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FULL PROJECT TITLE

'Design and Application of an Innovative Composting Unit for the Effective Treatment of Sludge and other Biodegradable Organic Waste in Morocco, MOROCOMP'

Task6:
Development of guidelines and specifications covering the sludge composting process -Characterization and use of compost as soil improver

Deliverable 18C:
Development of manuals for testing and analysis of compost

composting
MOROCOMP

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Table of contents

1 Introduction

To evaluate the quality of the produced compost it is necessary to quantify the characteristics of the end product and to do so there are series of experimental procedures that must be followed. Therefore the aim of this work is to present the basic principles of compost sampling as well as the test methods that need to be carried out in order to specify the properties of any given compost. The chemical analysis of compost is considered crucial since the analysis determine its quality and the possibility of the end product to be marketed based on its characteristics. Therefore the provided manual must be followed with caution in order to acquire representative results for the characterization of compost. The test methods include the determination of the moisture content, pH, electrical conductivity and the percentage of organic matter that the compost incorporates. The analysis of the basic macro-elements (total N, P, K, Na, Ca , Mg) and microelements (Cd, Cr, Cu, Fe, Mn, Ni , Pb, Zn) and a phytotoxicity test procedure are also presented.

2 Principles of compost sampling

Prior to the performance of compost chemical analysis it is necessary to take representative test samples of the compost that is under investigation. Obtaining a truly representative sample of compost under observation is not a simple task. Frequently sampling errors are commonly much greater than analytical errors. The extent to which the result of an analysis identifies a real characteristic of the whole compost depends upon the accuracy of sampling. When the sample analyzed is not representative, the result of the compost chemical analysis will yield a value that does not necessarily describe the property of compost as a whole. Rather, it delineates specifically a characteristic in the small test sample analysed. This analytical value can serve as an accurate description of the compost property under investigation only if:

- The gross sample accurately represents the whole compost from which it was taken
- No changes occur in the gross and subsamples prior to analysis,
- The subsample analyzed represents the gross sample accurately,
- The analysis determines a true value of the compost characteristic under investigation.

Of the four points, the first is the most troublesome because of compost variability. Differences in compost composition and properties are presented even in compost originated from the same feedstock material. The second and third requirements can be controlled fairly well during sample preparation. The fourth point is primarily related to the selection of the proper analytical procedure. Frequently, selecting the right procedure is more difficult than performing the actual analysis.

2.1 Types of compost sampling

The following sections describe several general principles of how representative compost samples can be obtained. These sections will consider several options in cost-effective techniques as well as accuracy and precision in the sampling process.

2.1.1 Simple random sampling

Of all sampling methods in use, random sampling is considered to be the simplest. The selection of samples is left completely to the luck of the draw without regard to the variation in a compost population. This is a method by which every sampling unit has an equal independent probability of being drawn. In a highly homogeneous field, it is often a satisfactory method. However, if the variability in the population is known, one of the following methods presented below might be considered more appropriate.

2.1.2 Systematic sampling

As the name implies, sampling is performed systematically. Systematic sampling can often provide more accurate results than random sampling, because with this method the samples are distributed more evenly over the population. However, if the compost population contains a periodic (systematic) variation, and if the interval between successive sampling happens to coincide with that of the compost variations, biased samples may be obtained. Therefore, it is advisable to study in advance the nature and occurrence of compost variability before a decision is made to use this method.

2.1.3 Stratified sampling

This type of sampling is commonly employed in heterogeneous compost. More precise sampling can be achieved if subpopulations are formed so that a heterogeneous population is divided into parts, called strata, each of which is fairly homogeneous. The field is thus divided into a number of strata, and a sample or samples are drawn independently from each stratum.

2.1.4 Compositing

Compositing is the mixing of sampling units to form a single sample which is used for chemical analysis. This method offers the advantage of increased accuracy through the use of large numbers of sampling units per sample. In compositing, the fundamental assumption is that analysis of the composited sample yields a valid estimate of the mean, which would be obtained by averaging the results of analysis from each of the sampling units contributing to the composite. This assumption is valid only if:

- The sampling volume represents homogeneous population
- Equal amounts of each sampling unit contribute to the subsample analysed,
- No changes have taken place in the composite and subsample prior to analysis that would affect the analytical results and
- An unbiased estimate of the mean is the only objective.

Compositing enables to obtain reliable means for a large number of composts at relatively small expense. However, only the mean value is obtained. Thus, no measure of variability is possible, except if analysis is performed to individual sampling units contributing to the composite.

2.2 Size and accuracy of sampling

The size of sampling can be distinguished into (1) size (quantity) of samples and (2) number of samples to be taken.

The size of the sample or the size of the sampling unit to be drawn depends upon three factors: (1) coarseness of the material, (2) objective of the analysis, and (3) the desired accuracy. Generally the coarser the material the larger the sample size required. On the other hand, the number of samples to be taken is determined principally by the objective and the costs. The more heterogeneous the compost population, the more intense must be the sampling rate to attain a given accuracy. The greater the number of samples and the smaller the sampling volume, the more precise will be the estimate of the compost characteristic analysed. Although, frequently the number of samples is decided arbitrarily, economic considerations often restrict both the quantity and the number of samples taken. In such cases, a suitable balance must be found between the amount and number of samples and the sampling costs. Therefore, the question that rises is how many samples should be taken and analysed in order to make the error as small as possible. A statistical approach has been developed according to which a decision must be made on the magnitude of the error that can be tolerated, called the acceptable error (E). This error can be calculated by:

$E = \pm t(V)^{0.5}$
$V = S^2/n$
In which t = t-test, V = variance, S^2 = sum of squares, and n=number of samples

The required number of samples dictated by the acceptable error can then be calculated by using one of the following formulas:

$n = 4\sigma/E^2$ or $n = t^2S^2/E^2$
in which n = number of samples and σ = standard deviation

The second equation ($n = t^2S^2/E^2$) is preferable, since it takes into consideration the probability level by including the t-test. The standard deviation is calculated by the usual formula:

$$\sigma = \sqrt{\sum \left(x_i - \bar{x}\right)^2 /(n-1)}$$

Whereas the sum of squares, S^2, is found by using the equation:

$$S^2 = \sum \left(x_i - \bar{x}\right)^2 /(n-1)$$

The above formulas can be used for determining the number of samples that should be taken in random, systematic or stratified sampling methods according to limits set for acceptable error.

3 Methods of compost analysis

3.1 Determining Moisture Content

Compost moisture content influences crop growth not only by affecting nutrient availability, but also nutrient transformations and compost biological behavior. Therefore, compost moisture is routinely measured in most compost trials. Moisture content can be evaluated in-situ by neutron probe however the gravimetric approach is more flexible. All analyses in the laboratory are related to an air or oven dry basis, and therefore must consider the actual compost moisture content.

Apparatus
1. Electric oven with thermostat

2. Desiccator

Procedure

1. Weigh 10g of compost (<2mm) into a previously dried (105^{o}C) and weighed metal can with lid.
2. Dry in an oven, with the lid unfitted, at 105^{o}C overnight.
3. Next day, remove from oven; fit the lid, cool in a desiccator for at least 30 minutes, and re-weigh.

Calculations

Calculation:

$$\% \text{ Moisture in compost} = \frac{\text{Wet soil (g)} - \text{Dry soil (g)} * 100}{\text{Dry soil (g)}}$$

3.2 Determining pH

Compost pH is a measure of hydronium ion (H_3O^+, or more commonly the H^+) activity in the compost solution. Compost pH influences crop production and soil chemistry, including availabilities of nutrients and toxic substances, activities and nature of microbial populations, and activities of certain pesticides. Compost pH is defined as the negative logarithm (base 10) of the H+ activity (moles per liter) in the compost solution. As the activity of H+ in the compost solution increases, the compost pH value decreases. Composts with pH values below pH 7 are referred to as "acid" and those with pH values above pH 7 as "alkaline"; composts at pH 7 are referred to as "neutral".

Compost pH is usually determined potentiometrically in a slurry system using an electronic pH meter. The proliferation of pH meters in recent years precludes an in-depth discussion of meter operation; specific instructions are provided with individual units. However, several precautions are presented as general considerations for all labs. First, electrodes should be checked and maintained frequently to prevent residue buildup which may affect operation. Rinsing between each compost sample, however, is not usually necessary. Electrodes should be protected in a way which prevents insertion to the very bottom of a slurry vessel which will cause abrasion of the sensing surface, decreasing electrode life and causing inaccurate readings. All meters should be calibrated routinely at two points before operation. One point of calibration should be at pH 7, while the other should be chosen based on the range of compost pH normally encountered by the lab. Reference and/or combination electrodes for measuring compost pH should be chosen carefully because flow rates at the liquid junction can affect the accuracy of compost pH readings. Labs may wish to use a set of reference compost samples of known pH to evaluate the performance of electrodes. Such samples should be stored and handled under carefully controlled conditions to prevent changes in compost properties over time. The reference compost pH of these samples should be determined using the average reading of several values. Electrodes which fail to produce pH readings consistent with established values when calibrated with clear buffers can be calibrated successfully using the reference composts themselves, but only if compost storage and handling are well controlled. Procedures for each method are given below.

Equipment:

1. pH meter with appropriate electrode(s).
2. Electronic balance or standard, 5 cm^3 stainless steel scoop.
3. Pipettes or automatic dispensers.
4. 1-oz. paper cups or equivalent.

Reagents:
1. Distilled or deionized water.
2. 0.01 *M* $CaCl_2$: Completely dissolve 1.47g $CaCl_2.2H_2O$ in 1 L of distilled or deionized water.
3. 1.0 *M* $CaCl_2$: Completely dissolve 14.7g $CaCl_2.2H_2O$ in 100 mL of distilled or deionized water.
4. Standard buffer solutions for calibrating pH meter, usually pH 4.0 and pH 7.0.

Procedure:
1. Calibrate the pH meter over the appropriate range using the standard buffers.
2. Weigh 50g air-dry compost (<2mm) into a 100mL glass beaker.
3. Add 50mL DI water using a graduated cylinder or 50mL volumetric flask
4. Stir vigorously for 15 seconds and let stand for 30 minutes.
5. Place electrodes in the slurry, swirl carefully, and read the pH immediately. Ensure that the electrode tips are in the slurry and not in the overlying solution.
6. Remove the combined electrode from the suspension, and rinse thoroughly with DI water in a separate beaker, and carefully dry excess water with a tissue
7. For the $CaCl_2$ pH measurement, add 1 drop of 1.0 *M* $CaCl_2$ solution to the previous sample. Alternatively, prepare a sample as was done in steps 2 and 3, using 0.01 *M* $CaCl_2$ instead of water. Stir vigorously and let stand for 30 minutes, stirring occasionally. Read the pH as in step 5.

Note
1. Make sure that the combined electrode contains saturated KCl solution and some solid KCl
2. Calibrate the pH meter using at least two buffer solutions of different pH values, usually 4.0 and 7.0. First, measure the temperature of the solution and adjust the "temperature" knob. Second, dip the combined electrode in pH 7.0 buffer solution, check for actual pH at measured temperature, and adjust with the "buffer" knob. Then, dip the combined electrode in the pH 4.0 buffer solution and adjust with "sensitivity" knob. Repeat until pH meter gives correct reading of both buffer solutions.
3. For compost samples which acquire very high organic matter a 1:2 or 1:5 (compost:water) ratio is used while for soils pH is measured in a 1:1 (soil:water) suspension. For special purposes, pH can be measured in a saturated compost paste, or in more dilute suspensions. In some laboratories, pH is a measured of compost and 1N KCl or 0.01M $CaCl_2$. The main advantage of the measurement of compost pH in salt solution is the tendency to eliminate interference from suspension effects and from variable salt contents, such as fertilizer residues.
4. Air-dry composts may be stored several months in closed containers without affecting the pH measurement.
5. If the pH meter and combined electrodes are not to be used for extended periods of time, the instructions for storage published by the instrument manufacturer should be followed.

3.3 Determination of Electrical Conductivity

Compost salinity refers to the concentration of soluble inorganic salts in the compost. It is normally measured by extracting the compost sample with water (1:1 or 1:5 compost:water ratio, w/v) or in an saturated paste extract. However, compost:solution ratios of a 1:1 or wider ratio are more convenient where the compost sample is limited. Such extracts are rapid and salinity is measured by electrical conductivity (EC) using a

conductivity bridge. The total salt content of compost can be estimated from this measurement. A more precise method involves evaporation of the aqueous extract and weighing the residue.

Salinity is an important laboratory measurement since it reflects the extent to which compost is suitable for growing crops. On the basis of a saturation extract, values of 0 to 2 dS/m (or mmhos/cm) are safe for all crops; yields of very sensitive crops are affected between 2 to 4 dS/m; many crops are affected between 4 and 8 dS/m; while only tolerant crops grow well above that level.

Apparatus
Vacuum filtration system
Conductivity bridge

Procedure
1. Prepare a 1:1 (compost:water) suspension, as for pH determination
2. Filter the suspension using suction. First, put a round Whatman No. 42 filter paper in the Buchner funnel. Second, moisten the filter paper with DI water and make sure that it is tightly attached to the bottom of the funnel and that all holes are covered.
3. Start the vacuum pump
4. Open the suction, and add the suspension to Buchner funnel
5. Continue filtration until the compost on the Buchner funnel starts cracking.
6. If the filtrate is not clear, the procedure must be repeated.
7. Transfer the clear filtrate into a 50-mL bottle, immerse the Conductivity Cell in the solution, and take the reading.
8. Remove the conductivity cell from the filtration, rinse thoroughly with DI water, and carefully dry excess water with a tissue.

Note
1. Readings are recorded in milli-mhos per centimeter (mmhos/cm) or deci-Diemens per meter (dS/m). The use of the unit deci-Siemens is preferred over the unit milli-mhos. Both units are equal, that is, **1dS/m = 1mmho/cm**.
2. Reading are usually taken and reported at a standard temperature of 25^{o}C.
3. Check accuracy of the EC meter using a 0.01N KCl solution, which should give a reading of 1.413 dS/m at 25^{o}C.

3.4 Determination of compost organic matter (Walkley – Black Wet Combustion Method Equipment)

Apparatus
1. 500-mL Erlenmeyer flasks.
2. 10-mL pipette.
3. 10-and 20-mL dispensers.
4. 50-mL burette.
5. Analytical balance.
6. Magnetic stirrer.

Reagents:
1. Potassium dichromate, $K_2Cr_2O_7$ 1N. (=1/6M): Weigh 49.00 g $K_2Cr_2O_7$ in a 1L volumetric flask. Dissolve with 800 mL tilled water, and make up to volume
2. Sulfuric acid, H_2SO_4 96% reagent grade.

3. Ferrous sulfate, $FeSO_4.7H_2O$, reagent grade, 0.5N: Weigh 140g $FeSO_4.7H_2O$ in a 1L volumetric flask. Dissolve in 800 ml distilled water under constant stirring. Ass 15ml of concentrated H_2SO_4, cool the mixture, and make uo to volume with distilled water.
4. Ferroin indicator. This indicator is available as a solution under the trade name, *1,10-phenanthroline Ferrous Sulfate Complex.*

Procedure:
Weigh 500.0 mg of compost, that has passed a 100 mesh sieve, in a 500-mL Erlenmeyer flask. Add with a burette 10 mL 1 *N* K_2Cr207 solution. Swirl to mix. Add carefully from a dispenser 20 mL concentrated H_2SO_4, Extreme caution should be exercised in dispensing the acid, since H_2SO_4 is very dangerous. Swirl gently to mix, while holding the mouth of the Erlenmeyer flask away from your body. Do not touch the bottom of the flask, since the reaction creates enormous amounts of heat. Allow the solution to cool to room temperature (20-30 minutes), after which 20 mL distilled water are added. Swirl again to mix. Add 5 drops offerroin indicator, and titrate the excess (unused part) of chromic acid with ferrous sulfate or ferrous ammonium sulfate to a clear blue-brown endpoint (T mL). Run a blank using the same procedure (B mL).

Calculations

$\%C_{org}$= [(B-T)*N*3*1.14*100] / mg dry compost

%OM = $\%C_{org}$*(100/58)

3.5 Determination of Macro-elements

3.5.1 Determination of Total Nitrogen (N)

The determination of total N is a complicated process, because of the presence of various forms of N. Several methods are available for the determination of total N in composts: (l) Kjeldahl method, (2) Dumas method, (3) NIRS (Near Infrared Reflectance Spectroscopy) method, and (4) Direct Distillation method. The Dumas method is a dry combustion method, and is the traditional and oldest method for measuring total-N in composts. However, of all the methods used, the Kjeldahl method, which is a wet oxidation method, is perhaps the most common method for the determination of total-N. The advantages of the Kjeldahl method, (l) the ease of running multiple analyses, and (2) applicability to samples low in N, mark it as a method of first importance.

Kjeldahl Method
The Kjeldahl method was reported by Johan Kjeldahl in l883, two years after Dumas (l83l) has introduced his dry combustion method. In outline, the Kjeldahl method consists of three phases: (l) the digestion phase, (2) the distillation of NH_4, and (3) the determination of NH_4. In the digestion phase, the sample is decomposed by H_2SO_4, with Na_2SO_4 and a suitable catalyst. The N and C in the sample are converted into $(NH_4)_2SO_4$ and CO_2, respectively. In the distillation phase, the NH_4^+ is converted to NH_3 and distilled into N_3BO_3 or a standard HCl solution. The determination of NH_4^+ is usually done by titration.

Accuracy and precision of Kjeldahl analysis depend on the digestion phase, since a number of organic compounds can not be broken down completely by the procedure. Several organic compounds are refractory to Kjeldahl digestion. Conspicuous among these substances are complex heterocyclic N substances, such as pyridine, nicotine acid, quinoline, certain alkaloids, and several amino acids, e.g., lysine, histidine, tyrosine, and

tryptophane. Azo-, diazo-, nitro-, and nitroso-compounds, hydrazines, hydrazones, and oximes are additional compounds that are refractory to Kjeldahl digestion. Fortunately, in many compost samples these compounds are either absent or present in minor amounts, and if present are accompanied by other organic compounds that assist in their decomposition by Kjeldahl digestion.

Because of the difficulties in breaking down several of the organic compounds, it is the digestion procedure that has undergone many revisions since the introduction of the Kjeldahl method. During the years, the method of analysis has also been changed from a macro into a semi-micromethod. The usual 800-mL Kjeldahl flasks heated by a gas flame have been replaced by 50-mL electrically heated flasks. Today *block digestors,* which can accommodate a large number of 50-mL tubes, are preferred. Block digestion systems are commercially available in various sizes, and require only a small space in the laboratory. This semimicro Kjeldahl method requires smaller amounts of reagents, such as smaller amounts of concentrated H_2SO_4, heavy metals used in the catalyst, and NaOH, which make disposal of these reagents after use more convenient.

Digestion Phase

A number of reagents are used in the digestion phase, such as salicylic acid, Zn dust, K_2SO_4 or Na_2SO_4, a suitable catalyst, and concentrated H_2SO_4.

1. Salicylic Acid and Zn Dust

For total N determination special provision must be made to include NO_3-N and NO_2-N. NO_3-N will not be recovered in the Kjeldahl digestion process, whereas NO_2^- will decompose by the addition of concentrated H_2SO_4 and is lost in the air. When NO_3-N must be included, the sample is pretreated by soaking in the cold, preferably overnight, in concentrated H_2SO_4 in which salicylic acid has been dissolved. The resulting nitro-salicylic acid is reduced by heating gently with $Na_2S_2O_3$. Zn dust may also be used for reduction of nitro-salicylic acid. Considerable care and attention are required during the reduction process with $Na_2S_2O_3$ because the mixture tends to froth badly and some losses of N may occur accordingly. Where the addition of salicylic acid and reducing agent can be avoided, a considerable saving of time is affected. It should be noted that this method of pretreatment for including NO_3-N cannot be used on wet samples.

2. *Potassium Sulfate or Sodium Sulfate*

K_2SO_4 or Na_2SO_4 is added to increase the boiling temperature of H_2SO_4 (330°C) to 360-390°C. In addition, the SO_4^{2-} anion is needed for preventing loss of NH_3 from the mixture, by conserving it as $(NH_4)_2SO_4$. The use of K_2SO_4 is preferred, since Na_2SO_4 tends to result in spattering, although Na_2SO_4 will yield a higher boiling temperature than K_2SO_4. The temperature is very important in the digestion process for N evaluation since loss of N will likely occur when the digestion temperature exceeds 400°C. The digestion temperature is controlled largely by the amount of K_2SO_4 used. With the addition of low, amounts of K_2SO_4 (0.3 g/mL of H_2SO_4) a longer digestion time is needed to reach the temperature for obtaining accurate results. In the presence of higher amounts of K_2SO_4 (1.0 g/mL of H_2SO_4) shorter digestion periods will produce satisfactory results. Even shorter digestion time using high amounts of K_2SO_4 is considered of more advantage over longer digestion time. Bumping, spattering, and other problems may arise from using longer digestion periods. However, the use of high amounts of K_2SO_4 may create frothing during digestion, and may tend to solidify the digest upon cooling. When solidification occurs after cooling, more time is perhaps required to take up the digest with water for further analysis. Solidification does not necessarily result in a loss of N.

3. *Catalyst*

Originally, $KMnO_4$ (potassium permangate) was used by Johan Kjeldahl as a catalyst to speed up the digestion process. However, during the years this has been replaced by a number of other catalysts. Mercury, HgO, HgI_2, Se, $SeOCl_2$, $CuSO_4$, V_2O_5, or Fe is found to be more effective as a catalyst than is $KMnO_4$. The addition of phosphate, H_2O_2, $K_2S_2O_8$, or $HCIO_4$ as aids in digestion has also been proposed. The catalyst most commonly employed is Hg, HgO, Se or $SeOCl_2$, or mixtures of these metals or compounds. Mercury, Hg, and selenium, Se, are considered to be the most effective catalysts, and the combination of Se, $CuSO_4$ and HgO appears to have the best catalytic action. However, both Hg and Se are very toxic metals, and pose serious problems for their safe disposal in the environment. Titanium oxide, TiO_2, is suggested as a substitute for HgO. When used in combination with $CuSO_4$, it is reported that digestion time for animal feeds is only 40 minutes. Today, H_2O_2 is frequently used for digestion of plant samples, and the use of catalysts containing hazardous metals is hereby completely eliminated. Commercially prepared mixtures are available in the form of pellets *(Kjeltabs),* tablets *(Kelmate),* or digestible bags. Although these mixtures still contain Hg and Se, they are very convenient for use in Kjeldahl digestion.

4. *Sulfuric Acid*

Sulfuric acid, H_2SO_4, is the essential reagent for the digestion process. It is consumed during the oxidation of organic matter, and during the reaction with mineral compost constituents. Excessive losses of H_2SO_4 should be avoided since this may result in the decomposition of $(NH_4)_2SO_4$ with a consequent loss of NH_3. More H_2SO_4 may be added at any time as becomes necessary. Excessive losses of H_2SO_4 may arise from (l) excessive heat, which results in unnecessary volatilization of the H_2SO_4 and (2) underestimation of the consumption of H_2SO_4 by either the organic matter or K_2SO_4. However, with proper regards to principles and techniques, excessive losses of H_2SO_4 due to volatilization and decomposition can be prevented.

The amount of H_2SO_4 needed for the oxidation varies with the type of sample analyzed. Estimates of the quantities of H_2SO_4 consumed by various materials during the digestion process are listed below.

Sample	**H_2SO_4 (36 N)**
1g carbohydrates	4.0 mL
1g protein	5.0 mL
1g fat	10.0 mL
1g K_2SO_4	0.5 mL
1g salicylic acid	6.8 mL
1g $Na_2S_2O_3.5H_2O$	0.5 mL
1g soil organic matter	5.8 mL

From this list, it is obvious that more H_2SO_4 will be required for the digestion of samples high in fat.

The loss of N on continued digestion is perhaps a general phenomenon in Kjeldahl digestion. These losses seem to be more pronounced when Se is used, and to be somewhat reduced when HgO accompanies Se. The only means for reducing such losses is to use adequate amounts of H_2SO_4.

5. *Digestion Time*

Digestion occurs in a two-phase sequence. In the first phase of digestion, the mixture turns black in color, and large amounts of fumes are produced. During the second phase, the mixture first turns light brown in color, to become almost colorless at the end.

However, complete digestion is not coincident with clearing time, since as much as l0% of the N in the samples has yet to be converted into NH_4. For complete digestion, the process of digestion is allowed to continue after clearing time has been reached. Usually the total time required is taken as l.5 times the clearing time. If Cu is used in the catalyst mixture, the digest has a light blue-green color, mixed with white precipitates, after cooling.

3.5.1.1 Determination of Ammonium

The determination of ammonium in the digest is traditionally done by distillation and titration. NH_3 is removed from the digest by distillation from alkaline solution:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

The NH_3, collected in a saturated solution of H_3BO_3, is determined by titration with a standard solution of H_2SO_4 or HCl. However, the ammonium in the digest is more conveniently determined by spectrophotometry or colorimetry. Of the three methods stated above, the most popular method today is the colorimetric method. Two major colorimetric methods are available: (l) the *phenol- hypochlorite method,* and (2) *salicylate-hypochlorite method.* The basic principle in the colorimetric method is, that in alkaline solution, NH_3 forms a blue color with a phenol-sodium hypochlorite reagent. The intensity of the blue color is proportional to the amount of NH_3 present. This analytical technique, known later as the *indophenol blue* or *phenate method,* forms the basis for the rapid analysis of NH_3 with automated colorimetric instruments, e.g., Technicon AutoAnalyzer, or Flow-injection Analyzer. The color intensity is measured by a colorimeter, and the results printed by a recorder. Routine analysis by the traditional Kjeldahl digestion-distillation method requires the close attention of a skilled technician. With the development of these automated instruments, the Kjeldahl analysis can be performed just as effectively and with just as much precision and accuracy by far less skilled personnel. The automated procedures have gained considerable popularity because of their speed, sensitivity, and ease of use. They are also frequently combined for determination of nitrite, nitrate, and phosphorus.

Since phenol is known today as a harmful carcinogenic compound, this reagent tends to be replaced by less toxic compounds, such as in the *salicylate-hypochlorite method.* The color reaction is based on the reaction of NH_4 with a weakly alkaline mixture of sodium salicylate ($NaC_7H_5O_3$) and chlorine (Cl), supplied in the form of Clorox. Sodiumnitroprusside [$Na_2Fe(CN)_5NO.2H_2O$] is added to speed up the color reaction at room temperature. When a Technicon AutoAnalyzer is used, a wetting agent is added to the buffer solution and the sodium salicylate mixture.

1. *Alkaline Distillation and Titration of Ammonium*

Reagents
1. Concentrated H_2SO_4.
2. Standardized 0.01 N H_2SO_4.
3. Kjeltab, Kjelmate, or make your own catalyst mixture by mixing 200 g K_2SO_4, 20 g $CuSO_4.5H_2O$, and 2 g Se.
4. NaOH 10 N.
5. Saturated boric acid, H_3BO_3, solution (50 g/L).
6. Mixed indicator. Dissolve l00 mg of methylene blue and 66 mg methyl red in l00 mL ethanol. This indicator is green in alkaline solution, gray at the neutral point, and purple in acid solution.

Procedure

Weigh l.000 g of compost to pass a 0.4-mm sieve, in a filter paper. More sample can be weighed for composts low in N. Fold the filter paper into a small bag, and drop it in a digestion block tube. Add Kjeltab, Kjelmate, or l.5 g catalyst mixture, and 5 mL concentrated H_2SO_4. Place the tube in a digestion block, and heat to boiling until green. Continue heating for another hour. After digestion is completed, allow the tube to cool, and slowly add 20 mL distilled water. The digest is ready for NH_4 determination by conventional distillation-titration procedure, or by automated colorimetric methods.

In case of plant samples, weigh 250 mg of sample, ground with a Wiley mill, add 10 mL H_2SO_4 and proceed as discussed above. (Frequently, the catalyst mixture can be replaced and digestion is performed by adding 2 mL 30% H_2O_2.) Continue heating for 30 more minutes, until white fumes are produced. Then cool the tube, and repeat digestion with 1 mL H_2O_2 until the digest iB clear and remains clear on cooling. Dilute with l5 mL distilled water, and the digest iB ready for NH_4 determination.

Distillation and Titration Process

Place a 50-mL Erlenmeyer flask containing l0 mL H_3BO_3 and a few drops of mixed indicator under the condenser stem of the distillation apparatus, so that the end of the stem is touching the surface of the H_3BO_3 solution. Then, transfer and wash the entire content of the digestion tube into the steam chamber of the distillation apparatus. Add slowly 20 mL of l0 N NaOH into the digest. When about l ml of NaOH remains in the funnel stem, rinse the funnel quickly by spraying with water, and close the chamber rapidly. Start distillation by allowing steam to flow through the digest, and as soon as NH_3 starts to collect, the boric acid solution turns green. When the distillate reaches the 35 mL mark on the receiver Erlenmeyer flask, stop the distillation. Rinse the end of the condenser stem, and titrate the distillate with standardized 0.0l N H_2SO_4, dispensed from a l0-mL microburette. Run also a blank analysis. The total N content in percentages is calculated as follows:

$$\% N = [(T - B) * N * 1.4]/s$$

in which: T = sample titration, mL standard acid, B = blank titration, mL standard acid, *N* = normality of standard acid, and s = sample weight, g.

2. ***Colorimetric Determination* of *Ammonium***

In the absence of automated instruments, the NH_4^+ in the digest can also be measured manually with a colorimeter. This manual procedure is in fact the basic principle for use in the automated instruments.

Reagents

1. Sodium salicylate-Sodium nitroprusside solution: Dissolve 300 mg of Na-nitroprusside [$Na_2Fe(CN)_5NO*2H_2O$] and l50 g Na- salicylate (NaC_7H_5O3) in 600 mL water.
2. Sodium hypochlorite (NaOCl, or Clorox): Measure 6 mL Clorox, containing 5.25% Cl, in a l00-mL volumetric flask, and dilute to the mark with distilled water.
3. Buffer solution: Dissolve 50g of Na-K-tartrate ($NaKC_4H_4O_6 \cdot H_2O$) and 26.8 g disodium phosphate (Na_2HPO_4) in 600 mL distilled water in a 1-L volurnetric flask. Add 54 g NaOH, and allow this to dissolve by constant stirring, before the volume is made up to 1 L with distilled water,
4. Standard NH_4^+ solution: Dissolve 412.5g of ammonium sulfate [$(NH_4)_2SO_4$] in 1 L of distilled water. This solution contains l00 µg of NH_4^+/mL. Pipet 5 mL of this solution

in a 250-mL volumetric flask, and dilute to the mark with distilled water, The resulting solution, containing 2 µg NH_4^+ per mL, is used for making a standard (calibration) curve.

Procedure
Pipet l mL of the diluted Kjeldahl digest into a colorirnetric tube or icuvette. Add 5.5 mL buffer solution, and 4 mL Na- salicylate-nitroprusside solution and stir to mix, Add 2 mL Na- hypochlorite solution and mix. Allow the solution to stand for 45 minutes at 25°C for complete color development, and measure the absorbance of the colored solution at 650 nm.

Prepare a standard curve with 4 or 5 different known amounts of NH_4^+ using the same procedure as outlined above.

3.Spectrophotometry Determination of Ammonium

Method:
Photometric determination using the indophenolblue method subsequent to extraction with calcium chloride solution.

Reagents:
1. Spectroquant Ammonium Test, Cat. No. 1.14752.0001 OR
   Spectroquant Ammonium Cell Test, Cat. No. 1.14558.0001, 1.14544.0001, 1.14559.0001
2. Calcium chloride solution 0.025 mol/l $CaCl_2$ → 2.778g
3. Dissolve 3.68g Calcium chloride dehydrate GR, Cat. No 1.02382.0500 with deionized water in a 1000 ml standard volumetric flask and fill up to volume.
4. Charcoal activated for soil tests, Cat. No. 1.02181.1000

Sample preparation:
In a glass bottle mix 50 g of naturally moist sample, free from coarse stones, with 100 ml of a calcium chloride solution. Add 1 spatula – tipfull of charcoal activated for soil tests and shake the closed bottle in a shaking machine for 1 hour (alternative: stir in a beaker). Let the suspension settle and filter through a fluted filter.
For the determination of the water-content dry a similar sample to constant weight in the drying kiln at 105°C. A drying of the sample before determination is not advisable because of the fast change in the nitrogen – forms. The analysis should take place very quickly. Storing the sample in plastic bags at room temperature changes the analysis values after a short time already.

Analysis:
Determine by means of Spectroquant Ammonium-Test or cell test.

Calculation:
NH_4^+-content in mg/kg = analysis value in mg/l x 2

3.5.1.2 Determination of NO_3^-

The accurate determination of NO_3-N in composts requires that considerable care must be taken in handling the samples between the field and the laboratory. Unless special provisions are made, NO_2-N is usually included as NO_3-N. The determination of NO_3^- can be performed by *indirect* and *direct* methods:

***Indirect Methods Through Conversion* of NO_3^- *to* NH_4^+**

1. Distillation with and without MgO or Devarda alloy. The distillation procedure as described above for the determination of total N is performed with and without the addition of Devarda alloy. The difference in results equals the NO_3^- concentration in the sample.
2. Cu-Cd reductor method. NO_3^- is reduced into NO_2^- by passing the extract through a Cu-Cd reductor column. The NO_2^- is then determined by the *Griess-Ilosvay* method employing manual or automated procedures.

Direct Methods

1. ***Colorimetric method*** using the phenol disulfonic acid procedure. This method is based on the development of a yellow color, when l,2,4 phenol disulfonic acid is nitrated and the resulting 6-nitro-l,2,4 phenol disulfonic acid is neutralized with NH_4OH. The intensity of the yellow color is proportional to the NO_3^- concentration in the sample.
2. ***Nitrate specific-ion electrode***

Extraction of NO_3^-

Since nitrate, NO_3^-, is soluble, all reagents are suitable for extraction of nitrate from compost, even water. Acidified l N KCl, pH 1.0, and neutral l N KCl or NaCl are preferable.

Procedures

Phenol Disulfonic Acid Procedure

Reagents

1. Phenol disulfonic acid solution: Dissolve 25 g of pure phenol (crystals) in l50 mL concentrated H_2SO_4 in a 500-mL beaker. Add 75 mL fuming H_2SO_4 mix the solution, and place the beaker in boiling water for 2 hr. Store the resulting phenol disulfonic acid, $C_6H_3OH(HSO_3)_2$ in an amber colored bottle. This reagent is highly corrosive.
2. Powdered $CaCO_3$, reagent grade.
3. Powdered $CaSO_4*2H_2O$, reagent grade.
4. Ag_2SO_4, for removal of Cl^-, which interferes with the color reaction.
5. Powdered $Ca(OH)_2$, reagent grade.
6. Powdered $MgCO_3$, reagent grade.
7. Standard NO_3^- solution: Weigh 72l.4 mg of pure dry KNO_3 in a 1-L volumetric flask, and dissolve with distilled water to the mark. This solution, containing l00 mg N/L, is called the stock solution. Prepare a working solution by pipetting 25 mL of the stock solution into a 250-mL volumetric flask. This working solution contains l0 mg N/L, and is used for making a standard curve.

Preparation of Cl^- *free Extract*

Weigh 50.0 g of compost in a 500 mL Erlenmeyer flask, and add 500 mg $CaSO_4$ and 250 mL distilled water. Shake the flask mechanically for l5 min, and centrifuge the content to separate the supernatant from the compost. Filter the clear supernatant liquid into a clean 500-mL Erlenmeyer flask, add 200 mg $AgSO_4$, and shake the flask mechanically for l5 min. Add 200 mg $Ca(OH)_2$ and 500 mg $MgCO_3$ to precipitate Ag^+. After shaking the mixture for 5 min, filter the suspension into a polyethylene vial for further analysis.

Analysis of the Extract

Pipet 20 mL of the clear extract into a l50-mL Pyrex beaker, and evaporate the extract to dryness. Allow the beaker to cool to room temperature, and add rapidly 3 mL of phenol disulfonic acid. Rotate the beaker to effect complete contact, and allow the color reaction

to develop (l0 min). Add 20 mL distilled water and stir to dissolve. Transfer the colored solution into a l00-mL volumetric flask, dilute to the mark with distilled water, stir thoroughly, and measure the color intensity at 420 mμ against a blank solution.

Prepare a standard curve by pipetting different aliquots of the working solution and proceeding according to the procedure as described above.

3. Spectrophotometry Determination of NO_3^-

Method:
Photometric determination with Nitrospectral subsequent to extraction with Calcium chloride solution.

Reagents:
1. Spectroquant Nitrate Test, Cat. No. 1.14773.0001 or
2. Spectroquant Nitrate cell test, Cat. No. 1.14542.0001
3. Calcium chloride solution: Dissolve 3.68 g calcium chloride dehydrate, Cat. No. 1.02382.0500 with deionized water in a 1000 ml standard volumetric flask and fill up to volume.
4. Charcoal activated for soil tests, Cat. No. 1.02181.1000

Sample preparation:
In a glass bottle mix 50 g of naturally moist sample, free from coarse stones, with 100 ml of a calcium chloride solution. Add a spatula – tipfull of charcoal activated for soil tests and shake the closed bottle in a shaking machine for 1 hour (alternative: stir in a beaker). Let the suspension settle and put it through a fluter filter.
For determination of the water – content dry a similar sample to constant weight in the drying kiln at 105^{o}C. A drying of the sample is not advisable because of the fast change in the nitrogen – forms. The analysis should take place very quickly. Storing the sample in plastic bags at room temperature changes the analysis values after a short time already.

Analysis:
Determine by means of Spectroquant Nitrate-Test or cell test.

Calculation:
NO_3-content in mg/kg = analysis value in mg/l x 2

3.5.1.3 Determination of NO_2^-

Nitrite can be determined by using indirect and direct methods.

Indirect Procedure: distillation and titration method.
1. Determine (NH_4^+ + NO_3^- + NO_2^-) by adding Devarda alloy and MgO in the distillation process.
2. Deterrnine (NH_4^+ + NO_3^-) by adding sulfamic acid before the addition of Devarda alloy and MgO to destroy NO_2^-.
3. The difference between analyses (l) and (2) is the NO_2^- concentration.

Direct Procedure:
1. Griess - Ilosvay method.

Reagents

1. Diazotizing solution: Weigh 500.0 mg of sulfanilamide in a 100-mL volumetric flask. Add 80 mL 2.4 N HCl solution to dissolve the reagent under constant stirring, after which the volume is made up to the mark with the HCl solution. Store this solution at 4°C in a refrigerator.
2. Coupling agent: Weigh 300.0 mg N-(1-napthyl)-ethylene-diamine hydrochloride in a l00-mL volumetric flask. Add 80 mL of 0.12 N HCl and dissolve the reagent under constant stirring, after which the solution is made up to volume with 0.l2 N HCl. Store the solution in an amber colored bottle in a refrigerator at 4°C.
3. Standard NO_2^- solution: Weigh 246.4 mg $NaNO_2$ in a 1-L volumetric flask and add 800 mL distilled water to dissolve, after which the volume is made up to the mark. This solution, containing 50 mg N/L, is the stock solution and must be stored in a refrigerator at 4°C. To prepare a working solution for a standard curve, pipet 20 mL of the stock solution into a l-L volumetric flask. The volume is made up to the mark with distilled water.

Analysis of the Extract

The 2 M KCl extract can be used for the determination of NO_2^-. The extract is sometimes colored, but this does not interfere with the analysis. Pipet 2 mL of the 2 M KCl extract into a 50-mL volumetric flask, and add 40 mL of distilled water. Add 1 mL of diazotizing reagent, mix by swirling the flask, and allow the mixture to react for 5 min, after which 1 mL of coupling agent is added. Mix the solution and allow it to stand for 20 min. Make up to volume with distilled water, mix thoroughly, and measure the color intensity at 540 nm against a blank solution. Prepare a standard curve, by pipetting 0, l, 2, 4, and 5 mL of the working solution into 50-mL volumetric flasks, and proceed as described above.

2. Spectrophotometry Determination of NO_2^-

Method:

Photometric determination using the Griess-method subsequent to extraction with Calcium chloride solution.

Reagents:

1. Spectroquant Nitrite Test, Cat. No. 1.14776.0001 or Spectroquant Nitrite cell test, Cat. No. 1.14547.0001
2. Calcium chloride solution: Dissolve 3.68 g calcium chloride dihydrate, Cat. No. 1.02382.0500 with deionized water in a 1000 ml standard volumetric flask and fill up to volume.
3. Charcoal activated for soil tests, Cat. No. 1.02181.1000

Sample preparation:

In a glass bottle mix 50 g of naturally moist sample, free from coarse stones, with 100 ml of a calcium chloride solution. Add a spatula – tipfull of charcoal activated for soil tests and shake the closed bottle in a shaking machine for 1 hour (alternative: stir in a beaker). Let the suspension settle and put it through a fluter filter.

For determination of the water – content dry a similar sample to constant weight in the drying kiln at 105°C. A drying of the sample before determination is not advisable because of the fast change in the nitrogen – forms. The analysis should take place very quickly. Storing the sample in plastic bags at room temperature changes the analysis values after a short time already.

Analysis:

Determine by means of Spectroquant Nitrite-Test or cell test.

Calculation:
NO_2-content in mg/kg = analysis value in mg/l x 2

3.5.2 Determination of Total Phosphorus (P)

1. Fluoro-boric acid digestion procedure

The *fluoro-boric acid digestion procedure*, employing specially designed acid digestion vessels, called bombs, is presented here because of its rapidity, simplicity, and the need of only small amounts of reagents. The traditional method by fusion with NaOH or Na_2CO_3 needs expensive Pt crucibles and a variety of chemicals. The digest obtained by the bomb procedure can also be used for analysis of other elements (total elemental analysis of soil and plant samples).

Reagents

1. Hydrofluoric acid, HF, 48%
2. Aqua regia: Mix 1 part of concentrated HNO_3 with 3 parts of concentrated HCl. The present author noticed that this reagent can be deleted without interfering in the digestion process.
3. Powdered boric acid, H_3BO_3
4. Ammonium molybdate solution: Weigh 25 g $[(NH_4)_6Mo_7O_{24}.\ 4H_2O]$ in a beaker, and add 200 mL distilled water. Heat at 60°C to dissolve under constant stirring. The solution is filtered to remove any coarse residue. Dilute 275 mL concentrated H_2SO_4 (cp grade) with distilled water to 750 mL, and allow the solution to cool. The molybdate solution is then poured slowly into the sulfuric acid solution.
5. Antimony: Dissolve 667.0 mg of potassium antimony tartrate, $KSbO.C_4H_4O_6$ in 250 mL distilled water.
6. Ascorbic acid: Weigh 10 g of ascorbic acid in a l00-mL volumetric flask. Add 80 mL distilled water to dissolve, and dilute to the mark. Store this reagent in a cold room at 2°C.
7. Mixed reagent: Mix equal volumes of the ascorbic acid and the Sb solution before use. Prepare a fresh solution when required.
8. Standard solution of KH_2PO_4: Weigh 2l9.4 mg of KH_2PO_4 in a l-L volumetric flask, and dissolve with 500 mL distilled water. Add 25 mL 7 *N* H_2SO_4, and dilute to the mark with distilled water. This stock solution contains 50 mg P/L. Pipet 20 mL of this stock solution into a 500-mL volumetric flask, and dilute to the mark with distilled water. This is the working solution, which contains 2 µg P/mL.

Digestion and Extraction

Since HF is used in the digestion procedure, polyethylene beakers, stirrers, and volumetric flasks have to be employed in collecting and processing the results.

Weigh 50 mg sample, ground to 150 mesh, into the teflon vessel of the acid digestion bomb (20 mg for samples high in total P). After adding 0.5 mL aqua regia and 3.0 mL HF (48%), the vessel is closed and reassembled in the stainless steel body of the bomb. The bomb is sealed by turning the knurled cap, placed in an oven, and heated at ll0°C for 40 min. Cool and unscrew the bomb. Wash the content with 4-6 mL distilled water by spraying into a l00- mL polyethylene beaker. Washing should not exceed l0 mL. Take care to wash all precipitates into the beaker. Add 2.8 g H_3BO_3, and stir with a plastic stirrer to dissolve the boric acid. Add 5-l0 mL H_2O and any precipitate should dissolve at this time. Continue adding distilled water to about 40 mL. Transfer the solution into a l00-mL polyethylene volumetric flask, make up to volume, and store the solution in a plastic vial for further analysis. This solution can be used for total P determination and other macro-, and microelement analyses.

Ascorbic Acid Sulfomolybdo-Phosphate Blue Color Analysis of P

Pipet 5 mL of the digest into a 50-mL polyethylene volumetric flask. Aliquots of <2 mL or >2 mL can also be used, depending on the intensity of the blue color developed. Add 10 mL of the ammonium molybdate solution containing H_2SO_4 and swirl the flask to mix. Add 4 mL of the antimony-ascorbic acid mixture, and dilute with distilled water to the mark. Maximum color intensity develops within 10 min, and remains stable for several hours. The absorbance is measured at 840 nm.

Prepare a standard curve by pipetting 0, 1, 4, 5, and l0 mL of the KH_2PO_4 working solution into a series of 50-mL volumetric flasks. Proceed according to the procedure described above.

2. Spectrophotometry Determination of P

Method:

Photometric determination as orthophosphate according to the vanadate-molybdate method. Extraction with CAL buffer analogous to the LUFA methods.

Reagents:

CAL-Extraction solution: Dissolve 15.4 g calcium lactate for soil analysis, Cat. No. 1.02103.0250, and 7.9 g calcium acetate for soil analysis, Cat. No. 1.09325.0500 separately in 300 ml of water GR., Cat. No. 1.16754.9010 whilst warming. Combine the two solutions in a 1-l volumetric flask and add 17.9 ml acetic acid 100% GR, Cat. No. 1.00063.1000. Allow to cool, make up to the mark with water and mix well.
Spectroquant Phosphorus Test(VM), cat No. 1.14842.0001

Sample preparation:

Air-dry the compost sample, subsequent to removing course particles such as stones and plant material. Sieve the sample through 2 mm mesh. Place 5 g of sample in a glass bottle of approx. 300 ml volume and add 100 ml of extraction solution. Shake the closed flask for 90 minutes at 175 shakes per minute. Filter the extract through a fluted filter and dispose of the first 10 ml filtrate.

Analysis:

The determination should be carried out with Spectroquant Phosphorus Test(VM) as P_2O_5. Samples containing more than 14 mg/l P_2O_5 should be diluted with extraction solution. If the extract has a yellow colour, it should be measured, without the addition of reagent, as a blank and the result subtracted from the sample value. Measurement should be carried out in 10 mm rectangular cells at 405 nm against a blank comprising extraction and reagents in equal proportions.

Calculation:

$$\text{mg } P_2O_5 \text{ per kg air-dried compost} = \frac{\text{Measured value (mg/l } P_2O_5) \text{ x } 100}{\text{Sample weight in g}}$$

3.5.3 Determination of Total Potassium (K)

1.Fluoro-boric acid digestion

Fusion of samples with solid NaOH or $NaCO_3$, and digestion using mixtures of concentrated H_2SO_4 and $HCIO_4$ have been employed for total elemental analysis.

However, as indicated in an earlier section, the fluoro-boric acid digestion method employing specially designed bombs is the most rapid and convenient method for total elemental analysis of soil, mineral, and plant samples. The procedures discussed below are also applicable to determination of Na.

Reagents
1. Hydrofluoric acid, HF, 48%
2. Aqua regia: mix 1 part of conc HNO_3 with 3 parts conc HCl
3. Powdered boric acid, H_3BO_3
4. Stock K solution containing 1000 µg K/mL. Pipet 2 mL from the stock solution into a 1-L volumetric flask, and dilute to the mark with distilled water. This solution, called the standard solution, contains 2 µg K/mL.

Extraction of Total K
The following procedure of the fluoro-boric acid digestion method has been discussed in the determination of total P (section 3.5.2). However, for reader's convenience, it is discussed again below with some minor revisions. Polyethylene beakers, stirrers, and volumetric flasks have to be employed with this method, because HF is used.

Weigh 50 mg sample, ground to 150 mesh, into the Teflon vessel of the acid digestion bomb (20 mg for samples high in total K). After adding 0.5 mL aqua regia and 3.0 mL HF (48%), the vessel is closed and reassembled in the stainless steel body of the bomb. The bomb is sealed by turning the knurled cap, placed in an oven, and heated at 110°C for 40 min. Cool and unscrew the bomb. Wash the content with 4-6 mL distilled water by spraying into a 100- mL polyethylene beaker. Washing should not exceed 10 mL. Take care to wash all precipitates into the beaker. Add 2.8 g H_3BO_3, and stir with a plastic stirrer to dissolve the boric acid. Add 5-10 mL H_2O and any precipitate should dissolve at this time. Continue adding distilled water to about 40 mL. Transfer the solution into a 100-mL polyethylene volumetric flask, make up to volume, and store the solution in a plastic vial for analysis of major compost K and other macro-, and micro-elements.

Analysis of Total K by Atomic Absorption Spectroscopy
Calibrate the atomic absorption spectrophotometer, provided with a hollow cathode K tube, with the standard containing 2 µg K/mL. This standard should give a reading of about 0.22 absorbance units with the Perkin-Elmer. Other models require different standards and readings. Check your instrument's manual for the correct settings.

Aspirate your extract into the instrument and read the absorbance. If your reading is >0.22 absorbance, dilute your extract so that the reading is below or equals 0.22 absorbance. The working range for K is linear from 0 to 2 µg K/mL.

2. Spectrophotometry Determination of total K

Method:
Photometric determination using sodiumtetraphenyl borate. Extraction with CAL buffer analogous to the LUFA methods.

Reagents:
1. CAL-Extraction solution: Dissolve 15.4 g calcium lactate for soil analysis, Cat. No. 1.02103.0250, and 7.9 g calcium acetate for soil analysis, Cat. No. 1.09325.0500 separately in 300 ml of water GR., Cat. No. 1.16754.9010 whilst warming. Combine the two solutions in a 1-l volumetric flask and add 17.9 ml acetic acid 100% GR, Cat. No. 1.00063.1000. Allow to cool, make up to the mark with water and mix well.

2. Spectroquant Potassium Cell Test, Cat No. 1.14562.0001
3. Sodium hydroxide solution 32% GR, Cat. No. 1.05590.2500

Sample preparation:
Air-dry the compost sample, subsequent to removing course particles such as stones and plant material. Sieve the sample through 2 mm mesh. Place 5 g of sample in a separating flask of approx. 300 ml volume and add 100 ml of extraction solution. Shake the closed flask for 90 minutes at 175 shakes per minute. Filter the extract through a fluted filter and dispose of the first 10 ml filtrate. Adjust the clear filtrate to pH 5-7 with 32% sodium hydroxide solution. Samples containing more than 35 mg/l K_2O should be diluted with extract solution.

Analysis:
The determination should be carried out with Spectroquant Potassium Cell Test as K_2O. Measurement should be made against the pure extraction solution. If the sample extract has a yellow colour, it should be measured, without the addition of reagent, as a blank and the result subtracted from the sample value. The parameters for calibrating the method in the SQ 118 photometer are given on page 2.

Calculation:

$$\text{mg } K_2O \text{ per kg air-dried compost} = \frac{\text{Measured value (mg/l } K_2O) \times 100}{\text{Sample weight in g}}$$

3.5.4 Determination of Total Sodium (Na)

1. Fluoro-Boric Acid Digestion Method

Fusion of samples with solid NaOH or $NaCO_3$, as suggested for determination of total K, cannot be used in the determination of Na. Digestion using mixtures of concentrated H_2SO_4 and $HClO_4$ as for total elemental analysis is a possibility. However, the most convenient method for determination of total Na content in composts is the fluoro-boric acid digestion method employing specially designed bombs. For the procedure, reagents, and extraction of total Na by the fluoro-boric acid method reference is made to section 3.5.2.

Analysis of Total Na by Atomic Absorption Spectroscopy
Calibrate the atomic absorption spectrophotometer, provided with a hollow cathode N a tube, with a standard containing 0.8 µg Na/mL. The standard is prepared by pipetting 0.8 mL from of stock solution, containing 1000 µg Na/mL, into a 1-L volumetric flask, and diluting it to volume with distilled water. This standard should give a reading of about 0.23 absorbance units with the Perkin-Elmer instrument. Other models require different standards and readings. Check your instrument's manual for the correct settings.

Aspirate your extract into the instrument and read the absorbance. If your reading is >0.23 absorbance, dilute your extract so that the reading is below or equals 0.23 absorbance. The working range for N a is linear from O to 1 µg N a/mL.

3.5.5 Determination of Total Calcium (Ca)

1. Fluoro-Boric Acid Dillestion Method

The procedures and determination of total Ca are exactly the same as those for total potassium. The extract for determination of K obtained by the fluoro-boric acid method is also used for total Ca analysis by atomic absorption spectrophotometry. In this case, the instrument, provided with a hollow cathode Ca tube, is calibrated with a standard solution containing 4 µg Ca/mL, which should give an absorbance reading of 0.20 units with a Perkin-Elmer AA spectrophotometer. The working range of Ca is linear from 0 to 4 µg Ca/mL. The standard solution is prepared by pipetting 4 mL of a stock solution (1000 µg Ca/mL), into a 1-L volumetric flask, and diluting it to the mark with distilled water.

2. Spectrophotometry Determination of total K

Method:
Photometric determination with Glyoxal bis(2-hydroxyanil) subsequent to acid extraction.

Reagents:
Spectroquant Calcium-Test, cat No. 1.14815.0001
Hydrochloric acid 1 mol/l, Cat. No. 1.09970.1000
Hydrochloric acid 25% GR, Cat. No. 1.00316.1000
Sodium hydroxide solution 10% GR, Cat. No. 1.05588.1000
Acilit indicator strips pH 0-6, Cat. No. 1.09531.0001

Sample preparation:
In an Erlenmeyer flask mix 10 g of air-dry, finely-ground, stone-free material with 50 ml hydrochloric acid 1 mol/l and held at a gentle boil for 5 minutes on a heating plate. The pH range of the supernatant solution should be 1 – 3 (test with Acilit indicator strips pH 0 – 6). If necessary, adjust by using hydrochloric acid 25%. Heat the material once again, for a few minutes. After cooling, adjust the mixture to ph 7 – 7.5 using a pH – meter by addition of sodium hydroxide solution 10%.
Transfer the suspension, with deionized water, into a 1000 ml standard volumetric flask, fill up to the mark with deionized water, and mix.

Analysis:
Determine by means of Spectroquant Calcium – Test.

Calculation:
Ca – content in mg/kg = analysis value in mg/l x 100

3.5.6 Determination of Total Magnesium (Mg)

The extract obtained by the fluoro-boric acid digestion procedure for total K determination (section 3.5.2) is used for determination of total Mg by atomic absorption spectrophotometry. The instrument, provided with a hollow cathode Mg tube, is calibrated with a standard solution containing 0.3 µg Mg/mL, which gives an absorbance reading of 0.19 units with a Perkin-Elmer AA spectrophotometer. The working range of Mg is linear from 0 to 0.5 µg/mL. The standard solution is prepared in two steps as follows. In the first step: pipet 3 mL of stock solution (1000 µg Mg/mL), into a 1-L volumetric flask. Dilute to volume and a solution containing 3 µg Mg/L is obtained. In the second step: pipet 10 mL of this solution, containing 3 µg Mg/mL, into a 100-mL volumetric flask and dilute to volume with distilled water. This solution, containing 0.3 µg Mg/mL, is the standard solution for calibration of the AA instrument.

3.6 Determination of Micro-elements

Preparation of sample: pretreatment of sample with the process digestion

- 0,5g of compost are placed in special conical flask
- Transfer 0.50 g or less of sample into a 100-mL Digesdahl digestion flask. Note: Be sure you have a homogenous sample. Solid sample should be finely ground or chopped and mixed well.
- Add 4 mL of concentrated sulphuric acid (spec. gravity 1.84) to the digestion flask. Note: Use only Hach Digesdahl digestion flasks. Volumetric flasks with concave bottom should not be used. Note: Safety glasses and a safety shield placed between the operator and the Digesdahl are required
- Turn the temperature dial to a heat setting of 440 °C (825 ° F). When the proper temperature is reached, turn on the water to the aspirator and make sure there is suction to the fractionating column. Note: Wait for the proper temperature to be reached before sample is placed on the heater
- Place the flask weight followed by the fractionating column with funnel on the flask. Place the flask on the heater and boil 4 minutes. Do not boil to dryness.
  Note: If sample foams up into the neck of the flask, lower temperature to 335 °C (635 ° F).Continue heating at lower temperature until all water is evaporated. Then return to original digestion temperature.
  Note: White acid vapors accompanied with a reflux line indicate that the sulfuric acid is boiling
- Do not proceed if sulphuric acid is not visible in the flask. Add 10 mL of 50% hydrogen peroxide to the charred sample via the funnel on the fractionating column.
  Note: Do not heat to dryness. Note: Visually confirm the presence of sulfuric acid in the flask before adding hydrogen peroxide.
  Note: If the digest does not turn colorless, add 5 mL increments of peroxide until the digest becomes clear or does not change color.
  Note: If sample foams during hydrogen peroxide addition, stop the peroxide flow and remove the digestion flask and fractionating column (use finger cots). Cool for 30 seconds and return apparatus to the heating block. Start peroxide addition with 2 mL, then follow with the remaining peroxide.
- After addition of hydrogen peroxide is complete, boil off excess hydrogen peroxide by heating for one more minute. Do not heat to dryness.
  Note: If the sample goes to dryness, turn off the Digesdahl and air cool to room temperature. Add water to flask before handling. Repeat the digestion from the beginning using a new sample.
- Take the hot flask off the heater and allow the flask to air cool. Remove the fractionating column from the digestion flask.
  Note: Use finger cots to remove the digestion flask. Place it on a cooling pad for at least one minute. Then remove the column. Do not add water to the flask until it has cooled.
- Dilute the digest to approximately 70 mL with deionized water.
  Note: Add deionized water slowly at first. Cool the flask if necessary for handling.

After digestion the heavy metals concentration of the diluted solution can be measured through atomic absorption. Based on the Perkin Elmer 2380 AAS – EM shown in Figure 1 the standard conditions of atomic absorption for each heavy metal is presented in Table 1. The basic steps followed during the method of atomic absorption is (1) Lamp alignment (2) Burner adjustment (3) Calibration (4) Measurement

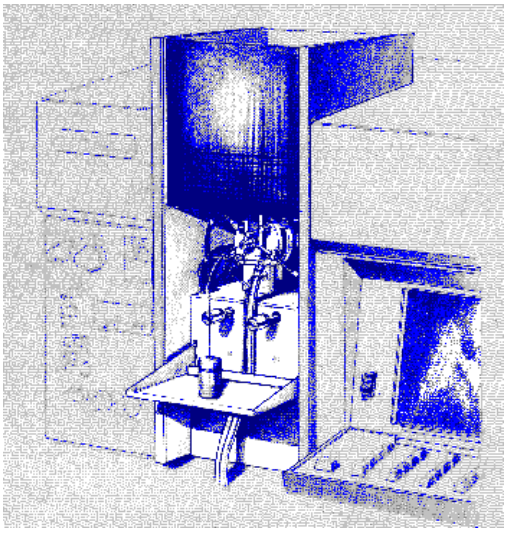

Figure 1: Perkin Elmer 2380, AAS – EM Atomic Absorption Spectrophotometre

Table 1: Standard Conditions for atomic absorption

Element	**λ (nm)**	**SBW (nm)**	**Flame Gases**	**Sens. Check mg/L (1), (2)**	**Linear Range mg/L (4)**	Interferences
Cd	228.8	0.7	A - Ac	1.5	2.0	
Cr	357.9	0.7	A - Ac	4.0	5.0	
Cu	324.8	0.7	A - Ac	4.0	5.0	
Fe	248.3	0.2	A - Ac	5.0	5.0	0.2% $CaCl_2$
Mn	279.5	0.2	A - Ac	2.5	2.0	0.2% $CaCl_2$
Ni	232.0	0.2	A - Ac	7.0	2.0	
Pb	283.3	0.7	A - Ac	20.0	20.0	
Zn	213.9	0.7	A - Ac	1.0	1.0	

3.7 Determination of phytotoxicity level of compost

Reagents:

- Petri dishes
- lettuce seeds
- Filter papers (Whatman #1)
- Distilled water
- Dark incubator (25^{o}C)
- Ruler

Procedure:

1. Weigh 10gr of each compost sample
2. Add 100 ml of distilled water and agitate the solution for 30min
3. Centrifuge the solution at 3000rpm for 10min and then filter it using Whatman 41 (20 – 25 μm)

4. Add 10 ml aliquot or extract to a petri dish with a Whatman no.1 ashless filter paper and place 20 seeds in each dish (three replicates for each sample)
5. Place Whatman no.1 ashless filters in two Petri dishes adding 10 ml Distilled water. These dishes are used as a control
6. The plates are incubated at 25 ◦C in the dark for 5 days.
7. Seed germination and root length in each plate are measured on the 5^{th} day.
8. In both germination tests, the percentages of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) after exposure to sludge extracts were calculated as follows

Calculations:

$$\text{RSG}(\%) = \frac{\text{number of seeds germinated in sludge extract}}{\text{number of seeds germinated in control}} 100$$

$$\text{RRG}(\%) = \frac{\text{mean root length in sludge extract}}{\text{mean root length in control}} 100$$

$$\text{GI}(\%) = \frac{\text{RSG x RRG}}{100}$$