of composting process

Per cent seed germination in 2-phase olive husk compost (trial batch compost 7) was lower than that in the potting mix; however, it was over 80% of that of the control (Table 10). Seed germination in both the 2-phase olive husk compost trial batch 6 and the 3-phase olive husk compost trial batch 4 were higher than that in the control.

Table 10 – Germination of cress (*Lepidium sativum* L.) seeds in 2- and 3-phase olive husk composts after completion of composting process

Medium	% seed germination at seven days		
	2-phase olive husk compost trial batch 7	2-phase olive husk compost trial batch 6	3-phase olive husk compost trial batch 4
Potting mix (Control)	87.0	79.4	79.4
Olive husk compost	70.0*	87.8	88.6

2- and 3-phase olive husk compost ingredients: Olive husk + wheat straw + chicken manure
* Seed germination in the olive husk compost = >80% of that of the control.

The lengths of shoots and roots of 7 days old cress seedlings were higher in 2-phase olive husk compost than in the raw olive husk (Figs.19 and 20).

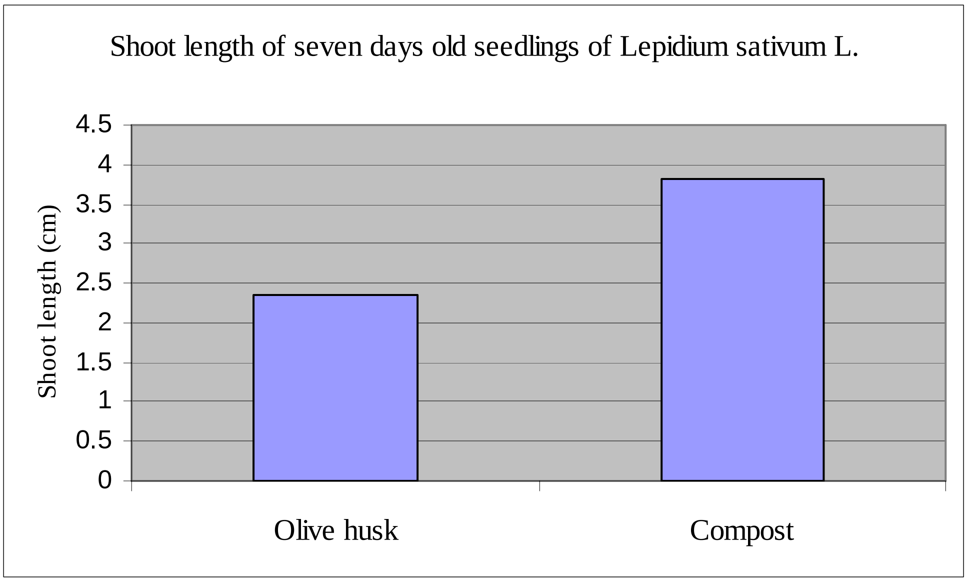


Figure 19 – Shoot length of seven days old seedlings of Lepidium sativum L.

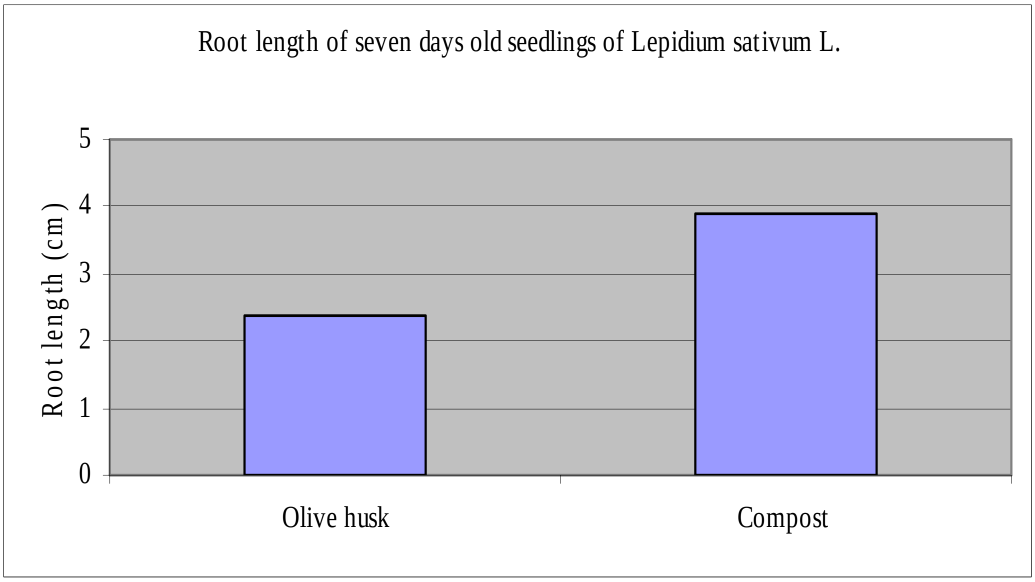


Figure 20 – Root length of seven days old seedlings of Lepidium sativum L.

Microbial decomposition of compost ingredients

The decomposition of the compost ingredients due to microbial activities were observed on the composted samples. The electron scanning micrographs show the growth of fungi and bacteria in the compost (Figs.21-23).

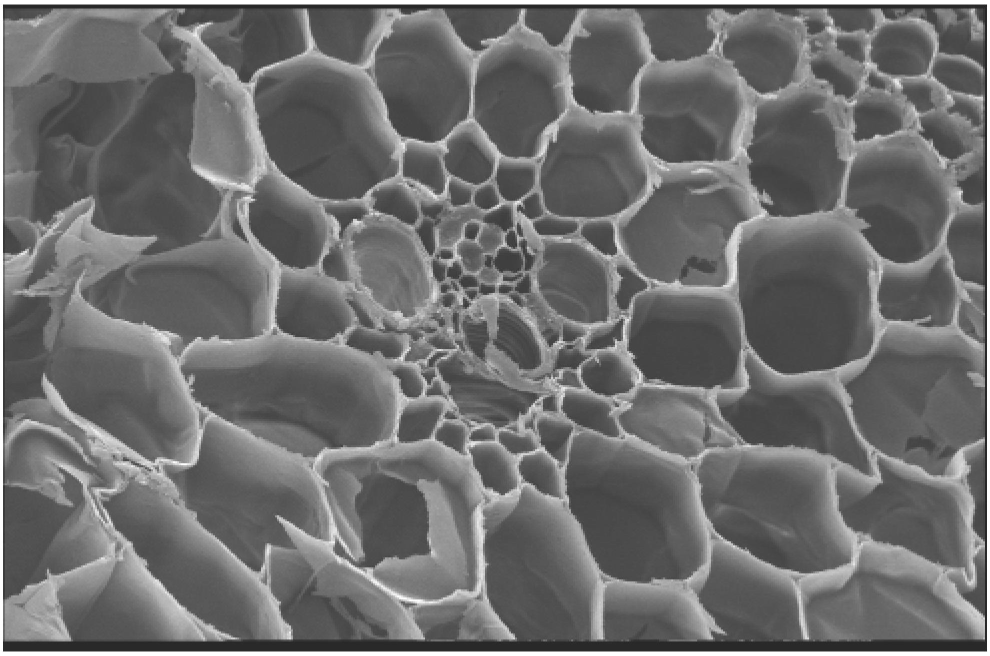


Figure 21 – Cross section of un-composted wheat straw showing structure of internal cells

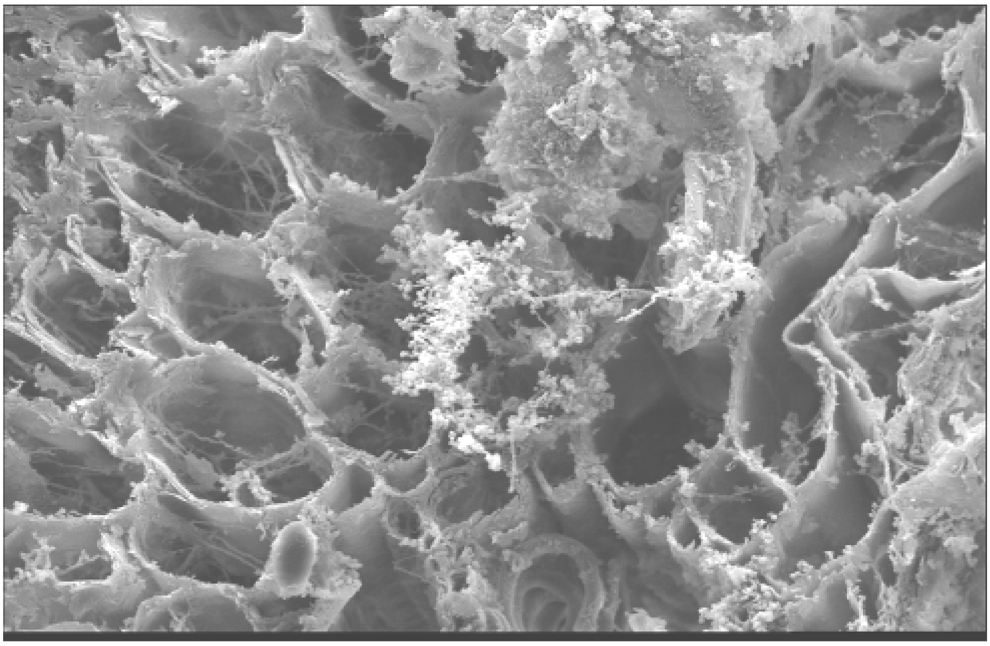


Figure 22 – Beginning of the break down of cell walls by microorganisms. Note the presence of fungi and bacteria

Figure 23 – Colonies of bacteria in the process of breaking down olive husk compost ingredients

Discussion

The problem - The olive mill waste

The Australian production of olive oil in 2007 was estimated to be 8700 metric tonnes. The local olive industry has been expanding at the rate of about 9% per annum over the recent years (Fig. 1). In contrast to Australia, the average annual olive production growth rate in the Mediterranean and Middle East countries has been 5% over the last fifteen years (Raviv *et al.* 2007). The prediction is that in excess of 7.5 million olives will be planted in Australia in the immediate future. Consequently, over 25,000 metric tones of oil may be available per annum from Australian producers by 2011 (Miller 2007).

The amount of solid (olive husk) and liquid waste (olive mill wastewater) produced is directly proportional to the production of olives. These olive mill wastes are produced in significantly large quantities in short periods of time. In spite of predictions of significant increase in fruit production the Australian olive industry does not appear to recognise the vast quantities of solid and liquid wastes that will be generated to the detriment of the environment. The continued rapid expansion in the Australian olive industry can be expected to result in the production of over 62,500 tonnes of solid and over 350 x 10^6 litres of liquid wastes per annum. The industry is therefore faced with the challenge to manage these wastes in order to achieve sustainable production under clean environment. The Second RIRDC Plan for the Australian Olive Industry for the five-year period 2003 to 2008 has identified olive processing solid and liquid wastes as a key environmental issue for the industry to address.

The solution to the problem

There are currently over one hundred patented techniques covering various aspects of olive waste management (Niaounakis and Halvadakis 2004). These patents were granted over a period of about thirty four years from 1969 to 2003. As far as we know, none of these techniques are universally in use. There are two main reasons for this:

(1) Most of the existing technologies are sophisticated and expensive for commercial use
(2) The technology required for olive waste management needs to be site-specific

This project was carried out to address the need for a waste management strategy for the rapidly accumulating olive mill waste in the Australian industry. The project has scientific, commercial and social elements embedded in it. The scientific element deals with a technology for converting olive solid waste into humified compost. The commercial element has the potential to establish regional composting cooperatives. The social element involves overcoming health implications resulting from the inherently odorous and bio-toxic solid wastes from olive oil extraction process.

The project has three major rationales. These are:

- To develop a no-waste strategy that involves recycling the end products without creating any further waste
- To use a natural remediation process of microbial composting that removes phytotoxic compounds from olive husk
- To give the olive growers the option of a low cost technology

The olive oil extraction methods versus the olive mill waste

Three methods of olive oil extraction are used in the olive growing regions of the world. These are pressing (traditional or classical method), three-phase system, and the two-phase system (both are continuous or centrifugal systems). The 3-phase system was introduced in the 1970's to reduce labour costs and increase processing capacity and yield. The 2-phase process was first introduced in Spain in 1991. It reduces the water requirement for the process, but creates a solid waste that is significantly different to one produced by the 3-phase system.

The wastes produced by these systems are different in their characteristics, and consequently, the waste management system needs to be designed accordingly.

The Australian olive growers use either the 2-phase or the 3-phase process for extraction of olive oil. However, majority of these growers use the 2-phase system which produces more solid but significantly less water waste than the 3-phase process (Table 1).

Physical characteristics of olive husk

Two types of olive husk from the 2005, 2006 and 2007 harvests were characterised in this project. One type of olive husk was collected from the 3-phase oil extraction process at Rylstone, NSW, and the other type of olive husk was collected from the 2-phase oil extraction process at Camden, and Clandulla, NSW. Differences were observed in the general physical characteristics of olive husk waste generated from the 2- and 3-phase processes. The consistency of the 2-phase olive husk was that of a thick paste. It dried to a hard solid, and had low porosity and small particle size. The olive husk from the 3-phase process had a consistency that was not pasty. It hardened into a loose solid with relatively higher porosity and larger particle size. These differences in physical characteristics of the solid wastes had an impact on the care taken to mix the ingredients uniformly during the composting process. The amount of water required to maintain moisture during composting was, in general, higher in the 3-phase olive husk mix.

Olive husk composting process

There has been much work on decontamination of olive mill waste water by using biological processes such as anaerobic digestion (Tekin and Dalgic 2000), biofilm (Bertin *et al* 2001) activated sludge, oxidation ditch sequencing batch reactor, controlled wetlands (Niaounakis and Halvadakis 2004) etc. However, there appears to be relatively little work on using composting as a bioremediation process for management of the olive husk waste from oil mills.

Work carried out in this project has demonstrated that composting is an efficient and low cost technology for olive solid waste management.

The durations of the composting process were different for the different 2- and 3-phase olive husk compost trial batches. The composting period ranged from 21 to 80 days, and the results showed that it is possible to produce mature olive husk compost in twenty one days. However, longer duration is required for the preparation of fully humified compost. Previous attempts by several workers required considerably longer duration of composting ranging from 90 to 224 days (to achieve satisfactory detoxification of olive husk (Vlyssides *et al.* 1996, Benitez *et al* 2004, Baeta-Hall *et al* 2005).

The addition of different carbon sources such as lignocellulose residues (wheat straw, olive pruning) and nitrogen sources such animal manure (chicken manure) to 2- and 3-phase olive husk facilitated the composting process. Other workers have used different ingredients such as crude olive waste, cow manure; fresh olive tree leaves etc. to compost olive husk (Sciancalepore *et al* 1994, 1995, 1996; Niaounakis and Halvadakis 2004). Wheat straw and chicken manure were mainly used in this project. Olive pruning was also used as an ingredient in one batch of compost. The quantities and C:N ratios of the individual ingredients used to make up the two experimental composting mixes were computed to fall within the limits set by the Australian Certified Organics guidelines and the US standards.

The starting C:N ratios of the olive husk compost trial batches used in the project fell within the range of 25 to 35 as specified in the Australian Certified Organic guidelines (Figs. 10-15). Some workers add fertilisers such as urea to olive husk to ensure a C:N ratio of 35 (Tomati *et al* 1995). However, fertilisers were not considered necessary as an ingredient in the make up of the compost mix in this project and satisfactory results were obtained without it. This will enable organic olive growers to use the composting technology developed in this project.

The composting process followed a general pattern of an initial rise in compost temperature to a maximum of 63 -66^{0}C and a gradual decrease in temperature to 18-29^{0}C (Figs. 4-6). The maximum temperature was reached at different days in the different compost batches. Similarly, the time taken for the compost temperature to drop to the minimum level also varied between compost batches. This is a reflection of the differences in microbial activities in 2- and 3-phase olive husk composts. Thermophilic phases in the 3-phase compost trial batch 4 and 2-phase compost trail batch 6 were 13 and 20 days respectively. The mesophilic stage in the 3-phase compost trial batch 4 and 2-phase compost trail batch 7 were 6 and 28 days respectively. These thermophilic and mesophilic periods are suitable for microbial activities to convert proteins and carbohydrates in the compost. The extended time taken for the drop in temperature in 2-phase olive husk compost trial batches 6 (Fig. 4) and 7 (Fig. 5) was possibly due to incomplete conversion of ammonia and other chemical activities in the compost.

Regular turning of the olive husk compost heap was found to be essential for microbial thermogenesis which allowed the compost to reach desired temperature, aeration, and dissipation of ammonia. Our method confirms the work of Baeta-Hall *et al* (2005) that mechanical turning of the compost adopted by us in this project was more feasible on a commercial scale than forced air injection.

pH of the compost is an important factor in achieving process efficiency. The compost pH was at the required levels at all stages in the process (Figs 7 – 9). One of the roles of compost pH is to enable the evolution and dissipation of ammonia (NH_3). Although the presence of NH_3 in the compost plays a role in pasteurisation, it can be toxic to several beneficial microorganisms in the compost. Optimum evolution of NH_3 occurs at pH 8.3. The composting process used in this project has maintained pH levels of 8.0 - 8.4 at the required processing time.

The percentages of organic matter in 2-phase olive husk compost trial batch 6 at day 29 (end of composting) and in compost trial batch 7 at day 56 (of 70 days composting) were 69.2 and 36.0 respectively (Table 9). These levels of organic matter are significantly higher than the desirable level of 20% suggested by (Blosser and Korpf 2006). Humates were higher in compost of trial batch 7 (70.1%) than in that of trial batch 6 (61.2%). Humic acid content of compost trial batches 6 and 7 were 0.60% and 0.62% respectively. The desirable levels of humus complex and humic acid in composts are 25% and 1.9% Blosser and Korpf (2006). The levels of humic acid in the compost can be increased by the addition of microorganisms such as aerobic and facultative aerobic, sporing and non-sporing and nitrogen fixing bacteria such as *Azotobacter* and *Nitrosomonas* groups responsible for transformation to humus.

One of the main aims of composting is to remove phytotoxicity associated with olive husk. The composting technology developed in this project has significantly reduced the level of phenols within the duration of the process. As far as we are aware, there are no standard published methods for quantitative analysis of solvent soluble phenols in olive husk compost. In order to track the removal of phenolic compounds in the olive husk compost, we developed an extraction method for the determination of phenols in the compost. There was a gradual reduction in the level of phenol from start to finish of the composting process. In 2-phase olive husk compost trial batch 6 the phenol levels dropped from 2.97 mg/g on day 11 to 0.89 mg/g on day 44 (Fig. 16). Similar patterns of significant reduction in phenol levels at the end of the composting process were observed in the other 2- and 3-phase compost trial batches Figs. 17 and 18).

One of the markers of maturity in olive husk compost is the absence of phytotoxicity. It is known that olive waste inhibits germination of seeds of different plant species (Della Greca *et al* 2001, Niaounakis and Halvadakis 2004). The presence of phenols as well as short and long chain fatty acids is considered to be responsible for the phytotoxic (Cassa *et al*. 2003, Kistner *et al.* 2004, Isidori *et al.* 2005) and antimicrobial (Fiorentino *et al.* 2003, Isidori *et al.* 2005) nature of these wastes. The results obtained in this project have shown that the 2- and 3-phase olive husk composts were not phytotoxic at the completion of the composting process. Tests showed that germination of cress (*Lepidium sativum* L) in both the 2-phase olive husk compost trial batch 6 and the 3-phase olive husk compost trial batch 4 were higher than that in the potting mix control (Table 11). Our results agree with that of Raviv *et al* (2007) who showed that olive waste inhibited per cent germination and root elongation of cress.

The olive husk compost developed in this project has levels of per cent nitrogen (0.6 – 1.2), carbon: nitrogen ratio (15 – 20), pH (7.3 – 8.1), and per cent seed germination (80) that are considered desirable in good quality compost. The only fraction whose level in the compost could be further improved is humic acid. While the range in the level of humic acid in our compost is 0.51 to 0.62%, the desirable level of humic acid is considered to be 1.9%. This can be achieved by inoculating the compost with suitable microorganisms during the composting process when ammonia has been dissipated. However, this was beyond the scope of this project.

The cost of production of the composting process developed in this project is relatively low. An indicative budget for the production of the 2- and 3-phase olive husk composting process is approximately $50 (fifty dollars) per tonne. This cost has been based on the following assumptions:

- Olive husk = no cost
- Olive pruning = no cost
- Chicken manure = $50.00/Tonne
- Wheat straw = $7.00/bale (20kg/bale)
- Microbial mix
  (e.g. Actizyme®) = $30.00/kg

(excluding labour cost)

Conclusions

This project has demonstrated that 2- and 3-phase olive husk waste can be successfully converted into compost by using relatively low cost bioremediation technology. It has further shown that the resultant compost is non-phytotoxic and can therefore be recycled as an organic amendment to olive grove soil. A successful composting model was developed that can be applied as an olive mill waste management tool.

The next step in the bioremediation process of olive husk waste is to achieve a higher level of humification of the compost than that obtained in this study. Research by Tomati *et al* (2000) on olive husk composting confirm the evolution of humic acid towards a more complex, stable structure, and supports the validity of composting in approaching humus-like fractions from organic wastes to native soil humic substances. Factors such as moisture, temperature, pH, aeration, microbial activity, and soil fertility levels affect the conversion of organic matter such as the olive husk compost to humus. Custom-made composting facilities that are strategically located in the different olive growing regions will enable the industry to grow olives in an environmentally friendly and sustainable manner.

Implications

The main impact of the outcomes of this project on the Australian olive industry is the application of an efficient and economical olive mill solid waste management technology. It is suggested that olive mills adopt the composting process developed in this project either individually or collectively as regional enterprises. The consequence will be the ability to convert liabilities (waste) into assets (value-added compost) thus contributing to sustainable and responsible farming practices.

The composting technology is economical and commercially feasible. An indicative budget for the production of the 2- and 3-phase olive husk composting process is approximately $50 (fifty dollars) per tonne. This cost has been based on the assumption that there is no cost for olive husk and olive pruning and that the ingredients, chicken manure, wheat straw and microbial mix cost $50/T, $7/bale and $30/kg respectively. Labour cost has been excluded.

Recommendations

Composting recommendation

It is recommended that olive mills practice the composting model developed in this project. It is important to closely follow the process described in this project. Olive growers will be able to convert olive husk (liability) into value-added compost (asset) by successful practice of composting oil mill waste. This will aid in sustainable and responsible farming practices.

Steps to disseminate the results of the project

There was a significant response from olive growers to the 2- and 3-phase olive husk composts produced in this project when they were displayed at the 2007 Australian Olive Expo in Canberra. It is recommended that the Rural Industries Research and Development Corporation consider allocation of funds to organise an olive waste composting demonstration field day. Theoretical and practical aspects of the composting process can be explained and demonstrated during two days of activities.

Further development to maximise the results of the project

The benefit of using the compost as an addition to olive grove soil can be increased to the maximum degree by improving the concentration of humus in the compost. The different characteristics of normal compost and humified compost are:

- Normal compost enables the plant to grow, but they may not be able to reach their potential
- Humified compost enables the plant to grow and helps them to reach their potential

Two examples of the capacity of humus are: ability to retain nutrients on demand for plants due to its high cation exchange capacity (150-300 compared with 2 -120 for clay and 1-4 for sand), and water-holding capacity (helping the plants to be more drought resistant). It is recommended that the Rural Industries Research and Development Corporation consider funding a project on extending the quality of olive waste compost and its application in olive grove soil. The main aim of such a project will be to achieve a net accumulation of humus in the soil and its effect on olives.

Well processed composts can be suppressive to a variety of soil-borne pathogens, including those of olives; however, little is known about the suppressive capacity of compost made from olive solid waste. The proposed project would aim to further develop the results achieved in the present project and to develop a composting system that would break down the recalcitrant olive solid waste. Part of this technique will involve the use of specific microorganisms. These microorganisms, along with the resident microflora in the compost, will assist in the breakdown of organic compounds as well as selected macromolecules to different degrees. The resultant product would be compost having the potential to suppress organisms causing especially root diseases of olives.

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Appendices

Appendix 1 - Development and validation of a method of solvent extraction and determination of total phenols in olive mill waste compost

There were four major stages in the method development which will be described briefly here and in more detail in a manuscript that is currently being prepared for submission to Journal of Agricultural and Food Chemistry.

Selection of a standard

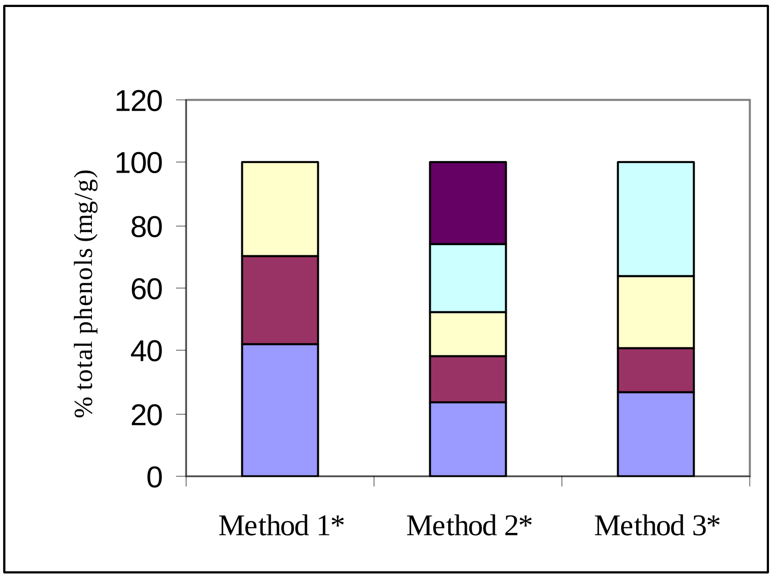
This method does not allow an absolute measurement of phenols, but rather a measurement of equivalents to selected standard. This is acceptable when using the method for comparison of samples, or to monitor the reduction in phenols during, for example, a composting process. There is no universally accepted standard, which means that concentrations of phenols in different studies are not directly comparable.

Although there are reports of tannic acid (Farag, 2003), syringic acid (Romero et al, 2002) and oleuropein (Cardoso et al, 2005), caffeic acid (eg Blekas et al, 2002) and gallic acid (eg McDonald et al, 2001) are the most commonly used. Oleuropein is present in high quantities in olives, but it is neither stable nor commercially available, and hence is not a suitable choice. In this study both caffeic and gallic acid were tested and, although caffeic acid was selected for use throughout the study, both gave consistent results and linear standard curves over the concentration range 0-20mg/L (see Fig 3).

Extraction protocols

There are a plethora of extraction protocols in the literature for solvent extraction of phenolic compounds from different plant materials and they have been reviewed by various authors (see, for example, Waterman and Mole, 1994 and Robards, 2003). These reviews highlight the difficulties associated with analysis of complex mixtures of compounds and emphasise that no extraction protocol will extract with 100% efficiency all the phenolics in such a sample. However, methods can be developed for a particular sample type that will give reproducible results.

In this study we selected methanol-water (80:20, v/v) in conjunction with acetone-water (60:40, v/v) as solvents and evaluated the effect of extraction volume, number of extractions and extraction time. Initially, extractions were performed using 50ml of solvent and 5g of dried compost. Successive extracts were combined and reconstituted to 250ml. This was subsequently modified to 30ml volumes for each extraction, and reconstitution of pooled extracts to 100ml. Although the total phenol concentration was significantly lower by this method (average of 3.1mg/g for 50ml extraction compared to 2.5mg/g for 30ml extraction volumes for one compost sample, $P<0.05$), this was considered acceptable, because the purpose of this study was to monitor relative concentrations of phenols during the composting procedure. In addition, there were several advantages to using the lower extraction volume. These included the reduction in the use of organic solvents (reduction in cost and waste disposal) and the increase of sensitivity of the assay when a smaller extraction volume was used. This is important in the latter stages of composting when the levels of phenols are low. Figure 1 shows the results obtained when three different extraction protocols were compared. Details of each protocol are provided in Table 1. The total phenols, measured with the Folin-Ciocalteau reagent, were comparable in each case: 3.2mg/g using Method 1, 3.5mg/g using Method 2 and 3.6mg/g using method 3.



Appendices Figure 1 – Comparison of protocols for extraction of phenols from olive mill compost

* Details of each Method are provided in Table 1. Fraction 1 (the first methanol extraction) is at the bottom of the graph. 5g samples of dried compost were extracted with 30ml solvent at 20^{o}C.

Appendices Table 1 – Extraction protocols

Fraction*	Method 1	Method 2	Method 3
1	80% methanol/20h	80% methanol/1h	80% methanol/3h
2	80% methanol/48h	80% methanol/1h	80% methanol/2h
3	60% acetone/3h	80% methanol/1h	80% methanol/20h
4		80% methanol/20h	60% acetone/3h
5		60% acetone/3h	

The method adopted for the testing of compost samples was Method 1, which satisfied the following criteria:

- it reproducibly extracted the phenolic compounds present in the waste (when the same sample was extracted on different days, there was no significant difference in the results)
- it minimised the workload
- it was suitable for use with a large number of samples, and
- it was sufficiently sensitive to detect low levels of phenols at the end of the composting procedure.

The use of the longer extraction times is supported by Waterman & Mole (1994) who have reported that short extractions with dried materials are not adequate.

Reproducibility of the phenol assay

Some samples were subjected to repeated analysis for phenol content to determine the reproducibility of the assay. The results analysed by Minitab 14 fully nested ANOVA. From multiple data sets, there was no significant difference between replicates tested on the same day, or between different extracts of the same sample, at a significance level of $P = 0.05$, but there was a significant difference if extracts were stored and tested on different days ($P<0.05$). However, there was no definite trend in the values with increase in storage time (values fluctuated marginally) and this probably reflects the effect of temperature on the assay, which was performed at room temperature (approx 20^{o}C), rather than a degradation of the sample on storage.

Recovery of caffeic and gallic acids from compost samples

The protocol was further evaluated by spiking the Folin-Ciocalteau assay with both caffeic and gallic acids and spiking samples of the compost with caffeic acid. To test recovery of the standards from the assay, extracts of four different composts with various levels of phenols were spiked by adding either gallic acid (8mg/L) or caffeic acid (6mg/L and 12 mg/L) to the assay tubes. Recovery of gallic acid in the four samples ranged from 90-94%, and recovery of caffeic acid from 94-102% (6mg/L spike) and 92-93% (12mg/L). The higher recovery of caffeic acid supported its selection as the standard for the assay.

To test the efficiency of the extraction procedure, 5g samples of dried compost were moistened with 100mg or 50mg of caffeic acid and dried at 25^{o}C. Samples were then extracted using the standardised protocol (Method 1) and assayed by the Folin-Ciocalteau procedure as described in the Methods section. At both levels of spike, 89-92% recovery was achieved.

Recommendations

To ensure reproducible results, use of a consistent, reproducible protocol, such as the one described in this study is essential. Important aspects to consider include:

- storage of samples
- taking a representative sample, especially in early phases of composting when material is not homogeneous
- drying the sample to constant weight before extraction
- extracting in glass containers as solvents can degrade some plastics during lengthy extractions
- selection of solvent(s)
- ratio of solvent to dry material
- the number and length of extractions and temperature

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Appendix 2 - Figures

Appendices Figure 2 – Raw olive husk waste dumped on farm land

Appendices Figure 3 – Composted olive husk waste

Appendices Figure 4 – Storage of olive mill wastewater in anaerobic pond

Recycling Solid Waste from the Olive Oil Extraction Process

by Assoc. Professor N.G. (Tan) Nair and Dr Julie Markham

RIRDC Pub. No. 08/165

The report is about developing an environmentally sustainable system to manage solid waste from the 2- and 3- phase olive oil mill extraction processes. The Australian olive industry has been expanding at the rate of about 9% per annum over recent years. This significant increase in fruit production will result in vast quantities of solid and liquid wastes. The industry is faced with the challenge of managing these wastes in order to achieve sustainable production in a clean environment.

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