

APPENDIX 1: SELECTING THE APPROPRIATE SAMPLING POINTS AND COLLECTING SAMPLES

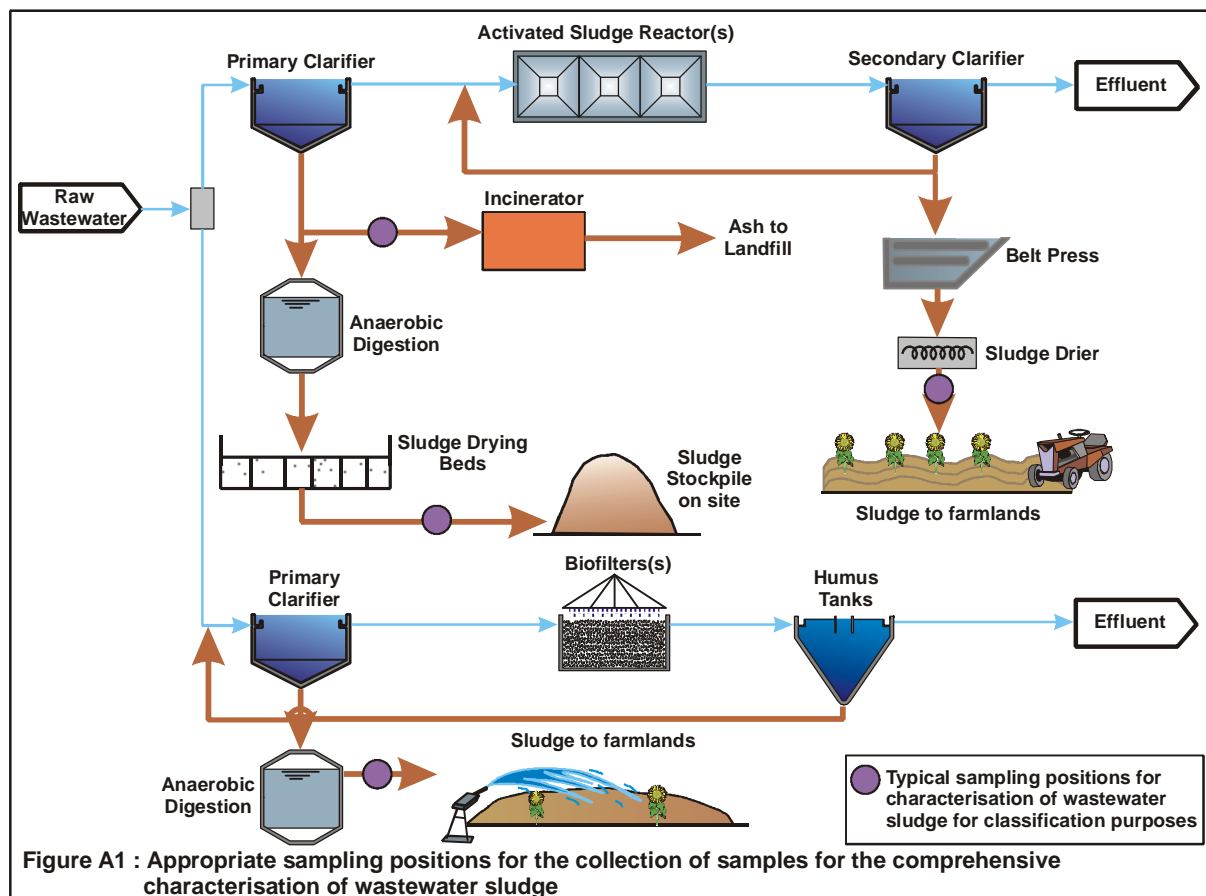
Collect at least three daily (24 hour) composite sludge samples from each sludge stream considered for use/disposal. If disinfection processes are employed, an additional three samples should be collected immediately after the disinfection process. The samples need to be cooled and sent to the laboratory as soon as possible to prevent contamination and re-growth. Figure A1 shows appropriate sampling positions for this purpose. It is important not to confuse plant operational sample points such as the clarifier underflow or the activated sludge return flow with the samples required for classification purposes, although in some cases these could be the same.

A laboratory will typically require at least 500 g _{dry mass} to perform all the required analyses. In other words, if the sludge destined for disposal has a solids content (SS – suspended solids) of 1%, the laboratory can require up to 50 l of sample. Discuss the requirements with the selected laboratory.



Plants that employ dewatering, stabilisation, conditioning and/or disinfection should collect the sludge samples in a glass container and keep it cool ($\leq 4\text{ }^{\circ}\text{C}$) as this will minimise microbiological activity. The 1l “Consol” preserve bottles are ideal but not required. Helminth ova adhere to glass. It is advisable to collect a separate Helminth ova sample in a plastic container.

1000 ml “Consol” preservative container



APPENDIX 2: ANALYTICAL METHODS

Appendix 2.1 Volatile acids and alkalinity

1. Scope

This method is applicable to the determination of volatile acids and alkalinity in sludge.

2. Principle

Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid to a pH 4.0. Evaporation of volatile material and titration to pH 6.2.

3. Interferences

Do not dilute, concentrate or alter sample. Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response.

4. Equipment

4.1 Where applicable use only class A volumetric glassware.

4.2 Centrifuge with centrifuge tubes.

4.3 Digital burette.

4.4 Pipette.

4.5 100 mL Erlenmeyer flask.

4.6 pH meter.

4.7 Magnetic stirrer with stirring bars.

5. Reagents and Standard Solutions

5.1 *Distilled water:* Conductivity should be less than $\leq 0.5 \text{ mS m}^{-1}$.

5.2 *Standard 0.1 mole per litre sulphuric acid H_2SO_4 .*

5.2.1 Prepare a 0.1 mL H_2SO_4 solution by diluting 5.6 mL concentrated H_2SO_4 (sg = 1.84) to 1000 mL with distilled water.

5.3 *Standardisation of 0.1 mole per litre H_2SO_4 .*

5.3.1 Standardise against 0.1 mol I^{-1} Na_2CO_3 . Dissolve 10.599 g Na_2CO_3 in 1000 mL of distilled/de-ionised water. Retention time: 3 months. Store in fridge.

5.3.2 Pipette 25 mL of the 0.1 mol I^{-1} Na_2CO_3 in an Erlenmeyer flask.

5.3.3 Dilute with ± 25 mL distilled/de-ionised water.

5.3.4 Add 3 drops BDH "4.5" indicator.

5.3.5 Titrate with 0.1 mol I^{-1} H_2SO_4 , colour change from blue to orange.

5.3.6 Determine the H_2SO_4 concentration. See section 8.1.

5.4 *0.05 M/L Sodium hydroxide (NaOH) solution.*

5.4.1 Prepare a 0.05 mol I^{-1} sodium hydroxide (NaOH) solution by dissolving 2.0 g NaOH in about 800 mL distilled water. Cool down and make up to 1 L.

- 5.5 *Standardisation of 0.05 mol I⁻¹ sodium hydroxide (NaOH).*
- 5.5.1 Standardise against 0.1 mol I⁻¹ H₂SO₄.
- 5.5.2 Pipette 25 ml of the 0.05 mol I⁻¹ NaOH in an Erlenmeyer flask.
- 5.5.3 Dilute with \pm 25 ml distilled/de-ionised water.
- 5.5.4 Add 3 drops BDH "4.5" indicator.
- 5.5.5 Titrate with 0.1 mol I⁻¹ H₂SO₄.
- 5.5.6 Determine the NaOH concentration. See section 8.1.
- 5.6 *Quality Control solutions for alkalinity: (QC 1: 1500 mg/l.)*
- 5.6.1 Dissolve 1.65 g Na₂CO₃ (dried for 4 hours at 105°C \pm 2°C) in an A grade 1L Volumetric Flask and bring to the mark with distilled/de-ionised water. Store in the fridge in a polyethylene bottle. Retention time: 1 month.
- 5.7 *Quality Control solutions for alkalinity: (QC 2: 500 mg/l.)*
- 5.7.1 Dissolve 0.55 g Na₂CO₃ (dried for 4 hours at 105°C \pm 2°C) in an A grade 1L Volumetric Flask and bring to the mark with distilled/de-ionised water. Store in the fridge in a polyethylene bottle. Retention time: 1 month.

6. Sampling and Storage:

Collect samples in polyethylene or borosilicate glass bottles and store at a low temperature. Fill bottles completely and cap tightly.

7. Analytical Procedure

- 7.1 Centrifuge the sludge sample for about 5 minutes at 4000 r.p.m.
- 7.2 Measure out 50 ml of the water into a 100 ml Erlenmeyer flask.
- 7.3 Titrate with 0.1 mol I⁻¹ H₂SO₄ solution to pH 4.0 and determine alkalinity by noting amount of acid titrated.
- 7.4 Add acid to bring pH to 3.2 to 3.5.
- 7.5 Evaporate on water bath for 30 minutes. Cool to room temperature and titrate with 0.05 mol I⁻¹ sodium hydroxide to a pH of 4.0. Zero the burette.
- 7.6 Titrate to a pH of 6.2 with 0.05 mol I⁻¹ sodium hydroxide and note titration volume needed.

8. Calculation of Results

8.1 Standardisation of acid or base

$$\begin{array}{lcl}
 C_a V_a & = & C_b V_b \\
 C_a & = & \text{Concentration of acid} \\
 V_a & = & \text{Volume of acid titrated} \\
 C_b & = & \text{Concentration of base: } \frac{\text{mass primary standard}}{\text{molar mass}} = \text{mol} \\
 V_b & = & \text{Volume of base used} \\
 MF & = & \text{Multiplication factor}
 \end{array}$$

8.1.1 Concentration of acid = $\frac{C_b V_b}{V_a}$
= $\frac{\text{Concentration} \times 25 \text{ ml}}{V_a \text{ (ml)}}$
= $\pm 0.1 \text{ mol l}^{-1}$

8.1.2 Concentration of base = $\frac{2(C_a V_a)}{V_b}$
= $\frac{2(\text{concentration} \times V_a)}{25 \text{ ml}}$
= $\pm 0.05 \text{ mol l}^{-1}$

8.1.3 Multiplication factor for alkalinity = $\frac{\text{Concentration of acid calculated in 8.1.1}}{\text{Actual concentration of acid}}$
= $\frac{\pm 0.1000 \text{ mol l}^{-1} \times 200}{0.1000 \text{ mol l}^{-1}}$
= ± 200

8.1.4 Multiplication factor for acid = $\frac{\text{Concentration of acid calculated in 8.1.2}}{\text{Actual concentration of base}}$
= $\frac{\pm 0.05 \text{ mol l}^{-1} \times 200}{0.05 \text{ mol l}^{-1}}$
= ± 1

8.1.5 Alkalinity determination = $\frac{\text{Concentration of acid calculated in 8.1.1} \times \text{normality} \times 50\ 000}{\text{Volume of sample}}$
= $\frac{\text{Titrated (ml)} \times (\pm 0.2\text{N}) \times 50\ 000}{50 \text{ ml}}$
= $\text{Titrated (ml)} \times (\pm 2000)$

8.2 Total alkalinity = (acid titration to pH 4) x (± 200)

To convert from molarity to normality:

$$\begin{aligned} M &= D \times V \\ &= 1.84 \times (5.6 \text{ mL}) \\ &= 10.304\text{g} \\ &\quad \underline{\text{molar mass}} \end{aligned}$$

BUT equivalent mass = Z

Z is the factor that depends on the chemical context (chemical reaction involved). For acids the value of Z is equal to the number of moles of H^+ , displaceable from one mole of acid, e.g. for HCl , $Z = 1$, while for H_2SO_4 , $Z = 2$.

$$\text{Molar mass of } \text{H}_2\text{SO}_4 = 89.08 \text{ g mol}^{-1}$$

$$\text{The equivalent mass of } \text{H}_2\text{SO}_4 = \frac{89.08 \text{ g mol}^{-1}}{2} = 49.04 \text{ g mol}^{-1}$$

The normality when 5.6 mL of H_2SO_4 is used will then be:

$$\begin{aligned} \text{Normality} &= \frac{\text{mass used}}{\text{g/equivalent}} \\ &= \frac{10.304 \text{ g}}{49.04 \text{ g/equivalent}} \\ &= 0.21 \text{ N} \\ \therefore 0.1 \text{ M } \text{H}_2\text{SO}_4 &= 0.21 \text{ N } \text{H}_2\text{SO}_4 \end{aligned}$$

If NaOH titration from 4.0 to 6.2 < 3.7, then x 50.

If NaOH titration from 4.0 to 6.2 \geq 3.7, then x 75.

9. Reporting of Results

9.1 Express results as Total Alkalinity in $\text{mg CaCO}_3 \text{ l}^{-1}$.

9.2 Express results as Volatile Acid in $\text{mg CH}_3\text{COOH l}^{-1}$.

Reference: Adapted from Standard Methods. 1998. American Public Health Association, American Water Works Association, Water Environment Federation. *Standard methods for the examination of water and wastewater*. Edited by M.A.F. Franson, A.D. Eaton, L.S. Clesceri, and A.E. Greenberg. 20th Edition. Washington DC. USA. American Public Health Association

Appendix 2.2 Procedure to determine Helminth ova in wastewater sludge

The method below was developed for the determination of *Ascaris* ova in wastewater and sludge samples. The method can also be used for the determination of all helminth' ova. The method is currently being optimised and adapted to ensure better recovery (Jimenez-Cisneros, 2005). The updated method will be communicated as soon as it becomes available. In the interim, laboratories are urged to adopt the method detailed below (adapted from EPA/625/R-92/013).

Test Method for Detecting, Enumerating, and Determining the Viability of *Ascaris* Ova in Sludge

1. Scope

This test method describes the detection, enumeration, and determination of viability of *Ascaris* ova in water, wastewater, sludge, and compost. These pathogenic intestinal helminths occur in domestic animals and humans. The environment may become contaminated through direct deposit of human or animal faeces or through sewage and wastewater discharges to receiving waters. Ingestion of water containing infective *Ascaris* ova may cause disease.

This test method is for wastewater, sludge, and compost. It is the user's responsibility to ensure the validity of this test method for untested matrices.

This standard does not purport to address all the safety problems associated with it use. It is the responsibility of the user of this standard to establish appropriate safety and health practices.

2. Terminology

The normal nematode life cycle consists of the egg, 4 larval stages and an adult. The larvae are similar in appearance to the adults; that is, they are typically worm-like in appearance.

Molting (*ecdysis*) of the outer layer (*cuticle*) takes place after each larval stage. Molting consists of 2 distinct processes, the deposition of the new cuticle and the shedding of the old one or escheatment. The cuticle appears to be produced continuously, even throughout adult life.

A molted cuticle that still encapsulates a larva is called a *sheath*.

Ascarid egg shells are commonly comprised of layers. The outer tanned, bumpy layer is referred to as the *mammillae* layer and is useful in identifying *Ascaris* eggs. The *mammillae* layer is sometimes absent. Eggs that do not possess the *mammillae* layer are referred to as *decorticated eggs*.

A potentially infective *Ascaris* egg contains a third stage larva encased in a sheath of the first larval stage.

3. Summary of Test Method

This method is used to concentrate pathogenic *Ascaris ova* from wastewater, sludge, and compost. Samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particulates. The solids in the screened portion are allowed to settle out and the supernatant is decanted. The sediment is subjected to density gradient centrifugation using magnesium sulfate (specific gravity 1.20). This flotation procedure yields a layer likely to contain *Ascaris* and some other parasitic ova if present in the sample. Small particulates are removed by a second screening on a small mesh size screen. Proteinaceous material is removed using an acid alcohol/ethyl acetate extraction step. The resulting concentrate is incubated at 26°C. until control *Ascaris eggs* are fully embrocated. The concentrate is then microscopically examined for the categories of *Ascaris ova* on a Sedgwick-Rafter counting chamber.

4. Significance and Use

This test method is useful for providing a quantitative indication of the level of *Ascaris ova* contamination of wastewater, sludge, and compost.

This test method will not identify the species of *Ascaris* detected nor the host of origin.

This method may be useful in evaluating the effectiveness of treatment.

5. Interferences

Freezing of samples will interfere with the buoyant density of *Ascaris ova* and decrease the recovery of ova.

6. Apparatus

6.1 A good light microscope equipped with bright field, and preferably with phase contrast and/or differential contrast optics including objectives ranging in power from.

6.2 Sedgwick-Rafter cell.

6.3 Pyrex beakers, 2 l. Coat with organosilane.

- 6.4 Erlenmeyer flask, 500 mL. Coat with organosilane.
- 6.5 A centrifuge that can sustain forces of at least 660 X G with the rotors listed below.
 - 6.4.1 A swinging bucket rotor to hold 100 or 250 mL centrifuge glass or plastic conical bottles.
 - 6.4.2 A swinging bucket rotor to hold 15 mL conical glass or plastic centrifuge tubes.
- 6.6 Tyler sieves.
 - 6.5.1 20 or 50 mesh.
 - 6.5.2 400 mesh, stainless steel, 5 inch (12.7 cm) in diameter.
 - 6.5.3 A large plastic funnel to support the sieve. Coat with organosilane.
- 6.7 Teflon spatula.
- 6.8 Incubator set at 26°C.
- 6.9 Large test tube rack to accommodate 100 or 250 mL centrifuge tubes.
- 6.10 Small test tube rack to accommodate 15 mL conical centrifuge tubes.
- 6.11 Centrifuge tubes, 100 or 250 mL. Coat with organosilane.
- 6.12 Conical centrifuge tubes, 15 mL. Coat with organosilane.
- 6.13 Stoppers.
- 6.14 Wooden applicator sticks.
- 6.15 Pasteur pipettes. Coat with organosilane.
- 6.16 Vacuum aspiration apparatus.
 - 6.16.1 Vacuum source.
 - 6.16.2 Vacuum flask, 2 L or larger.
 - 6.16.3 Stopper to fit vacuum flask, fitted with a glass or metal tubing as a connector for 1/4 inch tygon tubing.
- 6.17 Wash bottles (500 mL), label "Water".
- 6.18 Spray bottles (16 fl oz.) (2).
 - 6.18.1 Label one "Water".
 - 6.18.2 Label one "1% 7X".

7. Reagents and Materials

- 7.1 Purity of Reagents - Reagent grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.
- 7.2 Purity of Water - Unless otherwise indicated, references to water shall be understood to mean reagent water.
- 7.3 Preparation of Reagents
 - 7.3.1 Phosphate buffered water (1 L = 34.0 g KH_2PO_4 , pH adjusted to 7.2 ± 0.5 with 1 N NaOH).

- 7.3.2 1% (v/v) 7X ("Limbro" laboratory detergent) (1 l = 999 ml phosphate-buffered water, 1 ml 7X "Limbro", Adjust pH to 7.2 + 0.1 with 1 N NaOH).
 - 7.3.3 Magnesium sulphate, sp. gr. 1.20. (1 l = 215.2 g MgSO₄ check specific gravity with a hydrometer; adjust as necessary to reach 1.20).
 - 7.3.4 Acid alcohol. 0.1 N H₂SO₄, made in 35% ethyl alcohol. (100 ml = 35 ml EtOH, 0.9807 g H₂SO₄)
 - 7.3.5 Ethyl Acetate, reagent grade.
- 7.4 Organosilane. For coating glassware (This is to prevent the Helminth Ova from adhering to the glass). Coat all glassware according to manufacturer's instructions. (Alternatively, only use only plastic equipment)
- 7.5 Fresh *Ascaris ova* for positive control, either dissected from *Ascaris suum* gravid adult female worms or purified from *Ascaris* infected pig faecal material. (Other Helminth ova can also be used)

8. Precautions

- 8.1 When harvesting *Ascaris ova* (or any other Helminth ova) from gravid female worms, the analyst must wear latex gloves, a surgical mask and protective goggles or full face mask, and laboratory coat before dissecting the worms. Moreover, it is recommended that the *Ascaris ova* (or any other Helminth ova) (or any other Helminth ova) harvest be carried out either in a biological safety cabinet or minimally a chemical hood. These precautions are designed to prevent the development of an allergy to *Ascaris* pseudocoelomic fluid. If infective *Ascaris* ova are ingested they may cause disease.

9. Sampling

- 9.1 Collect 1 litre of compost, wastewater, or sludge in accordance with standard sampling methodology.
- 9.2 Place the sample container(s) on wet ice or around chemical ice and transport back to the laboratory for analysis within 24 hours of collection.
- 9.3 Store the samples in the laboratory refrigerated at 2 to 5°C. Do not freeze the samples during transport or storage.

10. Preparation of Apparatus

Follow the manufacturer's instructions

11. Calibration and Standardization

Follow the manufacturer's instructions

12. Procedure

- 12.1 The percentage moisture of the sample is determined by analysing a separate portion of the sample, so the final calculation of ova per gram dry weight can be determined. The concentration of ova in liquid sludge samples may be expressed as ova per unit volume.
- 12.2 Initial preparation:
 - 12.1.1 Dry or thick samples: Weigh about 300 g (estimated dry weight) and place in about 500 ml water in a beaker and let soak overnight at 4 - 10°C. Transfer to blender and blend at high for one minute. Divide sample into four beakers.
 - 12.1.2 Liquid samples: Measure 1000 ml or more (estimated to contain at least 50 g dry solids) of liquid sample. Place one half of sample in blender. Add about 200 ml water. Blend at high speed for one minute transfer to a beaker. Repeat for other half of sample.
- 12.3 Pour the homogenized sample into a 1000 ml tall form beaker and using a wash bottle, thoroughly rinse blender container into beaker. Add 1% 7X to reach 900 ml final volume.
- 12.4 Allow sample to settle four hours or overnight at 4 - 10°C. Stir occasionally with a wooden applicator, as needed to ensure that material floating on the surface settles. Additional 1% 7X may be added, and the mixture stirred if necessary.
- 12.5 After settling, vacuum aspirate supernatant to just above the layer of solids. Transfer sediment to blender and add water to 500 ml, blend again for one minute at high speed. (Aspiration can also be done manually using plastic pipettes)
- 12.6 Transfer to beaker, rinsing blender and add 1% 7X to reach 900 ml. Allow to settle for two hours at 4 - 10°C, vacuum aspirate supernatant to just above the layer of solids.
- 12.7 Add 300 ml 1% 7X and stir for five minutes on a magnetic stirrer.
- 12.8 Strain homogenized sample through a 20 or 50 mesh sieve placed in a funnel over a tall beaker. Wash sample through sieve with a spray of 1% 7X from a spray bottle.
- 12.9 Add 1% 7X to 900 ml final volume and allow to settle for two hours at 4 - 10°C.
- 12.10 Vacuum aspirate supernatant to just above layer of solids. (Aspiration can also be done manually using plastic pipettes). Mix sediment and distribute equally to 50 ml graduated conical centrifuge tubes. Thoroughly wash any sediment from beaker into tubes using water from a wash bottle. Bring volume in tubes up to 50 ml with water.

- 12.11 Centrifuge for 10 minutes at 1000 X G. Vacuum aspirate supernatant from each tube down to just above the level of sediment. (Aspiration can also be done manually using plastic pipettes). The packed sediment in each tube should not exceed 5 mL. If it exceeds this volume, add water and distribute the sediment evenly among additional tubes, repeat centrifugation, and vacuum aspirate supernatant.
- 12.12 Add 10 to 15 mL of MgSO₄ solution (specific gravity 1.20) to each tube and mix for 15 to 20 seconds on a vortex mixer. (Use capped tubes to avoid splashing of mixture from the tube.)
- 12.13 Add additional MgSO₄ solution (specific gravity 1.20) to each tube to bring volume to 50 mL. Centrifuge for five to ten minutes at 800 to 1000 X g.
- 12.14 Allow the centrifuge to stop without the brake. Pour the top 25 to 35 mL of supernatant from each tube through a 400 mesh sieve supported in a funnel over a tall beaker.
- 12.15 Using a water spray bottle, wash excessive flotation fluid and fine particles through sieve.
- 12.16 Rinse sediment collected on the sieve into a 100 mL beaker by directing the stream of water from the wash bottle onto the upper surface of the sieve.
- 12.17 After thoroughly washing the sediment from the sieve, transfer the suspension to the required number of 15 mL centrifuge tubes, taking care to rinse the beaker into the tubes. Usually one beaker makes one tube.
- 12.18 Centrifuge the tubes for three minutes at 800 X G, then discard the supernatant.
- 12.19 If more than one tube has been used for the sample, transfer the sediment to a single tube, fill with water, and repeat centrifugation.
- 12.20 Re-suspend the pellet in 7 mL acid alcohol solution and add 3 mL ethyl acetate.
- 12.21 Cap the tube with a rubber stopper and invert several times, venting after each inversion.
- 12.22 Centrifuge the tube at 660 x G for 3 minutes.
- 12.23 Aspirate the supernatant above the solids.
- 12.24 Re-suspend the solids in 4 mL 0.1 N H₂SO₄ and pour into a 220-mL polyethylene scintillation vial or equivalent with loose caps.
- 12.25 Before incubating the vials, mark the liquid level in each vial with a felt tip pen. Incubate the vials, along with control vials containing *Ascaris* or other Helminth ova mixed with 4 mL 0.1 N H₂SO₄ at 26°C for three to four weeks. Every day or so, check the liquid level in each vial. Add reagent grade water up to the initial liquid level line as needed to compensate for evaporation. After 18 days, suspend, by inversion and sample small aliquots of the control cultures once every 2 - 3 days. When the majority of the control *Ascaris* ova are fully embryonated, samples are ready to be examined.
- 12.26 Examine the concentrates microscopically using a Sedgwick-Rafter cell to enumerate the detected ova. Classify the ova as either unembryonated, embryonated to the first, second or third larval stage. In some embryonated *Ascaris* ova the larva may be observed to move.

See Figures below for examples of various *Ascaris* egg categories.

13. Calculation

13.1 Calculate % total solids using the % moisture result:

$$\% \text{ Total solids} = 100\% - \% \text{ Moisture}$$

13.2 Calculate categories of ova/g dry weight in the following manner:

$$\text{Ova/g dry wt} = \frac{(\text{NO}) \times (\text{CV}) \times (\text{FV})}{(\text{SP}) \times (\text{TS})}$$

Where:

NO = no. ova

CV = chamber volume(= 1 ml)

FV = final volume in ml

SP = sample processed in ml or g

TS = % total solids

14. Report

14.1 Report the results as the total number of *Ascaris* ova, number of unembryonated *Ascaris* ova, number of 1st, 2nd or 3rd stage larva; reported as number of *Ascaris* ova and number of various larval *Ascaris* ova per g dry weight. Representative reporting forms are shown in Figures below.

Reference: EPA Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge. EPA/625/R-921013, Revised October 1999

Note: This method is currently being revised and adapted and the most recent version will be communicated to interested parties as soon as it becomes available.



Figure 2. *Ascaris* ovum: fertile, note the bumpy outer mammilated layer.

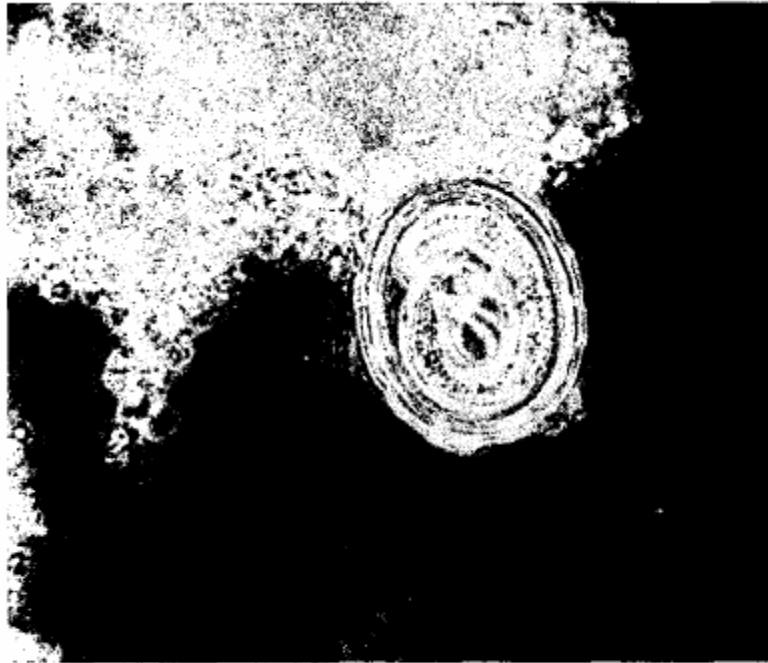
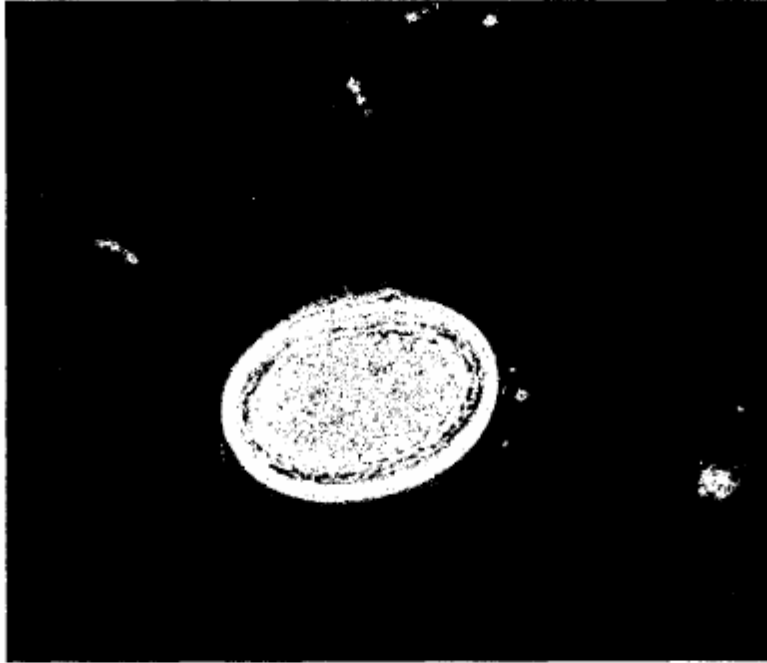


Figure 4. *Ascaris ovum*: decorticated and embryonated.

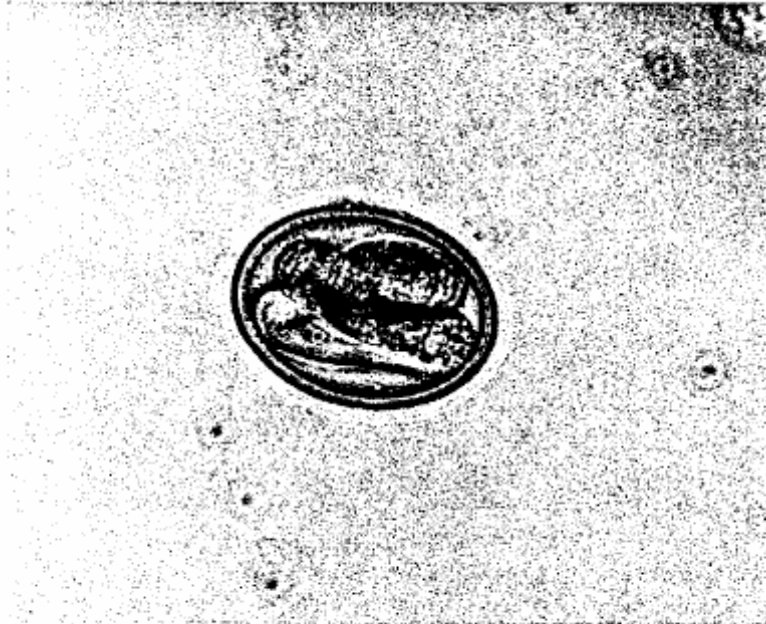


Figure 5. *Ascaris* ovum: decorticated, embryonated.

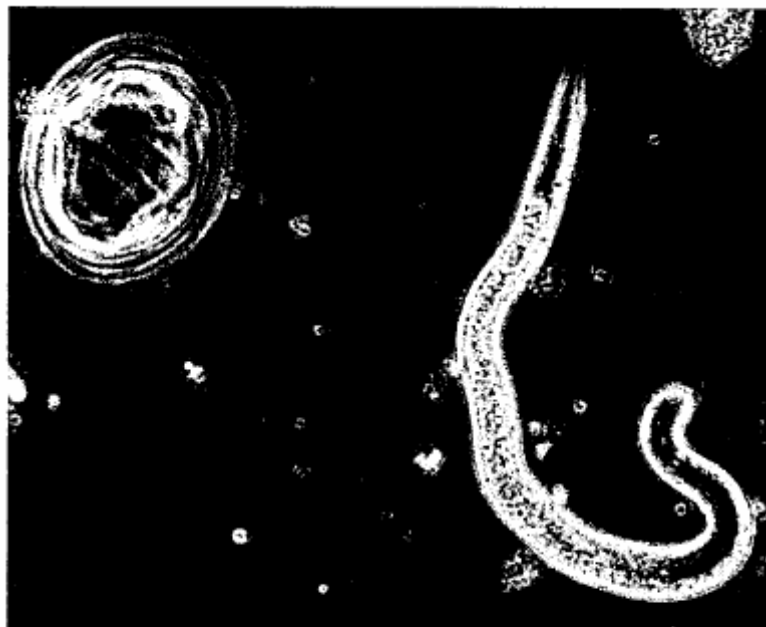


Figure 6. *Ascaris* ovum with second stage or potentially third stage larva; note the first stage larval sheath at the anterior end of the worm.

Appendix 2.3 Methods for the determination of Total Kjeldahl Nitrogen and Total Phosphorus in sludge samples

Kjeldahl digestion to determine N

REAGENTS: Concentrated Sulphuric Acid, (H_2SO_4) Digestion Mixture: Potassium sulphate (AR) very finely ground (1500g) mixed with 4g finely ground (AR) $Cu SO_4 \cdot 5H_{20}$.

Weigh out 1.000g of sludge into a digestion tube, and mix it with 2.0g of the digestion mixture. Place this on a block at $360^{\circ}C$, covered with glass "pears". Digest for 2 hours or until solution is clear. Do not allow to dry. Remove from the block and cool slightly, then add 5 ml deionised water before cooling completely. Once cooled, rinse into a 100ml volumetric flask, bringing it up to volume. Filter using Whatman no 2 filter paper.

Reference: Croll BT, Tomlinson T and Whitfield RW. 1985. Determination of Kjeldahl nitrogen in sewage effluents, trade effluent and sewage sludge. *Analyst* 110:861-866.

Method for Digestion and Determination of P and K

Sample Digestion: 1g of sample is digested with 7ml HNO_3 (conc. nitric acid) and 3ml $HClO_4$ (perchloric acid) at temperature up to $200^{\circ}C$ and brought to volume in a 100ml vol. flask. (Adapted from method for plant digestion at ISCW).

P and K Determination

The P and K can then be determined by ICP-AES (Inductively Coupled Plasma – Atomic Emission Spectroscopy). Values can also be confirmed by DCP (Direct Current Plasma Emission Spectroscopy) at a wavelength of 253.565 nm.

Reference: Zasoski, RJ and Burau, RG. 1977. A Rapid Nitric-Perchloric Acid Digestion Method for Multi-Element Tissue Analysis. *Communications in Soil Science and Plant Analysis. vol. 8* (5):425-436.

Appendix 2.4 Method for the determination of total metal content of sludge and soil samples (aqua regia digestion)

The method is summarized below.

- Weigh 3g of the <150µm sludge or soil sample into a 250ml reaction vessel.
- Moisten with 0.5-1ml water and add, while mixing, 21ml hydrochloric acid followed by 7ml nitric acid, drop by drop if necessary to reduce foaming.
- Stand for 16h at room temperature
- Boil under reflux for 2h
- Decant the sediment-free supernatant into 100ml flask through filter paper
- Fill the flask to the mark with 0.5mol/l nitric acid

The full method can be found in the reference below.

Reference: ISO 11466: 1995(E). Soil quality: extraction of trace elements soluble in *aqua regia*.

APPENDIX 3: VECTOR ATTRACTION REDUCTION OPTIONS

The following options are available to reduce the vector attraction potential. These options have been adopted from the US EPA Part 503 Rule.

Option 1: Reduction in Volatile Solids Content

Vector attraction is reduced if the fraction of volatile solids in the primary sludge is reduced by at least 38 percent during the treatment of the sludge. This percentage is the amount of volatile solids reduction that is attained by anaerobic or aerobic digestion plus any additional volatile solids reduction that occurs before the sludge leaves the treatment works, such as through processing in drying beds or lagoons, or by composting.

Digestion process efficiency can be measured by the reduction in the volatile solids content of the feed sludge to the digester and the sludge withdrawn from the digester. Anaerobic digestion of primary sludge generally results in a reduction of between 40 and 60% of the volatile solids.

O'Shaunessy's formula can be used to calculate the volatile solids (VS) reduction in a digester:

$$\text{VS reduction (\%)} = \{(V_i - V_o) / V_i - (V_i \times V_o)\} \times 100$$

Where V_i = volatile fraction in feed sludge

V_o = volatile fraction in digested sludge

Example of calculation of VS reduction

Assume volatile solids in feed sludge = 84%

Therefore volatile fraction of feed sludge = 0.84 = V_i

Assume volatile solids of digested sludge = 68%

Therefore volatile fraction of digested sludge = 0.68 = V_o

$$\text{VS reduction (\%)} = \{(0.84 - 0.68) / 0.84 - (0.84 \times 0.68)\} \times 100$$

$$= 59\%$$

Option 2: Additional Digestion of Anaerobically Digested Sludge

Frequently, primary sludge is recycled to generate fatty acids or the sludge is recycled through the biological wastewater treatment section of a treatment works or has resided for long periods of time in the wastewater collection system. During this time, the sludge undergoes substantial biological degradation. If the sludge is subsequently treated by anaerobic digestion for a period of time, it adequately reduces vector attraction. Because the sludge will have entered the digester already partially stabilized, the volatile solids reduction after treatment is frequently less than 38 percent.

Under these circumstances, the 38 percent reduction required by Option 1 may not be achievable. Option 2 allows the operator to demonstrate vector attraction reduction by testing a portion of the previously digested sludge in a **bench-scale unit** in the laboratory. Vector attraction reduction is demonstrated if, after anaerobic digestion of the sludge for an additional 40 days at a temperature between 30° and 37°C, the volatile solids in the sludge are reduced by less than 17 percent from the beginning to the end of the bench test.

Option 3: Additional Digestion of Aerobically Digested Sludge

This option is appropriate for aerobically digested sludge that cannot meet the 38 percent volatile solids reduction required by Option 1. This includes activated sludge from extended aeration plants, where the minimum residence time of sludge leaving the wastewater treatment processes section generally exceeds 20 days. In these cases, the sludge will already have been substantially degraded biologically prior to aerobic digestion.

Under this option, aerobically digested sludge with 2 percent or less solids is considered to have achieved vector attraction reduction, if in the laboratory after 30 days of aerobic digestion in a batch test at 20°C, volatile solids are reduced by less than 15 percent. This test is only applicable to liquid aerobically digested sludge.

Option 4: Specific Oxygen Uptake Rate (SOUR) for Aerobically Digested Sludge

Frequently, aerobically digested sludge is circulated through the aerobic biological wastewater treatment process for as long as 30 days. In these cases, the sludge entering the aerobic digester is already partially digested, which makes it difficult to demonstrate the 38 percent reduction required by Option 1.

The specific oxygen uptake rate (SOUR) is the mass of oxygen consumed per unit time per unit mass of total solids (dry-weight basis) in the sludge. Reduction in vector attraction can be demonstrated if the SOUR of the sludge that is used or disposed, determined at 20°C, is equal to or less than 2 milligrams of oxygen per hour per gram of total sludge (dry-weight basis). This test is based on the fact that if the sludge consumes very little oxygen, its value as a food source for micro organisms is very low and therefore micro-organisms are unlikely to be attracted to it. Other temperatures can be used for this test, provided the results are corrected to a 20 °C basis. This test is only applicable to liquid aerobic sludge withdrawn from an aerobic treatment process.

Option 5: Aerobic Processes at Greater than 40 °C

This option applies primarily to composted sludge that also contains partially decomposed organic bulking agents. The sludge must be aerobically treated for 14 days or longer, during which time the temperature must always be over 40°C and the average temperature must be higher than 45°C.

This option can be applied to other aerobic processes, such as aerobic digestion, but Options 3 and 4 are likely to be easier to meet than the other aerobic processes.

Option 6: Addition of Alkaline Material

Sludge is considered to be adequately reduced in vector attraction if sufficient alkaline material is added to achieve the following:

- Raise the pH to at least 12, measured at 25 °C, and without the addition of more alkaline material, maintain a pH of 12 for at least 2 hours.
- Maintain a pH of at least 11,5 without addition of more alkaline material for an additional 22 hours.

The conditions required under this option are designed to ensure that the sludge can be stored for at least several days at the treatment works, transported, and then used or disposed without the pH falling to the point where putrefaction occurs and vectors are attracted.

Option 7: Moisture Reduction of Sludge Containing no Un-stabilised Solids

Under this option, vector attraction is considered to be reduced if the sludge does not contain unstabilised solids generated during primary treatment and if the solids content of the sludge is at least 75 percent before the sludge is mixed with other materials. Thus, the reduction must be achieved by removing water, not by adding inert materials.

It is important that the sludge does not contain un-stabilised solids because the partially degraded food scraps likely to be present in such sludge would attract birds, some mammals, and possibly insects, even if the solids content of the sludge exceeds 75 percent. In other words, simply dewatering primary sludge to a 75% solid is not adequate to comply with this option. Activated sludge, humus sludge and anaerobically digested sludge can, however be dewatered to 75 % solids and comply with option 7.

Option 8: Moisture Reduction of Sludge Containing Unstabilised Solids

The ability of any sludge to attract vectors is considered to be adequately reduced if the solids content of the sludge is increased to 90 percent or greater, regardless of whether this contains primary sludge or raw unstabilised sludge. The solids increase should be achieved by removal of water and not by dilution with inert solids. Drying to this extent severely limits biological activity and strips off or decomposes the volatile compounds that attract vectors.

The way dried sludge is handled, including storage before use or disposal, can again create the opportunity for vector attraction. If dried sludge is exposed to high humidity, the outer surface of the sludge will increase in moisture content and possibly attract vectors. This should be properly guarded against.

Option 9: Sludge Injection

Vector attraction reduction can be demonstrated by injecting the sludge below the ground surface. Under this option, no significant amount of sludge can be present on the land surface within 1 hour of injection, and if the sludge is Microbiological Class A or B, it must be injected within 8 hours after discharge from the pathogen-reducing process.

The reason for this special consideration for Microbiological class A and B sludge (assuming vector attraction has not been reduced by some other means) is that pathogens could re-grow and Microbiological class A and B sludge has no site restrictions to provide crop, animal grazing or access protection.

Note:

Microbiological class A and B can be applied to soil much later than 8 hours after discharge from the pathogen-reducing process if another vector attraction reduction option such as dewatering and/or drying is applied. The times referred in Option 9 are intended for liquid sludge application of Microbiological classes A and B.

Injection of sludge beneath the soil places a barrier of earth between the sludge and vectors. The soil removes water from the sludge, which reduces the mobility and odour of the sludge. Odour is usually present at the site during the injection process, but quickly dissipates once injection is complete.

Option 10: Incorporation of Sludge into the Soil

Under this option, sludge must be incorporated into the soil within 6 hours of application to or placement on the land. Incorporation is accomplished by ploughing or some other means of mixing the sludge into the soil. If the sludge is Microbiological class A or B with respect to pathogens, the time between processing and application or placement must not exceed 8 hours – the same as for injection under Option 9. See the note under Option 9.

Note: Practical restrictions, such as the ability of the plough to function immediately after application, could cause delays in the incorporation of the sludge within the 6 hours. This could cause the development of odours and increase risk of vector attraction. In these cases the sludge producer need to monitor the development of odours and manage the situation diligently.

DEFINITIONS AND DESCRIPTION OF KEY TERMS

Agricultural land:	Land on which a food crop, a feed crop, or a fibre crop is grown. This includes grazing land and forestry.
Agronomic rate:	The sludge application rate (dry-weight basis) designed (i) to provide the amount of nitrogen needed by the food crop, feed crop, fibre crop, cover crop, or vegetation grown on the land and (ii) to minimise the amount of nitrogen in the sewage sludge that passes below the root zone of the crop or vegetation grown on the land to the groundwater.
Agricultural use:	The use of sludge to produce agricultural products. It excludes the use of sludge for aquaculture and as an animal feed.
Annual pollutant loading rate:	The maximum amount of a pollutant that can be applied to an area of land during a 365-day period.
Assimilative capacity:	This represents the ability of the receiving environment to accept a substance without risk.
Beneficial uses	Use of sludge with a defined benefit, such as a soil amendment.
Bioavailability:	Availability of a substance for uptake by a biological system.
Biosolids:	Stabilised Sludge. Organic solids derived from biological wastewater treatment processes that are in a state that they can be managed to sustainably utilise the nutrient, soil conditioning, energy, or other value.
BPEO:	Best Practicable Environmental Option. BPEO is the outcome of a systematic consultative and decision-making procedure that emphasises the protection of the environment across land, air and water. It establishes, for a given set of objectives, the option that provides the most benefit or least damage to the environment as a whole at acceptable cost in the long term as well as the short term.
Co-disposal (liquid with dry waste):	The mixing of high moisture content or liquid waste with dry waste. This affects the water balance and is an acceptable practice on a site equipped with leachate management measures.
Co-disposal (dewatered sludge with dry waste):	The mixing of dewatered sludge with dry waste in a general landfill site or hazardous landfill site without affecting the water balance of the site.
Composting:	The biological decomposition of the organic constituents of sludge and other organic products under controlled conditions.
Contaminate:	The addition of foreign matter to a natural system. This does not necessarily result in pollution, unless the attenuation capacity of the natural system is exceeded.
Controlled access:	Where public or livestock access to sludge application areas is restricted or controlled, such as via fences or signage, for a period of time stipulated by this guideline.

Cumulative pollutant loading rate:	The maximum amount of a pollutant that can be applied to a unit area of land.
Dedicated land disposal	Sites that receive repeated applications of sludge for the sole purpose of final disposal.
Delisting:	DWAF Minimum Requirements. If the EEC (Estimated Environmental Concentration) is less than the Acceptable Risk Level ($0,1 \times LC_{50}$), the waste can be delisted, i.e., be moved to a lower Hazard Rating or even disposed of at a General Waste landfill with a leachate collection system (G:B+landfill).
Dewatering:	Dewatering processes reduce the water content of sludge to minimise the volumes for transport and improve handling characteristics. Typically, dewatered sludge can be handled as a solid rather than as liquid matter.
Disinfection:	A process that destroys, inactivates or reduces pathogenic microorganisms.
Domestic septage:	Liquid or solid material removed from a septic tank, cesspool, portable toilet, marine sanitation device, or similar treatment works that receives only domestic sewage. Domestic septage does not include liquid or solid material removed from a septic tank, cesspool, or similar treatment works that receives either commercial or industrial wastewater and does not include grease removed from a grease trap at a restaurant.
Domestic sewage:	Waste and wastewater from humans or household operations that is discharged to, or otherwise enters a treatment works.
Drying:	A process to reduce the water content further than a dewatering process. The solids content after a drying process is typically > 75%.
Dry-weight (DW) basis:	The method of measuring weight where, prior to being weighed, the material is dried at 105°C until reaching a constant mass (i.e., essentially 100 % solids content).
<i>E. coli</i>:	A subset of coliforms found in the intestinal tract of humans and other warm-blooded animals. They can produce acid and gas from lactose at 44 to 44.5°C; hence the test for them is more specific than for total coliforms and selects a narrower range of organisms. They are a more specific indicator of faecal contamination than total coliforms.
Estimated Environmental Concentration (EEC):	The Estimated Environmental Concentration represents the concentration of a substance to the aquatic environment when introduced under worst-case scenario conditions, i.e., directly into a body of water. It is used to indicate possible risk, by comparison with the minimum concentration estimated to adversely affect aquatic organisms or to produce unacceptable concentrations in biota, water or sediment.

Faecal coliform:	<i>Faecal coliforms</i> are the most commonly used bacterial indicator of faecal pollution. <i>Faecal coliforms</i> are bacteria that inhabit the digestive system of all warm-blooded animals, including humans.
General Waste:	Waste that does not pose an immediate threat to man or to the environment, i.e., household waste, builders' rubble, garden waste, dry industrial and commercial waste. It may, however, with decomposition, infiltration and percolation, produce leachate with an unacceptable pollution potential.
Hazardous Waste:	Waste, other than radioactive waste, which is legally defined as Hazardous in the state in which it is generated, transported or disposed of. The definition is based on the chemical reactivity or toxic, explosive, corrosive or other characteristics which cause, or are likely to cause, danger to health or to the environment, whether alone or when in contact with other waste. After UNEP definition.
Helminth ova:	The eggs of parasitic intestinal worms.
Immobilisation:	Immobilisation (or chemical stabilisation) is a process in which the material is converted to a more chemically stable or more insoluble or more immobile form.
Incineration:	Incineration is both a form of treatment and a form of disposal. It is simply the controlled combustion of waste materials to a non-combustible residue or ash and exhaust gases, such as carbon dioxide and water.
Industrial wastewater:	Wastewater generated in a commercial, industrial, or manufacturing process.
Land application:	The spraying or spreading of wastewater sludge onto the land surface; the injection of wastewater sludge below the land surface; or the incorporation of wastewater sludge into the soil so that the wastewater sludge can either condition the soil or fertilise crops or vegetation grown in the soil.
Land disposal	Application of sludge where beneficial use is not an objective. Disposal will normally result in application rates that exceed agronomic nutrient requirements or cause significant contaminant accumulation in the soil.
Landfill:	To dispose of waste on land, whether by use of waste to fill in excavation or by creation of a landform above grade, where the term "fill" is used in the engineering sense.
LC₅₀:	The median lethal dose is a statistical estimate of the amount of chemical which will kill 50% of a given population of aquatic organisms under standard control conditions.
LD₅₀:	The median lethal dose is a statistical estimate of the amount of chemical which will kill 50% of a given population of animals (e.g., rats) under standard control conditions.

Maximum available threshold (MAT):	The maximum available (NH_4NO_3 extractable) metal concentration allowed for soils receiving sludge.
Monthly average:	The arithmetic mean of all measurements taken during a given month.
Most probable number (MPN):	A unit that expresses the amount of bacteria per gram of total dry solids in wastewater sludge.
Pathogenic organisms:	Disease-causing organisms. This includes, but is not limited to, certain bacteria, protozoa, viruses, and viable helminth ova.
pH:	The logarithm of the reciprocal of the hydrogen ion concentration. The pH measures acidity/alkalinity and ranges from 0 to 14. A pH of 7 indicates the material is neutral. Moving a pH of 7 to 0, the pH indicates progressively more acid conditions. Moving from a pH of 7 to 14, the pH indicates progressively more alkaline conditions.
Precautionary principle:	Where a risk is unknown; the assumption of the worst-case situation and the making of provision for such a situation.
Receptor:	Sensitive component of the ecosystem that reacts to or is influenced by environmental stressors.
Recycle:	The use, re-use, or reclamation of a material so that it re-enters the industrial process rather than becoming a waste.
Residue:	A substance that is left over after a waste has been treated or destroyed.
Restricted agricultural use:	Use of sludge in agriculture is permitted but restrictions apply (crop restrictions, access restrictions etc).
Sludge-amended soil:	Soil to which sludge has been added.
Sludge:	Solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works. Wastewater sludge includes, but is not limited to, domestic septage; scum or solids removed in primary, secondary, or advanced wastewater treatment processes; and material derived from wastewater sludge in a wastewater sludge incinerator. It does not include the grit and screenings generated during preliminary treatment of domestic wastewater in a treatment works.
Soil organisms:	A broad range of organisms, including microorganisms and various invertebrates living in or on the soil.
Specific oxygen uptake rate (SOUR):	The mass of oxygen consumed per unit time per unit mass of total solids (dry-weight basis).
Stabilisation:	The processing of sludge to reduce volatile organic matter, vector attraction, and the potential for putrefaction and offensive odours.
Stabilised sludge	Organic solids derived from biological wastewater treatment processes that are in a state that they can be managed to utilise the nutrient, soil conditioning,

	energy, or other value.
Sterilise:	Make free from microorganisms.
Supplier:	A person or organisation that produces and supplies sludge for use. This includes a water business producing and treating sludge and processors involved in further treatment.
Sustainable use:	The use of nutrients in sludge at or below the agronomic loading rate and/or use of the soil conditioning properties of sludge. Sustainable use involves protection of human health, the environment and soil functionality.
Total investigative level (TIL):	The total metal concentration in soils where further investigation is necessary before sludge application can commence.
Total maximum threshold (TMT):	The maximum total metal concentration allowed in soils receiving sludge.
Toxic:	Poisonous.
Toxicity Characteristic Leaching Procedure (TCLP):	A test developed by the USA Environmental Protection Agency to measure the ability of a substance to leach from the waste into the environment. It thus measures the risk posed by a substance to groundwater.
Unrestricted agricultural use:	Sludge is of such good quality that it can be used in agricultural practices without any restrictions.
VAR:	Vector Attraction Reduction.
Vector attraction:	The characteristic of wastewater sludge that attracts rodents, flies, mosquitoes, or other organisms capable of transporting infectious agents.
Vectors:	Any living organisms that are capable of transmitting pathogens from one organism to another, either: (i) mechanically by transporting the pathogen or (ii) biologically by playing a role in the lifecycle of the pathogen. Vectors include flies, mosquitoes or other insects, birds, rats and other vermin.
Waste:	An undesirable or superfluous by-product, emission, or residue of any process or activity, which has been discarded, accumulated or stored for the purpose of discarding or processing. It may be gaseous, liquid or solid or any combination thereof and may originate from a residential, commercial or industrial area.
Wastewater Sludge	The material recovered from predominantly domestic wastewater treatment plants. (Also see Sludge)
Wastewater Treatment Plant (WWTP):	Any device or system used to treat (including recycling and reclamation) either domestic wastewater or a combination of domestic wastewater and industrial waste of a liquid nature.
Wet weight:	Weight measured of material that has not been dried (see Dry-weight basis).



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