Recycled Organics Unit
PO Box 6267
University of New South Wales
Sydney, NSW 1466
Phone 0414 385 226
a.campbell@recycledorganics.com

ROU Information Sheet No. 3-11

Sampling and Sample Management for Consistent Analysis of Recycled Organics Products

The Australian Standard AS4454 (2012) Composts, soil conditioners and Mulches includes a new *Appendix A*: Sampling, Sample Handling and Preparation which provides general guidance on sampling, and specific guidance on sample quantities (derived from international standards). However, *Appendix N*: Maturity Index in the standard provides specific guidance on sample handling and preparation for biological stability tests, without which test results will be unreliable.

Neither appendix provides information in a form that can be directly implemented.

This ROU information sheet provides principles and operational practice for sampling, sample handling and shipping methods that are consistent with the requirements of AS4454 (2012) standard.

These methods are consistent with the requirements for sampling, handling and analysis of samples for the respirometry test options specified in the *Maturity Index* of the revised AS4454 (2012) standard for the assessment of biological stability (O2 uptake, CO2 evolution, NDI, Solvita ®).

Section 1 Sampling and Sample Management

1.1 Purpose of sampling

The first step in analyzing compost quality is to obtain a representative sample from the compost pile. The sample (and sampling points) should reflect the overall or average characteristics of the material being tested. Due to natural variation in raw materials and in environmental conditions within the compost pile (albeit within an expected range) properly representative sample must be formed by mixing numerous incremental samples to form a composite sample for analysis.

Laboratory testing is expensive. Tests performed on a sample that is not representative of the bulk of material, or that has not been correctly handled will produce unreliable results that may misrepresent the characteristics of the compost batch as a whole. This is undesirable for manufacturer's quality control purposes and for generating product information; and for selection and use by customers.

1.2 Definitions

Batch: A quantity of goods manufactured from known materials by the same process under the same conditions and assumed to have the same characteristics. The source/s of supply and period over which the materials have been received for an individual batch should be known for traceability.

Incremental sample: a discrete quantity of material taken from one discrete **sampling point**.

Combined or **composite sample**: combination of mixed the incremental samples from a single batch.

Sampling site/sampling area: an area of a compost windrow or pile that is deliberately prepared to expose an internal cross section of the pile from which incremental samples can be taken.

1.3 Safety / disclaimer

Care should be taken when handling waste materials and samples that may contain sharps and sharp fragments, chemical contaminants or possible pathogenic organisms. Facility operators should ensure that sampling and associated operations are carried out in a safe manner that protects staff and minimises risks. Such risks are dependent upon engineering arrangement and equipment used at the facility, and should be addressed via risk assessment by managers of the facility.

1.4 Sample management for maturity test accuracy

Compost maturity is now assessed in AS4454 (2012) by assessing both the biological stability of a product, and also complementary assessment of plant growth response attributes relevant to the relative presence/absence of phytotoxic characteristics (see AS4454 Appendix N).

Biological stability can be defined as the extent to which readily biodegradable material has decomposed. A material is considered unstable if it contains a high proportion of biodegradable matter that can sustain high microbial activity. If the material contains mainly recalcitrant or humus-like matter, it is not able to sustain high levels of microbial activity under suitable environmental conditions (temperature and moisture), and therefore it is considered biologically stable.

Respirometry test methods assesses the level of biological activity in a sample of material under specified conditions that are conducive to microbial activity by measuring the rate of respiration in the form of carbon dioxide evolved or oxygen consumed by microorganisms, or in the form of the heat generated by this biological activity. Respiration is directly related to the metabolic activity of a microbial population, the micro-organism population will collectively respire at higher rates in the presence of higher levels of biologically available organic matter, while microbiological activity and total respiration will be lower where such material is scarce (under equivalent conditions).

Tests specify the quantity of suitably moist compost that is incubated at a specified temperature under specific conditions for measurement. Respirometry tests can provide a repeatable and quantitative or semi quantitative measure of the degree to which a material has been decomposed and stabilized.

Respiration determination is reported to be sensitive to the time lag between sample collection and analysis. To minimize changes in the sample due to microbial activity over time, samples should be analysed as soon as possible after collection. For reliability of results, ideally samples should be prepared and assessment begun within 48 hours of sampling. This requires prior arrangement with the laboratory so that they are expecting the sample to arrive and have the test apparatus available to conduct the test in a timely manner.

Temperature and moisture content during sample transport, handling and preparation are widely reported throughout the literature as critical for reliability of respiration test indices as biological activity is a function of both parameters.

Adequate sample moisture is critical. Test methods specify sample preparation requirements including moisture and temperature adjustment, and lag time for microbial acclimatisation. Acclimatisation is particularly important where samples have dried to < 40% moisture, or where samples have been enclosed in a sealed container without adequate air for an extended period of time, or have been frozen during transport.

Samples for microbial testing must not be frozen. To minimize changes in the sample due to microbial activity with samples maintained at temperatures of >1°C and <4°C during handling and transport; and packaged and handled in a manner to best avoid risk of freezing, high temperatures, drying out, and the development anaerobic conditions.

The methods described aim for increased validity and consistency in laboratory testing by ensuring a representative sample is obtained and by minimising risk of damaging impact on samples in transport.

There are known interferences that can distort respirometry test results, including the following:

a) **Time lag between sampling and testing**: respiration determination is reported to be sensitive to the time lag between sample collection and analysis. To minimize changes in the sample due to microbial activity over time, samples should be analysed as soon as possible after collection. Ideally samples should be prepared and assessment begun within 48 h of sampling. If this is not possible, a sample management plan should be agreed with the laboratory conducting the test.

b) **Temperature and moisture content during sample transport, handling and preparation** are widely reported throughout the literature as critical parameters for respiration test indices, as biological activity is a function of both temperature and suitable moisture content. To minimize changes in the sample due to microbial activity, samples should be maintained at temperatures of

>1°C and <4°C during handling and transport, and packaged and handled in a manner that best avoids both drying out and the onset anaerobic conditions. Test methods specify sample preparation requirements, including moisture and temperature adjustment, and lag time for microbial acclimatization. Acclimatization is particularly important where samples have dried to < 35% moisture, have been enclosed in a sealed container without adequate air for an extended period of time, or have been frozen during transport.

c) **Volatile ammonia** can be a significant cause of noxious odours and is known to be phytotoxic and to inhibit microbial activity at elevated levels. Trials and laboratory experience have shown false positive results from respirometry-based tests due to the suppression of biological activity from elevated volatile ammonia levels. Volatile ammonia assessment should be implemented to inform the validity of results from respirometry tests.

d) **Particle size for test:** respirometry tests for composts in UK, EU and USA standards are commonly conducted on compost products of ≤ 10 mm particle size grade. As requirements in Table N3.2 are based on international standards and research, it is recommended that respirometry assessments for compliance with this Standard be carried out on a ≤ 10 mm particle size fraction of the specimen for test.

e) **Acclimatization and rewetting of dry samples**: where the received sample is assessed to have a moisture content below 40%, the sample should be remoistened and thoroughly mixed to a moisture content of 50%. To acclimatize microbial activity prior to testing, place the remoistened sample in an unsealed container that avoids contamination, is out of direct sunlight and is in a draught-free environment at a temperature of (25°C ±2°C) for 48 hours prior to conducting tests specified for biological stability. NOTE: this requirement applies irrespective of any other requirement specified in an individual method.

Laboratory tests where respiration is calculated per quantity of volatile solids

f) Carbonates can increase volatile solids determinations due to the release of CO2 during sample combustion at 550°C in the LOI (Loss on Ignition) method specified for BVS. Samples with significant carbonate content must be pre-treated (leached with acid) prior to analysis to remove carbonates.

g) Inert materials, including petroleum-based materials such as film and hard plastics can increase volatile solids determinations due to the release of CO2 during sample combustion at 550°C in the LOI method specified for BVS, therefore plastics and other contaminants should be removed to the greatest extent possible prior to combustion. The mass of contaminants removed prior to sample combustion should be recorded and test results adjusted to account for the mass of inert materials in the original sample.

1.5 Working with your lab

Sample management practices should be agreed with the laboratory conducting the test, and should be documented on the purchase order. In addition, the laboratory should be advised upon dispatch of the sample from the facility and arrangements made for the laboratory to confirm receipt of sample.

One advantage of the current AS4454 (2012) standard is that simple tests are now available for on-site assessment of product maturity. This includes TMECC 05-05B bioassay, and the Solvita ® maturity index test kit (which provides two tests, assessing both CO2 evolution and also volatile ammonia).

This allows manufacturers to readily test products prior to sale for compliance with maturity specifications. To best correlate the results with full characterisation test conducted by your laboratory service provider, check and ensure the temperature settings in your on-site “lab” room are equivalent to those of your independent laboratory service provider for more comparable biological stability results; and make sure you are using the same seed variety and conditions as your lab for the bioassay.

1.6 Determining the number of incremental samples required per batch

The number of sampling points required to obtain a representative sample of compost from a batch is calculated from international standards[1], note that these quantities

A minimum of 12 and a maximum of 30 *incremental samples* shall be obtained from a compost batch of defined volume (of up to 5,000 m^3), then thoroughly mixed into a representative *composite sample*:

Number of incremental samples required for piles of different size

Windrow or pile volume	No. of increment samples required
≤ 575 m^3	12
600 m^3	13
1000 m^3	16
1500 m^3	20
2000 m^3	23
2500 m^3	25
3000 m^3	28
≥ 3600 m^3	30

The international standards for sampling from which these calculations are obtained is relevant for sampling materials where the properties being tested for are consistently distributed throughout the material.

The AS4454 (2012) standard provides guidance for the minimum required size of each *incremental sample* on the basis of the maximum particle size range of the material being sampled, as shown in the table below. The objective is to ensure the sampling method is not biased towards avoiding larger particles:

Minimum incremental sample size for materials of different particle size

Largest size mm	70	50	20	10	5
Litres per increment sample	6	4	2.5	1.25	0.6

Note that all incremental samples should all be of a consistent size.

Sampling operations shall be carried out over a sufficiently short period of time and in such a way as to avoid any alteration in the characteristics of the product. During sampling, all incremental samples shall be handled and stored in a manner that avoids contamination and maintains their characteristics.

[1] British Standards Institution. (2000). British Standard BS EN 12579:2000 Soil improvers and growing media: Sampling. British Standards Institution (BSI), London, United Kingdom www.bsi-global.com This is the English language version of the DIN EN 12579 : 1999 European Standard.

1.7 Sample division method: coning and quartering

The result of collecting and mixing the specified number of *incremental samples* each of consistent volume of the specified minimum required size may result in a composite sample of significantly larger quantity than is required for testing.

After thorough mixing, the resulting representative *composite sample* (or *combined sample*) can be divided into representative sub-samples via the coning and quartering method.

1.7.1 Apparatus

All apparatus must be clean

- 20 L plastic pails with lids; Spade/s; Plastic sheet (use the thickest available polyethylene builders plastic); Means of cleaning the apparatus

1.7.2 Method – coning and quartering

a) Empty the increment samples onto a clean plastic sheet to avoid contaminating the sample with material from the ground. Using a clean spade, arrange into a conical pile and thoroughly mix by working the pile whilst moving through two full 360^0 circuits around the pile.

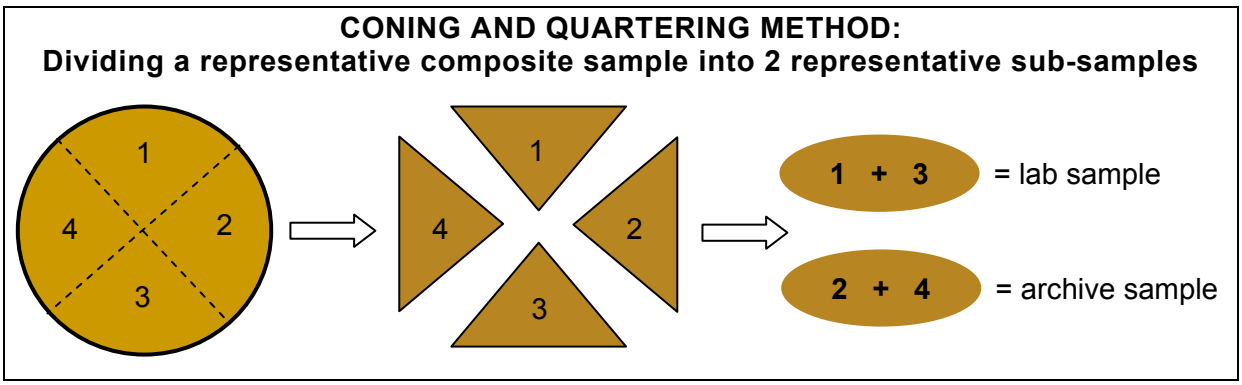
b) Cut the pile into 4 segments of equal size using the spade (as though cutting slices of a cake), and pull the segments apart with the blade to separate from each other;

c) Take two equally sized opposite segments and combine them to form a representative sub sample (*final sample*) that is approximately half of the composite sample in volume. This combined *final sample* for test is then packaged and transported to the laboratory.

d) Take the other two equally opposite sized segments and combine them and retain this composite *final sample* (approximately half of the composite sample in volume) at the facility as an '*archive sample*'. This is important in case the *final sample* sent to the laboratory is lost in transit or the laboratory experiences difficulties, or if re-testing of the sample is required for any reason. If possible it is preferable to refrigerate the archive sample in a suitably labelled and sealed container (ideally less than 4^oC but not below 1^oC); otherwise store the container in a dark, dry, cool location out of direct sunlight at a temperature of less than 10^oC but not below 0^oC to reduce any change in characteristics over time.

e) Coning and quartering can be repeated sequentially to halve the sample again as required to further reduce the *composite sample* into smaller representative *final samples* of suitable quantity.

f) Discard the unneeded or remaining material, or otherwise add back to a new batch of incoming raw materials for reprocessing.



Section 2 Windrow sampling methods and diagrams

2.1 Method A: sampling completely mixed compost piles

2.1.1 Apparatus

- All apparatus must be clean
- 20 L plastic pails with lids.
- Trowels or trenching shovel.
- Means of cleaning the apparatus.
- Suitable means of turning the compost pile.

2.1.2 Sampling method A

Arrange for the pile to be turned to break up clumps and completely mix and homogenize the materials.

For a pile that has been so thoroughly mixed with clumps broken up and distributed with a windrow turner (or equivalent), *increment samples* of consistent size can be taken from random positions (*sampling sites*) from along the exterior of the length and height of the pile immediately after mixing.

Ideally sample within 2 hours of mixing as a range of characteristics such as moisture distribution, O2 availability and biological distribution can alter rapidly, and certainly within a few hours of mixing.

Use a clean trenching shovel or trowel to extract *incremental samples* of consistent volume at various, evenly spaced locations from the sides of the windrow. Dig a plug from the pile for each *incremental sample* rather than simply scaping from the surface.

During sampling take care to handle and store *incremental samples* in a manner that maintains their characteristics, being careful to avoid cross contamination of the *sampling sites* prior to sampling (eg with turner wheels or mechanism), and of the *increment samples* during sampling and mixing (eg. from windblown materials).

Avoid sampling from the ends of the windrow where conditions are unique (in terms of surface area to volume ratio) and are not representative of conditions in the greater composting mass.

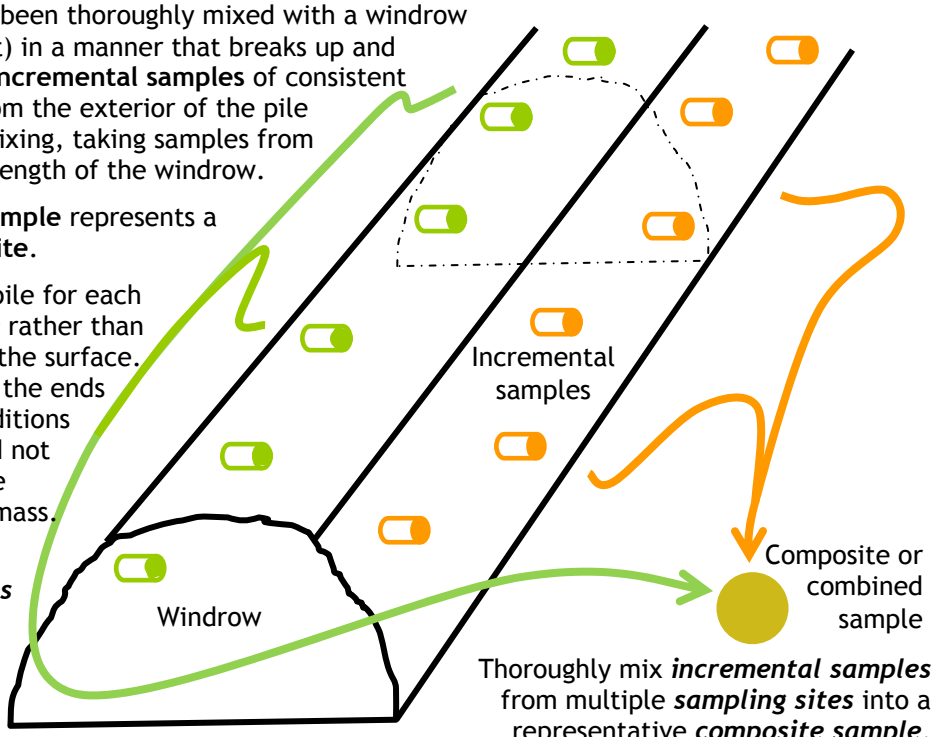
Method A: compost sampling diagram

For a batch that has been thoroughly mixed with a windrow turner (or equivalent) in a manner that breaks up and distributes clumps, **incremental samples** of consistent size can be taken from the exterior of the pile immediately after mixing, taking samples from each side along the length of the windrow.

Each **incremental sample** represents a different **sampling site**.

Dig a plug from the pile for each ***incremental sample*** rather than simply scaping from the surface. Avoid sampling from the ends of a windrow as conditions there are unique and not representative of the greater composting mass.

The number of ***incremental samples*** required for a representative ***composite sample*** depends on the size of the compost pile.



Recycled Organics Unit © (2011)

Use a clean trenching shovel or trowel to extract *incremental samples* of consistent and adequate volume, taking care to handle and store samples in a manner that maintains their characteristics, and being careful to avoid cross contamination of the *sampling sites* prior to sampling (eg with turners); and of the increment samples during sampling (eg. from windblown materials).

2.2 Method B: Sampling compost piles without complete mixing

2.2.1 Apparatus

- All apparatus must be clean
- 20 L plastic pails with lids. Trowels or trenching shovel. Means of cleaning the apparatus.
- Suitable means of turning the compost pile.

2.2.2 Method B: sampling compost piles without adequate mixing

A different sampling method is required where the windrow or pile is unturned, or turning methods do NOT completely break up and distribute clumps and homogenize the materials.

Arrange for a wheel loader or excavator to dig or back-blade half way in to a section of the pile exposing a near vertical cross section face (alternatively, for smaller piles, a loader may cut a cross section through the entire windrow).

This vertical face represents a *sampling site* that allows *increment samples* to be extracted from the cross section of the windrow. Sampling should occur immediately after opening and exposing the sampling site with the machinery (ideally within 2 hours as a range of characteristics such as moisture distribution, O2 availability and biological distribution can alter rapidly, and certainly within a few hours of mixing).

Use the clean trenching shovel or trowel to extract between three and five *increment samples* of consistent volume at various, evenly spaced locations from the exposed cross section face of the windrow.

Use a clean trenching shovel or trowel to extract *increment samples* of consistent volume at various, evenly spaced locations from the exposed cross section faces of the windrow.

Dig a plug from the pile for each incremental sample rather than simply scaping from the surface. During sampling take care to handle and store *incremental samples* in a manner that maintains their characteristics, being careful to avoid cross contamination of the *sampling sites* prior to sampling (eg with turners), and of the *increment samples* (eg. from windblown materials).

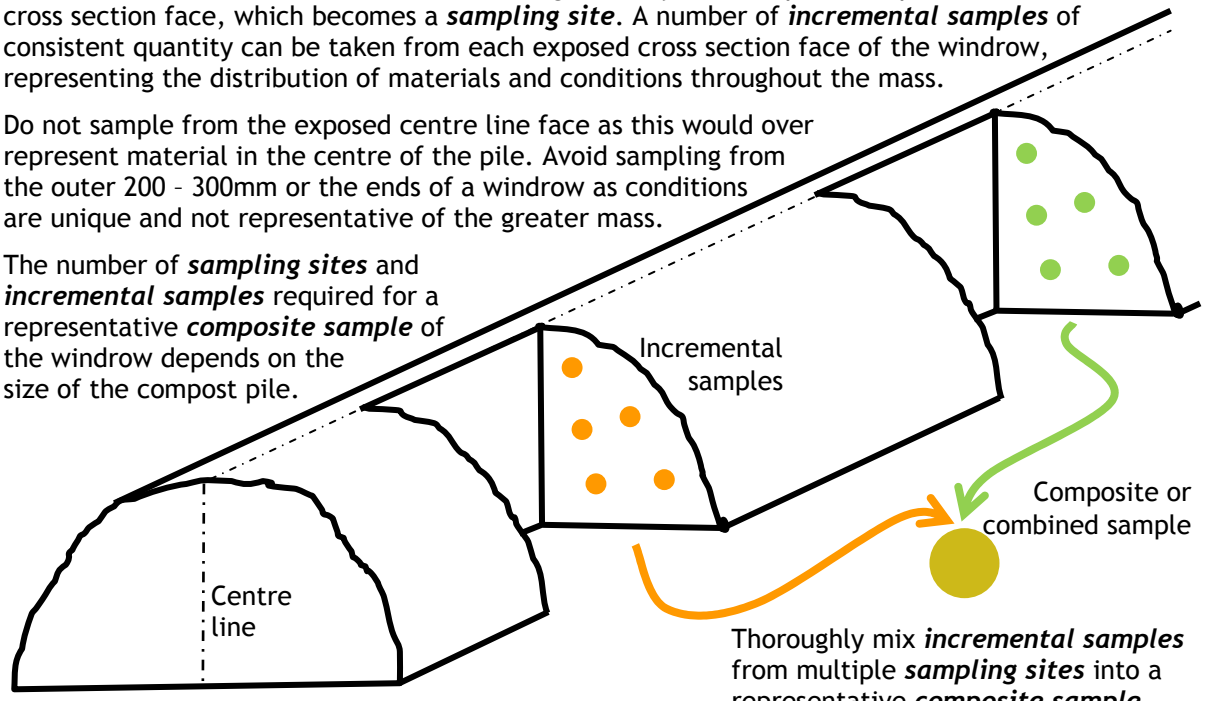
Note that samples are extracted from the cross section face, and NOT the centre line face within the windrow (as this would over represent material from the centre of the pile). Avoid sampling from the outer 200 - 300mm layer when sampling (top, bottom and sides), and avoid sampling from the ends of the windrow where conditions are unique (in terms of surface area to volume ratio) and are not representative of conditions in the greater composting mass.

Method B: Windrow compost sampling diagram

For use where a windrow or pile is unturned, or turning methods do **NOT** completely homogenize the materials. Use a loader or excavator to dig half way into the pile to expose a near vertical cross section face, which becomes a ***sampling site***. A number of ***incremental samples*** of consistent quantity can be taken from each exposed cross section face of the windrow, representing the distribution of materials and conditions throughout the mass.

Do not sample from the exposed centre line face as this would over represent material in the centre of the pile. Avoid sampling from the outer 200 – 300mm or the ends of a windrow as conditions are unique and not representative of the greater mass.

The number of ***sampling sites*** and ***incremental samples*** required for a representative ***composite sample*** of the windrow depends on the size of the compost pile.



Recycled Organics Unit © (2011)

Use a clean trenching shovel or trowel to extract *increment samples* of consistent volume, taking care to handle and store samples in a manner that maintains their characteristics, and being careful to avoid cross contamination of the *sampling sites* prior to sampling (eg with loader or excavator), and of the increment samples (eg. from windblown materials).

2.2.3 Method C: sampling compost piles assessment of beneficial microorganisms

Note, this is not relevant for pathogen or indicator pathogen assessment, which must be conducted on a representative sample obtained in accordance with methods above.

In the instance that a customer requests an assessment of the microbial communities of a compost for identification of microorganisms that are beneficial to soil health, such as counts of total or active fungal and/or bacterial communities:

a) Samples should preferably be taken from locations in the pile that most closely represent environmental conditions (moisture, air, heat) that will occur when the compost has been applied. This will better represent the microbial activity that the compost is able to support once applied.

b) There is little value in assessing compost sampled from locations in the pile where environmental conditions are irrelevant to application conditions and may be entirely unsuitable for the organisms being tested for.

c) Generally follow method B above, sampling only from areas of the cross section face of the compost pile that best represent relevant environmental conditions.

2.3 Packaging and shipping

The *composite sample* for testing by the laboratory must be packaged in such a way that characteristics are unaltered on arrival at the laboratory, and must be clearly labelled for identification and to maintain chain of custody.

Arrangements for testing should be made with the laboratory prior to sampling and shipping, including written confirmation of the analysis to be conducted, how the sample is to be managed on arrival, and expected date of sample delivery (to ensure test apparatus is available).

- Use a 10 litre sealable container (a robust plastic "handy pail" with sealable lid is suitable where samples are sent and received on the same day by road transport. Where samples are sent by air or overnight transport a fully enclosed EPS (polystyrene) container with tight sealing lid that can be securely taped to seal is preferred to avoid extremes of temperature. A small EPS esky is suitable for small samples, or a fully enclosed "ice pack broccoli" EPS vegetable box for larger samples.
- Load sample into strong high density polyethylene sampling bags then place bags into the container. Where containers have been previously used, thoroughly clean the containers[2], and rinse 3 times with clean water drain and air dry prior to loading the combined sample (with clean shovel) directly from the coning and quartering process above. For new containers, clean and then rinse with clean water prior to use.
- Securely attach shipping labels with tape to the outside of the container (1 on top, 1 on side).
- The label should clearly identify the delivery address and contact details for the laboratory.
- Place a similar label inside the container, with a letter providing sample and client details, and specifying the analysis to be conducted enclosed in a zip-lock plastic lunch bag.
- For chain of custody, the labels and/or enclosed paperwork should also clearly identify:
    - The source facility (facility name);
    - A unique batch identification number;
    - The type and grade of material;
    - The number of incremental samples combined for this representative laboratory sample;
    - The date and time of sampling;
    - The person responsible for sampling and packaging (name and signature); and
    - The facility contact person/number.
- Place pre-frozen, sealed gel type ice-pack/s on top of the sample in the packaging container (do not include ice that can melt and leak), then immediately prior to shipping securely install the lid to seal, and tape around the lid to ensure it cannot be dislodged.
- For respirometry tests, samples should be received by the lab and sample preparation begun within 48 hours of sampling. During this period, some methods specify that samples must not be frozen, and that temperatures above 4^{o}C are permitted for no more than 24 hours.
- Keep the container and sample in a dark, dry, cool location out of direct sunlight whilst awaiting collection (ideally refrigerated between 1 and 4^{o}C, or otherwise less than 10^{o}C, but not frozen).
- Arrange for collection on the same day of sampling, or the morning following.
- Use a delivery service that will deliver it to the laboratory within 24 hours.

[2] Where containers are simply soiled washing and brushing with mild detergent solution prior to triple rinsing is sufficient. Cleaning solutions such as *Decon* are suitable for sterilising containers where there is concern over the potential presence of chemical or biological residues, see www.sterile.com

- Upon dispatch or collection by delivery courier, contact the testing laboratory to confirm that the sample is in transit and to provide an ETA for delivery (eg. *tomorrow*).

2.3.1 Packaging for analysis of chemical contaminants

Where a sample is to be tested for potential presence of chemical contaminants, ideally pack a portion of the laboratory sample into a glass jar sealed with a metal cap or with aluminium foil under the cap to avoid inadvertent secondary contamination as organic pesticides can migrate through plastic lids.

500 ml is sufficient for mulch samples and 250 ml for soil conditioner samples.

2.3.2 Shipping label: top of container:

Print and complete for each sample, and attach to sample for transport to lab, consistent with the packaging and shipping instructions above.

DELIVERY ADDRESS

Laboratory delivery address:

Lab contact person: _____

Lab contact number: _____

Sample for analysis:

Please keep cool and out of direct sunlight

Project code: _____ Sample #: _____

Please advise by email when sample is received to confirm analysis required.

Contact email: _____

2.3.3 Shipping label: side of container:

Print and complete for each sample, and attach to the side of the package.

DELIVERY ADDRESS

Laboratory delivery address:

Lab contact person: _____

Lab contact number: _____

Sample for analysis:

Please keep cool and out of direct sunlight

Please open samples when received to allow exchange of air. Sample preparation and analysis should begin within 48 hours of sampling.

Samples should be kept contained in a dark, dry, cool location out of direct sunlight (ideally less than 10^{o}C but not below 1^{o}C. Do not freeze samples).

Sample has been shipped on day of collection, and should arrive at the laboratory by courier the day after sampling.

Sample details (and chain of custody):

Please advise by email when sample is received to confirm analysis required.

Contact email: _____

Project/batch code: _____ Sample #: _____

Details to be completed by person responsible for sampling and packaging:

Facility name: _____

Batch number: _____

Material type and grade: _____

_____

Sample quantity: _____

Number of incremental samples combined in this sample: _____

Sampling date and time: _____

Name: _____ Signature: _____