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Research Report

ASSESSMENT OF OPTIONS AND REQUIREMENTS FOR STABILITY AND MATURITY TESTING OF COMPOSTS

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ASSESSMENT OF OPTIONS AND REQUIREMENTS FOR STABILITY AND MATURITY TESTING OF COMPOSTS

Contents

FOREWORD

Commentary on Report provided by the WRAP Steering Committee

This report details work undertaken by ADAS Consulting Ltd on behalf of WRAP to assess testing options and to develop a test(s) for compost stability and maturity. Following assessment of a range of potential options, the approach taken by ADAS was to develop a carbon dioxide evolution test for measuring compost stability and a growing test to measure compost maturity. The results presented in this report advance the knowledge of characterising the stability of composted materials and also serve a useful function in identifying whether phytotoxins may be present in a sample of compost.

Readers are cautioned that the methods described should not be considered as standardised tests because their precision (repeatability and reproducibility) has not yet been quantified. Before the methods detailed in this report could be considered as standardised tests, clarification of the following critical aspects is required and inter-laboratory evaluation undertaken to quantify precision. These are aspects which have equally not been adequately investigated by most workers reporting methods in the literature on stability and maturity testing:

Microbial Respiration as measured by carbon dioxide evolution (Proposed test for Compost Stability)

Moisture Content

This report shows that moisture content is a critical factor. If reproducible results are to be obtained it is essential that the moisture contents of samples are optimised and prepared in a way that is reproducible. Different materials and particle size grades are able to hold different amounts of moisture. For example, composted wood chips will hold much less moisture than the <10mm fraction of composted greenwaste. The composting process, like any biological process, will stall if there is insufficient moisture. It is important that the material being incubated has optimum moisture for biological activity and that this can be achieved reproducibly. Given the above, it is likely that the moisture content should be related to the Water Holding Capacity (WHC) of the sample, which is a characteristic of the material. Further work is needed to standardise the optimal proportion of WHC and also how this can be achieved.

Incubation Temperature

Biological activity is temperature dependent; this report confirms that it is the most critical parameter determining CO_2 evolution. There is no consensus on the optimum temperature for respirometry. In the USA 35ºC is generally used, while some countries use 30ºC. Further work is needed to characterise the optimum temperature and the tolerance with which it needs to be controlled in order to obtain results of acceptable precision.

Pre-incubation Conditioning

There is a flush of biological activity when composted materials with low moisture or that have dried out are wetted, e.g. for incubation. This flush appears to last for 3 to 5 days (see figure 9.1 of this report) before 'equilibrium' metabolic activity is re-established. Further work is needed to standardise the conditions (time, temperature, moisture content) under which samples should be equilibrated prior to measuring the respiration rate.

Nutrient Supplementation

Biological activity might be very low in some materials because an essential nutrient is deficient, although this phenomenon was not apparent in any of the samples used for the experimental work described in this report. For example, papermill sludge contains abundant available carbon but negligible available nitrogen.

The respiration rate of this material would be quite low because it is limited by the lack of nutrients. However, if nitrogen and possibly phosphate, were supplied it would degrade and respiration would increase. Further work is needed to test this aspect, develop protocols for adding supplementary nutrients and then to standardise the procedure.

Growing Test (Bio-Assay) (Proposed test for Compost Maturity)

The information about growing trials is valuable, although the work to develop a test for maturity was inconclusive as the trials did not discriminate between samples, quite possibly because a range of other factors was affecting plant growth.

The steering committee is of the opinion that compost maturity can be adequately characterised from the CO_2 evolution rate and the nitrate:ammonia ratio, without the need for further growing trials. However, the growing trials are valuable in assessing the overall suitability of composted materials in supporting plant growth and for determining the presence of phytotoxins. The steering committee agrees that a bio-assay test is currently the only viable test for phytotoxicity.

WRAP Steering Committee

Mr B. Cooper	Consultant
Dr T. Evans	Tim Evans Environment
Miss L. Hollingworth	WRAP
Miss E. Nichols	The Composting Association

1. Summary

1.1. Introduction

The purpose of the work described is to assist in refining the existing standards for high quality composted material, by assessing testing options for stability and maturity. Stability and maturity are recognised as important parameters when assessing the quality of composts.

The work undertaken involved

- Literature search for the definitions and methods used for the testing of composts for stability and maturity.
- Selection and development of a laboratory method of testing for compost stability and maturity.
- Laboratory testing the compost stability and maturity method over a range of compost types and ages.
- Evaluation of phytotoxicity testing for compost using indicator species.

1.2. Definitions

An exhaustive literature search established there were no universally accepted definitions for compost stability or maturity. Working definitions for compost stability and maturity were defined for the purpose of evaluating, developing and recommending methods for measuring them.

- Compost stability was defined as 'the rate of biological activity'
- Compost maturity was defined in its simplest terms as 'readiness for use'.

1.3. Methods Selection

Aerobic respiration activity was identified as the stability parameter that was most directly correlated to biological activity in an aerobic matrix. This is universal to all composts and could be easily measured. Analytical techniques and methods for measuring respiration activity were compared. CO_2 evolution methods were considered to be the most direct and accurate measurements of aerobic respiration rate and hence biological activity.

Stability and phytotoxicity were recognised as the universally applicable maturity parameters for composts intended for agricultural or horticultural use. Different methods of assessing phytotoxicity were considered. A plant bioassay approach was recognised as the only direct way to measure phytotoxicity.

A discussion paper was presented for peer review detailing the logic and decision-making leading to recommendations. The views of the peer group were incorporated into the test methods to be carried forward into laboratory research and testing.

1.4. Laboratory Determination of Compost Stability

The method developed and tested was based largely on the carbon dioxide evolution method of BS ISO 14855:1999 *Determination of the Ultimate Aerobic Biodegradability and Disintegration of Plastic Materials Under Controlled Composting Conditions – Method by Analysis of Evolved Carbon Dioxide.* The method is compatible with procedures using automated respirometers measuring oxygen consumption. It is a great deal simpler and it was relatively inexpensive and simple to construct the test apparatus. A set of 10 test vessels for concurrent evaluation of different composts was made up.

Samples of green waste compost at 1 week, 1 month, 2 months, 6 months and over 6 months age were used in the laboratory evaluation of the method of determining stability. The method was sensitive at all stages of composting and at all respiration rates and levels of stability. Trials were undertaken involved testing green waste compost and biodegradable municipal waste at three stages of composting. These demonstrated the range, sensitivity and repeatability of the method.

- The developed method is repeatable, with consistent results for the same samples and significant differences between samples tested. Replicates agree with a precision of better than 1 mg CO_2/g VS/day.
- The method's main strengths are simplicity and ruggedness, without reliance on complicated or expensive instrumentation. The method is cheap, convenient and easy to set up and use almost anywhere where temperature can be controlled.

The commercially available rapid field test was evaluated with the developed stability method. In the small range of samples tested within this research the results obtained indicate that the Solvita^{TM} method is convenient and adequate for routine uses during production monitoring. In a statistical comparison, the Solvita test correlated well to the laboratory CO_2 results and the inter-laboratory trials showed a high degree of reproducibility.

1.5. Growing Test to Assess the Compost Readiness for Use

The method of phytotoxicity testing for determination of maturity used:

- composts from green waste and biodegradable municipal waste
- sample dilution with vermiculite to achieve a starting electrical conductivity (EC) of 400 $\mu S\ cm^{-1}$
- 9 cm (3.5-inch) plastic plant pots as the containers
- light at an intensity of 10,000 Lux, with a 16 hour day at 25^{o} C minimum and 8 hour night at 15^{o} C minimum.
- indicator species radish and lentils
- assessments after 7 days for radish and 7 and 14 days for lentils

An extensive growth trial involving green and biodegradable municipal waste composts, at 3 maturity ages and involving 144 pots was then conducted, to calibrate the test. Statistical analysis of the results indicates there were no significant differences between any of the composts over all the indicator species.

The conclusion from this result was that a growth test should not be looked on as a stand-alone quantitative test of maturity but should, at best, be used to support other tests.

With regard to readiness for use, the green waste compost and compost prepared from biodegradable mixed waste sources performed equally at a wide range of maturities.

2. Objectives

The purpose of the research was to assist WRAP in refining the existing standards for high quality composted material by assessing testing options for stability and maturity.

To facilitate project management the overall objective was subdivided into tasks and milestones in logical progression:

- Conduct a thorough literature search for definitions of compost stability and maturity, of parameters used to assess them and of analytical methods used to measure those parameters.
- Decide on working definitions of compost stability and maturity.
- Evaluate and recommend assessment parameters.
- Evaluate and recommend analytical techniques and test methods.
- Present a discussion paper for peer review detailing the logic and decision-making leading to recommendations.
- Review the sensitivity of the existing phytotoxicity test as a measure of stability and maturity
- Amend recommendations to take account of feed back from peers.
- Test the performance of selected analytical procedures on a range of composts.
- Modify and improve analytical procedures in the light of performance tests.
- Carry out an evaluation of commercially available field-based test methods for stability.
- Recommend selected and tested analytical methods to WRAP for adoption as standard reference procedures and/or validation by inter-laboratory testing (which was not included within the scope of this project).
- Publish accounts of the project and recommendations in recognised scientific journals.

3. Literature Search

A comprehensive search was made for information on testing procedures for compost stability and maturity using modern information technology and personal communication. Particular importance was given to:

- Existing and proposed statutory and voluntary compost quality standards and guidelines such as the British Standards (2002) Publicly Available Specification (PAS) 100 Specification for Composted Materials.
- Conference proceedings.
- Peer reviewed research papers.
- Publications from standardisation bodies.
- Personal communications from recognised experts.

References are made where relevant throughout this document. A full bibliography is given.

4. Definitions

The need for working definitions of certain terms was recognised at the outset of the project. 'Compost', 'composting', 'stability' and 'maturity', were terms essential to the project. Working definitions were needed.

For the purpose of conducting this research the following definitions were adopted as a result of evaluation and interpretation of the literature.

Composting The aerobic biodegradation of solid organic materials in a managed process that generates heat and a sanitised and stabilised solid residue that is much reduced in weight and volume.

Compost The solid residue resulting from a composting process.

Stability The rate of biological activity.

Maturity Degree of biodegradation at which compost is free of phytotoxic substances that cause delayed seed germination, inhibit plant growth or have other adverse effects on plants in any growing situation when used as directed. Readiness for use.

These might be considered by some to be an over simplification. The information leading to these is analysed in the sections below.

When defining a property, the ability to measure it is a factor that needs to be considered. Certain criteria for acceptable working definitions were set:

- It should make sense in plain English.
- Vague or ambiguous wording should be avoided.
- The property name should agree with the plain English or dictionary meaning of the word or words used, e.g. a property called maturity should refer to qualities related to ageing.
- A definition of a property should, as much as possible, be worded in terms of parameters that can be directly measured or demonstrated.
- The definition should agree with a consensus of informed opinion and be technically defensible.
- It should be as short and simple and as universally applicable as possible.

4.1. Composting

The term composting should be confined to aerobic processes, which result in heating of the organic matter mass as carbon compounds are aerobically respired as energy sources by micro-organisms. The process also involves considerable chemical, physical and microbiological changes such that the resulting product bears little resemblance to the original feedstock. The process can usually be divided into various stages. Initially the readily available carbon and energy sources comprising soluble carbohydrates, starch and soluble proteins are consumed by micro-organisms, principally bacteria, resulting in rapid growth of microbial biomass and considerable heating, consumption of oxygen and generation of carbon dioxide, ammonia, amides and other volatiles, often resulting in malodours. The composting then continues at a steadier pace during which period the less easily consumed cell wall carbohydrates, cellulose and hemicelluloses, are broken down and metabolised. The process slows and begins cooling as inhibiting waste metabolites accumulate, the readily available energy sources start to run out and micro-organisms are left with increasingly resistant structural ligno-cellulose compounds. Eventually the only carbon sources left are highly resistant lignin and its polyphenol derivatives, commonly grouped under the names humic and fulvic acids, and the compost becomes more aerobically stable and enters the maturation stage. Chemical and microbiological activities continue but at a much slower pace. During maturation the compost becomes less phytotoxic as catalytic enzymes and other biologically active substances are denatured and waste metabolites are degraded. There is a continuous succession of microbial populations during the composting process.

Zucconi (1986) defined composting as 'a controlled bio-oxidative process leading to the production of carbon dioxide, water, minerals, and a stabilised organic matter defined as 'compost' '.

Leege and Thompson (1997) defined composting as 'a managed process that controls biological decomposition and transformation of biodegradable material into a humus-like substance called compost'. This definition fails to differentiate aerobic composting from anaerobic fermentation or digestion. It also implies that the primary function of composting is to produce compost. This may not always be the case.

The potentially most valuable, but currently very poorly utilised, product of composting is the heat produced. This could be utilised via hot beds or by using heat pumps. Other valuable functions, and sometimes, primary purposes, of composting are sanitisation, stabilisation, reduction in methane producing potential and reduction in bulk of organic wastes.

The 2nd draft of the EU working document on Biological Treatment of Biowaste, CEC(2001), defined composting as 'the autothermic and thermophilic biological decomposition of separately collected biowaste in the presence of oxygen and under controlled conditions by the action of micro- and macro-organisms in order to produce compost'. This again implies that production of compost is the primary purpose which is not necessarily so.

There is no agreed definition of the composting process. Our working definition for composting is 'the aerobic biodegradation of solid organic materials in a managed process that generates heat and a sanitised and stabilised solid residue that is much reduced in weight and volume'.

4.2. Compost

Compost is the solid residue resulting from a composting process. It primarily refers to organic matter but can include inert contaminants. Composts are commonly used as soil improvers, but a small proportion is used as growing media or as a constituent of growing media. Some is landfilled as mechanically and biologically treated waste with a reduced potential to produce methane.

Zucconi (1986) defined compost as 'the stabilised organic matter resulting from a controlled bio-oxidative process (composting)'.

Leege and Thompson (1997) defined compost as 'the product resulting from the controlled biological decomposition of organic wastes that have been sanitised and stabilised to a degree which is potentially beneficial to plant growth when used as a soil amendment; compost is largely decomposed organic matter and is in the process of humification.

The 2nd draft of the EU working document on Biological Treatment of Biowaste (2001), defined compost as 'the stable, sanitised and humus-like material rich in organic matter and free from offensive odours resulting from the composting process of separately collected biowaste, complying with listed environmental quality clauses given in Annex III of the document.

This seems to suggest that material not meeting required quality standards could not be defined as compost. For this material a stabilised biowaste category is included.

Our preferred working definition for compost is simply 'the solid residue resulting from a composting process'.

Note that this definition excludes any horticultural growing media that has not passed through a composting process. The regrettable use of the term 'compost' to describe all horticultural container-growing media is common in English vernacular. It continues to cause considerable confusion.

4.3. Stability

There is no universally accepted definition of compost stability.

Leege and Thompson (1997) offer two definitions for stability: 1. A *stage* in the decomposition of organic matter during composting, and a function of biological activity; 2. The *level* of biological activity in a moist, warm, and aerated biomass sample'.

Bernal *et al*. (1998) related stability to compost microbial activity.

Stentiford (2000) described stability as '…the actual point reached in the biodegradation process; the degree of decomposition, that is, the extent to which the composting reaction has advanced'.

The UK Composting Association (2001) defined stability as 'the degree of biological decomposition that composting feedstocks have achieved'.

Hue and Liu (1995) related stability to microbial activity and hence the potential for unpleasant odour generation.

McAdams and White (1996) proposed a theoretical stability definition as 'the point where readily degradable substrate is diminished so that its decomposition rate does not control the overall rate of decomposition'.

The Californian Compost Quality Council (2001) defines stability as 'a stage or state of organic matter decomposition during composting which is related to the type of organic compounds remaining and the resultant biological activity in the material'.

Brewer and Sullivan (2001) related stability directly to microbial activity.

Butler *et al*. (2001) defined stability as the level of activity of the microbial biomass.

Iannotti *et al*. (1993) defined stability as the degree to which composts have been decomposed.

Iannotti *et al*. (1994) defined stability as 'the degree to which the biodegradable fraction in solid wastes has been diminished during composting.

Haug (1986) defined stability as 'the point at which the rate of oxygen consumption is reduced so that anaerobic or odorous conditions are not produced to the extent that they cause problems with storage and end use of the product'.

Lasaridi and Stentiford (1999) defined stability as 'the extent to which readily biodegradable organic matter has been consumed'.

Eggen and Vethe (2001) defined stability as the degree of microbial activity and potential for producing volatile malodorous components'.

The 2nd draft of the EU working document on Biological Treatment of Biowaste (CEC 2001), did not use the terms stability or maturity, but defined stabilisation as 'the reduction of the decomposition properties of biowaste to such an extent that offensive odours are minimised and that either the Respiration Activity after four days (AT_4) is below 10 mg O_2/g dm or the Dynamic Respiration Index is below 1000 mg O_2/kg VS/h'. Material with these properties is virtually inert and suitable for placement in a landfill.

Bragg (2002) proposed that from the standpoint of commercial compost based products which are formed by blending with other nutrients to produced a balanced product then the stability resulting from any additions was an important consideration.

In all, our literature search found 49 references offering uncited definitions for compost stability, using 12 parameters either singly or in combination:

Stability Parameter	Score (%)
Biological activity or respiration	35
Degree or stage of decomposition	20
Malodours	14
Nitrogen consumption	8
Nutrient availability	6
Phytotoxicity	4
Available carbon or other energy sources	2
Colour	2
Heavy metal dissolution	2
Water content	2
Environmental health risks	2
Texture	2

Biological activity is the most cited parameter, because stability increases as biological activity decreases. Degree, point, stage or state of decomposition is the second most popular definition, but this is highly dependent on the original feedstock and the process used and, therefore, unsuitable as a definition of stability for describing or assessing the quality of composts of unknown origin. Malodour is also frequently cited but is subjective and not really suitable as a reference parameter. The other cited parameters are indirect or the result of the failure of the authors to differentiate stability and maturity.

Thus stability is usually defined either as a *stage* in the composting process, **or** as a *rate* of activity. The stage or degree of decomposition is a difficult property to measure accurately in composts of unknown origin. As a stand alone measurement, the rate of activity is not universally applicable to all feedstocks or all composting processes as an accurate determination of the stage or state of decomposition. There is no universal scale of stage of the decomposition process. We need a definition for stability, which is universally applicable to all composts and particularly to composts of undeclared feedstocks or processes. Stability is almost always measured and reported as a *rate* of activity measured as O_2 uptake, CO_2 evolution or self-heating, although process researchers and operators often use this to predict the decomposition stage because they know the process and feedstock and have previous data enabling them to relate activity to a time scale.

Units would be required. It was agreed with the peer review group that the rate of CO_2 evolution be reported on a mass of organic matter basis. The organic matter (volatile solids) is determined by loss on ignition.

Our working definition for compost stability is 'the rate of biological activity'. This meets all our definition criteria.

As a stand-alone property, stability is of most importance to those concerned with composting operations and composting research who need to monitor the process. Stability is important for differentiation of material fit for landfilling and for those producing soil improvers. Those concerned with quality testing of the product and end users of the compost should be more concerned with maturity, which combines stability with other parameters.

4.4. Maturity

Maturity implies improved qualities resulting from 'ageing' or 'curing' of a product. In this sense the word maturity is the same as when used to describe other products that improve with age, e.g. malt whisky, fine wine or cheese. In other words maturity is a measure of a product's readiness for use. Initially 'fitness for purpose' was considered as a definition, but fitness is not necessarily time related and can be reliant on factors unrelated to an ageing or curing process. The factors used to define maturity should relate directly to fitness for purpose but should be factors, which may change during ageing. In practice, maturity is usually assessed by a combination of factors. An end product can be "mature" when it is ready for its intended use. It must be clearly understood that this does not make the product fit for all possible uses.

Leege and Thompson (1997) offered two definitions for compost maturity: '1. An organo-chemical *condition* of the compost, which indicates the presence or lack of organic phytotoxic chemicals in generally stable to very stable compost; 2. the *degree* to which a biomass sample is free of organic phytotoxic substances that can cause delayed seed germination or inhibit plant growth when used as directed ….no longer consumes nitrogen or oxygen ….is no longer highly active and will not cause depletion of nitrogen in the soil with which it is mixed'. They also offered three definitions for mature compost: '1. a generally stable to very stable compost with little or no phytotoxic chemicals present; 2. any organic material which has undergone a biological decomposition process complying with the Process to Further Reduce Pathogens …. and is in the process of humification; 3. the point at which a compost will not act detrimentally when used as a soil amendment'.

The UK Composting Association (2001) define maturity simply as 'the degree to which a compost has matured', but define mature compost as 'compost that does not have a negative affect on seed germination or plant growth'.

Bernal *et al*. (1998) described maturity as implying 'a stable organic matter content and the absence of phytotoxic compounds and plant or animal pathogens.

Iannotti *et al*. (1993) described maturity as being associated with plant growth potential or phytotoxicity.

Mathur *et al*. (1993) listed the adverse features of immature composts as foul odours, fire risk due to emission of flammable gases, environmental pollution, nuisance insects, bursting of bags and phytotoxicity.

Butler *et al*. (2001) defined maturity as 'the degree of humification of the material', but qualified this by describing the effects this can have on nutrient availability and plant growth.

Chen and Inbar (1992) stated that 'compost maturity should be defined as the degree of decomposition of organic matter during composting' but also stated that 'any definition of maturity must be based on the potential utilisation of the compost'.

Chen and Inbar (1993) defined maturity as ' the condition where compost poses no adverse effects on plants and is determined using bioassays'.

Brinton (2000) defined maturity as 'the degree of completeness of composting' and stated that it must be assessed by measuring two or more parameters of compost in addition to C:N ratio.

Hue and Liu (1995) described maturity as associated with plant growth and phytotoxicity as distinguished from stability, which is related to microbial activity.

The California Compost Quality Council (2001) defined maturity as 'the degree or level of completeness of composting' and further stated that 'maturity is not described by a single property and therefore is best assessed by measuring two or more parameters describing stability and the impact on plant development'.

In all, our literature search found 44 references offering uncited definitions for compost maturity, using 7 parameters either singly or in combination:

Maturity Parameter	Score (%)
Adverse effects on plants (including nutrient imbalances and C:N)	45
Degree of decomposition	23
Biological activity/respiration	11
Agricultural benefits (texture, water retention, nutrient release, pathogen suppression)	9
Odour	9
Pathogens	4
Colour	2

Adverse effect on plants, including phytotoxicity, nitrogen immobilisation and nutrient imbalance is the most cited parameter at 45%. Stability, combining biological activity and degree of decomposition is next at 34%. Odour is again cited but not suitable as a parameter for standardised testing. Agricultural benefits and colour are indirect qualities not necessarily related to an ageing or curing process. Pathogen reduction can be an important function of the composting process and presence of pathogens can certainly affect fitness for purpose. However sanitisation should have occurred during the early heating stages, so it is debatable whether it should be considered as a maturity parameter.

It is clear that maturity is usually defined in terms of stability and adverse effects on plants, but different maturity parameters and criteria may be applicable to different end uses.

For our purposes we have defined compost maturity in its simplest and most universally applicable form as 'readiness for use'. This corresponds to the definition proposed by Itavaara *et al* (1998). If the intended purpose is known this can be expanded in terms of parameters important to that purpose. The maturity of composts intended for agricultural or horticultural use is defined as 'the condition of composts which poses no adverse effects to horticultural or agricultural crops'. If the compost is intended for landfilling as mechanically and biologically treated waste, then the definition might be 'the condition of composts which pose negligible risk of methane production when landfilled'.

5. Evaluation and Selection of Parameters

Following the search and evaluation of working definitions, the literature evaluation was expanded in order to identify methods which would be suitable as base tests for stability and maturity. This was not assisted by the lack of differentiation between stability and maturity in many of the reference publications. The relevant aspects of the literature search were considered and are discussed individually in Annex E.

5.1. Nitrogen immobilisation

This is considered to be a serious problem with many composted wastes. It is debatable whether it is actually a maturity issue since it is chiefly dependent on the original feedstock. Never the less it forms part of the compost quality standards of several countries and states so must be considered.

The literature search produced many examples of attempts to improve on the ratio of total C to total N, which is now widely accepted as too simplistic for a single end-product test. Readily available C to readily available N would be a much better ratio but there is no consensus on methods to determine these fractions. The ratio of lignin to cellulose might be a useful way of assessing C availability but these are not easy compounds to measure accurately. Ligno-cellulose complexes would be particularly problematic.

Prasad (1997) evaluated three 'nitrogen fixation' tests: The Nitrogen Drawdown Index (NDI) developed by Handreck (1992) taking a few days; the N-immobilisation test developed by Zottl (1981) taking two weeks; the N-retention test developed by Prasad (1997) taking several weeks. The author concluded that short-term N fixation tests were unlikely to be reliable on a wide range of materials.

The NDI, adopted in the Australian standards, has been criticised for being too variable.

Some very rapid non-destructive techniques including NIRS, FT-IR and thermal analysis techniques have shown great potential for resolving this problem. Work at the ADAS Laboratories has already established that NIRS spectra of composts can be calibrated directly against bioassay data on growth and nitrogen uptake. This could almost certainly be combined with 'wet chemistry' data on stability and bioassay data on maturity in a single calibration. Thus, once calibrated, a single NIRS scan taking approximately one minute could supply all necessary information on stability, temporary phytotoxicity due to immaturity, and the potential for nitrogen immobilisation (plus other valuable information on major components including total-N, organic-N, ammonium-N, organic carbon, humic substances, cellulose, lignin, etc). However, such a project would need NIRS scanning plus chemical and bioassay data of several hundred composts from as wide a range of stages, feedstocks and processes as the calibration would be required to test. This would be a large and demanding project but, in our opinion, worthwhile.

Near Infra Red Spectroscopy (NIRS) is a rapid non-destructive technique that compares the spectrum of the sample with that of a library of spectra of samples of known composition. It is accepted as a standard method for forage evaluation, successfully predicting several factors simultaneously. The potential for prediction of manure quality and composted material quality has been indicated in literature, e.g. Sharma *et al.* (2000) and by work undertaken by ADAS laboratories.

In order to exploit the potential of the technique it is necessary to agree calibration methods, to analyse samples by the agreed methods and present these samples to the NIRS, to evaluate the resulting prediction equations and then validate them. The robustness of the NIRS prediction grows with the number of samples analysed.

The consequences of development of successful NIRS prediction equations are:

i) a number of quality parameters of composted materials e.g. stability, organic matter, dry matter nitrogen can all be predicted simultaneously.

ii) the main source of variability (sampling) can be addressed by taking several samples of composted material and analysing all these at low cost to give a much better overall picture of quality.

iii) more sophisticated properties such as potentially available nitrogen can be investigated.

5.2. Discussion

Many parameters have been proposed for assessing compost stability and maturity although the distinction between these two properties has often been unclear. Almost all of these other parameters have been indirect, i.e. they could not be used to assess stability or maturity directly as we have defined them but might have potential to indicate or predict those properties. These include:

- Acidity
- Electrical conductivity
- CEC,
- Colour
- Humic compounds and ratios
- Enzymes and ratios
- Lipid solubilities and ratios
- Nitrogen compounds and ratios
- Nitrogen fraction solubilities and ratios
- Volatile organic acids

Indirect parameters all need to be referenced to direct parameters. If they cannot be referenced to direct parameters that describe the properties as defined, then they are unsuitable even as indirect predictive parameters.

The criteria for selection of a suitable means of determining stability were identified as:

- Directness
- Universal applicability
- Ease of measurement
- Cost of measurement
- Speed of results

Aerobic respiration rate is the only parameter that directly measures biological activity in all composts at relatively low cost and is easily and quickly measured. It has also gained widespread acceptance. As such, aerobic respiration rate is the only parameter that meets all our criteria for assessing compost stability as we have defined it.

Stability and phytotoxicity are parameters universally important for assessing the readiness for use of composts destined for horticulture or agriculture and they can be directly measured. As such, stability (by respirometry) and phytotoxicity (by bioassay) were selected as universal maturity parameters. This does not exclude other direct parameters that may be relevant for specific end-uses.

Nitrogen immobilisation potential was carefully considered as a maturity parameter because it is often used for this purpose and forms part of the Canadian CCME (1999) and California Compost Quality Council (2001) compost standards in the form of C:N ratio. The potential for nitrogen immobilisation is certainly a factor which affects fitness for purpose, but the literature review indicates that the ratio of organic C to total N is over simplistic and unreliable for all compost types. It is too dependent on the nature of the initial feedstock. Carbon and nitrogen are essential for microbial metabolism and proliferation, but it is the availability of C and of N for microbial nutrition that is critical. If there is a relative excess of available-C, the microbial biomass will consume available-N to make cell protein; if there is a relative excess of available-N it is likely to be lost as ammonia gas.

The presence of pathogens is another factor affecting fitness for purpose but not really a maturity issue, because sanitisation should occur at earlier stages of the composting process.

6. Evaluation of Analytical Techniques and Test Methods

6.1. Stability

Methods of test are to be examined and proposed for materials as sampled. Amendments or blends with other materials may affect stability.

Aerobic respiration rate was previously selected as the most suitable parameter to assess aerobic biological activity and hence stability. In aerobic conditions, one carbon atom derived from catabolism is attached to two oxygen atoms to form carbon dioxide, releasing energy, including heat, in the process. Therefore, respiration can be measured several ways, broadly split into

- carbon dioxide evolution
- oxygen consumption
- self-heating.

If all three techniques were accurate measures of aerobic respiration then the graphs of respiration plotted against time would be identical. This is not the case. Possible explanations for this are discussed below.

- Self-heating is widely used in Europe and North America, using the well established Dewar flask (Rottegrad) method. This is a convenient method for routine operations but it is the least direct of the three main respirometry techniques. It actually measures temperature rises due to all exothermic biological and chemical activity, so is not strictly a true measure of respiration because many biological and chemical reactions not connected to respiration are exothermic.
- Oxygen uptake and carbon dioxide evolution are more direct and have been described as opposite sides of the same equation under aerobic conditions. However, oxygen may be consumed by non-biological oxidation of organic matter and minerals, so oxygen uptake is really a measure of biological/chemical oxygen demand (BOD).
- Carbon dioxide evolution is the most direct technique because it measures carbon derived directly from the compost being tested. Thus CO_2 evolution directly correlates to aerobic respiration and, of the three techniques considered, is the truest measure of respiration and hence aerobic biological activity.

6.2. Summary of respirometric techniques

- **Dewar self-heating** measures total exothermic biological and chemical activity. It is a convenient method of testing compost for end-use quality but is relatively indirect and slow and does not lend itself to high precision measurements. It has been described as unsuitable for the purpose of monitoring composting processes during the early stages due to poor discrimination. However it may be superior to oxygen uptake or CO_2 evolution for process monitoring during late stages of composting because it is sensitive to non-respiratory exothermic activity which may continue to decline while O_2 uptake and CO_2 evolution show little rate change. Some reports suggest the self-heating test may suffer from poor inter-laboratory ruggedness, because it is apparently affected by small changes in conditions. However, it has scored well in German interlaboratory trials (Bundesgutegemeinschaft Kompost 1994). Weppen (2002) demonstrated that the lack of precision in self-heating resulted from the location of the temperature probes not being defined precisely. Becker (1998) emphasized that moisture content of the compost should be carefully adjusted to 50% of the WHC prior to testing. Brinton (2004) has recently emphasized that the heat-loss factors of Dewar flasks must be carefully monitored, and may change over time andbetween flask manufacturers. If the thermal characteristics and size of flask, location of the probes and proper moisture level for the test were defined, the precision of the method would probably be acceptable.

- **O_2 uptake** measures biological/chemical oxygen demand. It can be very precise and convenient but requires the use of instrumentation, which needs frequent maintenance and calibration. Several techniques have been described for measuring oxygen consumption in composts and some automated commercial systems are available. Operating principles including electrolytic techniques, oxygen electrodes and pressure reduction have been employed. Some techniques measure gaseous oxygen

directly while others measure dissolved oxygen in water suspensions. Respiring micro-organisms might be expected to behave differently in water suspensions than in moist compost but this does not appear to be a problem.

- **CO_2 evolution** is the most direct and accurate measure of respiration and aerobic biological activity. Alkali traps are widely used as a standard technique for measuring carbon dioxide evolution and are well proven. The use of CO_2 gas detection tubes or CO_2 sensitive colorimetric indicators are related, alternative approaches. Alkali trap methods can be made very sensitive by adjusting incubation times and reagent strengths and volumes and this may compensate for relatively low CO_2 rate changes during maturation. The technique is also very rugged and inexpensive to set up, because it is largely insensitive to the design of the apparatus used and the measurements require only very basic equipment and chemicals.

If stability is defined as aerobic biological activity and aerobic respiration rate is selected as the parameter to assess it, then there is little argument that CO_2 evolution is the most suitable technique. Taking into account the observations made by researchers it is concluded that CO_2 evolution provides a rugged and robust method, which is accurate and precise over a wide range of compost materials. The approach also provides results with good sensitivity and can be undertaken without recourse to proprietary equipment and at relatively low cost by technicians who do not require high skill levels.

The advantages are:

- It is the most direct indicator of biological activity
- Universally applicable
- Ruggedness
- Precision
- Sensitivity
- Simplicity of equipment
- It is the easiest to use
- Low costs of measurement

Self-heating and O_2 uptake have their advantages, especially for process monitoring. Oxygen sensors, as used in continuous monitoring, are inexpensive and easily obtained. A combination of O_2 uptake in the early stages and self-heating in later stages might provide the best compromise for monitoring the entire composting process, even though the data produced by both techniques is not directly comparable. There is insufficient evidence in the literature to evaluate CO_2 evolution as a tool for process monitoring at all stages of composting.

Process monitoring is unlikely to need standardisation to the same extent as product testing. Researchers need the freedom to continually improve and develop new techniques which standardisation would inhibit.

6.3. Recommendation of stability testing laboratory techniques

For development of a universal standard method for quality testing of finished composts we recommend CO_2 evolution. There are advantages in being able to define stability in terms of a definitive and measurable parameter, which we have selected as aerobic biological activity. Similarly it is advantageous to be able accurately to measure this as aerobic respiration rate using a standard technique and method. CO_2 evolution methods are very rugged and likely to be superior to other techniques in terms of intra-laboratory repeatability and inter-laboratory reproducibility.

The ability to detect changes in activity rate are unimportant for product testing because there is no need to relate this back to the stage of composting. The activity rate itself is sufficient as a measurement of stability in end-products. Therefore we consider the ability of continuous automated measurements to detect changes in activity rate to be superfluous to the main purpose and application of the test.

6.4. Test method for CO2 evolution

British Standard ISO 14855:1999 (British Standards Institution 1999) describes a dynamic test apparatus in which carbon dioxide free air is continuously passed through aerated test compost mixture in the evaluation of biodegradability of plastic materials. Measurement of carbon dioxide concentration and flow is optional by alkali trap or by instrumentation.

Switzenbaum, *et al* (2002) evaluated carbon dioxide tests for biosolids stability. The tests used pre-incubation periods of 3 days at 20 ^{o}C. Prior to incubation the sample was readjusted to 50%DS. The incubation was a static method at 35ºC, and CO_2 was absorbed in NaOH and titrated. The final result was the average of determinations taken after 2, 3, 4 and 5 days. They also reported that SOUR test is applicable to aerobic liquid sewage sludge with 2% solids or less that has not been deprived of oxygen for more than two hours prior to the test. Thus, the SOUR test was not considered appropriate for dewatered sewage sludge or compost.

Various versions and adaptations of CO_2 evolution techniques have been reported in recent years by Crawshaw *et al*. (1980), Iannotti *et al*. (1994), Leege and Thompson (1997), Hue and Liu (1995), Popp and Fischer (1997), Rajbanshi *et al*. (1998), Brewer and Sullivan (2001) and the Californian Compost Quality Council (2001).

Crawshaw *et al*. (1980) wrapped samples loosely in muslin and transferred to wide neck jars containing 25ml of 20% w/v KOH solution. The sample was held above the solution on upturned plastic cups and the jars sealed with synthetic rubber stoppers allowing entry of CO_2 free air via a tube packed with soda lime. Jars were maintained at 20^{0}C for 7 days. Evolved CO_2 was measured by direct pH titration of the carbonate after neutralisation of the excess hydroxide. An important feature of this method is that it incorporated a means of introducing CO2 free air into the vessel to replace depleted oxygen and maintain atmospheric pressure.

Iannotti *et al*. (1994) sealed screened compost samples in a 3.8 litre vessel with 1.0 M NaOH solution to absorb CO_2. After 24, 48 and 72 hours incubation at 25^{0}C, absorbed CO_2 was precipitated with barium chloride as barium carbonate. Remaining base was titrated with 0.5 M HCl. The quantity of acid required for neutralisation was used to calculate CO_2-C evolved per gram dry weight of compost per 24 hour. The total amount of CO_2 evolved during 3 days was determined.

Hue and Liu (1995) moistened duplicate 10g samples of finely screened (<4 mm) compost to approximately 60% water content. These were placed in 500 ml Erlenmeyer flasks and incubated for 3 days at room temperature (24 $\pm$ 1^{0}C). After passing through a 10 cm column of CaO powder to strip out initial CO_2, pressurised air was metered into the incubating flasks at approximately 2.5 l/hr. The outgoing air from incubation flasks was bubbled through 20 ml of 1 M NaOH. Each day the spent NaOH was titrated potentiometrically to pH 8.30 with 1.0M HCl, then with 0.10 M HCl to pH 3.70. The amount of CO_2 evolved from compost during the incubation was calculated from the volume of 0.10 M HCl required to bring the pH from 8.30 to 3.70.

In the method of Leege and Thompson (1997) the total solids and biodegradable volatile solids content of the compost are determined separately. Samples are pre-incubated at room temperature for 3 days, then moisture content is readjusted to 50%. This ensures that the micro-organisms in the compost are acclimatised to the mesophilic environment. Pre-incubated sample (25 $\pm$ 2g, weighed to nearest 0.01g) is placed in an incubation vessel and 20 ml of 1 M NaOH (30 ml initially for unstable composts), contained in a 50ml beaker, is added to the vessel. The vessel is sealed and incubated at 37^{0}C. A blank, containing NaOH alone, is also incubated. The amount of CO_2 absorbed by each NaOH trap is determined daily over a 4 day period by a back titration of the residual with normalised HCl following addition of 0.5 M barium chloride. The CO_2 evolution rate is calculated as mg CO_2-C g^{-1} VS-C d^{-1} (VS-C = volatile solids). No data is available on the precision and bias of the method.

Popp and Fischer (1997) measured pressure-drop due to microbial oxygen consumption and absorbing CO_2 released by micro-organisms with NaOH. Compost (<10mm) was brought to a water-tension of ca. 30 hPa and filled in a perforated pot on the basis of 250 g dry matter. A maximum distance between the innermost of the compost and the surrounding gas atmosphere was kept to 2.5 cm to give advantageous conditions for

gas exchange. The compost was placed in a pressure tight vessel with 10g NaOH and 10 ml H_2SO_4 (30%) and incubated at 38^0C. The NaOH captured CO_2 under the compost and the H_2SO_4 captured ammonia above to avoid pressure changes not due to carbon dioxide/oxygen. Before incubation the vessel was enriched with oxygen to partial pressure 890 kPa. Pressure decrease was logged for 12 hours.

Rajbanshi *et al*. (1998) measured CO_2 evolution rate using beakers containing 5g moist sample and a NaOH trap (0.2-0.5 M; 30 ml) in an airtight bottle incubated at 30^0C for 24h. Moisture content of composts was pre-adjusted to 600 g kg^{-1}. The amount of trapped CO_2 was determined by back-titration of NaOH with HCl. The amount of CO_2 present in the sample before incubation was corrected by using blanks.

Brewer and Sullivan (2001) described two CO_2 evolution methods. In the first method, they investigated using industrial safety gas detection tubes (Draeger CO_2 Detection Tubes 0.1-6 vol%) to measure the CO_2 content in the headspace above an enclosed sample of compost. The test gave results after a four-hour incubation period. A sample weight of 500 g compost was placed in a 3.8L incubation vessel. The CO_2 detection tubes changed colour upon exposure to CO_2 and were calibrated as % carbon dioxide. In the second method they sprayed samples to renew moisture concentration to approximately 500 g $kg^{-1,}$ which were then held at 37^0C for 36 h in unsealed bags to promote microbial activity. 25 g was placed in a 0.5 L glass canning jar with an airtight lid and 20 (or 30) ml of 0.1 M NaOH was placed in the jar before sealing. The larger volume was used when respiration rates were high. The jars were incubated at 37^0C in a water bath. Carbon dioxide was trapped for two periods; 0-24 h and 24-48 h. Jars were opened briefly to exchange vials at 24 h. Triplicates of each composite compost sample were incubated. Each incubation included a blank comprising a sealed jar with NaOH and no compost.

The California Compost Quality Council (2001) describes 2 methods: (i) *Carbon Dioxide Evolution Rate*. Remove large pieces of inerts. Adjust samples to approximately 50% moisture and pre-incubate in bags in a chamber at 37^0C and 100% humidity for 3 days. Transfer the sample to a sealed container with appropriate monitoring equipment to allow daily measurement of CO_2 evolved for a 4-day period. (ii) *Respiration Rate*. Similar to (i), removal of large particles (>4mm) and inerts and mixing with saturated sand (4:1 ratio) to adjust moisture and ensure uniform release of CO_2. Three day incubation at 37^0C and addition of Hoagland's nutrient solution and mesophillic microbial inoculation to remove any biological limitation. After 3 days sub-samples are aerated and then incubated for 1 hour at 37^0C and the resulting CO_2 concentration in the air space of the container is determined. The results are calculated as CO_2 evolution per unit of volatile solids. Compost samples that have a moisture content below 30-35% may be biologically dormant; thus respiration rates will be artificially low without additional water. Therefore, a standard adjusted moisture content must be applied to all samples. Previously dried or cold stored samples may support uncharacteristically high biological activity (respiration) following moisture adjustment or increased temperature. Therefore a pre-incubation or equilibration of each sample must be employed to assure accurate measurement of respiration activity.

It is important to ensure that the results of the procedures lead to consistent and scientifically sound interpretation. There is some variation in the units used to express CO_2 evolution and in the values used to categorise compost stability. However each is measuring a weight of CO_2 per weight of sample per unit of time and these should be inter-convertible. It is recommended that mg CO_2 /kg volatile solids/hr is used as a standard unit. The recommendation made by Hue and Liu, that 120 mg CO_2/kg/hr should be the cut off mark below which composts can be considered stable, requires further validation but provides a good starting point.

The approach of measuring CO_2 evolution by alkali trap provides a good basis for a standard method for the assessment of compost stability. A number of the variations were used by different researchers. Factors include the following:

- choice of alkali, i.e. NaOH or KOH;
- sealed vessel or equilibration of oxygen and pressure;
- direct titration of carbonate or precipitation with barium chloride, followed by back titration;
- pH range of the titre, e.g. from 8.3 to 3.7 for sodium carbonate;
- incubation temperature, e.g. ambient, 20^oC, 24^oC, 25^oC, 37^oC, etc;
- incubation time e.g. 4 h, 12 h, 24 h, 48 h, 36 h, 72 h, etc;
- single titration or titration of aliquots of the solution.

There are a number of methods such as AT_4 and SOUR which have been developed, where factors such as temperature, incubation time, etc, have been examined. The validation and acceptance of these tests, and their outputs, has taken many years and, in the case of AT_4, has seen a large financial investment in evaluation and development. That method utilises instrumentation in the form of an automated respirometer. The AT_4 method's general principles are fundamentally the same as our alkali trap method with the exception of the wet chemistry titration, (Binner E, Zach A. 1999). The four day test period can be adopted.

Commercial test kits are available for routine testing of composts for stability and maturity. One maturity test kit (Solvita™) measures carbon dioxide and ammonia evolution in sealed jars using simple colour indicator strips. Another, called Oxitop, uses the pressure drop principle. Commercial test kits are convenient and have been shown to be adequate for routine uses and as field kits.

7. Evaluation of Phytotoxicity Techniques and Test Methods

Many methods for assessing stability or maturity of composts by using plants are reported in the literature. Generally, most authors do not differentiate between stability and maturity. Plant tests are widely viewed as providing a direct measure of stability or maturity, because composts can be considered "ready" when they are fit-for-purpose and this latter objective nearly always involves use with plants.

Much work on this subject includes use of the word phytotoxicity (toxicity to plants). It is important to differentiate between persistent phytotoxicity and temporary effects of compost instability or immaturity. Instability or immaturity may produce phytotoxic effects. However, phytotoxicity may occur with stable or mature composts due to substances which are not removed in the composting process, (e.g. heavy metals, persistent herbicides). Hence, phytotoxicity testing is not always the same as testing for stability or maturity. Most papers reviewed do not differentiate between the two and plant tests for stability/maturity are generally considered to be tests for phytotoxicity.

Plant tests used in research and in quality standards can be divided into four broad categories:

- germination tests (sometimes including root assessments)
- growth tests (assessment of top-growth and sometimes root mass)
- combinations of germination and growth
- other biological methods.

This grouping is presented partly for convenience, because the boundaries between the different categories can overlap. For example, the time period after which germination is assessed in some methods might be considered as growth in others. In the following review, work is discussed under these separate headings where this is appropriate, to simplify the presentation.

7.1. Germination tests

One of the most significant germination tests is that reported by Zucconi *et al.* (1981), and Zucconi *et al.* (1985), because many later tests are developed from this. These workers devised a test to measure the phytotoxicity of immature compost. The test was designed to be simple, rapid and requiring only basic equipment. Cress (*Lepidium sativum*) was the selected plant, because of its rapid growth and response. A water extract of the test compost was prepared by pressure, (15 minutes at 250 atmos., in the 1981 paper, 5 minutes at 2.5 atmos/cm^2 in the 1985 paper), which was sterilised by millipore filtration. The 1985 paper appears to suggest that the moisture content of the compost was first adjusted to 60% and that a number of concentrations of the extract were prepared, (100, 30, 10 and 3%, with 30 and 10% considered the most indicative). One ml of extract was applied to filter paper in a petri dish and the seeds sown. Seeds were incubated for 24 hours at 27 deg. C, in the dark, then growth stopped with 50% alcohol. A 24-hour growth period was found to reduce variability and accelerate the test. A germination index (GI) was determined by multiplying germination and root growth, (both as % values, the roots as a % of the control) and dividing by 100. The index was considered able to account for both low toxicity - which affects root growth - and high toxicity, which affects germination. The authors stressed, however, that a single test was unlikely to cover all aspects of phytotoxicity.

Lasaridi and Stentiford (1998) used the GI for cress seeds (*Lepidium sativum*) in a comparative study of respirometric techniques for evaluating MSW compost stability. GI was measured using a method based on that of Zucconi *et al.* (1985), using a 1:10 (dry weight /volume) compost water extract. This employed a relatively simple extraction method, not the pressure extraction method used by Zucconi.

The GI was found to be highly significantly correlated with compost age, oxygen uptake measurements and significantly correlated with maximum temperature. However, GI was less sensitive as a measure of stability than the respirometric measurements, especially in the earlier stages of composting, remaining below 15% for more than three weeks. The compost also remained more phytotoxic than the control samples, as measured by GI, throughout the whole composting period, (71-81 days).

In discussing this work, Lasaridi and Stentiford (1998) interpreted the increase in GI with composting time as indicating metabolism of phytotoxic substances. They concluded that GI is more suitable for assessing compost stability during maturation, when respirometric parameters tend to stabilise and lose sensitivity, rather than during the active, thermophilic phase. The authors also concluded that the connection between phytotoxicity, as measured by GI, and the effect of composts on plants, is still unproven.

Fang and Wong (1999) used cress seed (*Lepidium sativum*) germination to determine maturity, when studying the effects of lime amendment on sewage sludge composting. GI was determined, based on the method of Zucconi *et al.* (1981), with a compost:water extraction ratio of 1:2 (w/v). The authors considered that the GI reflected maturation well, increasing from about 12% at the beginning of composting to 80% at between 49 and 100 days. Maturity as indicated by GI agreed well with the $C/N_{organic}$ ratio. Ammonia and low molecular weight organic acids were two phytotoxic substances proposed and plant growth increased as these disappeared.

Germination of a range of weed seed species was studied by Ozores-Hampton *et al.* (1999), using MSW and biosolids composts of different maturities. GI was measured (Zucconi *et al.* 1981), using a pre-determined optimum compost:water extraction ratio of 20g (dry weight):50 ml. Compost maturity affected final germination, root length, GI and mean days to germination (MDG) for the four species studied. However, percent germination was reduced more by 8-week old compost than 3-day and 4-week old compost. Root length and therefore GI also produced inconsistent results over this period. The authors considered that the time of the phytotoxic stage can vary among MSW composts, being affected by compost substrate, composting methods and pile management. The compost used retained high levels of volatile fatty acids after eight weeks.

Wu *et al.* (2000) found a modified Zucconi *et al.* (1981) method, with tomato seed (*Lycopersicon esculentum*) and 1:10 compost water extraction, was successful in monitoring stabilisation and maturation in most cases. However, compost from one site produced low germination, despite low CO_2 evolution. The authors concluded that CO_2 evolution tested stability, whereas phytotoxicity based on germination tested maturity and that low respiration rates did not necessarily reflect low phytotoxicity.

In a further, related study, Wu and Ma (2001) discuss some of the substances in immature composts, which can affect germination. A variety of organic compounds, including short- and long-chain fatty acids and phenolic acids have been proposed. The authors report that Manios *et al.* (1987) found a combination of volatile acids in an immature compost extract was phytotoxic to lettuce seedlings (*Lactuca sativa*) at concentrations far below the minimum levels at which individual acids, such as formic, acetic, benzoic, salicylic and tannic acids, exerted any harmful effect. In other words, the effects of these phytotoxic substances was additive.

Sanchez-Monedero *et al.* (2001) compared the germination test of Zucconi *et al.* (1985) with the water-soluble organic carbon to water soluble organic nitrogen ratio (C_{OW}/N_{OW}) and the water soluble organic carbon to total organic nitrogen ratio (C_{OW}/N_{OT}) of composts, both of which were considered to be suitable indices of stabilisation. Departing from Zucconi *et al.* (1985), a 1:10 (w:v) aqueous extract was used. The authors found that the germination index was in good agreement with C_{OW}/N_{OW} and C_{OW}/N_{OT} and maturity of the composts. Germination inhibition by uncomposted wastes was thought to be due to low molecular weight organic acids, (acetic, propionic and butyric).

Brewer and Sullivan (2001) investigated germination as a maturity indicator for composted green waste. Seeds of rye (*Secale cereale*), barley (*Hordeum vulgare*) and zucchini (*Cucubita pepo*) were sown in pots containing a 1:1 mixture of compost and potting soil. Germination was recorded on day 5. The method did not indicate compost maturity. The authors suggested that degradation of phytotoxic organic acids occurred rapidly while handling the composts.

A draft of a proposed Belgian method has been considered, (Ministerie van Middenstand en Landbouw; Cooper, personal communication). In this, the electrical conductivity of the compost is determined. The conductivity is then adjusted by mixing with high-purity sand. Specific proportions of sand (and water) are stated in the method, according to the initial conductivity of the compost. Germination of cress (*Lepidium sativum*) is then assessed after ten day's growth in covered 1200 cm^3 plastic boxes and compared to a pure sand control.

A proposed Irish method has also been examined., (M. Prasad, Bord na Mona Horticulture; Evans, personal communication). The test material, after adjustment to a target moisture content, is placed in petri dishes and seed of cress (*Lepidium sativum*), mustard (presumably *Sinapis alba*) or radish (*Raphanus sativus*) are sown into shallow wells of the pure test material. Germination, length of roots and (optionally) fresh weights of tops are recorded after four days of growth (with lids on) and compared to controls. The control should be conducted with a similar material to the test substance, where possible, or a non-fertilised or fertilised peat with electrical conductivity below 500µS/cm.

7.2. Growth tests

7.2.1 The Composting Association CATM/01/2000 Test

The UK's Composting Association CATM/01/2000 test (Composting Association, 2000) specifies a reduction in plant growth of 20% compared to the control, as the maximum acceptable adverse effect in its growth test. This is measured as germination after 14 and 28 days and fresh weight of plant tops after 28 days, for tomato plants (*Lycopersicum esculentum*) grown in a compost/peat mix. The ADAS Laboratories conduct the test by adjusting the electrical conductivity of the compost to a normal level for peat-based growing media (300 - 500 µS/cm), using sphagnum peat. In operation, ADAS have found the test to be a reasonable indicator of compost readiness-for-use, but with certain major limitations. Firstly, the test gives only minor consideration to the early stages of growth (within the first 7 days). Many other tests reviewed suggest this is the most important stage to assess. Secondly, the species grown is known to be very sensitive to residues of hormone herbicides (e.g. clopyralid) in growing media, but is considered not to be sufficiently sensitive to other adverse growing conditions, both physical and chemical. In the Composting Association tests run by ADAS, germination (measured at 14 and 28 days) is rarely reduced appreciably, compared to the controls and reductions in growth are usually only minor on test plants. This may partly be because 14 days is too long and some effects will have disappeared. It may also reflect the generally good quality of the samples tested, but the appearance of some of these samples would lead to the expectation of poorer growth than is often found, suggesting tomatoes are too robust. Tomatoes are also quite slow to grow, when raised from seed. If the objective is to develop short and medium duration growing tests, (maximum 14 days duration), the growth of tomatoes is too slow. In contrast, as a longer term test, specifically for residues of hormone herbicides, the test is considered satisfactory, with the proviso that the test is run for at least 42 days, levels of other major nutrients in addition to nitrogen are adjusted at the start and a larger growing volume per plant is used, (e.g. 4 plants in a 15 cm (6-inch) pot).

7.2.2. Combinations of germination and growth

Other procedures have been tested and proposed. These will help the development and understanding of limitations and advantages of growth tests.

Morel *et al.* (1985) proposed a pot test, growing two species (maize, *Zea mays* and beans - not specified) as a better test of phytotoxicity than can be obtained via analysis or respirometry. Plants were grown for 18 days in pots of pure compost, 90% compost + 10% peat and 75% compost + 25% peat; growth was compared to a 100% peat control. No thresholds were given of what constituted satisfactory growth.

Keeling *et al.* (1994) used fresh (unstable) refuse-derived compost in extended growth trials. Germination of several plant species after 22 days was inhibited by compost alone. However, in pot trials with ryegrass (*Lolium perenne*), a slow nutrient release effect was observed. After six month's growth, identical yields were obtained with unamended compost and 150 kg/m^3 compost in a sand-grit substrate.

Hauke *et al.* (1996) considers that not all plants used in growth tests indicate phytotoxicity sufficiently well. In growth tests with ornamentals (begonia, pelargoniums and petunia), ryegrass (*Lolium perenne*) and spring barley (*Hordeum vulgare*), ornamentals showed greater reductions in growth than ryegrass and barley, at compost addition rates of 50% and over.

Container-grown woody ornamentals were grown by Raymond *et al.* (1998) in 12 different, immature (non-aged) composts, prepared from various proportions of spent mushroom substrate, waxed corrugated cardboard and pulverised wood wastes. Despite the compost immaturity and the presence initially of high

levels of soluble salts, plant top dry weights exceeded those of the control growing medium. This was attributed to rapid leaching of soluble salts within days of planting.

Zucconi and Bertoldi (1986) discuss germination and growth tests and the differences in what they reveal. Germination tests provide an instant picture of phytotoxicity, whereas growing tests will be affected by continuing changes in the stability or maturity of the compost tested: there may be damaging effects on growth in the earlier stages, but beneficial effects later on, with different conclusions depending on the time of assessment.

Garcia *et al.* (1992) investigated barley (*Hordeum vulgare*) germination in petri dishes, using either acid-washed silica sand mixed with various fresh and composted urban wastes, or 1:10 w/v solid/liquid water extracts on filter paper. Germination %, shoot weight and (water extracts only) root weights were recorded after 5 days. Secondly, ryegrass (*Lolium perenne*) was grown in pots of local soil amended with the wastes at 30 or 180 t/ha, recording weight of tops at one and two months. The highest correlation with germination was found for samples with the most stable organic matter, (a smaller proportion of labile carbon, carbon extracted with $Na_4P_2O_7$, carbon precipitated at pH 2 and water soluble carbon). Inhibition was increased by NH_4^+, polyphenols and organic acids of low molecular weight. Overall inhibition was less with water extracts. The authors suggested that either some phytotoxic substances are not extracted by water, or there are intrinsic negative effects from direct use of the wastes, e.g. temperature increase, O_2 depletion, etc. In the ryegrass test, application of all wastes, including composts, at the higher rate, had a negative effect after one month, but mostly a positive effect after two.

Cress (*Lepidium sativum*) germination and radish *(Raphanus sativus*) growth were investigated as indicators of phytotoxicity and maturity by Grebus *et al.* (1994), using composted green waste. The cress was used in a modified version of the Zucconi *et al.* (1981) germination test. Water extracts of the compost were prepared by a saturated paste method and then diluted 0, 3x and 10x before use. Germination and radical length were assessed by stopping growth with ethanol after 24 hours and the germination index was calculated. For the radish growth test, plants were grown in a potting mix, prepared by mixing the compost with peat and perlite (1:2:2 v/v). Dry weight of tops was measured after 7 days. The authors found the cress germination test to be unsuitable. The undiluted and 3x diluted compost extracts remained toxic to cress at all maturity levels (up to 125 days after starting composting). Only the 10x dilution became significantly less phytotoxic as maturity increased. High ammonium-N concentrations and possibly electrical conductivities were thought to be partly responsible. In contrast, the radish growth test was considered to be a good indicator of maturity. Radish dry weights increased in compost samples taken until 55 days from the start of composting. This suggested that 60-70 days of composting were required to destroy the inhibitory growth effects.

Bidlingmaier and Maile (1996) describe an interlaboratory test of compost analyses, including plant growth tests. A total of 95 laboratories took part and a broad range of chemical analyses were undertaken, as well as growth tests, (though not all laboratories conducted all tests). Details of the growth tests used are not reported. However, a relative plant tolerance test, using 25% compost in a growing medium, proved to have the largest variation in results between laboratories of any test investigated. The minimum and maximum yields reported, excluding outliers, varied between about 2 and 16 g. The authors concluded that numerous laboratories were not in a position to conduct this test correctly. Results for a second plant test, “germinable seeds”, was similarly found to show amongst the greatest variation of all tests between laboratories.

The Canadian Council of Ministers of the Environment (CCME), Composting Subcommittee, (1996) sets the following guidelines for maturity. It is considered that no single test of maturity is reliable and sufficient by itself, so that more than one test is recommended. Four options are presented, one of which the compost should conform to. Options 2-4 involve different curing periods. Option 1 specifies that two out of the following three test requirements shall be met:

1. testing for the ratio of carbon and nitrogen, which must be C/N $\leq$ 25;
2. oxygen uptake, which shall be: < 150 mg O_2/kg organic matter (volatile solids) per hour;
3. the germination of cress (*Lepidium sativum*) seeds and radish (*Raphanus sativus*) seeds in compost shall be greater than a value corresponding to at least 90% of the germination rate of the control sample and

plant growth rate of the compost-soil mix shall not be less than 50% in comparison to the control sample.

The sensitivity of different species in germination and growing tests was investigated by Ortega *et al.* (1996), using cork oak bark (*Quercus suber*). The study was not concerned primarily with compost maturity, but the methods used are relevant. Two germination bioassays were investigated. In the first, aqueous extracts were prepared using a 1:1 substrate:water ratio (v/v) and either cold water (15°C) or hot water (70°C). Two ml of extract were then placed on filter paper in petri dishes. In the second, 15 ml of substrate was placed directly in petri dishes. In both tests seeds were then sown and the germination index (according to Zucconi *et al.* 1981) was determined. In the growing test, seeds were sown onto 56 ml containers of the substrate and grown for 30 or 45 days, depending on the species. The species investigated in both germination and growth tests were pepper (*Capsicum annum*), Chinese cabbage (*Brassica pekinensis*), cauliflower (*Brassica oleracea*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), watermelon (*Citrullus vulgaris*) and tomato (*Lycopersicon esculentum*). The authors found that hot water extracts reduced radicle growth compared to cold water. Further, tomato and lettuce were the most sensitive species to the phytotoxic phenolic substances present, (salinity was not a problem). Finally, the germination test directly on the substrate was recommended because of its simplicity and ability to predict the results of the growth test, provided sensitive species were chosen.

Leege and Thompson (1997), for The US Composting Council, give detailed instructions for two germination tests and one growth test. In their preliminary discussion, they recommend that tests should use a sample of the compost-*amended* growing media which it is planned will finally be used, along with the plant species it is intended to grow. It is not entirely clear from the text, but they are probably referring to extended growth tests here, rather than germination tests. The three methods are as follows:

1. Germination and root elongation in water extract (500 ml water:400g compost as received), centrifuged and filter-sterilised (0.45 µm); sow rapidly-germinating plant species and grow for two to three days. Determine germination index, according to Zucconi *et al* (1981). Root (= radicle) length considered more sensitive than germination.
2. Quick-test for *in-vitro* germination and root elongation. Germination and root elongation of cucumber seed in water extract (two parts water to one part compost, dry weight basis), filtered (filter paper). Assess relative germination rate and root length after 5-7 days, compared to deionised water control.
3. Quick-test for emergence and relative growth (direct seeding). Seedling emergence and relative vigour in 50:50 blend of compost and vermiculite. Cucumber seed grown for 14 days. Assessment made of percent emergence and relative vigour (at least 7 cm height, turgid and no deformity).

The authors state that some species, including cucurbits, are relatively salt tolerant and so not susceptible to serious salt damage, but are susceptible to various toxic organic compounds found in some immature composts.

Erhart and Burian (1997) compared a germination test, using compost extracts, with a growth test, using composted household biowastes of varying maturity. For the germination test, 240 ml compost was wetted to 100% water capacity, then an additional 150 ml water added. This was shaken then centrifuged. Three ml of extract was placed on filter papers in petri dishes then cress seed (*Lepidium sativum*) sown. Germination was recorded after 24 hours and radicle length after 48 hours. In the growth test, compost was mixed at 0, 15, 30, 50 and 100% with standard growing media (undefined) and brick dust. Germination rate and fresh weight was recorded after 10 days. The authors report that germination in the extracts was in good agreement with germination and relative yields in the growing test. However, unlike germination, radicle length was strongly impaired by the salt content of the extracts. The authors concluded that germination rate was a better indicator of phytotoxic substances than radicle length, except in the case of inorganic salts.

Johanson *et al.* (1997) discuss maturity and national standards. The authors consider growth tests to be outstanding predictors of compost *maturity*, but consider that the test plants, seeds or seedlings must have approximately the same sensitivity to phytotoxic substances, salts, nutrients etc. as the plants the compost will be used on. They also state that growth tests need to be combined with another test, to verify compost *stability*. The authors then list the following national requirements for growth tests:

1. Germany - suitability as a growing medium must be proven with a germination test and with regard to nitrogen dynamics (RAL quality symbol);
2. Italy - germination test >70% compared to control; if >40% but <70%, compost must be subject to a growth test; can be considered not phytotoxic if a growth test with cress (*Lepidium sativum*) results in >100% relative growth;
3. Netherlands - at least 90% germination of spring barley, compared to a control and no visual damage, in a 25% mix.

Germination index, weed seed survival and suitability as a constituent in a potting mix were investigated, amongst other measures, as predictors of compost maturity of composted green waste by Hartz (1997). GI was determined using a method similar to Zucconi *et al.* (1981). 20 g air-dried compost was extracted with 100 ml water, then the filtrate further diluted 2:1 water:extract. Germination and radicle length of tomato seed (*Lycopersicon esculentum*) were determined after three days. Weed seed survival was assessed by placing compost 2.5 cm deep in seed trays and keeping it moist for six weeks. The growth test involved planting plug-raised periwinkle (*Vinca minor*) in pots of 1:1 compost and perlite and growing them for six weeks, with weekly nitrogen feeds. In all composts tested, there was a clear trend of increasing GI with increasing duration of composting, (from 0 up to 82-118 days). However, statistically, there were few differences between GI at different composting times, suggesting the method was not very sensitive. The author suggested high electrical conductivity may have been a confounding factor. Few viable weeds survived the initial weeks of composting. The author presented no data, but his comments suggest weed seed survival was a poor indicator of maturity. Growth of periwinkle showed a similar result to the tomato GI, with a clear trend of increasing dry weight with increasing composting time, but a lack of sensitivity in terms of differentiation between times. The author concluded that there are no easily performed laboratory procedures, which can identify maturity, though some chemical methods are good indicators of immaturity.

Warman (1999) discusses the limitations of and developments from the method of Zucconi *et al.* (1981). For example, many researchers have proposed seed soaks or direct sowing into compost and compost-peat mixtures as alternatives to the pressure extraction method of Zucconi *et al.* and other plant species besides cress (*Lepidium sativum*) have been used. Warman (1999) considers that Zucconi *et al.*'s written procedure is difficult to duplicate and as a result there is no universal growth test in use world-wide. Also, there has been little work to determine whether some seed species are more sensitive to phytotoxic substances in immature compost than others. Warman (1999) points out that controversy exists whether a test for compost phytotoxicity is equivalent to a test for compost maturity. He is one of very few authors to consider this issue.

Warman (1999) addressed these concerns by comparing three plant tests, with a range of composts. The three test methods were as follows:

1. the Canadian germination test, above (CCME, 1996): compost mixed 1:2 v/v with various soils, seeds grown for 14 days after 50% of control seeds germinated, % germination + seedling length + fresh weight of tops recorded;
2. direct sowing onto pots of compost: % germination and seedling length measured after 10 days;
3. a modified Zucconi *et al.* (1981) method: 1:2.5 w/v fresh compost:water extract (by centrifugation) used without further dilution, % germination measured at 24 and 48 hours.

Cress (*Lepidium sativum*), radish (*Raphanus sativus*) and Chinese cabbage (*Brassica chinensis*) were compared. In test 1 germination was always equal or higher in the immature composts compared to the control (soil). The only significant result was that length and weight of seedlings was higher in one immature compost than the control. The characteristics of the soil mixed with the composts greatly influenced germination and growth. The author believed that the composts were improving the soil qualities. Test 2 did not separate germination in two out of three immature composts from the control. Similarly seedling length did not reflect compost maturity. Test 3 also provided little indication of compost maturity or phytotoxicity. The author concluded that tests 1 and 3 were not sensitive enough to detect differences in maturity; test 2 was more sensitive, but other test(s) were needed to evaluate maturity.

Brinton and Traenkner (1999) investigated the relationship between plant growth and volatile organic acids (VOA) and CO_2 evolution. VOA and CO_2 were measured in a range of composts, some known to have

caused phytotoxicity problems. Two growth tests were conducted. In one, compost extracts and standardised acetic, butyric and propionic acids were added to nutrient solutions, to examine effects on roots. In the second, cress (*Lepidium sativum*) and wheat (*Triticum aestivum*) were grown for seven days in 1:3 (v/v) blends of compost and peat, recording % germination and fresh weight. Both wheat and cress yield showed negative correlations with VOA concentration and CO_2 rate, indicating that mature composts were more beneficial to plants. There was a significant negative correlation between wheat seedling yield and VOA and a less significant correlation for cress: the latter was more sensitive to conductivity. CO_2 evolution rate had no significant effect on germination for either species: CO_2 rate depressed plant growth, which was attributed to oxygen deprivation in the root zone. Critical levels of VOA in compost, which resulted in observable seedling growth depression were reported as approximately 5,000 mg/kg in the original compost. Critical respiration rates associated with observable depression were about 0.4% CO_2-C loss/day.

Brinton (2000) reviewed compost quality standards and guidelines, both within the US and internationally, for the New York State Association of Recyclers. He describes the European ECO-Label for Soil Improvers, which includes a declaration of no phytotoxic effects. His review of plant phytotoxicity tests is brief. He mentions

- German tests with 25 and 50% compost plus peat or soil, or 100% compost using cress, barley or radish (must pass >90% for barley) and a requirement that compost must be tested for the use recommended on the bag;
- Australian tests with compost leached before testing, presumably to remove salts and eliminate the need for media dilution, plus two use categories with specific limits for agriculture and garden use;
- Swiss tests with 100% compost, plus the "closed cress test", which distinguishes gaseous phytotoxicity, in addition to that compost-borne, (see below);
- Austrian tests with a range of compost/peat dilutions, using cress and barley seeds (must pass >80%).

The author then describes a new approach being taken by the California Compost Quality Council (CCQC), in conjunction with Woods End Laboratory (Brinton's base) and others. The same is reported by Brewer and Sullivan (2001). In this approach, *maturity is no longer viewed as a single property that can be singly tested for*. Instead, the group consider it should be assessed by two or more parameters, drawn from two groups of tests, e.g. CO_2 evolution (in Group A), VOA concentration or plant tests (in Group B). Composts are then placed in one of three maturity classes: very mature, mature and immature. For plant tests, the appropriate thresholds are as follows:

	Very mature	**Mature**	**Immature**
Seed germination % of control	>90	80-90	<80
Plant trials % of control	>90	80-90	<80

In discussing these, Brinton (2000) again stresses his belief that germination tests are very poor indicators of compost quality. Growing plants in compost mixtures with soil or other media and germination and root elongation measurements, perhaps with water extracts, are all mentioned. However, the author states that plant tests may indicate either none or any one or more of the factors termed phytotoxic. Results are dependent on preparation of the media, especially concentration or blending and these - as well as the plant species - must be clearly stated.

The effects of VOA and oxygen levels on plant growth were considered further by Brinton and Evans (2001), investigating container plant performance in relation to compost quality and maturity. No correlation had been found between germination and several parameters, including CO_2 evolution and VOA. Composts were mixed with peat and sand to obtain low electrical conductivities of about 200 µS/cm (ADAS Index 1). Oxygen concentration in the containers was measured. This was found to diminish with depth and correlated closely with compost maturity. Uncured composts remained low in oxygen, whereas semicured composts improved after the second week. The oxygen levels corresponded to growth differences observed for tops and roots, with much reduced top and root growth as the level of compost curing decreased. The most significant statistical correlation was between VOA and root length. However, VOA was not present at the end of the trial (<500 ppm), indicating that the effects of immature, oxygen-depleting composts were

very persistent. The authors concluded that a number of interrelated factors cause plant effects resulting from maturity and that separating stability and maturity factors may be irrelevant. They proposed that the pathway of effects starts with elevated CO_2 evolution, translated into elevated VOA. The authors reported that VOA levels as low as 500 ppm in growing media and 100 ppm in nutrient solutions may exert phytotoxic effects, though they did not present the data, which support this. Effects of VOA and ammonia may be stronger at root emergence and early growth; oxygen deprivation and hydrogen sulphide effects may be longer lasting or occur later during growth. Referring to this study and earlier work of Brinton and Traenkner (1999), the authors concluded that germination was a poor predictor of maturity.

Garcia-Gomez *et al.* (2001) also looked at both germination index and pot trials. Growing ryegrass (*Lolium perenne*) in soil amended with 2% (w/w) composted waste, sampled at various stages, the yield of 28-day old ryegrass showed phytotoxic effects from nine-week old compost even when the germination index of cress (*Lepidium sativum*; Zucconi *et al.* 1985 method) was above 87%.

The conclusion that more than one method of measuring compost stability or maturity is required was also reached by Reinikainen and Herranen (2001). They looked at different methods of measurement, including germination percentage of Chinese cabbage (*Brassica pekinensis*) after 7 and 14 days, plus fresh weight after 28 days. Germination reached 70%+ after the thermophilic phase, whereas fresh weights were still low and increased considerably with older compost. The authors concluded that stability and maturity need to be assessed by a combination of methods, depending on the intended use of the compost. In their work, the growing trial combined with other measurements (self-heating, residual oxygen and the development of electrical conductivity, acetic value and NH_4-N/NO_3-N) gave satisfactory results. Fuchs, et al.(2001) in The Swiss ASAC Guidelines 2001 , specify tests with lower and higher compliance thresholds for horticultural use (lower) or protected horticulture and private gardening (higher), as shown below:

	Parameter measured	Compost for horticultural use	Compost for protected crops and private gardening
Cress (open)	Seedling weight after 7 days	>70% of ref.	>90% of ref.
Cress (closed)	Root length after 7 days	>25% of ref.	>50% of ref.
Salad	Seedling weight after 10 days	>50% of ref.	>70% of ref.
Bean	Root weight after 10 days		>70% of ref.
Ryegrass	Seedling weight after 14 days		>70% of ref.

Test methods are described by Fuchs and Bieri (2000) and were selected on the basis of their sensitivity and reproducibility in a ring test, using composts of different maturities. Seeds of each species are sown in pots of test and control compost. For the closed cress test sealed, air-tight containers are used. This test is considered by the authors to be the most sensitive to compost maturity. They do not discuss why, but Brinton (2000) states that it distinguishes gaseous phytotoxicity. Wheat and barley were also investigated in the ring test, but were found not to respond to compost quality and the roots were difficult to wash out. We concur with both these points.

Sullivan (2000), in a brief report, describes a comparison of various chemical tests plus seed germination and growth for assessing stability and maturity. Detailed results are not reported, but respiration rate was found to be the most reliable test.

Florida's On-line Composting Centre (2002) publishes two germination methods and a growing test for use by compost producers and users. The first method assesses whether the compost is "finished" by measuring plant germination in compost extracts, using kitchen-type equipment. A 1:2 compost:water extraction is conducted, with radish (*Raphanus sativus*) or some other fast germinating seed. Germination after 24, 48 and 72 hours is compared to a control (water only); 80% germination is considered satisfactory. In the second method, maturity is assessed by sowing seeds of radish or other fast-germinating species, into pots of the finished compost and recording germination after seven days. The result is compared to a control and the compost is considered immature if germination is "significantly less" (undefined). The growing test measures the "quality" of the compost and whether it is providing plant nutrients adequately. The 7-day old seedlings from the germination in compost test are grown on for 21 days, then plant tops and washed roots

are harvested. Fertilisation of half the test pots is allowed, to demonstrate any nutrient deficiencies in the compost. No thresholds are given for interpretation of results, other than that poor growth indicates that the compost is "unfinished" and microbes are still using plant nutrients to decompose the compost.

7.3. Other biological methods

Herrmann and Shann (1993) considered enzyme activities as indicators of compost maturity. The principle investigated was that if the microbial community structure stabilises along with the compost's physical and chemical composition, their enzymatic activities should reflect this; "a uniform enzymatic signature for the utilisation of the recalcitrant humic and cellulosic fractions of the compost should emerge...to provide an easily accomplished method to indicate maturity". The main enzymes assayed were alkaline phosphatase, acid phosphatase, endo-cellulose, glucosidase and lipase (C10). Biomass was measured using lipid phosphate as a measure of the amount of cellular membranes and metabolism by the amount of ^{14}C-acetate incorporated into the lipid phosphate pool. All the tests followed steady trends during composting. Cellulase activity was found to be a good indicator of stability and lipase activity a good indicator of maturity. A proprietary test strip ("Api-Zyme") also gave comparable results to the standard enzyme tests. The authors concluded that the use of specific enzymatic activities present an inexpensive and fast method, possibly combined with other methods, to predict stability and maturity.

Helfrich *et al.* (1998) looked at oxygen consumption and fluorescene measurements of isolated, freshly suspended or freeze-dried chloroplast thylakoids (constituents of plant chloroplasts), from *Vicia faba* (beans). These were compared with traditional germination index and plant growth bioassays, for detecting phytotoxicity in composts. The germination test was based on the method of Zucconi *et al.* (1981). The growth test involved planting cucumber (*Cucumis sativus*) seeds in 50/50 mixes of compost and perlite. The new techniques proved suitable for evaluating the function of photosynthetic electron transport (PET) between photosystem II and photosystem I. When PET was inhibited by phytotoxic substances, fluorescence increased whereas oxygen consumption decreased. There were significant correlations between germination index and plant growth and the new techniques. The authors concluded that the methods represented rapid and sensitive tests for compost phytotoxicity.

Belete *et al.* (2001) investigated microbial community level physiological profiles as indicators of compost maturity. Microbial community structure changes during the composting process, interacting with factors such as temperature, oxygen gradients, redox potential, pH, and moisture. The authors used microbiological techniques including single-point readings at certain well colour densities, estimation of areas under activity curves, calculations of kinetic parameters and estimates of functional diversity. Comparisons were made to respiration and microbial biomass, as maturity indices. Community level physiological profiles proved suitable for identifying different stages of compost maturity and the authors considered the technique to be a promising tool.

The paper by Herrmann and Shann (1993) suggests enzyme-based methods in particular have potential as a rapid and cheap way of measuring stability and maturity. The three approaches reviewed above are not discussed further here, but it is recommended that the further development of enzyme-based methods is monitored.

7.4. Discussion on phytotoxicity testing literature review

It can be seen that the approaches used in plant tests fall into two broad groups, those using compost extracts and those using compost directly, the latter with or without dilution by another growing medium. Some authors have found their tests to be good indicators of stability or maturity, some have not. A number of key variables have been highlighted, which will affect the results obtained, including:

- volume of water used in extraction
- plant species
- dilutent used in growing tests (soil, peat, vermiculite etc.)

- time allowed for growth
- plant attribute assessed.

None of the authors, with the exception of Warman (1999) and Brinton (2000), appear to have given consideration to what plant tests are really measuring and what we want them to measure. This is probably because authors do not define the terms stability and maturity and tend to use them loosely. Defining stability as the rate of biological activity and maturity as readiness for use, plant tests are affected by both these properties. However, as compost use will almost always involve plant growth, plant tests are most relevant to maturity and assessing this is their logical objective.

All plant tests reviewed respond to phytotoxicity present at the moment of assessment. However, do any of the tests demonstrate that a compost is or is not mature, i.e. ready for use? The answer is almost certainly no for those using compost extracts, because the tested material is too far removed from what plants will experience in real use. Extract-based tests may detect one or more phytotoxic effects, but are unlikely to detect all effects and the magnitude of effects will be different from real use. Growth tests are likely to detect many more of the phytotoxic effects which will be experienced in real use, provided their design reflects that real use. They still have the limitation, though, that the results may depend on their duration and the moment of assessment, in addition to the differences from real use imposed by experimental conditions, (temperature, light regime, watering, etc). Pesticide bioassay work at the ADAS Laboratories has established that some phytotoxic effects may take weeks to develop. Many persistent herbicides used in amenity weed control, such as atrazine and diuron, are photosynthesis inhibitors and do not show effects on plants until the true leaves are well established and functioning. Persistent hormone herbicides, such as clopyralid and picloram, have sometimes been found to produce their effects in bioassays several weeks after plant establishment. Conversely, some of the authors reviewed have found that weeks or months are needed before phytotoxic effects present at the start of growth disappear.

Can plant tests ever adequately indicate readiness for use? Several authors have concluded that, when used alone, they cannot, though they may do so when used in conjunction with other, chemical tests. This seems to be a sensible suggestion, in the light of the evidence and it is recommended by this review author that we do not seek a stand-alone plant test for maturity, but rather a test which reflects real use, to be used in conjunction with other tests. It must be accepted that any such test will not assess all aspects of readiness for use, i.e. will not measure all possible phytotoxic effects, but only those it is considered most important to seek. As plant establishment is the most sensitive time to most phytotoxic effects involved in maturity, assessments during this stage will be important.

7.5. Requirements for standardised phytotoxicity testing

There are a number of key requirements of the plant test, if it is to be useful and widely adopted, as follows:

- rapid (though extended duration might be an option);
- easy to perform;
- reproducible between different testing centres;
- reflects intended use;
- easy to interpret.

BSI document PD CR 13455:1999 "Soil improvers and growing media - guidelines for the safety of users, the environment and plants" also suggests criteria to consider in tests, (specifically tests for phytotoxic factors). These criteria are broadly similar to the above, though in addition, BSI highlights the ability to differentiate nutrient shortage from phytotoxicity, responsiveness to herbicides and other organic contaminants and the need to be unaffected by high ammonium and salinity levels. The nutrient issue will depend on how long the test is conducted, because deficiencies will not affect germination and early establishment. The ability to detect herbicides will influence the duration of the test and will require consideration of priorities. High ammonium and salinity levels are addressed below.

There are several major uses of composts involving plants, which present the plant with different levels of exposure to the effects of immaturity. These are:

1. soil incorporation as a soil amendment or fertiliser;
2. as a surface mulch;
3. as a constituent of a growing media.

It seems logical that the testing requirements should be different according to use, as stipulated by Fuchs *et al.* (2001) in the Swiss ASAC Guidelines 2001. In the case of soil incorporation, it is questionable whether any plant testing of maturity is necessary anyway. Application rates will be controlled by various legislation, based on parameters such as nitrogen or heavy metal content. If a high-value or sensitive field crop is to be grown and there is particular concern about phytotoxic effects not addressed by analysis, this is most realistically assessed by a pot trial, run for perhaps four weeks, in which the compost is added to soil at the actual rates planned and the species of concern is grown.

Application as a surface mulch is probably a minor use for compost. Compost rapidly becomes attractive to weed seeds and so performs less well than other mulching materials. It is also hard to envisage a plant test which reflects this use in any meaningful way, unless plants are grown in pots of soil, surface-dressed with compost at the intended rate. Even then, the real use species are likely to be hardy ornamentals (shrubs and trees), which do not lend themselves to rapid greenhouse growth tests. It is suggested at this stage that a specific test for this use is not sought. Where particular concern exists, plant tests for the third use (below) could be used, because this is investigating the highest exposure situation.

The third use, then, is the one discussed below and for which a plant test is proposed.

We previously expressed the opinion that a compost extract based test should not be used, primarily because the results are so difficult to relate to real use of the compost being tested. Some of the test methodologies of Zucconi *et al.* (1981) and others are also quite complex to follow, requiring pressure plate extraction or centrifugation, millipore filtration, etc. Instead, a test growing plants directly in the compost is preferred. This immediately raises the issue of high electrical conductivity in the compost. In the Composting Association samples tested by the ADAS Laboratories, conductivity values of ADAS Index 7 - 9 (901 -1300+ µS/cm) is normal. Dilution of the compost with another growing medium is essential. Arguably, this weakens the test, because in diluting the compost to reduce the conductivity, other phytotoxic factors will also be diluted. However, in reality this is not an issue. If a compost has a high conductivity, it will be diluted in its real use also, so the test is only reflecting this. The only consideration is that dilution in the test should match closely the dilution in real use. High ammonium levels have not, so far, been an issue. They have been elevated in some samples, but dilution to control conductivity has also reduced ammonium levels. If ammonium levels required separate consideration (and the level is always determined in the preliminary analysis) it is suggested that the compost should be diluted accordingly, as it would in real use.

Many authors have found the early stages of growth to be especially sensitive to compost phytotoxicity, i.e. germination and especially root (radicle) development. Effects also can become apparent within a few days. It is proposed that these growth stages are assessed. Continuation beyond early establishment increases the likelihood of detecting other phytotoxic factors, such as herbicide effects. However, this would be at the expense of speed. One solution is that results are reported in two stages, with establishment results available within a few days of starting the test, then growing on results reported later. Growing on will, however, require consideration of nutrient levels and whether fertilisation should be used.

7.6. Recommendations for standardised phytotoxicity testing

It is recommended that a test is developed, similar to the draft Belgian 10 day test and, to a lesser extent, the proposed Irish test. Plants should be grown from seed sown directly in samples of the compost being tested, diluted with another growing medium according to the electrical conductivity. This is the approach used in the Belgian method, but not the Irish method, which uses undiluted sample. It is considered that growth in undiluted compost samples will, in most cases, be predominantly controlled by the electrical conductivity of the sample and this effect can be predicted from the preliminary chemical analysis. Container size should be sufficient to allow for the courser-sized composts: it is unlikely that some of the woody green waste composts would fit into petri dishes, without milling. Milling and any other processing should only be conducted if it will be used in real use. Covered (but not airtight) growing containers are recommended, since cover is sometimes used in real use. The nature of the diluting substance requires

careful consideration. The dilution method must achieve reliably the target conductivity in the final mix, (a problem with the current Composting Association method). Sand, as in the draft Belgian method, is a material likely to achieve this. However, it is unlikely to be used in real use and well-defined peat, (e.g. uniform size for ease of mixing) might be considered as an optional dilutent. Vermiculite or perlite can also be used.

8. Recommendations Arising From The Peer Review

The outcome of the literature review and its recommendations were circulated to members of the BSI Technical Committee AW/20 - Top soil and other growing media for peer review with presentation and discussion at the BSI AW 20 meeting held on 17th May 2002. The outcome of the views of the meeting was summarised in the minutes and is shown below:

8.1. Definitions

It was generally agreed that the words “stability” and “maturity” were inappropriate.

“Instability” may be more appropriate, however they were in universal use and it would be difficult to introduce new words to define what was meant by the various stages of the composting process. The definitions as presented were generally accepted.

8.2. Stability

It was agreed that the rate of CO_2 be reported by mass on an organic matter basis. The organic matter would be determined by loss on ignition.

8.2.1 Maturity

Discussion took place as to what was meant by “readiness for use”. The use could be as a hot bed and therefore at a very early stage in the composting process. If the product was used as a mulch, then some phytotoxicity may be advantageous in weed suppression. If landfill was the final destination then, so long as no more gasses would be evolved, it was ready for its intended use. For soil incorporation or as a growing media very low aerobic activity and phytotoxicity had to be considered. It was agreed that an end product was “mature” when it was ready for its intended use. It must be clearly understood that this did not make the product fit for all possible uses. Maturity could also be time related hence although the product is ready to use it cannot be said to be mature.

8.3. Analytical Methods

8.3.1 Respiration

From the literature research three methods were presented as being in regular use as a measure of stability: calorimetry (Dewar self heating), oxygen demand and CO_2 evolution. The self-heating method was not suitable for the initial stages of composting.

It was agreed that the proposed ADAS method was simple and did not require sophisticated equipment (although this was considered by some to be a drawback) and was claimed to be rugged. The ADAS method could use any weight of sample and could be conducted over any specified time period. The AT_4 method was strongly supported as this was established on the European mainland. The AT_4 method was a 4-day test measuring both CO_2 and O_2 to indicate stability.

Interest was shown in the possibility of a future indirect method, near infrared spectroscopy (NIRS). This method required “wet chemistry” data for calibration purposes, but once calibration had been achieved the method was exceptionally quick, taking only minutes to prepare the sample and determine the results.

8.3.2 Phytotoxicity

It was agreed that biological assay was the best way to determine possible phytotoxic effects. Various methods and plant species had been used. All had a use but none cover all the aspects required, i.e. speed

of test; assessment of germination and early root growth, top growth and finally fruit development. Electrical conductivity was a problem and it was agreed that some dilution of the test sample may be necessary; However, there was no agreement on possible diluents.

From these discussions the following recommendations were carried forward to the design and implementation of experimental work:

- Stability is defined as 'the rate of biological activity' and measured as the rate of aerobic respiration using a standardised CO_2 evolution procedure incorporating the best features of the various procedures reported in the literature.
- Maturity is defined as 'readiness for use' and assessed by stability and phytotoxicity, plus other direct parameters relevant to the intended use.
- A phytotoxicity test is to be developed which involves dilution of the test compost with a suitable material to take account of electrical conductivity. Different test species were to be considered, comparing their germination, root development and weight of plant tops after a short and a longer growth period.

The following individuals received the peer review report and attended the AW 20 meeting

Mr T Evans Chairman

Dr J Terry BSI

Mr G Brightman Institute of Groundsmanship

Mr H Burnett LACOTS/TSI (Convenor of CEN/TC 223 WG 3 Sampling & Quantity)

Mr B Cooper Co-opted (Convenor of CEN/TC 223 WG 4 Analytical methods)

Miss E Nichols Composting Association

Mr A Snarey BSI Consumer Policy Committee

Dr C Turner John Innes Manufacturing Association

Mr P Wheeler AEA Technology Environment

Miss R Collyer BSI Editing Consultant

Dr J Frederickson Open University

Mrs S Holmes ADAS rep. DEFRA

Dr A Keeling Harper Adams University College

Mr E Papadimitriou University of Leeds

Dr E Stentiford University of Leeds

Dr M Wood Reading University

Comments were also received from N Bragg, (Growing Media Association).

9. Laboratory Evaluation of a Method for Determination of Stability

9.1. Determination of Compost Stability

The method developed and tested was based largely on the carbon dioxide evolution method 09.09-C published by the U. S. Composting Council *Test Methods for the Examination of Composting and Composts*, first edition, 22 December 1997 but with important differences. The biggest and most important difference was the change from a sealed passive system to a dynamic system with constant air flow. This made the method principle comparable with BS ISO 14855:1999 *Determination of the Ultimate Aerobic Biodegradability and Disintegration of Plastic Materials Under Controlled Composting Conditions – Method by Analysis of Evolved Carbon Dioxide.* It also made the method broadly compatible with procedures such as AT_4 or AT_7 using automated respirometers.

Stability (biological activity) can be reported directly in units of carbon dioxide evolved (mg CO_2 g^{-1} VS d^{-1}) or can be calculated and expressed in units of oxygen consumption (O_2 g^{-1} VS d^{-1}).

9.2. Principle and Brief Method Description

A representative sample of the compost is coarsely screened to pass 20 mm and well mixed. Water is added to bring the moisture content to 400 – 600 g/kg. 100 g $\pm$ 2g of the wetted compost is lightly placed without compaction into a 1 litre vessel. Air is pumped at a rate of 2 – 4 l/hour, initially through a 1 M sodium hydroxide solution to remove atmospheric carbon dioxide, then through the compost incubation vessel and finally through a diffuser at the base of a 150 ml test tube filled with 50 ml 1 M NaOH. Any carbon dioxide produced by the compost is flushed through and trapped by the 1 M NaOH as sodium carbonate. For standardisation purposes the method is best carried out in a 25°C constant temperature room or incubator.

For the first 72 hours the sodium hydroxide trap is omitted to allow the sample to equilibrate. After 72 hrs (T_0) the test tube trap containing sodium hydroxide is attached and measurement of carbon dioxide evolution begins. At timed intervals (24 hours is convenient) the test tube containing sodium hydroxide is replaced. The carbonate is precipitated as barium carbonate by an addition of excess barium chloride and the residual sodium hydroxide is titrated with 1 M hydrochloric acid using a phenolphthalein indicator. The carbon dioxide produced within the timed period is calculated from the sodium hydroxide removed as barium carbonate and sodium chloride.

$$2\ NaOH + CO_2 \ ==>\ Na_2CO_3 + 2H_20$$

$$Na_2CO_3 + BaCl_2 \ ==>\ BaCO_3 + 2NaCl$$

$$NaOH + HCl \ ==>\ NaCl + H_2O$$

$$1\ ml\ M\ HCl \times 0.022 = g\ CO_2$$

The volatile solids content (organic matter) is determined by loss on ignition (dry matter – ash) at 450°C. The total solids (dry matter) content is determined by loss on oven drying (wet weight – dry weight) at 102°C. The total carbon dioxide evolved over 96 hours is used to calculate daily carbon dioxide evolution rate expressed as mg CO_2 g^{-1} VS d^{-1}.

One small aquarium pump can supply enough air to run 10 units simultaneously. No other electrically operated equipment or electronic instrumentation is needed. A cylinder of compressed air could be an alternative air supply. 10 units occupy no more space than 0.5 m x 0.5 m. Figure 9.1 illustrates the equipment required as a 10 unit set. A single unit is shown in Annex A.

Figure 9.1 Stability Test Units.

9.3. Experiment 1. Recovery of Carbon Dioxide

It was important to establish at the outset that the method was capable of full recovery of the carbon dioxide evolved. Recovery of carbon dioxide evolution resulting from the reaction of pure calcium carbonate with hydrochloric acid over a short time period was considered to be a good test of the method recovery.

A unit was prepared with a burette sealed into the vessel lid. Accurately weighed amounts of oven dried analytical grade calcium carbonate were placed in the vessel with 50 ml of deionised water and the lid sealed. The test tube containing 50 ml of 1 M NaOH was attached and the air flow was set at 2 litres/hour. 1 M HCl was dripped into the vessel at a rate of 1 ml per minute for 1 hour with gentle swirling. The air flow continued for 2 hours after the last addition. Duplicate aliquots of 0, 1.0, 2.0 and 4.0 grams of calcium carbonate were run this way.

The test tube was removed with washing of the air line and diffuser into the NaOH solution. 20 ml of 1M $BaCl_2$ was added and the solution titrated with 1 M HCl using a phenolphthalein indicator. The resulting recoveries are shown in Table 9.1.

Table 9.1: Carbon Dioxide Recovery

$CaCO_3$ (g)	Titre M HCl (ml)	Blk-Titre (ml)	CO_2* (g)	$CaCO_3$** (g)	Recovery (%)
0	50.0	0	0	0	-
0	50.0	0	0	0	-
0.5	40.2	9.8	0.216	0.49	98
0.5	40.1	9.9	0.218	0.50	100
1.0	30.2	19.8	0.436	0.99	99
1.0	30.2	19.8	0.436	0.99	99
2.0	11.0	39.0	0.858	1.94	97
2.0	11.4	38.6	0.849	1.93	97

* ml M HCl x 0.044/2 = g CO_2
** g CO_2 x 100/44 = g $CaCO_3$

The recoveries of evolved carbon dioxide were excellent. Even at carbon dioxide evolution rates exceeding 80% of the capacity of the NaOH trap, and in a time period much faster than that required for the method, it still achieved 97% recovery.

9.4. Experiment 2. Testing Composts of Different Maturities

The method was tested using real composts of different types and ages. Five composts at 1 week, 1 month, 2 months, 6 months stages of maturity plus another very mature screened compost of unspecified age were sampled from one site. One 6 month old and screened green waste compost was obtained from another site. 50 kg plastic sacks of the samples were transported overnight to the laboratory and stored refrigerated at 4°C. The sacks were sealed inside outer plastic sacks to prevent gaseous exchange and drying. Additional information on sources of all the compost samples are shown in Annex C

Table 9.2. lists some of the major properties of the composts tested in solution.

Table 9.2: Compost Parameters

Sample	1	2	3	4	5	6
pH	8.1	7.3	5.5	8.7	8.7	8.0
Conductivity (µs)	501	419	673	484	876	1610
Density (g/l)	270	236	247	413	574	615
Dry Matter (%)	51.17	70.5	73.2	59.8	70.2	74.0
Loss on ignition (%)	33.9	54.2	35.2	43.6	37.9	24.6
Phosphorus (mg/l)	45	15	20	19	29	3
Potassium (mg/l)	752	581	558	740	1460	1110
Magnesium (mg/l)	18	26	79	17	20	98
Ammonium-N (mg/l)	3	2	120	4	65	8
Nitrate-N (mg/l)	37	14	5	4	83	422
Calcium (mg/l)	59	82	273	71	69	585
Sodium (mg/l)	86	97	136	131	135	761
Chloride (mg/l)	381	320	332	481	6778	1119
Sulphate (mg/l)	70	78	84	41	98	538
Boron (mg/l)	0.47	0.55	0.75	1.18	1.38	1.11
Copper (mg/l)	0.31	0.16	0.15	0.20	0.30	0.53
Manganese (mg/l)	0.60	1.20	6.60	0.60	0.60	0.20
Zinc (mg/l)	0.75	0.71	2.14	0.46	0.60	0.72
Iron (mg/l)	8.5	17.7	11.9	12.1	16.8	2.42

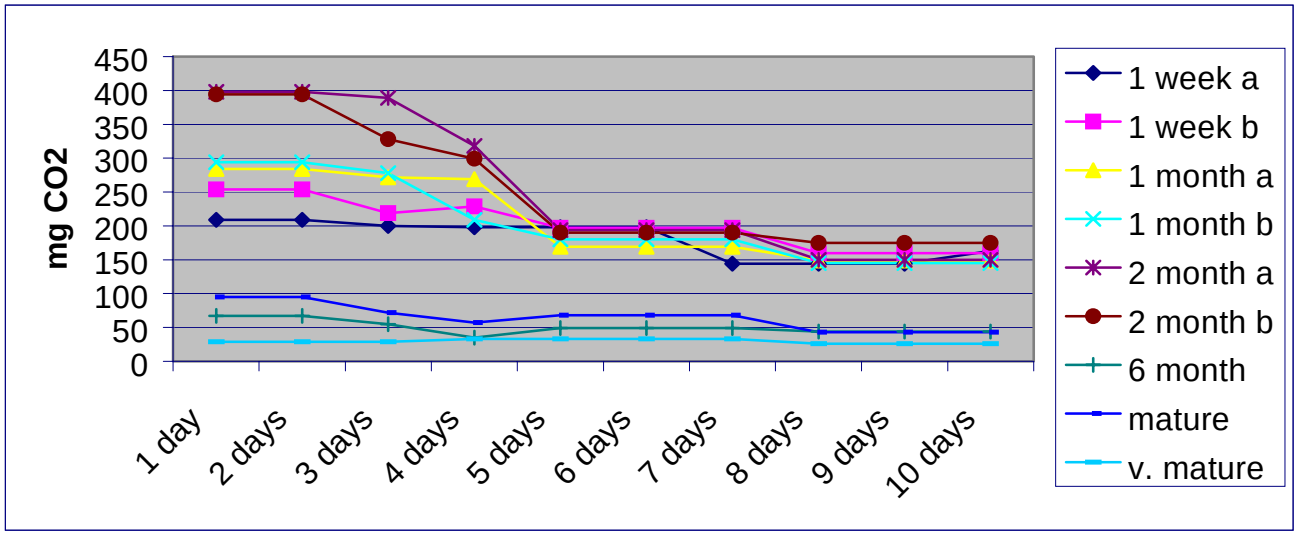
These composts were tested for stability simultaneously. The three youngest composts were tested in duplicate as an initial assessment of the repeatability of the method.

The carbon dioxide evolution was measured from the time the composts were moisture adjusted and placed in the apparatus. The three-day equilibration stage was omitted to allow us to monitor the changes during this period and assess the benefit of such a stage. In Table 9.3 results are expressed as 'mg CO2/100 g of wet compost/day' in table 9.3 and as 'mg CO2/g VS/day' in table 9.4 to demonstrate the difference this makes.

Table 9.3: Respiration mg CO2/100 g of wet compost/day

Compost	No.	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
1 week a	1	209	209	200	198	198	198	144	144	144	163
1 week b	1	254	254	219	229	197	197	197	160	160	160
1 month a	2	284	284	272	269	169	169	169	149	149	149
1 month b	2	294	294	278	209	180	180	180	145	145	145
2 month a	3	398	398	389	318	194	194	194	150	150	150
2 month b	3	394	394	328	299	190	190	190	175	175	175
6 month a	4	67	67	55	35	49	49	49	44	44	44
mature	5	95	95	72	57	68	68	68	43	43	43
v.mature	6	29	29	29	33	33	33	33	26	26	26

Chart 9.1: Plot showing mg CO_2/100 g of wet compost/day



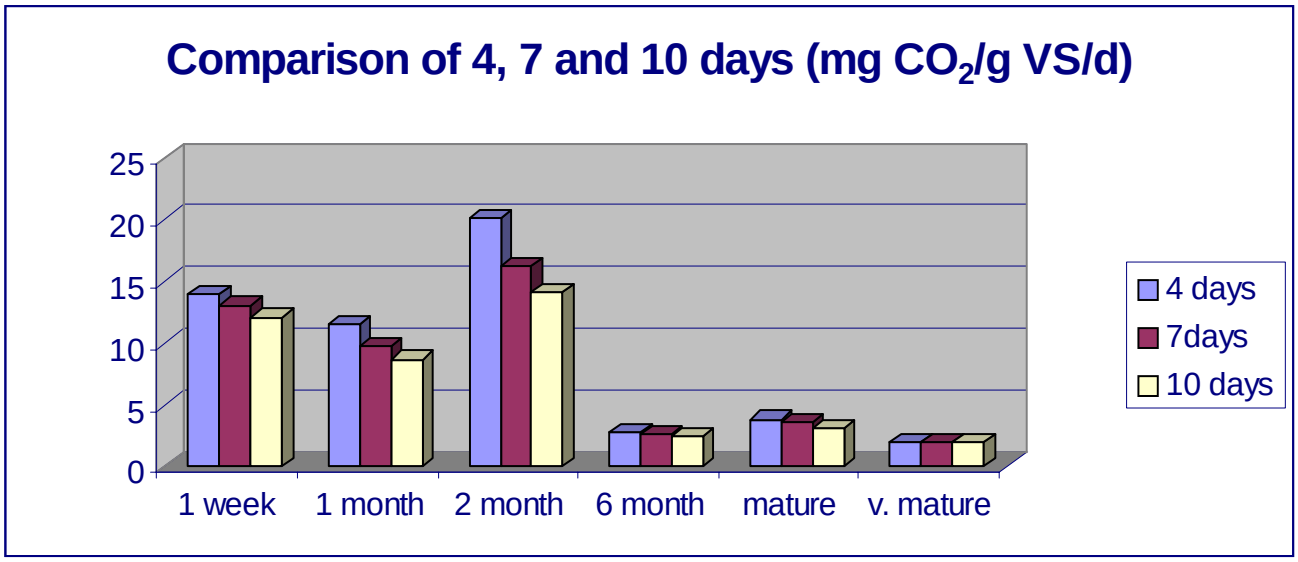
This data was further calculated as mg CO_2/g VS/day averaged over periods of 4, 7 and 10 days. This is shown in Table 9.4.

Table 9.4: mg CO_2/g VS/d averaged over 4, 7 and 10 days.

Compost	No.	4 Days	7 Days	10 Days
1 week a	1	12.85	12.22	11.40
1 week b	1	15.06	13.92	12.79
1 month a	2	11.67	9.73	8.68
1 month b	2	11.33	9.73	8.63
2 month a	3	20.04	13.26	13.97
2 month b	3	20.26	16.26	14.37
6 month	4	2.84	2.69	2.55
Mature	5	3.86	3.62	3.15
Very mature	6	1.94	2.01	1.93

Chart 9.2 shows this same data in a different graphical format. Duplicates of the 1 week, 1 month and 2 month old composts have been averaged.

Chart 9.2. CO_2 evolution (mg CO_2/g VS/d) averaged over 4, 7 and 10 days.



There are clear differences in the CO_2 evolution from the younger unfinished composts and more mature material. The 2 month old compost was more biologically active than the 1 month old or even the 1 week old compost showing that age alone is not a reliable indicator of stability. Feedstock and process are also important factors. The two-month old compost had a much lower pH and a higher ammonium-N concentration than the other composts, which probably had a significant effect on its aerobic biological stability.

The line graph of CO_2 evolution expressed as a concentration of the wet compost shows wide differences in CO_2 evolution at the early stages, which rapidly converge and show less differentiation after 4 days. However, when corrected for moisture and ash and expressed as a concentration of dry volatile solids, the differences in stability between composts is as clear at 10 days as it is at 4 days or earlier. Therefore the method developed can be used with accuracy over 4 days following an initial equilibration period. Use of 4 days would also give compatibility with AT_4 methods.

The rate of CO_2 evolution decreases with time. The rate of evolution did not fully stabilise at any stage. The sensitivity of the method is estimated at 0.01 mg CO_2/g VS/day.

For subsequent experiments we standardised on a 4 day incubation and measurement period, following a three day equilibration stage. This made the test broadly compatible with AT_4 and the timing proved very convenient for laboratory operations. The sub-samples were prepared and wetted on Fridays and allowed to equilibrate at the chosen temperature before incubations and carbon dioxide collection started on the following Mondays. The final measurements were made on the following Fridays and the equipment could be cleaned and recycled ready for the next batch of samples.

9.5. Experiment 3. Ruggedness Testing of the Method

Analytical methods need to be rugged, i.e. immune to modest (and inevitable) departures from the conditions specified in the method. A Youden ruggedness test applies a “fractional factorial” design to evaluating a number of variables in a relatively small number of analytical experiments. A seven-factor plan was devised varying the following parameters. The mature compost from the previous experiment was used throughout.

Table 9.4 Factors selected for ruggedness testing of future method.

Factor	Base condition	Alternative condition
A- Weight of wet compost	100 g	50 g
B- Dry matter percentage	54 %	62 %
C- Air flow rate	1 litre/hour	2 litre/hour
D- Temperature	25°C	20°C
E- Pre-incubation	2 days	0 days
F- Measurement time	4 days	2 days
G- NaOH trap	50 ml 1 M NaOH	50 ml 0.5 M NaOH

Table 9.5 Results (duplicate determinations) from introducing factor combinations into analysis of compost. [mg CO_2/g VS/day]

Test	Combination	i	Ii	Mean
1	no factors	13.81	14.23	14.02
2	cefg	21.53	20.43	20.98
3	bdfg	7.87	7.10	7.49
4	bcde	11.62	12.39	12.01
5	adeg	9.49	9.18	9.34
6	acdf	6.22	5.80	6.01
7	abef	28.69	25.34	27.02
8	abcg	15.94	15.40	15.67

The effect of Factor A is given by the difference between the mean of Tests 5-8 and the mean of Tests 1-4. Similarly the effect of Factor B is given by the mean of Tests 3,4,7, and 8 minus the mean of tests 1,2,3, and 6, etc.

Table 9.6 Effect of individual factors on results [mg CO_2/g VS/d].

Condition altered			
	Base method	Alternative	Difference
A- Weight of wet compost	14.51	13.63	0.88
B- Dry matter percentage	12.59	15.54	-2.95
C- Air flow rate	14.47	13.66	0.81
D- Temperature	19.42	8.71	-10.71
E- Pre-incubation	10.80	17.34	-6.54
F- Measurement time	12.76	15.38	-2.62
G- NaOH trap	14.76	13.37	1.40

Table 9.7 Ranking of method alteration effects.

	% Effect	Ranking
A- Weight of wet compost	-6.06	6
B- Dry matter percentage	+23.43	3
C- Air flow rate	-5.60	7
D- Temperature	-55.15	2
E- Pre-incubation	+60.56	1
F- Measurement time	+20.53	4
G- NaOH trap	-9.48	5

Pre-incubation equilibration had the greatest effect with a mean 60% increase in results if samples are not equilibrated prior to incubation. Changing the temperature from 25°C to 20°C depressed the measured CO_2 evolution by 55%. Decreasing the moisture content from saturated increased respiration by 23%. Averaging

the daily respiration rate over 2 days instead of 4 days enhanced the measured respiration by 20%. Halving the NaOH trap concentration resulted in a 9% drop in CO_2 recovery. Doubling the air flow rate resulted in a 5.6% drop in CO_2 recovery. Halving the weight of compost incubated resulted in a 6% reduction in measured respiration rate.

Duplicates were close demonstrating good precision. The large percentage effect of certain changes in conditions demonstrated the need for tight standardisation of the method.

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9.6. Experiment 4. Repeatability Testing of the Compost Stability Method

A method repeatability trial was conducted using the chosen optimal conditions, i.e.:

- 100 g wetted compost
- 40%-60% DM range
- 1 litre/hour air flow
- 25°C constant temperature
- 3 days pre-incubation time
- 4 days measurement time
- 50 ml 1M NaOH CO_2 collection trap

The trial involved testing green waste compost from one producer and municipal solid waste compost from another, at three stages of composting: two weeks; two months; >six months. 50 kg plastic sacks of the samples were transported overnight to the laboratory and stored refrigerated at 4°C. The sacks were sealed inside outer plastic sacks to prevent gaseous exchange and drying.

The compost properties are shown in Table 9.8. In each case there were 8 replicates plus two blanks. We were forced to extend the range of DM% wider than 40-50%, because some twiggy samples failed to hold more 40% water while others absorbed more than 60% water before reaching field capacity. The results are shown in table 9.9. Further information on the samples used for these tests is included at Annex C.

Table 9.8 Properties of Composts used for Method Repeatability Assessment

Compost	**green 2 weeks**	**green 8 weeks**	**green >6 month**	**mixed 2 weeks**	**mixed 8 weeks**	**mixed >6 month**
No.	**7**	**8**	**9**	**10**	**11**	**12**
pH	8.2	7.3	8.7	8.2	8.3	8.1
EC (µs)	396	454	801	1085	833	353
Dens (g/l)	293	289	577	330	435	355
P mg/l	45	27	32	32	21	21
K mg/l	724	732	1140	1020	907	545
Mg mg/l	22	30	23	41	24	13
NH_4N	6	1	185	206	70	2
NO_3N	2	1	2	3	9	2
Ca mg/l	63	78	83	189	136	63
Na mg/l	73	52	127	437	352	61
Cl mg/l	258	290	655	908	660	234
SO4 mg/l	45	67	68	327	223	48
B mg/l	0.37	0.63	1.00	0.93	0.68	0.31
Cu mg/l	0.10	0.10	0.30	0.73	0.11	0.10
Mn mg/l	0.50	0.60	1.10	0.70	0.30	0.30
Zn mg/l	0.32	0.63	1.01	1.36	0.72	0.39
Fe mg/l	2.9	9.6	37.1	6.9	2.9	2.2
LOI %	59.3	46.0	30.4	49.2	43.8	53.7
DM %	28.84	40.76	57.23	52.88	45.34	34.18

The loss on ignition percentage for the green >6 month is very high suggesting that composting had stalled for some reason. Ammonia is also high and nitrate is low showing that nitrifying activity is slow.

Table 9.9 Repeatability of the Compost Stability Assessment Method

Stability values expressed as mg CO2/g VS/day over the 4 day test

Compost	green 2 weeks	green 8 weeks	green >6 month	mixed 2 weeks	mixed 8 weeks	mixed >6 month
No.	**7**	**8**	**9**	**10**	**11**	**12**
	10.08	28.7	5.15	27.7	16.9	14.95
	11.23	28.1	4.10	13.5*	18.0	18.10
	10.68	29.1	5.63	28.8	19.3	17.95
	9.70	28.7	6.33	24.6	19.9	15.39
	8.23	29.3	5.88	24.0	18.1	15.27
	10.33	30.4	4.53	26.0	19.8	15.87
	11.00	30.0	5.48	25.0	19.2	16.82
	10.45	30.0	5.33	24.3	17.3	13.79
Mean	**10.21**	**29.29**	**5.30**	**25.77**	**18.56**	**16.02**
S_r	0.938	0.792	0.717	1.835	1.144	1.502
RSD_r	9.2	2.7	13.5	7.1	6.2	9.4
$r(2.8S_r)$	2.23	1.88	1.71	4.37	2.72	3.57

* discarded outlier.

9.7. Statistical Comparison of the Laboratory Stability Test with the Commercial SOLVITA Rapid test for Stability and Maturity

A commercial rapid test kit for compost stability and maturity is marketed by Woods End Research of the USA under the brand name Solvita™.

In parallel with the laboratory testing (experiment 4) for repeatability of the developed method for CO_2 as a measure of stability, the Solvita™ test kit was used on each one of the six samples. This trial was conducted independent of the above mentioned tests. This was used to give a direct comparison of the field test method and the proposed laboratory CO_2 test.

The instructions supplied with the kits were followed carefully. The basis of the test is that a 100cc loose volume of compost is incubated in a sealed flask in the presence of two colour indicators strips for carbon dioxide and ammonia. The indicators change colour indicating the concentrations of carbon dioxide and ammonia evolved by the compost. According to test literature, the colour changes obey the Beer-Lambert optical law, relating concentration of tested molecules to optical properties of the test. The colours are converted to Solvita standard units using colour comparison charts supplied with the test kit. A booklet is supplied enabling results to be reported using Woods End's own maturity index system, or as actual concentrations of carbon dioxide and ammonia.

The tests were carried out at 25°C, which was at the maximum of the recommended temperature range. The full statisitical report providing full details and results of the tests was provided as a separate report, as written by Phil Wallace, Enviros. The full report is reproduced below for continuity:

9.7.1 Objectives

To conduct statistical analysis of Solvita tests results obtained by WRAP and provide:

- The ranges of stability/maturity found for each test

- The interpretation of the results for each test
- Correlation between tests
- Variance between duplicates

9.7.2 Compost Samples

The compost samples that were used are described in Table 9.10. The samples had a range of age since window formation from 5 to 23 weeks. Three windrows were sampled from two sites rather than two windrows from three sites recommended in the site sampling protocol.

Table 9.10 Compost samples

Code	Screening	Windrow started	Compost sampled	Age
R3-GP	Unscreened	23/04/04	28/06/04	9 weeks
R16/1-GP	0 – 20 mm	16/01/04	28/06/04	23 weeks
R4-GP	Unscreened	14/05/04	28/06/04	6 weeks
W3 -WT	0 – 20 mm	01/03/04	29/06/04	17 weeks
W7/8 -WT	unscreened	24/05/04	29/06/04	5 weeks
W1/2-WT	0 – 40 mm	16/01/04	29/06/04	23 weeks

9.7.2.1 Protocols

Sampling and testing protocols were provided to the contractors. Direct Labs and SAC performed respiration tests according to 'Standardised method for the determination of compost stability by measurement of evolved carbon dioxide', a test developed by ADAS for WRAP. The other laboratory used their own methodology.

Solvita tests were to be carried out by all laboratories in accordance with the Solvita protocol. The samples were to have been pre-prepared by the labs in accordance to EN 13040 Section 8.5 (i.e. material passed through a 20 mm sieve). The samples were pre-conditioned by all laboratories apart from Woods End as the pre-conditioning is not part of their protocol.

The labs were also to record bulk density, pH and the temperature at which the test was conducted. The weight of each Solvita sample was to be recorded and the actual moisture content for the Solvita tested after adjustment (if necessary).
Samples were to be tested in triplicate. Respiration was to be reported as mg CO_2/g VS/day.

Data was provided from:

- Direct Labs
- SAC
- Woods End

Correlations were made between the data from Direct Labs and SAC as they were following the same methodology. Where possible, other correlations have been made.

Direct Labs

Measurements were made on the compost as received, and moisture contents at testing for both Solvita and respiration. The weight of compost used per Solvita test varied between 39 g and almost 80 g, reflecting the various bulk densities of the composts.

SAC

Incubation for respiration was carried out at 24.5^{o}C on 100 g compost. Compost sample weights for the Solvita tests ranged from 53 g to 82 g. Both tests were carried out at the same moisture contents. SAC reported four suspect titres that could be removed from the respiration data.

Woods End

The CONEG CO_2 evolution test was used to measure respiration rate of the compost samples. This was carried out at 34°C, some 9°C higher than the temperature used by ADAS. There is a commonly accepted relationship between biological activity and temperature, within this range, of a doubling in activity for every 10°C temperature increase. For comparison therefore, the Woods End data on respiration have been multiplied by a factor of 0.5 x 1.1 to account for the 9°C difference.

9.7.3 Statistical Methods

The stability tests across all laboratories have been analysed using Analysis of Variance (ANOVA) using the mean test result for each sample at each laboratory as a single sample of compost was provided to each laboratory with three replicate tests performed on each. Least significant differences (LSD) at P= 0.05 are shown to compare the mean values between samples and laboratories.

The results from Direct Labs and SAC have also been correlated using ANOVA utilising the replicate data supplied.

Regressions have been performed between test method results from Direct Labs and SAC.

9.7.4 Compost Properties

Compost pH

There appears to be a difference in method for pH testing between Direct Labs and SAC as the range of pH at Direct Labs was 8.3 to 8.9 and at SAC was 7.0 to 7.4. At Woods End the pH range was 7.8 to 8.1.

Loss on ignition (LOI)

Volatile solids were measured by each laboratory as loss on ignition and reported on a dry matter basis, see Table 9.11. The range of LOI measured for each sample was over 10% in two cases and LOI tended to be lower in the measurements made on the SAC samples.

Table 9.11 Loss on ignition % DM

	Direct Labs	SAC	Woods End	Max	Min	Mean
R3GP	41.8	31.0	34.0	41.8	31.0	35.6
R16\1GP	31.8	26.6	30.7	31.8	26.6	29.7
R4GP	31.7	24.5	33.4	33.4	24.5	29.9
W3WT	42.0	38.1	37.7	42.0	37.7	39.3
W7\8WT	34.6	30.0	40.4	40.4	30.0	35.0
W1\2WT	28.5	26.1	29.4	29.4	26.1	28.0
Mean	35.1	29.4	34.3			

Moisture content

Moisture content of the samples at the time of analysis was measured as shown in Table 9.12. There is good agreement between laboratories and all moistures are between 40 and 60%. Moisture can limit microbial activity when it is low.

Table 9.12 Moisture content %

	Direct Labs	SAC	Woods End	Max	Min	Mean
R3GP	47.9	45.3	46.7	47.9	45.3	46.6
R16\1GP	49.4	49.7	50.9	50.9	49.4	50.0
R4GP	46.2	46.1	44.5	46.2	44.5	45.6
W3WT	50.8	51.4	52.3	52.3	50.8	51.5
W7\8WT	42.7	44.7	45.7	45.7	42.7	44.4
W1\2WT	49.1	49.8	53.8	53.8	49.1	50.9
Mean	47.7	47.8	49.0			

9.7.5 Stability Tests

Each laboratory conducted tests using the Solvita kits. Pre-conditioning is not part of the Solvita protocol and so was not carried out at Woods End. In almost all cases at Direct Labs and SAC, the Solvita scores were identical for each replicate. Only single results were reported from Woods End.

Direct Labs and SAC carried out respiration tests according to 'Standardised method for the determination of compost stability by measurement of evolved carbon dioxide', a test developed by ADAS for WRAP. Woods End used the CONEG-USA CO2 method and the data has been transformed to units equivalent to those used by Direct Labs and SAC from mg CO2-C/g VS/day to mg CO2/g VS/day (single result reported).

Solvita CO_2

There was close agreement between Direct Labs, SAC and Woods End in the Solvita CO_2 scores.

Table 9.13 Solvita CO_2

	Direct Labs	SAC	Woods End	Max	Min	Mean	Age
R3GP	4.7	6	5	6.0	4.7	5.2	9 weeks
R16\1GP	7	7	7	7.0	7.0	7.0	23 weeks
R4GP	5	5	5	5.0	5.0	5.0	6 weeks
W3WT	7	6	6	7.0	6.0	6.3	17 weeks
W7\8WT	6	6	6	6.0	6.0	6.0	5 weeks
W1\2WT	7	7	6	7.0	6.0	6.7	23 weeks
Mean	6.1	6.2	5.8				
LSD (0.05) between samples =			**0.79**	**& between labs =**		**0.56**	

Solvita NH_4

There was relatively good agreement on Solvita NH_4 although on three samples Woods End was slightly lower.

Table 9.14 Solvita NH_4

	Direct Labs	SAC	Woods End	Max	Min	Mean	Age
R3GP	5	5	5	5.0	5.0	5.0	9 weeks
R16\1GP	5	5	5	5.0	5.0	5.0	23 weeks
R4GP	4.3	4	3	4.3	3.0	3.8	6 weeks
W3WT	5	5	4	5.0	4.0	4.7	17 weeks
W7\8WT	5	5	4	5.0	4.0	4.7	5 weeks
W1\2WT	5	5	5	5.0	5.0	5.0	23 weeks
Mean	4.9	4.8	4.3				
LSD (0.05) between samples =			**0.62**	**& between labs =**		**0.44**	

Solvita Index

For Direct Labs and SAC, the Solvita indices were the same or, on two samples, only one unit apart. Woods End results were also generally consistent with those of Direct Labs and SAC.

Table 9.15 Solvita Index

	Direct Labs	SAC	Woods End	Max	Min	Mean	Age
R3GP	4.7	6	5	6.0	4.7	5.2	9 weeks
R16\1GP	7	7	7	7.0	7.0	7.0	23 weeks
R4GP	5	5	4	5.0	4.0	4.7	6 weeks
W3WT	7	6	6	7.0	6.0	6.3	17 weeks
W7\8WT	6	6	6	6.0	6.0	6.0	5 weeks
W1\2WT	7	7	6	7.0	6.0	6.7	23 weeks
Mean	6.1	6.2	5.7				
LSD (0.05) between samples =			**0.82**	**& between labs =**		**0.58**	

Respiration

Utilising the data that included all of the replicates at SAC gave the results shown in Table 9.16. Omitting the four replicates gave the results shown in Table 7b. SAC recorded greater CO_2 evolution than Direct Labs. The Woods End method gave a much greater reading than from the other three laboratories due to temperature and so a correction factor was used. Statistics refer to the adjusted data.

Table 9.16 CO_2 evolution mg CO_2/g VS/day – all replicates

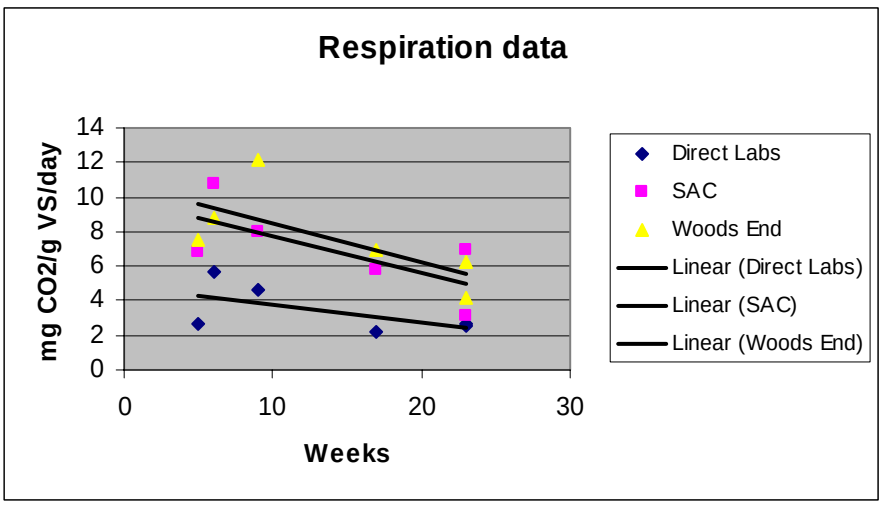
	Direct Labs	SAC	Woods End original	Woods End adjusted	Max	Min	Mean	Age
R3GP	4.6	8.0	22.1	12.2	12.2	4.6	8.3	9 weeks
R16\1GP	2.7	3.1	7.7	4.2	4.2	2.7	3.3	23 weeks
R4GP	5.7	10.8	16.0	8.8	10.8	5.7	8.4	6 weeks
W3WT	2.2	5.7	12.6	6.9	6.9	2.2	5.0	17 weeks
W7\8WT	2.7	6.8	13.7	7.5	7.5	2.7	5.7	5 weeks
W1\2WT	2.5	7.0	11.4	6.3	7.0	2.5	5.2	23 weeks
Mean	3.4	6.9	13.9	7.7				
LSD (0.05) between samples =			**2.47**	**& between labs =**		**1.75**		

Table 9.17 CO_2 evolution mg CO_2/g VS/day – omitting four SAC replicates

	Direct Labs	SAC	Woods End original	Woods End adjusted	Max	Min	Mean	Age
R3GP	4.6	8.0	22.1	12.2	12.2	4.6	8.3	9 weeks
R16\1GP	2.7	3.1	7.7	4.2	4.2	2.7	3.3	23 weeks
R4GP	5.7	10.2	16.0	8.8	10.2	5.7	8.2	6 weeks
W3WT	2.2	4.5	12.6	6.9	6.9	2.2	4.5	17 weeks
W7\8WT	2.7	6.0	13.7	7.5	7.5	2.7	5.4	5 weeks
W1\2WT	2.5	5.2	11.4	6.3	6.3	2.5	4.7	23 weeks
Mean	3.4	6.2	13.9	7.7				
LSD (0.05) between samples =			**2.28**	**& between labs =**		**1.61**		

Chart 9.3 shows how the trendlines for each laboratory against age of compost. The ADAS data set and trend line are lower than those for SAC and Woods End adjusted.

Chart 9.3 Respiration data



Comparison between Direct Labs and SAC results

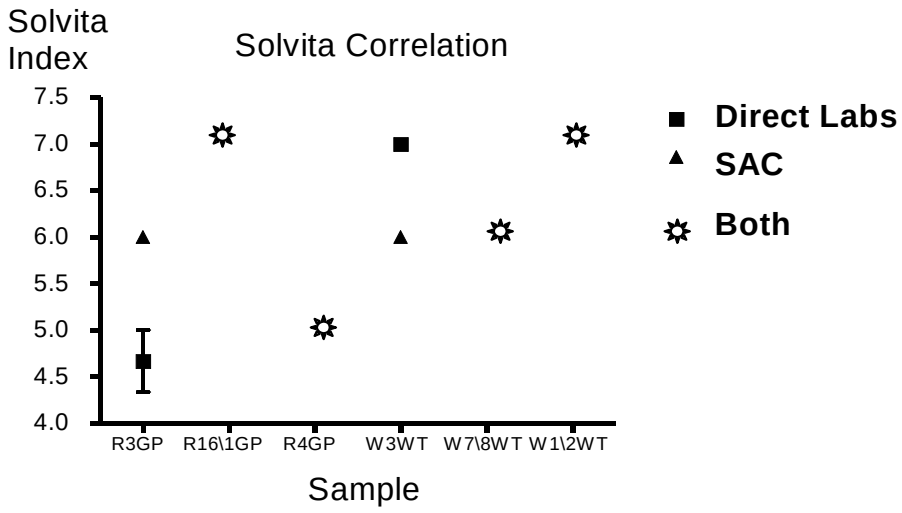
An analysis of the data generated by Direct Labs and SAC utilising the replicate data was carried out.

Solvita

Source of Variation	Degrees of Freedom	Sum of Squares	Mean square	Probability
Laboratories	1.0	0.02778	0.02778	not significant
Samples	5.0	21.47	4.294	< 0.0001
Interaction	5.0	4.139	0.8278	< 0.0001
Residual (error)	24.0	0.6667	0.02778	
Total	35.0	26.31		

The data showed that there was good agreement between laboratories and that the differences between the samples were significant and detected by the method. (In Chart 9.4 where a star is shown, both laboratories had the same result).

Chart 9.4 Solvita index

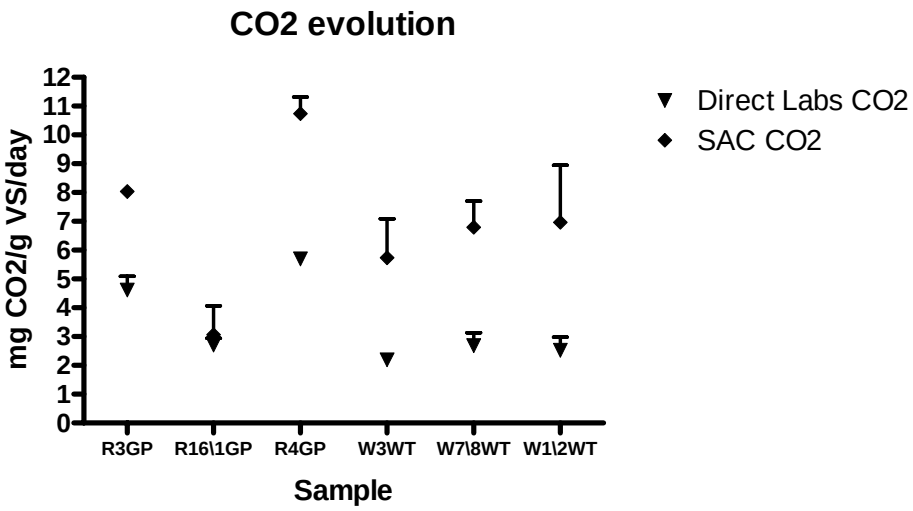


Respiration

Source of Variation	Degrees of Freedom	Sum of Squares	Mean square	Probability
Laboratory	1.0	109.4	109.4	< 0.0001
Sample	5.0	105.8	21.16	< 0.0001
Interaction	5.0	20.20	4.041	not significant
Residual (error)	24.0	52.24	2.177	
Total	35.0	287.7		

The respiration data confirms that there were significant differences between the two laboratories in the results as well as there being significant differences between samples.

Chart 9.5 CO_2 evolution



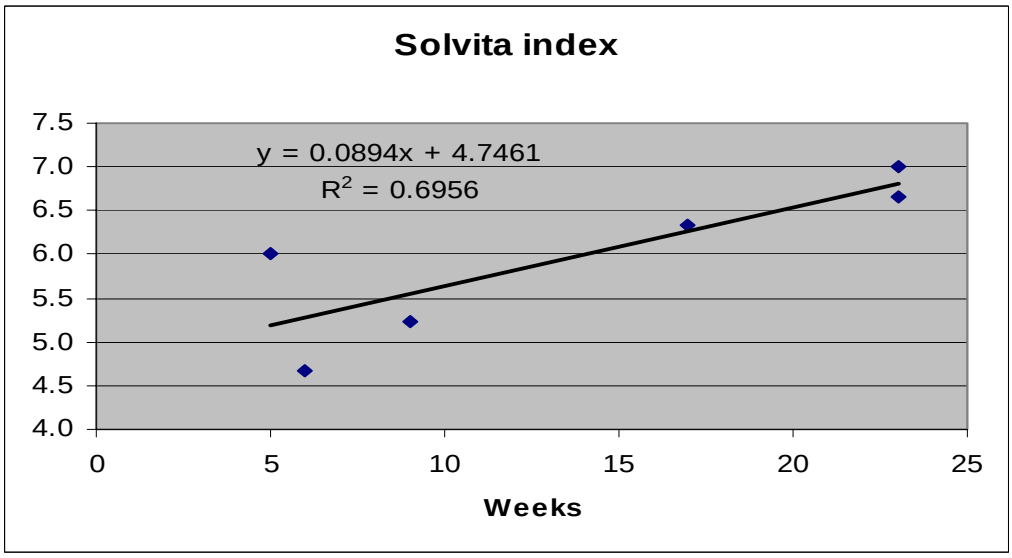
9.7.6 Interpretation

Although there was a large range in ages of the compost samples, from five to 23 weeks, the activity of the compost was not great in the young samples. Activity decreases greatly in the first few weeks of composting and a greater range of activity could have been usefully incorporated by using a very young compost, at about two weeks old.

Moisture content can also limit the activity of the microorganisms and, although moisture was above 40% in the test samples, it would be useful in the future to test samples at between 50 and 60% moisture with pre-conditioning.

The Solvita tests did pick out the oldest compost samples as shown in Chart 9.6 (averages from all three laboratories). The five week old compost was the driest sample which may have resulted in a higher index than expected.

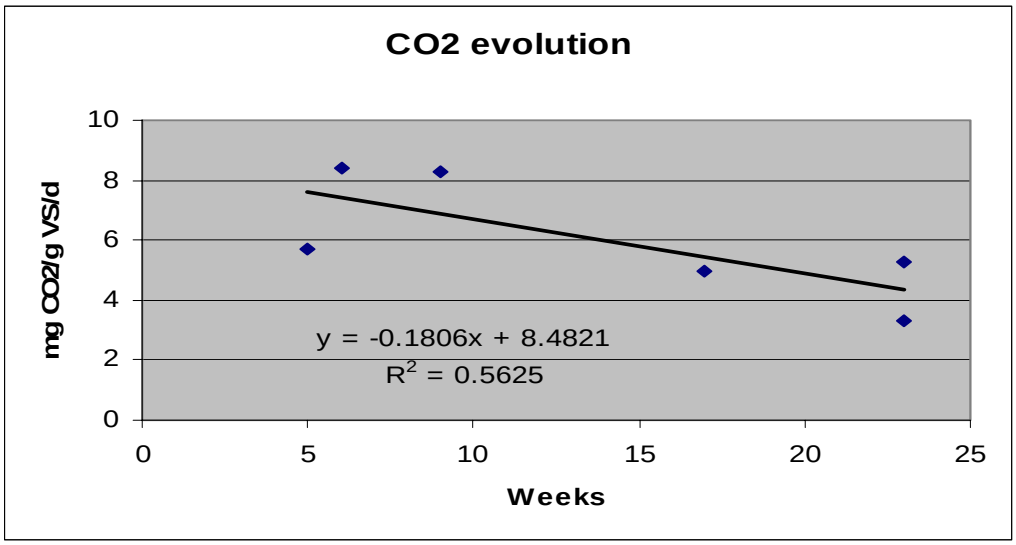
Chart 9.6 Solvita index – all laboratories



For respiration, measured as CO_2 evolution, the Direct Labs and SAC data show the trend of decreasing microbial activity with age of compost (Chart 9.7). Again the five week old sample seems to be lower in activity

than expected, possibly due to low moisture content. However, there are differences in test results between the laboratories for the samples and the reason for this needs to be determined if the method is to become a standard.

Chart 9.7 CO_2 evolution – all laboratories



9.8. Conclusions (Compost Stability Assessment Method)

A laboratory CO_2 method has been developed and tested that is sensitive at all stages of composting and at all respiration rates and levels of stability. This uses measurement of evolution of Carbon Dioxide which is in common with British Standards Institution and U. S. Composting Council Test Methods

As written, the method is capable of measuring stability as low as 0.01 mg CO_2/g VS/day. Replicates agree with a precision of less than 1 mg CO_2/g VS/day. However, in the statistical comparison between labs using the identical method, as reported under paragraph 9.7 above, the variation was higher.

The laboratory CO_2 method benefits from the ability to use a relatively large sample size. The method could be made even more sensitive by increasing the weight of compost taken or using a more dilute standard acid for titration.

The method described for determining compost stability by laboratory CO_2 technique combines features of several previous methods described by various authors.

The method is based on sound and fundamental scientific principles. Carbon dioxide evolution is a very direct measurement of aerobic respiration and therefore biological activity. It is also a direct measurement of carbon originating from the compost matrix.

The development of the continuous flow air replacement makes it a dynamic system, which several authors have demonstrated to be superior to static sealed systems.

The laboratory CO_2 method's main strengths are simplicity and ruggedness without reliance on complicated or expensive instrumentation. However, as it uses laboratory flasks and tubes and requires wet chemistry, an experienced chemist must conduct the procedure.

The method is inexpensive, convenient and easy to set up and use almost anywhere where temperature can be controlled.

The method is compatible with the BS method for assessing biodegradability of plastics and the AT_4 automated procedures, which are being strongly advocated in Europe.

The Youden ruggedness test showed it to be suitable for development as a standard procedure The Youden test also demonstrated the need for tight method standardisation to ensure intra-laboratory repeatability and inter-laboratory reproducibility. The Youden test was not however employed to assess interlaboratory variation after the preliminary in-lab test of the proposed method was completed. The interlaboratory trial (section 9.7) found considerably greater variation than the preliminary trial did.

We found that different types of composted wastes show different stability patterns over a composting period using both the laboratory CO_2 method a commercial field test kit.

10. Development of a Growing Test to Assess the Compost Readiness for Use

10.1. Introduction

Following submission of the ADAS report on test methods, it was agreed that a plant growth test would be developed, involving growth of plants directly in compost samples and not in compost extracts. This would reflect the way in which composts are likely to be used in practice. In particular -

- Compost samples would be mixed with a suitable diluent, to lower the electrical conductivity (EC) to a target value suitable for plant growth
- Plants would be grown in the diluted mixture, from seed, for a short and an intermediate period of time; a separate test, not covered here, would be needed for longer-term phytotoxic effects
- More than one plant species should be used, because of the differences in species sensitivity to phytotoxic factors

Vermiculite was suggested as the diluent, because this is used in real growing media and shows little chemical or physical variability. Perlite was also tested, for the same reasons and to see if one material gave better results than the other.

Radish (*Raphanus sativus*) was chosen as one species, because of its rapid growth. Lettuce (*Lactuca sativa*) was suggested as a second species to try, being sensitive to chloride and EC. However, little information is available on the sensitivity of these and other species to chemicals associated with compost immaturity, such as volatile organic acids.

10.2. Evaluation Tests

10.2.1 Adjusting sample electrical conductivity (EC)

In the current Composting Association test method for phytotoxicity, ADAS adjusts the EC of the sample by blending with sphagnum peat. The following formula is used:

Target EC 400* $\mu S\ cm^{-1}$

Peat EC 25 $\mu S\ cm^{-1}$

Example sample EC 1000 $\mu S\ cm^{-1}$

Then (1 x 1000) + (Y x 25) / (1 + Y) = 400

Where Y = parts of sphagnum peat, relative to one part of sample.

*****400 is used rather than 500 because the test runs for 28 days and the nitrogen level usually needs raising by addition of fertiliser: this will raise the EC above 400 μS.

Solving the equation in this example, Y = 1.6

It has been found that mixing peat **by weight** with the sample gives an EC fairly close to the target.

This method was tried with a sample of mature, composted green waste, with a high EC, 1606 $\mu S\ cm^{-1}$, using vermiculite and perlite. Initial analyses of the diluents gave average EC's of 30 and 22 $\mu S\ cm^{-1}$ respectively. (Major water extractable nutrient levels were all Index 0.) The target EC after adding the diluent was set as 450$\mu S\ cm^{-1}$. The actual EC's of the resulting mixtures obtained were as follows:

Table 10.1: EC following first trial dilution by weight (μS cm^{-1})

Diluent	Rep. 1	Rep.2
	μS cm^{-1}	
Vermiculite	171	158
Perlite	146	190

Clearly, the target was missed by a long way. However, the large proportions of diluents required to reduce such a high starting EC made uniform mixing difficult. The formula was therefore tested with a wider range of composts and with duplication of each mix.

Five composts, with a range of maturities, were obtained. Their initial EC's and those of the final, diluted mixtures using the above formula by weight, are shown below (target 400 μS cm^{-1}):

Table 10.2: EC following second trial dilution by weight (μS cm^{-1})

Sample	*Starting EC*	Diluent	Final EC	
			Rep. 1	Rep. 2
Compost No.				
1	501	V[1]	220	311
1	501	P[2]	301	292
2	419	V	210	298
2	419	P	246	353
3	673	V	300	170
3	673	P	277	274
4	484	V	280	279
4	484	P	297	282
5	876	V	152	138
5	876	P	254	241

[1] V = vermiculite; [2] P = perlite

The formula was obviously not working with vermiculite or perlite, in contrast to the previous findings with peat. It was decided to try once more, but mixing by volume not weight. In the example calculation above, this would mean mixing 1.6 volumes of vermiculite or perlite to one volume of sample. Using the same five samples of compost, the following final EC's were achieved, (target 400 μS cm^{-1}; no duplication).

Table 10.3: EC following first trial dilution by volume (μS cm^{-1})

Sample	Diluent	Final EC
Compost No.		
1	V	340
1	P	335
2	V	398
2	P	378
3	V	347
3	P	367
4	V	453
4	P	401
5	V	399
5	P	428

These results were much more satisfactory and similar to the success achieved with peat added by weight. Therefore, it was decided to proceed with the above formula, used on a volume basis. Further tests of the formula were made during two subsequent growth tests. Using samples 1 and 5 above only, the following results were obtained, (target EC 400 μS cm^{-1}):

Table 10.4: EC following second trial dilution be volume (μS cm^{-1})

Sample (growth test no.)	Diluent	Final EC
Compost No.		
1 (2)	V	386
1 (2)	P	380
1 (3)	V	509
1 (3)	P	552
5 (2)	V	416
5 (2)	P	356
5 (3)	V	384
5 (3)	P	479

This shows the variability of the method due to mixing and is considered to be satisfactory.

10.2.2 Growing trials

All growing trials were conducted in a heated glasshouse. The temperature control was set at 16 hours at 25^{o} C minimum / 8 hours at 15^{o} C minimum. Daytime temperatures on hotter summer days often rose into the 30's.

Growth test 1

The first growth trial was conducted with the five composts above, diluted with vermiculite or perlite by **weight**, (Table 10.2). To speed up testing, the growth test was set up before the EC results were available.

The mixtures were placed in 9cm (3.5 inch) plastic plant pots and sown with 10 radish seed (French Breakfast) or 10 lettuce seed, (All the Year Round). Seed were gently pushed into the growing media with a dibber. Pots were watered by fine rose, covered with plastic saucers, then stood in a plastic tray lined with capillary matting. Subsequent growth was observed.

Negative and positive controls were also set up. Negative controls consisted of 100% vermiculite or 100% perlite. Positive controls consisted of peat-based growing medium, prepared with added lime and nutrients.

When it was realised that the target EC's had not been achieved – and the test samples had been diluted too much – quantitative measurements on this trial were abandoned. However, several useful observations were made.

- Radish germinated quickly and was judged likely to produce sufficient plant material to weigh after seven days. Lettuce was much slower and even after 14 days had produced only small amounts of top growth.
- By growing in pots, emergence on selected dates could be assessed, but radical (root) length could not, without destructive sampling. This is in contrast to tests using compost extracts applied to filter paper.
- Fresh weights of whole plants (tops plus roots), on selected dates, could be assessed by destructive sampling. Roots generally needed rinsing off by dipping in water, to remove adhering growing media, then dabbing on absorbent paper to remove excess water. However, this was simple to perform and it is considered that the errors in the technique are less than those associated with trying to measure the length of very short, curling radicals.
- Vermiculite produced slightly more vigorous plants than perlite.

Growth test 2

The first and fifth compost samples above, identified as least mature (1) and screened/mature (5), were diluted with vermiculite or perlite **by volume**. The starting EC's of the mixtures are shown in Table 10.4

above, identified as Growth Test no. 2. Pots were sown with eight radish (French Breakfast), eight lettuce (Valdor, a winter hardy variety, which it was hoped might prove more vigorous), or six peas (Meteor, a hardy variety). Peas were added to see if sufficient top growth to weigh, of a second species in addition to radish, could be produced in seven days. Also, it had been suggested by one of the Peer Review panel, that pea radicals were sensitive to residues of hormone herbicides such as clopyralid.

In view of the previous difficulty in observing root development, all seed were placed on the growing media surface. It was anticipated that, by covering the pots, sufficiently high humidity would be maintained for germination.

Initial radish growth was good. However, some plants later died off, because their roots were not well anchored in the growing media. This problem did not occur in the positive controls (peat-based growing medium). Lettuce performed similarly, with quite good plant numbers after seven days, but subsequent loss of plants. Peas did not perform well. Germination was slow, probably because of insufficient seed / growing medium contact. Also many seed rotted without germinating. Peas which did establish, eventually grew away very well, but this was after 14 days.

Plant numbers on seven and fourteen days after starting (DAS) are shown in the tables below:

Table 10.5: Control plant numbers

DAS		100% V	100% P	Peat-based
7	Radish	5	7	7
	Lettuce	10	7	8
	Pea	1	5	3
14	Radish	4	4	7
	Lettuce	9	7	8
	Pea	4	5	2

Table 10.6: Sample 1 plant numbers

DAS		V	P
7	Radish	7	5
	Lettuce	7	5
	Pea	4	3
14	Radish	5	4
	Lettuce	5	5
	Pea	2	2

Table 10.7: Sample 5 plant numbers

DAS		V	P
7	Radish	5	7
	Lettuce	6	7
	Pea	5	4
14	Radish	3	4
	Lettuce	4	4
	Pea	3	3

Fresh weights of whole plants (roots plus tops) after 14 days are shown in the table below:

Table 10.8: Fresh weight per pot (g)

Sample	Radish	Lettuce	Pea
Compost No.			
100% V	0.7	0.4	3.1
100% P	0.6	0.3	4.4
Peat-based standard	1.7	0.9	5.2
1 V	2.2	0.5	4.1
1 P	2.4	0.6	2.8
5 V	1.5	0.2	11.6
5 P	1.3	0.8	6.5

The relatively-high pea weights were due partly to the seed.

The following observations were made:

- Final growth was better in the test mixes than the negative controls (100% vermiculite or perlite)
- Growth in vermiculite was slightly more vigorous than in perlite
- Pea roots became very extensive and required a lot of washing, to remove adhering growing medium
- Overwatering of the capillary matting must be avoided, because this leads to damping off of sensitive species, (lettuce and pea). It is recommended that the capillary matting is used only as an emergency water reservoir, but that watering is undertaken from the surface, with a fine rose. The matting should be kept moist, but water must not be allowed to pond above it.

Growth test 3

The first two tests had found that only radish produced sufficient plant material to weigh after seven days. Lettuce barely produced enough after 14 days. Therefore, it was decided to test a greater range of species.

The first requirement of a test species is that it grows sufficiently quickly to produce enough plant material for assessment in the time period used. However, the species chosen must also be sufficiently sensitive to the phytotoxic factors involved in compost immaturity to differentiate samples adequately. A number of species had been suggested in the previous ADAS literature review, or by members of the Peer Review committee, for example rapidly-growing brassica species. Brassicas, though, include radish: members of the same species are likely to be sensitive to similar phytotoxic substances. It is also likely that the more rapidly a species grows, the less sensitive it is. This can be seen with cereals and oilseed rape, which are used in existing ADAS bioassays. For this reason, radish was selected as the preferred brassica and an additional, unrelated species was sought.

Several ornamentals were selected on the basis that they were normally quite vigorous and easy to grow. These included Aster (*Callistephus chinensis*), which ADAS data suggested was sensitive to salt (sodium chloride). Green lentil (*Lens culinaris*) was also included. This is already used by ADAS in a bioassay to detect residues of sulfonylurea herbicides, in which very good growth is achieved after 14 days. Research-based agrochemical companies also use this species.

All seeds were covered by the growing media.

Composts 1 and 5 above were again used, diluted with vermiculite or perlite to give the starting EC's shown in Table 10.4 above, (Growth Test no. 3). Negative and positive controls were again included. One set of additional negative controls was added, in which 100% vermiculite or perlite pots were watered initially with a standard Phostrogen liquid feed, rather than deionised water, (0.5 g Phostrogen / litre deionised water, equivalent to 50 mg N / l). This was done because of the poorer growth found above in some of the negative controls compared to the test mixes. These pots were to be sampled at 14 DAS, but unfortunately this was not done until 17 DAS.

Species and seed numbers sown were as follows:

Radish 8

Lettuce 8

Lentil 8

Aster 8

Marigold (*Tagetes patula*) 8

Nasturtium (*Tropaeolum majus*) 4

Rhudbeckia (*Rudbeckia hirta*) 8

Plant numbers on 7 and 14 DAS are shown in the tables below:

Plant numbers

Table 10.9: Controls

Species	DAS	100% Ver	100% V+Phos	100% Per	100% P+Phos	Peat-based
Radish	**7**	8	8	8	8	8
	14	7	8	8	8	8
Lettuce	**7**	5	6	7	5	7
	14	4	7	5	6	7
Lentils	**7**	6	7	7	0	8
	14	5	7	7	4	7
Aster	**7**	0	0	0	0	0
	14	0	0	0	0	0
Marigold	**7**	6	7	7	8	8
	14	3	6	7	8	8
Nasturtium	**7**	2	3	3	3	2
	14	4	3	4	3	4
Rudbeckia	**7**	4	5	2	3	4
	14	7	7	3	4	5

Table 10.10: Test mixtures

Species	DAS	Sample 1/Ver	Sample 1/Per	Sample 5/Ver	Sample 5/Per
Radish	**7**	8	8	8	7
	14	7	8	8	7
Lettuce	**7**	8	6	3	4
	14	8	7	7	7
Lentils	**7**	5	6	8	7
	14	5	6	8	8
Aster	**7**	0	0	0	0
	14	0	0	0	0
Marigold	**7**	7	6	8	7
	14	6	7	8	8
Nasturtium	**7**	1	2	1	0
	14	1	2	4	4
Rudbeckia	**7**	2	3	1	1
	14	2	4	5	5

Fresh weights of whole plants (g)

Table 10.11: Controls

Species	DAS	100% Ver	100% V+Phos (17 DAS)	100% Per	100% P+Phos (17 DAS)	Peat-based
Radish	**7**	0.3		0.3		1.2
	14	0.9	2.9	1.2	3.1	5.1
Lettuce	**7**	<0.1		<0.1		<0.1
	14	0.1	1.2	0.6	0.9	1.1
Lentils	**7**	1.2		1.2		2.5
	14	2.5	3.6	1.8	1.5	4.3
Aster	**7**	0		0		0
	14	0	0	0	0	0
Marigold	**7**	0.2		0.1		0.3
	14	0.5	2.2	0.8	1.7	1.8
Nasturtium	**7**	0.6		2.4		1.1
	14	3.4	6.0	2.8	6.4	6.2
Rudbeckia	**7**	<0.1		0		<0.1
	14	0.5	0.6	0.1	0.6	0.6

Table 10.12: Test mixtures

Species	DAS	Sample1/Ver	Sample1/Per	Sample 5/Ver	Sample 5/Per
Radish	**7**	1.1	1.2	0.8	0.7
	14	2.0	2.4	5.3	5.1
Lettuce	**7**	<0.1	<0.1	0	0.1
	14	0.9	0.9	0.4	0.8
Lentils	**7**	1.4	1.4	1.9	1.2
	14	3.0	3.9	4.3	5.8
Aster	**7**	0	0	0	0
	14	0	0	0	0
Marigold	**7**	0.1	0.3	0.1	0.2
	14	1.0	1.0	1.8	1.6
Nasturtium	**7**	0.3	1.0	0	0
	14	1.8	3.5	7.2	7.3
Rudbeckia	**7**	0	0.1	0.1	0
	14	<0.1	0.7	0.6	0.1

The results suggest the following:

- Lettuce produces just sufficient plant material after 14 days to be useful as a test species
- Lentils perform sufficiently well to use as a test species, in particular for a 14-day growth period
- Nasturtium may be useful in a 14-day test, though growth was poor in sample 1 (immature compost)
- Marigold may be useful in a 14-day test
- Results were broadly similar between vermiculite and perlite
- Using a liquid feed with the negative controls is probably worthwhile – growth was generally better and sometimes similar to the positive (peat-based) control.

Proposed Method for Phytotoxicity as a Measure of Maturity

The growing tests up to this point led to the following proposed method:

A growing test should be run in which two unrelated plant species are grown and assessments are made after 7 days on one and 7 and 14 days on the second. Radish (e.g. French Breakfast) is the only species found suitable for assessment after 7 days. Lettuce (outdoor or cold-tolerant variety) or green lentils are considered suitable for assessment after 14 days. The test sample should be diluted to a target starting electrical conductivity of 400 $\mu S\ cm^{-1}$, using the formula described above (9.2.1). A higher figure could be used, up to 500 $\mu S\ cm^{-1}$ and this would allow more of the sample to be used. However, the lower figure allows for error and variation in the use of the formula, with a reasonable safety margin against EC's remaining too high.

Dilution should be conducted using vermiculite. A subsequent test, (conducted concurrently with Growth test number 4), compared lettuce and lentil growth in 100% fine versus 100% standard grade vermiculite. Lettuce growth was slightly better in standard vermiculite, whereas lentil growth was appreciably better in fine vermiculite. On this basis, it is recommended that fine vermiculite should be used, if available, but that standard grade is satisfactory.

The growing test should be run in new (previously unused) 9 cm (3.5-inch) plastic plant pots. Eight seed should be sown per pot. Seed should be covered by the growing medium, by gently pushing them below the surface until no longer visible. Pots should be covered with inverted saucers until emergence starts. Pots should be placed on capillary matting, isolating different test samples and positive and negative control samples from each other.

The growing test should be conducted under controlled light and temperature conditions, with a 16 hour day at 25^{o} C minimum and 8 hour night at 15^{o} C minimum, using artificial lighting to achieve at least a 16 hour

day. Illumination should equal at least 10,000 Lux at the bench top and be provided by high pressure sodium lamps. Lower temperatures and less light will reduce the amount of plant material available to assess, which is already low.

Negative controls should be set up, using 100% vermiculite of the same batch as used to dilute the sample. This should be watered initially (until fully wetted up) with a liquid feed, containing approximately 50 mg/l N, plus phosphorus and potassium at approximately a similar concentration to this. Proprietary feeds with N:P:K ratios such as 10.10.27 are acceptable. Subsequent watering of these controls should be with plain de-ionised water. The negative controls are used to compare growth in the assessments, but also demonstrate that the vermiculite and water is uncontaminated.

Positive controls should also be established, with a standard peat-based growing medium. These controls demonstrate that the environmental conditions in the glasshouse are satisfactory for plant growth.

Pots should be covered initially. Groups of pots can be covered by plastic sheeting. The cover should be removed from each pot as soon as the first seed germinates, to reduce the risk of damping off. This will require the use of individual pot covers from this point onwards, such as inverted plant pot saucers.

All water used should be de-ionised. Watering should be conducted from the surface, using a fine rose. The objective should be to keep the surface moist and not let the capillary matting become any wetter than moist: no water must pond above the matting.

Radish growth should be measured by counting number of plants emerged per pot on 7 days after starting (DAS), plus total weight of whole plants per pot on 7 DAS. The latter should be determined by removing the whole plants from the growing medium and removing adhering growing medium by gently shaking, then immersing in water, followed by dabbing on absorbent paper. All plants from all treatments must be treated in an identical manner.

The second species (lettuce or lentils) should be assessed on 7 days for numbers emerged, then 14 DAS, using the same measurements and procedures as for radish. In addition, any unusual foliar or root symptoms (compared to the negative controls) should be noted.

Growth test 4

The work so far had lead to detailed proposals for a plant growth test. A suitable methodology had been devised and tested, with appropriate test species – radish for the seven-day test and lettuce or lentils for the 14-day test. A further growth trial was then conducted, to calibrate the test. Various thresholds are proposed in the literature as the basis on which to interpret growth test results, e.g. the test sample should achieve at least 80% of the growth of the control, (Composting Association, 2000). Some methods, e.g. the Swiss ASAC Guidelines (2001), propose different thresholds according to the proposed use, with higher thresholds for more demanding uses such as protected horticulture and private gardening.

However, none of these methods report the scientific basis for the thresholds proposed and it is likely that they are based on previous practice or personal judgement. This raises the question as to how well the tests can differentiate between composts of different maturity. In particular, the concerns of authors such as Warman (1999) and Brinton (2000), that plant tests may not be satisfactory tests of compost maturity, are not addressed by such an approach.

Therefore a test was conducted to try to provide a scientific basis on which to set thresholds for interpretation, rather than adopting those reported in the literature. This was conducted using the proposed method, on six further composts with a range of maturities. The six composts and their starting plus diluted (final) EC's, are shown in Table 10.13. Further information on the sourcing of the composts used in the tests is provided at Annex C.

Table 10.13: Composts used in calibration trial

Sample no.	Compost no.	Type[a]	Maturity[b]	Starting EC	Final EC[c]	
				µS cm^{-1}	**Rep 1**	**Rep 2**
					µS cm^{-1}	µS cm^{-1}
1	10	BMW	Fresh	1085	497	587
2	7	GW	Fresh	396	429	473
3	11	BMW	Semi	833	553	590
4	8	GW	Semi	801	298	277
5	12	BMW	Mature	353	357	355
6	9	GW	Mature	454	270	195

[a] BMW = biodegradable municipal waste; GW = green waste
[b] Fresh = immature; semi = semi-mature; mature = composting finished
[c] Diluted with vermiculite, target 400 µS cm^{-1}

For each sample, three plant species were grown (radish var. French breakfast, Green lentil, lettuce var. Winter density), sowing eight seeds of each species per pot. Negative (100% vermiculite, watered initially with nutrient solution containing 50 mg l^{-1} N) and positive (peat-based growing medium) controls were set up. All treatments were replicated six times and arranged in six blocks, (total 144 pots, 90mm (3.5-inch)). All pots were placed in individual saucers, lined with capillary matting.

Radish was assessed on 7 DAS, (plant numbers per pot and fresh weight of whole plants per pot). Lentils and lettuce were assessed on 7 and 14 DAS, (plants numbers on 7 and 14 DAS, plus fresh weight of whole plants per pot on 14 DAS).

Results

Table 10.14 shows the results for individual composts plus controls and Table 10.15 shows results for each maturity category of the six test composts. Compost identifier numbers are shown in Annex C. The results for each individual pot are shown at Annex D.

Table 10.14: Analysis of plant numbers and yield for test composts in calibration test, showing means of six replicates for individual test composts

	Sample										
	1	2	3	4	5	6	7	8	F prob	sed	res df
Compost No.	10	7	11	8	12	9	-control	+control			
Plant numbers											
Radish (7 day)	6.50	7.67	7.33	7.17	7.67	7.33	7.00	8.00	0.036	0.417	35
Lentil (7 day)	6.83	7.17	7.33	7.83	7.50	7.67	7.83	7.50	* 0.182	0.388	35
Lentil (14 day)	7.33	7.33	7.67	8.00	7.33	8.00	7.67	7.83	* 0.280	0.357	35
Lettuce 7 day	*1.2*	*1.7*	*1.5*	*0.7*	*0.5*	*0.8*	*1.0*	*1.8*			
Lettuce 14 day	*2.2*	*1.5*	*3.0*	*0.5*	*1.7*	*1.3*	*1.7*	*1.5*			
Yield g											
Radish (7 day)	0.70	0.82	1.03	0.68	0.88	0.94	0.57	1.40	<0.001	0.159	35
Lentil (14 day)	3.43	2.13	3.00	3.53	2.82	3.75	3.22	5.08	<0.001	0.402	35
Lettuce 14 day	*0.028*	*0.010*	*0.052*	*0.003*	*0.037*	*0.010*	*0.028*	*0.032*			

* Not significant

Table 10.15: Analysis of plant numbers and yield for test composts in calibration test, showing effects of compost maturity groups (mean of 12 values)

	Fresh	Medium	Mature	F prob	sed	Res df
Plant numbers						
Radish (7 day)	7.08	7.25	7.50	* 0.462	0.333	28
Lentil (7 day)	7.00	7.58	7.58	* 0.069	0.278	28
Lentil (14 day)	7.33	7.83	7.67	* 0.176	0.265	28
Yield g						
Radish (7 day)	0.76	0.86	0.91	* 0.342	0.105	28
Lentil (14 day)	2.78	3.27	3.28	* 0.283	0.350	28

* Not significant

1) Radish

Plant numbers (7 days)

There were no significant differences between the negative control (Sample 7) and the six test composts ($P < 0.05$). Only the negative control and Sample 1 gave significantly lower plant numbers than the positive control (Sample 8) ($P < 0.05$).

There were no significant differences between the three compost maturity groups.

Yield (7days)

The negative and positive controls gave the lowest and highest yields respectively. The positive control had a significantly higher yield than all six test composts and the negative control, while the negative control was only significantly lower than samples 3 and 6.

There were no significant differences between the three compost maturity groups.

2) Lettuce

Lettuce growth was very poor in all treatments. Plant numbers and yields (means shown in Table 10.14) were considered to be too low for statistical analysis.

3) Lentils

Plant numbers (7 and 14 days)

There were no significant differences between any of the eight treatments, or between the compost maturity types.

Yield (14 days)

The positive control (Treatment 8) had a significantly higher yield than all the other seven treatments. Other than for sample 2, there were no significant differences between the negative control and the other five test composts, (samples 1,3,4,5 and 6).

There were no significant differences between the three compost maturity groups.

Discussion

1) Radish and lentils

The negative control does not appear to be suitable, despite satisfactory results in the earlier tests. On this basis, it is recommended that an "optimum" growing medium such as the positive control is used.

The most important result is that the test did not differentiate between composts of different maturity. There were very few differences between treatments in plant numbers. The positive control gave better yields than all six test samples and the negative control, for both species. The negative control did not give better yields than the test samples, except in the case of lentil yield and Sample 2.

There are two possible reasons why the test proved to be insensitive to compost maturity. Firstly, it is possible that an unfortunate group of composts were tested and the test samples were not good examples of the maturity classes ascribed. Their maturity classes were assigned on the basis of how long they had been composted. In the absence of a test to determine maturity, this is the obvious basis on which to select samples. It is possible that length of composting period did not, on this occasion, reflect the levels of phytotoxic factors present. It is possible that with other samples it may have done. The stability of the composts as measured by CO_2 production did show significant differences in table 9.9. However, in appearance, five out of six of the samples were course, fibrous and woody and this included the two mature samples. The possibility remains, therefore, that the samples were not representative of the maturity classes they were intended to represent.

A more profound explanation is that suggested by authors such as Warman (1999) and Brinton (2000), that plant growth tests may not be suitable for identifying compost maturity. Theories can be proposed as to why this might be the case. In particular, there is the dilemma with plant growth tests that plants will not normally grow in undiluted composts (principally because of the high electrical conductivity), yet if they are diluted to an appropriate conductivity, the phytotoxic factors may have been diluted to a level at which they no longer have adverse effects. The more accurately the sample conductivity is adjusted, the better the growth will be. There is also the possibility that the growth period is too short for some phytotoxic effects to become apparent and here it is important to consider Warman's comments (1999) that maturity and phytotoxicity may not be the same. The objective, agreed with the funders, was to develop a rapid test, yet important effects may be slow to develop.

The basis on which thresholds for interpretation were drawn up in other tests in the literature is not reported. As previously discussed, it is likely that most or all have no statistical basis. The results of the fourth ADAS growth test suggest that most or all of these other tests may be unable to differentiate compost maturity. Warman (1999), in contrast, does report significance when comparing three plant growth tests and found that none were sensitive enough to detect differences in maturity.

2) Lettuce

There are several possible reasons for such poor growth of the lettuce. Of the three test species used in Growth test no. 4, lettuce was expected to give the lowest plant weights, based on the previous tests, (see for example Tables 9.9 – 9.12). In the previous tests, weights were only just sufficient for the species to be considered suitable. The poorer growth in test 4 than previous tests may have been due to the lower light and temperatures of late Autumn, despite supplementary lighting and heating. Temperature records show a mean maximum of 23°C and mean minimum of 16°C were achieved during test 4, close to the targets of 25°C and 15°C respectively. Light and temperature conditions may still have been below those of early and mid-summer, when the previous tests were run.

A different lettuce variety, Winter density, was grown in test 4. It is possible than this is less vigorous than the earlier varieties, All the Year Round and Valdor.

Whatever the reason, the growth of lettuce is clearly inadequate and affected by too many external factors for it to be considered a reliable species in a 14-day growth test. It is recommended, therefore, that lettuce is discounted as a test species.

10.3. Conclusion

A plant growth test has been developed, but in a replicated calibration test over 7 and 14 days, several composts could not be differentiated on the basis of their apparent maturity. There is a possibility that the test samples used were not representative of the maturity classes sought. However, the results raise the possibility that plant growth tests may be unreliable for assessing compost maturity. This may be a result of diluting the sample with another growing medium, which could have the effect of diluting phytotoxic factors to non-significant levels. However, if composts were to be tested in the undiluted state, using plants, then in most cases growth effects would be dominated by the electrical conductivity and little would be learnt that could not have been predicted from the preliminary chemical analysis. As dilution with another medium reflects likely use in practice, this is considered to be the most relevant approach for a test.

There is also the possibility that the duration of the tests investigated was too short for phytotoxic effects to have become apparent. Dilution with another growing medium may minimise short-term phytotoxic effects, (dominated by electrical conductivity), but longer-term effects might still develop. Again, since dilution and longer-term growth reflect real use in practice, the investigations reported above may lead to the conclusion that plant growth tests are not appropriate as rapid measures of compost maturity. The work certainly suggests that plant growth tests should not be relied on as the sole method of assessing maturity.

11. Glossary of Abbreviations

AT_4	Respiration method over 4 days
AT_7	Respiration method over 7 days
ATP	Adenosine triphosphate
BOD	Biochemical oxygen demand (or biological/chemical oxygen demand)
BMW	Biodegradable Municipal Waste
C	Carbon
CCME	Canadian Council of Ministers of the Environment
CCQC	California Compost Quality Council
CEC	Cation Exchange Capacity
CIWMB	California Integrated Waste Management Board
DOC	Dissolved Organic Carbon
DSC	Differential Scanning Calorimetry
EC	Electrical Conductivity
FA	Fulvic Acid
FT-IR	Fourier Transform Infrared
GI	Germination Index
HA	Humic Acid
LOI	Loss On Ignition
NMR	Nuclear Magnetic Resonance
MDG	Mean Days to Germination
MSW	Municipal Solid Waste
NDI	Nitrogen Drawdown Index
NIR or NIRS	Near Infrared Reflectance Spectroscopy
PET	Photosynthetic Electron Transport
RAL	German quality symbol
SOUR	Specific Oxygen Uptake Rate
TOD	Total Oxygen Demand
TOC	Total Organic Carbon
VOA	Volatile Organic Acids
VS-C	Volatile Solids Carbon
WSC	Water Soluble Carbohydrate

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Annex A. Standardised Method for the Determination of Compost Stability by Measurement of Evolved Carbon Dioxide

A Method To Determine The Aerobic Stability Of

Composted Organic Materials

Contents **Page**

Safety warning
Care should be taken when handling samples that may contain sharp fragments, chemical contaminants or possible pathogenic organisms.

1. Scope and field of application

A method for the determination of aerobic stability of composted materials. The sample shall be obtained in accordance with SOIL IMPROVERS AND GROWING MEDIA - SAMPLING (EN 12579) The procedures described herein are not necessarily applicable to or suitable for all types of composted materials.

2. Normative references

This method incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this method only when incorporated in it by amendment or revision. For undated references the latest edition of the publications referred to apply.

ISO 5725:1994	Precision of test methods - determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.
EN 12579:2000	Soil improvers and growing media - Sampling
EN 13040:1999	Soil improvers and growing media - Sample preparation for chemical and physical test, determination of dry matter content, moisture content and laboratory compacted bulk density
EN 13039:2000	Soil improvers and growing media - Determination of organic matter content and ash
PAS 100:2002	Specification for composted material

3. Principle

Moisture adjusted compost is incubated at 25°C with continuous replacement of carbon dioxide free air. Carbon dioxide evolved from the compost is collected in a sodium hydroxide solution as sodium carbonate. The collected carbonate is precipitated as barium carbonate by the addition of excess barium chloride. The concentration of carbon dioxide evolved by the compost is measured by titration of the residual sodium hydroxide with standard acid.

NOTE Barium carbonate is not decomposed by the action of the acid when phenolphthalein is used as an indicator, colour change occurs at pH 8.5.

4. Definitions

For the purpose of this standard the definitions given in PD CR 13456, EN 12579, EN13040 and PAS 100 the following apply:

Maturity

Ready and suitable for its intended use.

5. Reagents

5.1 General

All reagents used shall be of recognised analytical quality. Use water of grade 2 complying with EN ISO 3696

5.2 Saturated barium chloride solution

c($BaCl_2$) = dissolve an excess of $BaCl_2.2H_2O$ in 1 litre of water and filter.

5.3 Hydrochloric acid

c(HCl) = 1mol/l; purchase this solution ready prepared

5.4 Phenolphthalein indicator solution

c($C_{20}H_{14}O_4$) = dissolve 1g of phenolphthalein in 100 ml of ethyl or isopropyl alcohol. Add 100 ml water. The indicator may be purchased ready prepared.

5.5 Sodium hydroxide

c(NaOH) = 1mol/l; purchase this solution ready prepared and
standardised in a collapsible airtight container. Discard when blanks turn cloudy after addition of barium chloride.

6. Apparatus

6.1 Constant temperature room or incubator

capable of maintaining a temperature of 25 ± 1 °C.

6.2 Carbon dioxide scrubbing vessel

500 ml Drechsel bottle design or similar fitted with a sintered disc e.g. aquarium air diffuser.

6.3 Carbon dioxide collecting vessel, 100 ml Drechsel bottle design or similar fitted with a sintered disc, e.g. aquarium air diffuser. A simple 150 ml test tube with rubber bung fitted with inlet and outlet tube connections is sufficient.

6.4 Incubation vessels, 500 ml – 1000 ml polyethylene jars with airtight screw top lids incorporating internal and external inlet and outlet tube connections.

6.5 Flexible tubing, narrow bore plastic**.**

6.6 Air pump, small aquarium type. Ability to adjust airflow is advantageous but not essential.

6.7 Dispensing pipette, 50 ml capacity, grade A.

6.8 Burette, 50 ml capacity, grade A.

6.9 Titration flask, Erlenmeyer type 500 ml

6.10 Magnetic stirrer, optional

6.11 Sieve, 20 mm apertures

6.12 Balance, capable of weighing 120 g with an accuracy of 0.1 g

6.13 Diffusers, Aquarium type or similar

6.14 Flow restrictor or bleed valve to adjust flow. Only needed if pump is not adjustable.

7. Procedure

7.1 Apparatus

Sequentially connect together with the flexible tubing **(6.5)** the air pump **(6.6),** the carbon dioxide scrubbing vessel **(6.2),** the incubation vessel **(6.4)** and the carbon dioxide trapping vessel **(6.3).**

7.2 Sample preparation

7.2.1 Prepare the test sample in accordance with EN 13040:1999, clause 8.5.

7.2.2 Determine the volatile solids in accordance with EN 13039:2000.

7.2.3 Determine the total solids of the sample (**7.2.1)** in accordance with EN 13040:1999, clause 10

7.2.4 Adjust the total solids concentration of approximately 500g of sample **(7.2.1)** to between 40 % and 60 % mass/mass by small additions of water. Add water gradually with mixing until compost is visibly moist but no free liquid drains. Compost must remain friable with plenty of air porosity. Seal the wetted sample in a plastic bag excluding most of the air. Leave to equilibrate at 25^{o}C for three days.

7.2.5 Determine the final total solids of the sample (**7.2.4)** in accordance with EN 13040:1999, clause 10.

NOTE 1 For a four-day test it is very convenient to adjust the moisture on a Friday afternoon, equilibrate over the weekend, and start the incubations on the Monday afternoon.

7.3 Determination of carbon dioxide evolution rate.

After the 3 days transfer 100 g ± 2 g of the sample **(7.2.4)** weighed to the nearest 0.1 g to the incubation vessel **(6.4).** Transfer approximately 250 ml of sodium hydroxide solution **(5.5)** to the carbon dioxide scrubbing vessel **(6.2)** and accurately pipette 50.0 ml of 1 M sodium hydroxide solution **(5.5)** into the carbon dioxide collecting vessel **(6.3).** Switch on the air pump and adjust the airflow rate **(6.14)** to approximately 1–2 l / hr. After 24 hrs wash the internal delivery tube and aerator into the collecting solution and transfer into a pre-prepared collecting tube containing a further 50 ml 0f 1M sodium hydroxide. Stopper the tube being removed to prevent absorption of atmospheric carbon dioxide. Note the times the first trap is removed and the replacement trap fitted. Repeat this process every 24 hours over a 4-day period. Do not turn off the air pump at any time or backpressure may cause NaOH to siphon back to the pump.

Transfer the contents of the carbon dioxide trapping vessel **(6.3)** into the titration flask **(6.9)** with water washing. Add 20 ml of barium chloride solution **(5.2)** to precipitate any carbon dioxide. Add two to three drops of phenolphthalein solution **(5.4)** and titrate with 1M hydrochloric acid **(5.3)** until the pink colour just changes to white (colourless in the case of blanks) with one drop of the acid**.**

NOTE 1 In the presence of strong alkali is better to use rubber stoppers than glass stoppers.

7.4 Determination of blank value

An apparatus and reagent blank test shall be carried out in parallel with the determination, by the same procedure using the same quantities of all reagents but omitting the test portion.

NOTE 1 If the apparatus has been set up correctly the titration value shall be very near to 50 ml indicating that all atmospheric carbon dioxide has been trapped in the first trapping vessel.

NOTE 2 It is preferable to set up a series of parallel tests using the same pump to facilitate running replicates and blanks simultaneously with the same batch of reagents.

8 Calculations and expression of results

The mass of carbon dioxide evolved over 4 days is given by the following equations

mg CO_2 evolved per 24 h time period = {[$B_{vol} - S_{vol}$] x 44.2} / 2

Total mg CO_2 = sum of mg CO_2 evolved over 4 days

mg CO_2/g VS/d = [Total mg CO_2] / [dry weight of compost x VS x t]

where
B_{vol} is the volume in ml M HCl for the blank titre
S_{vol} is the volume in ml M HCl for the sample titre
dry weight of compost is amended compost (**7.2.5**)
VS is the mass of volatile solids / g of compost
t is the time in days

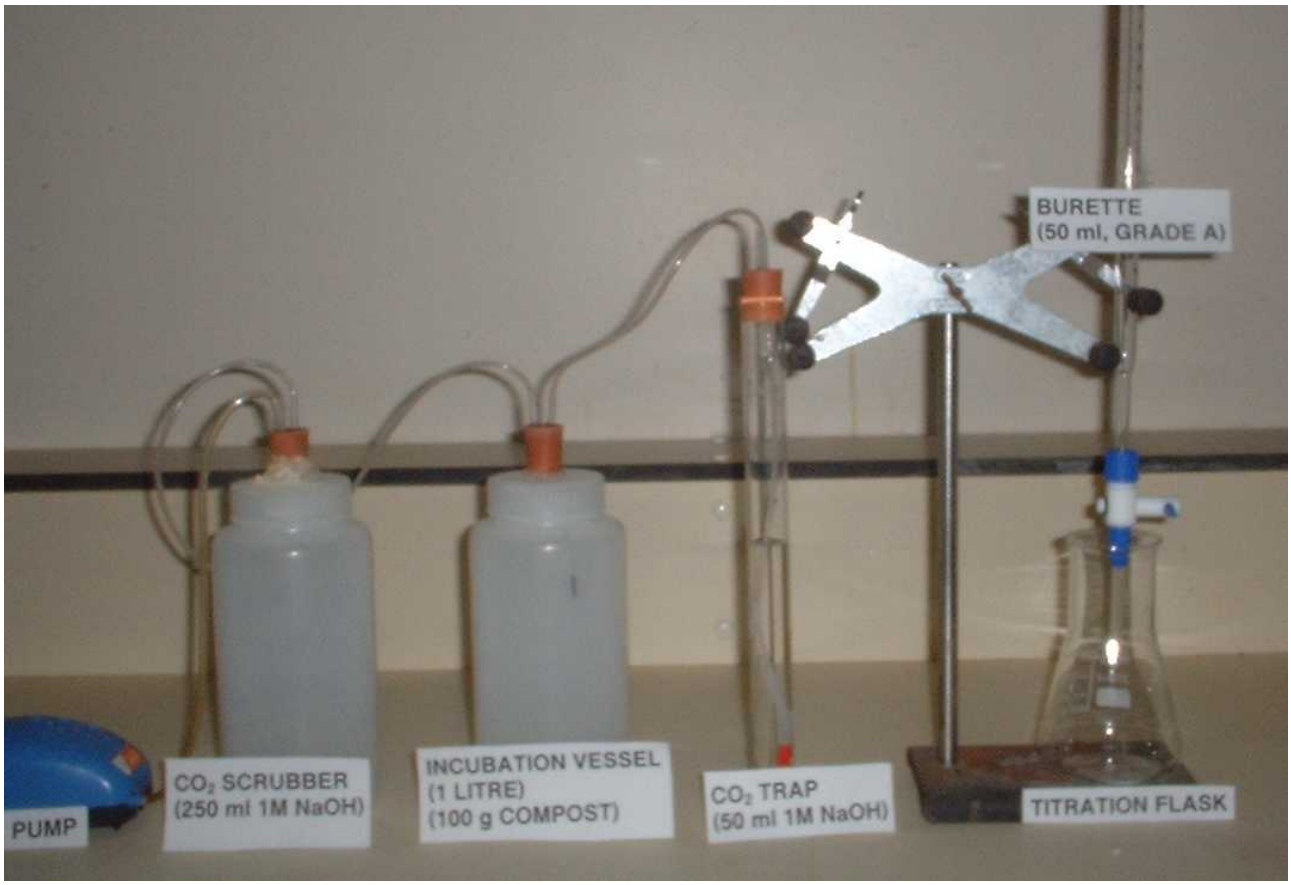
9. Precision

10. Test Report

The test report shall include the following information:

a) a reference to this Standard;
b) a complete identification of the sample;
c) the results of the determination expressed as mass/mass on dry matter basis
d) moisture content;
g) any details not specified in the Standard, or which are optional, as well as any other factor , which may have affected the results

Figure A1: Principal Components of a Single Test Unit



Annex B. Proposed Method for Phytotoxicity as a Measure of Maturity

A method to assess

Phytotoxins In Composted Organic Materials

Contents **Page**

Safety warning

Care should be taken when handling samples that may contain sharps or are of a dusty nature.

1. Scope and field of application

This Standard specifies a method to assess phytotoxic substances ("phytotoxins") by conducting a plant bioassay over a 2-week period on composted materials. The procedures described herein are not necessarily applicable to or suitable for all types of compost.

2. Normative references

This method incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this method only when incorporated in it by amendment or revision. For undated references the latest edition of the publications referred to apply.

BS EN 3696	Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)
BS EN 12579:2000	Soil improvers and growing media - Sampling
BS EN 13038:2000	Soil improvers and growing media – Determination of electrical conductivity
BS EN 13040:1999	Soil improvers and growing media - Sample preparation for chemical and physical test, determination of dry matter content, moisture content and laboratory compacted bulk density
BS EN 13652:2001	Soil improvers and growing media – Extraction of water soluble nutrients
PAS 100:2002	Specification for composted material

3. Principle

The response of indicator plants to phytotoxins is determined using an amended sample under controlled growing conditions.

4. Definitions

For the purpose of this method the definitions given in EN 12579, EN 13040 and PD CR 13456:1999 and PAS 100 apply.

5. Apparatus

5.1 Measuring jug, 1 litre capacity

5.2 Plastic plant pots, 9cm (3.5inch) (previously unused)

5.3 Plastic plant saucers, 9 cm (3.5 inch) (previously unused)

5.4 Capillary matting, 3mm thick

5.5 Greenhouse or plant growth chamber maintained in the range 15 -25 °C

5.6 Light source, equal to 10,000Lux at the bench top, provided by high pressure sodium lamps

5.7 Vermiculite, horticultural grade

5.8 Seed dibber, a round ended rod capable of going through the holes in the seed template

5.9 Balance, capable of weight to 1 mg

5.10 Sphagnum peat, medium grades; no higher than 2 on the von Post scale with a conductivity of < 5mS/m

5.11 Opaque plastic film, e.g. black polythene sheeting

5.12 Scoop, volume 250ml

5.13 Fine rose watering can

5.14 Absorbent paper

6. Fertilisers

6.1 General purpose mix 10 : 10 : 27 for example “Phostrogen”

6.2 Ground dolomite (magnesium) limestone, horticulture grade

7. Seeds

7.1 Radish, French breakfast

7.2 Green lentils, as available from supermarkets

8. Preparation of peat-based growing medium (PBGM)

Thoroughly mix 3 g of fertiliser **(6.1)** and 8 g of ground limestone **(6.2)** into 2 litre of peat **(5.10)** .

9. Preparation of the test sample

9.1 The sample shall be taken in accordance with EN 12579:2000 and prepared in accordance with EN 13040:1999 (Sample preparation for chemical and physical test, determination of dry matter content, moisture content and laboratory compacted bulk density up to and including clause 8.1).

9.2 Determine the EC of the sample **(9.1)** in accordance with BS EN 13038:2000

9.3 Determine the EC of the vermiculite **(5.7)** in accordance with BS EN 13038:2000

9.4 Determine the EC of the sphagnum peat **(5.10)** in accordance with BS EN 13038:2000

10. Adjustment of sample electrical conductivity (EC) to 400 µS cm^{-1}

10.1 Using the equation below determine the factor to calculate the volume of vermiculite required to dilute the sample to approximately 400 µS cm^{-1}

$$\text{Target EC (400)} = [(\text{Sample EC}) + (F \times \text{Vermiculite EC})] / (1 + F)$$

Where F = dilution factor **by volume**

which can be simplified to

$$F = \frac{(\text{Sample EC} - \text{Target EC})}{(\text{Target EC} - \text{Vermiculite EC})}$$

NOTE: If the dilution factor exceeds 2 (the quantity of added vermiculite is more than twice the quantity of compost) then the test shall not be carried out.

10.2 Mix by volume the quantity of vermiculite **(5.7)** determined by the above equation with an appropriate volume of sample **(8.1)** to give final mixed volume not less than 5 litres. Check the electrical conductivity and if necessary re-adjust to give a final electrical conductivity of 400 µS cm^{-1} ± 50 µS cm^{-1}

11. Procedure for the determination of phytotoxins

Cut the capillary matting **(5.4)** such that it is about 0.5cm greater in diameter than that of the 9cm pot **(5.2)** and place in the centre of the saucer **(5.3)**
With a scoop (**5.12**) fill to the top, 6 pots (**5.2**) with the sample (**9.2**). In a similar fashion fill 6 more pots **(5.2)** with PBGM (**10**). Firm the material in the pots by gently tapping of the pot on a hard surface. The final surface of the sample should be about 0.75 -1cm below the rim of the pot.

Evenly space onto the surface of 3 pots containing the sample **(9.2)** 8 seeds **(7.1)** into each pot. In the remaining sample pots evenly place on the surface of each pot 8 seeds of either **(7.2 or 7.3).** Using the seed dibber **(5.8)** press each seed to just below the surface until no longer visible. Repeat the process using the PMGM **(10)**
Place each pot **(11.2)** in a saucer **(5.3)** with the capillary matting **(11.1)** in the centre of the saucer.

Water the modified sample with deionised water, **(6.3)** using the fine rose watering can **(5.13)** until fully wetted up but stop as soon as water starts to seep from the base of the pot. The growing media must not be left saturated.

Water the modified sample with deionised water until they also are fully wetted up.
All subsequent watering to all the pots shall be with deionised water. The surface of all pots shall be kept moist and the capillary matting shall also not become any wetter than moist. No water must pond above the capillary matting.
Cover all the pots with the opaque plastic film **(5.11)** and leave in the green house or growth chamber **(5.5).**
Twice daily inspect each pot for plant growth and moisture content watering if necessary as described above. After plant emergence remove the opaque covering. Any pots not showing plant emergence shall be recovered either with the opaque covering or a saucer.

11.2 After 7 days, determine the number of radish and lentil seedlings per pot.

11.3 After 7 days remove each radish seedling removing any adhering growing medium by gentle shaking then washing the roots by immersing in a shallow tray of water. A plant saucer **(5.3)** has been found to be

suitable. Dry the plant by dabbing on absorbent paper (**5.14).** Combine the plants from each pot and weigh to two decimal places.

11.4 After 14 days, determine the number of lentil plants per pot. Remove and weigh the lentil plants as described in **(11.3).**

12. Calculation and expression of phytotoxin bioassay results

Record the number of seedlings that emerge and compare the growth characteristics between the PBGM and the amended test sample. Note any unusual symptoms.
Record the weights of the plants from the amended sample and the PBGM and determine the average weight per seedling.

13. Test Report

The test report can be prepared separately or in conjunction with the test report of any subsequent analytical results.

The test report shall include the following information:
a) a reference to this method;
b) a complete identification of the sample;
c) the total number emerged weed propagules per litre of sample, in both test sample and PBGM, (weeds in PBGM may indicate aerial contamination of the growth area);
d) any observed abnormalities;
e) the number of radish seedlings emerged after 7 days, for test sample and PBGM;
f) the number of lentil plants emerged after 7 and 14 days, for test sample and PBGM;
g) the total fresh weight of emerged plants after 7 days (radish) or 14 days (lentil), for test sample and PBGM;
h) any observed abnormalities;
i) the average fresh weight per seedling or plant for test sample and PBGM;
j) any details not specified in this method, or which are optional, as well as any other factors which may have affected the results.

Annex C. Compost Samples Sources and Treatment

Sample maturity	Number	Date of supply	Source	Selection	Transport/storage	Age	Date of tests
First set of trials for stability and phytotoxicity							August 2002
1 week	1	July 2002	Source segregated green waste	ADAS project manager	Same day to cold storage 4°C Double wrapped	1 week	
1 month	2						
2 month	3					4 weeks	
mature	4					8 weeks	
Very mature	5					26 weeks	
Very mature	6	August 2002	Source segregated green waste	ADAS labs	4°C Double wrapped	26 weeks	
Second set of trials for stability and photo toxicity							
Immature	7	October 2002	Source segregated green waste	Supplier	Carrier cold storage 4°C Double wrapped	2 weeks	November 2002
Mature	8					8 weeks	
Very mature	9					6 months	
Immature	10	November 2002	MRF separated organic fraction of mixed waste		Carrier cold storage 4°C Double wrapped	2 weeks	November 2002
Mature	11					8 weeks	
Very mature	12					6 months	

Green waste was obtained from a windrow composting operation building up one windrow per week of source segregated materials. The windrows were turned on a weekly cycle. Material was selected from the centre section of the windrow in order to give materials which was representative of materials of the age expected. This is particularly important for the materials of least age.

Biodegradable municipal mixed compost waste samples were obtained from the composting process output stream from a MRF. The organic fraction is then passed to the composting facility. The composted material is then processed in size reduction and screens to final quality product which is used successfully in soil manufacture for land restoration.

Annex D. Growth Trial Individual Pot Results

POT	BLOCK	CROP	COMPOST	SUB-COMPOST	Radish	Radish	Lentil - 7 day	Lentil - 14 day	Lentil - 14 day
					plant no.	yield g	plant no.	plant no.	yield g
1	1	Radish	BMWfr	Fresh	6	0.4	*	*	*
2	1	Radish	GREENfr	Fresh	8	0.8	*	*	*
3	1	Radish	BMWmed	medium	8	0.6	*	*	*
4	1	Radish	GREENmed	medium	6	0.5	*	*	*
5	1	Radish	BMWmat	mature	7	0.9	*	*	*
6	1	Radish	GREENmat	mature	8	1.05	*	*	*
7	1	Radish	Cneg	none	8	0.8	*	*	*
8	1	Radish	Cpos	none	8	1.1	*	*	*
9	1	Lettuce	BMWfr	Fresh	*	*	*	*	*
10	1	Lettuce	GREENfr	Fresh	*	*	*	*	*
11	1	Lettuce	BMWmed	medium	*	*	*	*	*
12	1	Lettuce	GREENmed	medium	*	*	*	*	*
13	1	Lettuce	BMWmat	mature	*	*	*	*	*
14	1	Lettuce	GREENmat	mature	*	*	*	*	*
15	1	Lettuce	Cneg	none	*	*	*	*	*
16	1	Lettuce	Cpos	none	*	*	*	*	*
17	1	Lentil	BMWfr	Fresh	*	*	7	8	3.5
18	1	Lentil	GREENfr	Fresh	*	*	7	7	1.8
19	1	Lentil	BMWmed	medium	*	*	8	8	3.1
20	1	Lentil	GREENmed	medium	*	*	8	8	4
21	1	Lentil	BMWmat	mature	*	*	8	8	2.7
22	1	Lentil	GREENmat	mature	*	*	8	8	4.2
23	1	Lentil	Cneg	none	*	*	7	7	3.3
24	1	Lentil	Cpos	none	*	*	7	7	3.8
25	2	Radish	BMWfr	Fresh	7	0.3	*	*	*
26	2	Radish	GREENfr	Fresh	7	0.8	*	*	*
27	2	Radish	BMWmed	medium	7	0.9	*	*	*
28	2	Radish	GREENmed	medium	7	0.6	*	*	*
29	2	Radish	BMWmat	mature	8	0.6	*	*	*
30	2	Radish	GREENmat	mature	8	0.9	*	*	*
31	2	Radish	Cneg	none	7	0.9	*	*	*
32	2	Radish	Cpos	none	8	1	*	*	*
33	2	Lettuce	BMWfr	Fresh	*	*	*	*	*
34	2	Lettuce	GREENfr	Fresh	*	*	*	*	*
35	2	Lettuce	BMWmed	medium	*	*	*	*	*
36	2	Lettuce	GREENmed	medium	*	*	*	*	*
37	2	Lettuce	BMWmat	mature	*	*	*	*	*
38	2	Lettuce	GREENmat	mature	*	*	*	*	*
39	2	Lettuce	Cneg	none	*	*	*	*	*
40	2	Lettuce	Cpos	none	*	*	*	*	*
41	2	Lentil	BMWfr	Fresh	*	*	7	7	3.5
42	2	Lentil	GREENfr	Fresh	*	*	8	8	2
43	2	Lentil	BMWmed	medium	*	*	8	8	2.9
44	2	Lentil	GREENmed	medium	*	*	8	8	2.6
45	2	Lentil	BMWmat	mature	*	*	8	8	1.9

46	2	Lentil	GREENmat	mature	*	*	8	8	3.8
47	2	Lentil	Cneg	none	*	*	8	8	3.1
48	2	Lentil	Cpos	none	*	*	7	8	4.9
49	3	Radish	BMWfr	Fresh	6	0.4	*	*	*
50	3	Radish	GREENfr	Fresh	8	0.6	*	*	*
51	3	Radish	BMWmed	medium	8	0.7	*	*	*
52	3	Radish	GREENmed	medium	8	0.5	*	*	*
53	3	Radish	BMWmat	mature	8	0.9	*	*	*
54	3	Radish	GREENmat	mature	8	0.6	*	*	*
55	3	Radish	Cneg	none	6	0.3	*	*	*
56	3	Radish	Cpos	none	8	1.1	*	*	*
57	3	Lettuce	BMWfr	Fresh	*	*	*	*	*
58	3	Lettuce	GREENfr	Fresh	*	*	*	*	*
59	3	Lettuce	BMWmed	medium	*	*	*	*	*
60	3	Lettuce	GREENmed	medium	*	*	*	*	*
61	3	Lettuce	BMWmat	mature	*	*	*	*	*
62	3	Lettuce	GREENmat	mature	*	*	*	*	*
63	3	Lettuce	Cneg	none	*	*	*	*	*
64	3	Lettuce	Cpos	none	*	*	*	*	*
65	3	Lentil	BMWfr	Fresh	*	*	7	8	3.9
66	3	Lentil	GREENfr	Fresh	*	*	7	8	2.2
67	3	Lentil	BMWmed	medium	*	*	6	8	1.8
68	3	Lentil	GREENmed	medium	*	*	7	8	2.2
69	3	Lentil	BMWmat	mature	*	*	7	7	2.9
70	3	Lentil	GREENmat	mature	*	*	8	8	3.2
71	3	Lentil	Cneg	none	*	*	8	8	2.4
72	3	Lentil	Cpos	none	*	*	7	8	3.8
73	4	Radish	BMWfr	Fresh	6	0.8	*	*	*
74	4	Radish	GREENfr	Fresh	8	0.8	*	*	*
75	4	Radish	BMWmed	medium	8	1.2	*	*	*
76	4	Radish	GREENmed	medium	8	0.9	*	*	*
77	4	Radish	BMWmat	mature	8	1	*	*	*
78	4	Radish	GREENmat	mature	7	1	*	*	*
79	4	Radish	Cneg	none	7	0.4	*	*	*
80	4	Radish	Cpos	none	8	1.1	*	*	*
81	4	Lettuce	BMWfr	Fresh	*	*	*	*	*
82	4	Lettuce	GREENfr	Fresh	*	*	*	*	*
83	4	Lettuce	BMWmed	medium	*	*	*	*	*
84	4	Lettuce	GREENmed	medium	*	*	*	*	*
85	4	Lettuce	BMWmat	mature	*	*	*	*	*
86	4	Lettuce	GREENmat	mature	*	*	*	*	*
87	4	Lettuce	Cneg	none	*	*	*	*	*
88	4	Lettuce	Cpos	none	*	*	*	*	*
89	4	Lentil	BMWfr	Fresh	*	*	6	6	2.8
90	4	Lentil	GREENfr	Fresh	*	*	6	6	1.8
91	4	Lentil	BMWmed	medium	*	*	8	8	3.9
92	4	Lentil	GREENmed	medium	*	*	8	8	3.8
93	4	Lentil	BMWmat	mature	*	*	6	6	2.9
94	4	Lentil	GREENmat	mature	*	*	7	8	3.3
95	4	Lentil	Cneg	none	*	*	8	8	3.5

96	4	Lentil	Cpos	none	*	*	8	8	5.1
97	5	Radish	BMWfr	Fresh	7	1.2	*	*	*
98	5	Radish	GREENfr	Fresh	8	1	*	*	*
99	5	Radish	BMWmed	medium	8	2.1	*	*	*
100	5	Radish	GREENmed	medium	7	1	*	*	*
101	5	Radish	BMWmat	mature	8	1.3	*	*	*
102	5	Radish	GREENmat	mature	8	1.6	*	*	*
103	5	Radish	Cneg	none	8	0.7	*	*	*
104	5	Radish	Cpos	none	8	2.2	*	*	*
105	5	Lettuce	BMWfr	Fresh	*	*	*	*	*
106	5	Lettuce	GREENfr	Fresh	*	*	*	*	*
107	5	Lettuce	BMWmed	medium	*	*	*	*	*
108	5	Lettuce	GREENmed	medium	*	*	*	*	*
109	5	Lettuce	BMWmat	mature	*	*	*	*	*
110	5	Lettuce	GREENmat	mature	*	*	*	*	*
111	5	Lettuce	Cneg	none	*	*	*	*	*
112	5	Lettuce	Cpos	none	*	*	*	*	*
113	5	Lentil	BMWfr	Fresh	*	*	6	7	3.6
114	5	Lentil	GREENfr	Fresh	*	*	7	7	2.2
115	5	Lentil	BMWmed	medium	*	*	8	8	3.5
116	5	Lentil	GREENmed	medium	*	*	8	8	5.2
117	5	Lentil	BMWmat	mature	*	*	8	8	2.2
118	5	Lentil	GREENmat	mature	*	*	7	8	3.2
119	5	Lentil	Cneg	none	*	*	8	7	2.9
120	5	Lentil	Cpos	none	*	*	8	8	6
121	6	Radish	BMWfr	Fresh	7	1.1	*	*	*
122	6	Radish	GREENfr	Fresh	7	0.9	*	*	*
123	6	Radish	BMWmed	medium	5	0.7	*	*	*
124	6	Radish	GREENmed	medium	7	0.6	*	*	*
125	6	Radish	BMWmat	mature	7	0.6	*	*	*
126	6	Radish	GREENmat	mature	5	0.5	*	*	*
127	6	Radish	Cneg	none	6	0.3	*	*	*
128	6	Radish	Cpos	none	8	1.9	*	*	*
129	6	Lettuce	BMWfr	Fresh	*	*	*	*	*
130	6	Lettuce	GREENfr	Fresh	*	*	*	*	*
131	6	Lettuce	BMWmed	medium	*	*	*	*	*
132	6	Lettuce	GREENmed	medium	*	*	*	*	*
133	6	Lettuce	BMWmat	mature	*	*	*	*	*
134	6	Lettuce	GREENmat	mature	*	*	*	*	*
135	6	Lettuce	Cneg	none	*	*	*	*	*
136	6	Lettuce	Cpos	none	*	*	*	*	*
137	6	Lentil	BMWfr	Fresh	*	*	8	8	3.3
138	6	Lentil	GREENfr	Fresh	*	*	8	8	2.8
139	6	Lentil	BMWmed	medium	*	*	6	6	2.8
140	6	Lentil	GREENmed	medium	*	*	8	8	3.4
141	6	Lentil	BMWmat	mature	*	*	8	7	4.3
142	6	Lentil	GREENmat	mature	*	*	8	8	4.8
143	6	Lentil	Cneg	none	*	*	8	8	4.1
144	6	Lentil	Cpos	none	*	*	8	8	6.9

Annex E. Stability And Maturity Laboratory Test Methods Discussion Of Literature

Morel *et al*. (1985) compared several indirect parameters (C/N, polysaccharides, ATP, chromatography, colour) against direct respiration and phytotoxicity tests to assess maturity. They concluded that no single test was sufficient but that simplified or predictive tests had potential if calibrated against direct or more sophisticated measurements.

Zucconi (1986) asserted that 'C/N analytical levels in the end product do not present *per se* an objective value for assessing stabilisation. Information on C/N should include initial and final values'.

Juergen *et al*. (1993) reported that microbiological measurements were useful in the estimation of compost maturity but chemical measurements were contradictory and depended very strongly on the original feedstock. They also asserted, in contrast to most other researchers, that respiration rate did not contribute to the assessment of compost maturity. They found that a combination of dehydrogenase activity and arginine ammonification data led to an unambiguous classification of the six different source composts tested.

Iannotti *et al*. (1993) investigated various chemical, physical and biological assays to assess stability and maturity of composted MSW. Respirometry, growth bioassays, water soluble organic C and water soluble organic C:organic N ratio correlated well with compost age, but germination tests revealed inhibition at all maturity levels.

Ciavatta *et al.* (1993) monitored compost stabilisation using the ratio between the humified fractions (HA+FA) and the total extractable carbon and by using an electrofocussing technique. Both techniques were reported to show potential, although no attempt was made to relate results to direct measurements of phytotoxicity or microbial activity.

Grebus *et al*. (1994) reported that composting time was highly significantly correlated to availability of plant nutrients (P, K, Ca, Mg) EC, Total C/N ratio, nitrate-N, CEC and O_2 respirometry.

Hue and Liu (1995) compared several methods of predicting stability in sixteen composts of differing type. Water soluble C:water soluble organic-N best separated stable from unfinished composts, but they reported problems with some composts due to the very low concentrations of water soluble organic-N. Water soluble C:total organic-N and NaOH soluble C:water soluble C were acceptable alternatives.

Paletski and Young (1995) reported that aerobic respirometry of solid samples of compost provided a precise measure of microbial activity.

Adani *et al*. (1995) proposed using humic substances in apolar and polar extracts to measure stability and maturity.

The Canadian national standards (1996) specify a rather complicated yet flexible list of alternatives to ensure maturity: **either** any two of C/N, O_2 uptake, germination of cress **or** a minimum curing time of 21 days with no reheating more than 20^{o}C above ambient **or** a minimum curing time of 21 days with an overall reduction of organic matter >60% by weight **or** the compost must be cured for 6 months after the pathogen reduction process is complete with conditions conducive to aerobic biological activity and with no reheating to thermophilic temperatures.

Dinel *et al*. (1996) developed an organic matter stability test for assessing compost maturity by measuring the relative solubilities of lipids in different solvents. This was compared to more traditional chemical tests (C, N, NH4-N, NO_3-N, C/N, E_4:E_6 optical densities), with the conclusion that it was scientifically sound and universally applicable, although it was not validated against direct measurements of biological activity or phytotoxicity.

Avnimelech *et al*. (1996) reported that simple chemical tests (pH, EC, NH_4-N and organic C) could be used to monitor composting processes and predict stability, although they were feedstock or process specific so were unreliable as general tests.

Popp and Fischer (1997) proposed measuring maturity using respiration in combination with an empirical pH drop method, which correlated well with self-heating.

Abate *et al*. (1998) reported that thermal analysis techniques, especially differential scanning calorimetry correlated well with humification parameters and could be used to distinguish between well and poorly stabilised composts.

Gattinger *et al*. (1997) showed that microbial biomass and activity, which declined with age, might be used to evaluate compost maturity.

Jackson and Line (1997) used FT-IR and NMR to characterise component change during maturation, but concluded that these techniques could not directly assess phytotoxicity.

Johansson *et al*. (1997) reviewed the techniques and procedures used to assess stability and maturity including direct and indirect methods and also reviewed national standards and limits. They concluded that more investigation into stability and maturity testing was needed, but methods were currently available which were "under any circumstances better than relying on the temperature and the C:N ratio as is common today".

Hartz (1997) evaluated several analytical tests for predicting maturity and concluded that, although there were no easily performed laboratory tests that can document maturity, there are several good indicators of immaturity: <2-3 months of composting, temperature above 50^{o} C, C/N ratio >16-18, high NH_4-N, low NO3-N.

Verdonk (1998) compared maturity parameters in Belgium, Holland and Germany. Phytotoxicity and pathogens were universally important and were supported by other parameters: nitrate:ammonia ratio (Belgium), temperature (Holland) and self-heating (Germany).

Lasaridi and Stentiford (1998) described respiration as 'a global measure of microbial activity, that can provide a reliable, repeatable and scientifically sound assessment of stability'. They further reported that 'C/N ratio...was too dependent on compost type, and was thus of limited usefulness'.

Helfrich *et al*. (1998) found good correlations between direct phytotoxicity measurements and indirect methods using oxygen consumption and fluorescence of lyophilised chloroplast thylakoids.

Bernal *et al*. (1998) compared a range of carbon and nitrogen fractions plus CEC to measure stability and maturity. C/N, water soluble C, water soluble C/organic N, ammonia/nitrate and ammonium-N could all be used to predict maturity. Some carbon mineralisation parameters showed some potential, but CEC and organic matter humication were too dependent on the original feedstock.

Rajbanshi *et al*. (1998) investigated the relationship between respiration, C:N ratio, pH, biomass (as total extractable lipid phosphate) and seed germination. They concluded that germination and respiration were sufficiently reliable indicators of maturity.

Gomez (1998) reported that compost maturity has a great influence on heavy metal solubilities.

Brinton and Traekner (1999) showed that carbon dioxide evolution and volatile organic acid concentration could predict phytotoxicity in young seedlings.

Lasaridi and Stentiford (1999) demonstrated the superiority of respiration over germination index as a diagnostic tool for process control and performance evaluation.

Namkoon *et al.* (1999) evaluated C/N, NH_4-N, CEC, volatile solids, humification index (HI) and water soluble Corg/Norg as maturity parameters. C/N and NH_4-N were reported to be unsuitable maturity parameters but VS, water-soluble Corg/Norg, CEC and HI were highly correlated.

Tiquia and Tam (2000) compared microbiological and chemical parameters for assessing the maturity of spent pig litter compost. They found that total N, P, K, C:N ratio, total heterotrophic numbers, ATP content and microbial biomass C and N were dependent on the initial feedstock or composting strategy or both, so were unsatisfactory as reliable parameters for assessing maturity. Dehydrogenase activity, pH, NH_4-N, water extractable Cu and water extractable Zn were recommended as maturity parameters.

Warman (1999) evaluated seed germination and growth tests for assessing compost maturity and concluded that, on their own, such tests were not sensitive enough to distinguish between mature and immature composts.

The UK Composting Association's Standards for Composting, Shields, S. (1999) did not mention stability or maturity as such but did include a full bioassay phytotoxicity test.

Vuoringen (1999) reported that phosphomonoesterase and beta-D-glucosidase enzymes were unreliable as maturation indicators due to several interfering factors.

Hsu and Lo (1999) compared several chemical methods, for assessing maturity of composted pig manures and reported that no single parameter was adequate to assess stability or maturity. They found that combinations of C/N ratio, ash, extractable metal contents and humic substances were good indicators of stability and maturity.

Carlsbaek and Broegger (1999) compared Corg/Norg in water extracts, total oxygen demand (TOD) in 96 hours, the 'Solvita' test kit and self-heating as methods for assessing stability when compiling a 'New Standardised Product Sheet for Compost in Denmark'.

Li *et al*. (2001) found that pH, EC plus changes in organic matter composition such as crude fat and carbohydrates, cellulose plus hemicellulose, and crude protein and lignin content were poor indicators of maturity but, in contrast to most other research, found that C/N and HA/FA were good maturity indicators.

Manna *et al.* (2000) investigated C:N, CEC, TOC, biodegradability, water soluble carbohydrate, P solubilities, nitrate and ammonia and concluded that C:N was an unreliable parameter but that WSC, CEC, CEC/TOC, biodegradability and lignin to cellulose ratios could be used as chemical parameters to predict maturity.

Ouatmane *et al*. (2000) compared differential scanning calorimetry (DSC) and FT-IR against pH, C/N, ash and humic substances and concluded that spectroscopic and thermal techniques are complementary to each other and to chemical tests and could be a powerful and fast approach for the study of compost maturity.

Wu *et al*. (2000) compared several indirect parameters (pH, EC, water holding capacity, total volatile solids, total P, total N, C/N ratio, humic/fulvic ratio and dissolved organic carbon) for evaluating maturity against direct measurements of respiration and phytotoxicity. They concluded that HA/FA and C/N are not accurate indicators of stability or maturity but pH, EC, respiration, phytotoxicity and DOC could be used to monitor stabilisation and maturation processes.

Tomati *et al*. (2000) proposed an index for the evaluation of compost maturity based on the evolution of molecular weights of humic acids during composting.

Shin *et al*. (2000) compared cellulose, lignin, organic acids, total sugar, reducing sugar concentration, optical density at 450 nm; phosphatase, urease, amylase and cellulase enzyme activities. They reported that only total sugar and reducing sugar concentration were any use for assessing maturity of composted pig manure.

Wu and Ma (2001) reported good correlation's between phytotoxicity, respiration rate and water soluble carbohydrate.

Abate *et al*. (2000) reported that thermal analysis methods showed potential for monitoring compost maturation.

Brinton (2000) described the work of the California Compost Quality Council (CCQC) and the California Integrated Waste Management Board (CIWMB) in redefining maturity and setting standards in a two level approach requiring more than one dissimilar test selected from respiration, C/N, NH_4-N, NH_4-N:NO_3-N, VOA and bioassays.

Sullivan (2000) asserted that respiration rate (CO_2 evolution measurement) was the most reliable test for compost maturity and stability, and compared Dewar, Solvita, Draeger-tube and laboratory CO_2 procedures.

Balis and Tassiopoulou (2001) reported an improved self-heating method to assess stability. They accelerated respiration by adding hydrogen peroxide and incubating in a microcosm thermally insulated system.

Wilkinson *et al*. (2000) criticised the nitrogen draw down index (NDI) which replaced C:N ratio in the Australian compost quality standard AS4454. C:N was replaced in 1999 because it was a poor predictor of N immobilisation when assessing composts containing resistant C compounds. NDI should theoretically have given a better prediction of plant effects, but it showed unacceptably high variability in performance tests.

Brewer and Sullivan (2001) compared several quick respiration tests for measuring stability and found that these correlated well with phytotoxicity tests to predict maturity.

Lasaridi and Stentiford (2001) demonstrated the value of respiration (O_2 uptake measurement) as a direct measurement of microbial activity.

Reinikainen and Herranen (2001) concluded that neither stability nor maturity could be assessed by a single method. They compared plant growth against pH, EC, water soluble nutrients, self heating, oxygen consumption and infrared spectroscopy and concluded that self heating, residual oxygen and the evolution of EC, acetic value and NH_4-N/NO_3-N plus growth trials gave a satisfactory picture of the progression of the maturation process.

Mari *et al.* (2001) investigated thermogradient respirometry as a tool for measuring stability with some success.

Butler *et al*. (2001) compared CEC, self-heating and oxygen uptake and concluded that Dewar flask self-heating was the most useful indicator of compost maturity.

Smith and Hughes (2001) proposed a technique to assess cellulolytic enzyme activity in composts which was suggested could be a factor responsible for phytotoxicity. The test was based on loss of weight of buried cellulose filter papers. This test was compared to several chemical parameters (pH, EC, LOI, Org C, N, C/N, NO_2-N, NO_3-N, NH_4-N), microbial activity measured using a fluorescein diacetate method and phytotoxicity measured using germination and growth tests. A positive correlation existed between the cellulolytic activity and the microbial activity.

Provenzano *et al.* (2001) investigated the use of EEM fluorescene spectroscopy and FT-IR to assess maturity of composts. They compared spectra against days of composting and reported a strong correlation between maturity and contour densities of spectral maps. They were confident that further refinement of these techniques should provide a relatively rapid method of assessing the suitability of the compost to land application.

Ranalli *et al*. (2001) evaluated various chemico-physical, spectroscopic (FTIR), thermal (DTG, DSC) microbiological, enzymatic analyses and phytotoxicity bioassays. No single measurements were adequate as stand alone assessments of maturity but enzyme activities plus FTIR and thermal techniques were promising tools for development.

Kapanen and Itavaara (2001) discussed the use of ecotoxicity tests for compost applications. The test methods discussed employed microbes, enzymes, soil fauna, and plants and these were related in some instances to chemical parameters in the compost.

Sanchez-Monedero *et al*. (2001) reported that the ratio of NH_4-N to NO_3-N was a clear indicator of compost maturity.

Madejon *et al*. (2001) found that germination inhibition correlated well with the activity of soil enzymes after compost application.

Eggen and Vethe (2001) investigated several chemical tests as faster alternatives to respirometric techniques and found that C:N and HA:FA ratios were insupportable but TOC (in the fulvic fraction) and water soluble N (total and organic) were correlated with respiration rate.

The UK Composting Association (2001) discussed the various approaches to assessing stability and maturity, which have been proposed and distinguished between direct and indirect methods.

Brinton *et al*. (2001) reported that VOA and respiration using CO_2 techniques correlated well with cress germination and container plant growth. Ammonia and VOA effects were stronger at root emergence stages while oxygen depletion and sulphide effects were longer lasting. Self-heating correlated to germination and growth over a limited range only. They suggested that maturity is best indicated by two or more unrelated analyses.

Smidt and Lechner (2001) demonstrated the potential of FT-IR to identify differences between composts of different ages. It may be possible to use these differences for calibration purposes.

Garcia-Gomez *et al*. (2001) suggested that maturity based on phytotoxicity should be tested in plant-soil systems to be meaningful.

Brewer and Sullivan (2001) found that compost maturity was strongly correlated with pH, total C and respiration but neither biological nor sensory measures were reliable indicators of maturity.

Johansson (2002) successfully evaluated the maturity of MSW compost using Near InfraRed Spectroscopy (NIRS).

Smith *et al*. (2001) demonstrated the power of NIRS to predict nitrogen mineralisation in composts intended for organic potting mixes by calibrating spectra directly against data on plant growth and nitrogen uptake.

Adani *et al*. (2002) repeated an argument originally made by Haug (1986) that O_2 uptake is preferable to CO_2 evolution for respiration purposes because O_2 uptake is affected by organic matter oxidation. Yet non-respiratory oxidation of organic matter represents an interference to the O_2 uptake techniques as a measure of respiration. The CO_2 evolution technique is not affected by this interference.

Lasaridi and Stentiford (1996) argued that CO_2 evolution does not distinguish between aerobic and anaerobic respiration. This has been quoted by many other authors as an argument against using CO_2 evolution techniques. Yet we are measuring aerobic respiration under aerobic conditions. In the presence of oxygen, facultative organisms employ only aerobic respiration and obligate anaerobes shut down completely.

Koenig and Bari (2000) compared self-heating and oxygen uptake respirometry and reported that self-heating was less complicated and expensive and more convenient than oxygen uptake and allowed larger and more representative amounts of sample to be tested.

Stentiford and Lasaridi (2000) demonstrated that, compared to oxygen respirometry, self-heating was relatively insensitive at differentiating composting stages during the first 2-3 weeks of composting but stated that, while oxygen respirometry was best suited as a diagnostic tool for process evaluation and performance, self-heating and germination tests were better suited for product maturity assessment.

Stentiford (2002) compared the performance of the Specific Oxygen Uptake Rate (SOUR) test against other 'respirometry' techniques. The Solvita test, which is a CO_2 evolution technique, was shown to be 'more suited to measurement of maturity whereas SOUR is a better stability indicator'. SOUR correlated very closely with a 'dry' O_2 uptake technique. O_2 uptake techniques and self-heating correlated well with germination but the O_2 techniques were more highly correlated. SOUR was a better process indicator for stability, whilst self-heating was better in the maturation phase.

Brinton *et al.* (2000) expressed the view that 'the Dewar test may be viewed as a "holistic" procedure, compared to laboratory respirometric techniques.....integrates a number of factors present in normal composts and therefore correlates well with field observations about compost behaviour. Its use, however, should not be viewed as replacing quantitative laboratory procedures'.

Brinton (2001) reported that 'the Dewar (self-heating) test is limited in the sense that it best distinguishes very mature from mature compost; it can not distinguish moderate maturity from high maturity, which may be important for potting mix use'.

Butler (2001) found that self-heating was better than oxygen uptake rate for monitoring respiration and hence stability throughout the composting process, because oxygen uptake did not change after 29 days while self-heating changed throughout the 57 day process.

Brewer and Sullivan (2001) compared several respirometry tests: self-heating; CO_2 colorimetric (Solvita); CO_2 alkali trap and CO_2 gas detection tube. All methods gave comparable results but self-heating was criticised for being slow and less sensitive to maturity than previously believed

Weppen (2002) reported that 'systematic errors occurred when (self-heating) assays were performed in Dewars of different sizes or at several packing densities and humidities of the compost'.

The California Compost Quality Council (2001) compared the relative merits of various respirometry techniques and commented that 'although oxygen consumption and carbon dioxide evolution are related, the measurements are not consistently equivalent. Generally the measurement of oxygen consumption requires more sophistication, time and quality control, in comparison to the more simple and often more precise measurement of carbon dioxide evolution'.

In some instances CO_2 evolution was used as a reference technique against which other techniques were compared. Brewer and Sullivan (2001), when evaluating various respirometric techniques, wrote '...the time-proven analytical laboratory technique to determine the rate of CO_2 release from compost always will be our preferred choice...'.

Scaglia *et al*. (2000), Adani *et al*. (2001) and Adani *et al*. (2002) emphasised the difference between dynamic and static respirometry techniques. Dynamic techniques incorporating constant aeration produced respiration values twice that of static techniques without constant aeration.

Norgaard *et al*. (1997) reported that the specific oxygen uptake rate (SOUR) test of Lasaridi and Stentiford correlated well with cress germination and an electrolytic oxygen consumption respirometer, but that Dewar self-heating did not clearly distinguish between different compost samples. Sikora (2003) compared Dewar witjh oxygen uptake and found Dewar to have a relatively high degree of reproducibility and concluded it was more reliable than oxygen uptake rate for evaluating biosolids compost. In Germany, Dewar testing is included in interlaboratory trials which perform statistical ranking based on three variance groups: Group 1 with variance less than 10%, Group 2 with variance between 10 and 20%, and Group 3, variance greater than 20%. In 1993, the Dewar test scored in Group 2 (Bundesgutegemeinschaft Kompost, 1994).

The ASTM standard test method (1996) measures oxygen consumption using gas chromatography or other unspecified oxygen detecting equipment in a very complicated, cumbersome and expensive method which involves mixing the test composts with a stabilised compost inoculum. The stabilised compost inoculum has to be made in bulk (100-200 litres) in the laboratory from similar feedstock to the test compost, which severely limits its usefulness as a standard test for laboratories required to test many different types of compost. Method performance data is not available but the method is unlikely to be very reproducible between laboratories. Validation of the method by interlaboratory testing would be a challenging prospect. Also it does not directly test the unknown compost but, instead, tests a 50/50 mixture of the test compost with the stabilised inoculum compost which has questionable value.

Iannotti (1993) described the importance of using an appropriate temperature to avoid shock to thermophiles, which could cause erroneously low values in respirometry procedures using short incubations.