Bio-solids Manual

Sampling and Laboratory Physical, Chemical and Microbiological Procedures and Quality Control

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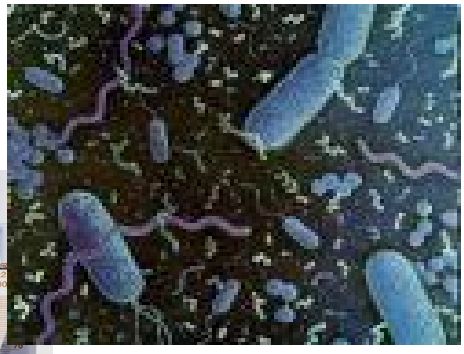

January - 2009

Bio-solids Manual (Volume III): *Sampling and Laboratory Physical, Chemical and Microbiological Procedures and Quality Control*

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ACKNOWLEDGEMENT

The Environmental Research Center ERC at Royal Scientific Society would like to acknowledge the United States Agency for International Development USAID (Washington and Jordan / Water Resources and Environment Office - Jordan), the International Arid Lands Consortium / the University of Arizona - USA and the Badia Research & Development Centre - Jordan for their contribution in the administrative and financial support preparing this manual.

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Scope

This technical manual provides laboratory procedures for bio-solids analysis generated by different wastewater treatment processes. It is based on current, state-of-the-art field and laboratory practices. In addition, bio-solids sampling and analysis are needed to assess compliance with current requirements as stated in Jordanian Standard No. (1145/2006).

The manual covers principles of sampling bio-solids, analytical procedures, some aspects concerning quality control of the carried out analyses as well as safety considerations associated with sampling and analysis of bio-solids samples. The methods of analysis, required in the annexed Jordanian Standard No. (1145/2006), include chemical and microbiological analyses. The chemical analyses discussed in this manual are Total Kjeldahl Nitrogen (TKN), Ammonia, Organic Carbon, Total, Fixed and Volatile Solids, Total Phosphorus and Heavy Metals. Concerning microbiological analyses the following analyses are described: Fecal Coliform Count TFCC, Intestinal Pathogenic Nematode IPN eggs and *Salmonella* spp.

Finally, this manual could be considered as a guide in developing and implementing a sampling and analysis program, to gather information on bio-solids quality, and determine compliance with permit conditions.

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Chapter One: Safety Considerations and Quality Control

1.1 Sludge and Bio-solids Sampling Safety Instructions

Safety is important in sludge and bio-solids sampling, especially since many sampling points preclude direct collection of grab samples. The following instructions should be taken into consideration while sludge and bio-solids sampling:

- Personal hygiene is important for all personnel involved in sludge and bio-solids sampling efforts.
- As a precautionary measure, inoculations are recommended for all personnel who have direct contact with sludge. As a minimum, inoculation should include diseases such as typhoid and tetanus.
- Avoidance of direct sludge contact is preferred, and is possible if proper precautions are taken.
- Rubber or latex gloves should be worn at all times; especially while collecting or handling samples, and waterproof garments are used when the risk of splashing exists.
- Wash any cuts or scrapes thoroughly and treat immediately.
- Gas production from biologically active sludge samples may cause pressure build-up. This is especially true if the samples are not stored at $4^{o}C$ as recommended.
- When sludge sampling is in confined areas, particularly around anaerobic digesters, dangerous gases may be present. These gases may include either explosive vapors (methane), poisonous mixtures (including hydrogen sulfide), or oxygen-deprived atmospheres (carbon dioxide). Explosive vapors require care to avoid sparks and possible ignition. These situations necessitate adequately ventilated equipment, gas detection meters and backup breathing apparatus. Exercise care around open pits or uncovered holes. Proper lighting increases the visibility of such hazards.
- Loose or dangling garments (ties, scarves, etc.) should not be worn around equipment with moving parts, especially pumps. Exercise extra awareness around pumps controlled by intermittent timers.
- Finally, be very careful when sampling high pressure sludge lines or lines containing high temperature thermally-conditioned sludge; in order to avoid injury by either high pressure streams or burns.

1.2 Laboratory Safety Instructions

1.2.1 General

1. Safety takes precedence over all other considerations.
2. Do not work alone while performing dangerous chemical procedures. Be sure there is someone in the immediate vicinity you can reach in case of emergency.
3. Know the location of, and how to use eye-wash fountains, showers, and fire blankets.
4. Be sure you understand the hazards involved in a procedure and take all necessary safety precautions before beginning your procedure.
5. Food products are **not** to be stored in laboratory refrigerators. Consumption of food or smoking is not permitted in laboratory operation areas.
6. Unsafe facilities, equipment, or behavior should be reported to your supervisor.
7. Unattended equipment and reactions are major causes of fire, floods, and explosions.
8. Be sure all utility connections are secure. Anticipate hazards that would result from failure of electrical, water, or gas supply.

1.2.2 Personal Protection

1. Wear approved eye and face protection suitable for the work at hand. Safety glasses or goggles should be worn at all times while working with chemicals at the counter or laboratory hood. A face shield should be worn when working with potentially eruptive substances.
2. Custodians, maintenance workers, and visitors must observe safety rules, including eye protection, while in the laboratory.
3. Wear protective gloves and clothing whenever handling corrosive or other hazardous chemicals.
4. Wear closed-toe shoes at all times in the lab.
5. Be sure that moving parts of mechanical apparatus are guarded to prevent hazardous contact.
6. Maintain your lab area reasonably neat and uncluttered.
7. Use the fume hood for all operations involving harmful gases or fumes and for flammable or explosive materials. Check the hood to see that it is operating adequately and has been inspected annually.

8. Use a safety shield or barrier to protect against explosion, implosion, and flash fires when performing reactions with large volume of flammable liquids or unstable materials.
9. Inspect glassware for cracks, sharp edges, and contamination before using. Broken or chipped glassware should be repaired and polished or discarded.
10. Broken glass should be put in impervious containers that are large enough to completely contain the glass. These containers are to be placed into the building trash dumpsters by laboratory personnel, not by custodians.

1.2.3 Chemical Handling

1. Use a safety pail for transporting dangerous or flammable liquids.. Secure containers to prevent tipping when transporting materials on a cart.
2. Do not work with large quantities of reactants without special precautions.
3. Never pour anything back into a reagent bottle.
4. Use caution when adding anything to a strong acid, caustic, or oxidant. Add slowly.
5. Never add solids to a hot liquid.
6. Never pipette chemicals by mouth. Use pipette filler.
7. Do not point the mouth of a vessel being heated toward any person, including yourself.
8. When working with hazardous material, guard against skin contact which might lead to infection. Also guard against inhalation of aerosols, and contamination of food and beverages.
9. Known carcinogens should not be used or stored in normal laboratory situations. Such substances require extreme precaution, tight security, limited access, and appropriate safety procedures.
10. Never heat a flammable solvent in an open vessel in the presence of sparks or flame. Use only steam, hot water or a grounded heating mantle.
11. When not in use, make sure natural gas lines in the laboratory are shut off at the line valve rather than at the equipment.
12. Always locate energized electrical equipment or other devices that may emit sparks or flames at least six inches above the floor.
13. All electrical apparatus must be properly grounded. All laboratory electrical apparatus should have a three-conductor cord that connects to a grounded electrical outlet. This is true except for dual-insulated equipment,

14. All electrical wiring for experiments, processes, etc. should be done neatly, and must conform to electrical safety code requirements.
15. All experiments involving volatile flammable liquids should be considered fire or explosive hazards.
16. Strong oxidants should be stored in a dry area apart from organic materials.
17. Acids digestion must be done in specially designed wash-down laboratory hoods.

1.2.4 Chemical Storage

1. All chemical substance containers shall be labeled to identify contents. All flammable liquid containers shall be labeled "Flammable" or "Ignitable".
2. Quantities of flammable solvents should be stored in approved, flammable-liquid storage cabinets, or in approved solvent-storage rooms.
3. Unsealed chemicals containers should not be stored in the lab. They may detonate by shock, friction, or heat.
4. Do not store acidic or caustic liquids above eye level.
5. Do not store glass containers of hazardous liquids on the floor unless they are inside protective containers or pans.
6. Inventory chemicals periodically and discard the old ones.

1.2.5 Container Handling

1. Be sure that all containers are properly labeled.
2. Do not reuse a container without first removing the original label completely.
3. Chemical transport containers are not to be used for non-compatible chemicals or for food products at any time.
4. Never place uncapped vessels of chemicals in a refrigerator, on benches, or in hoods.
5. Refrigeration of flammable materials must be done in spark-proof or explosion-proof refrigerators.

1.2.6 Chemical Spills and Disposal of Chemical Wastes

1. Devise a plan to deal with spills before one occurs. Post the plan in the lab. Quickly and thoroughly clean up any liquid or solid chemical spill in the laboratory or area of operations. If any uncertainty exists, seek assistance of supervisor or call Environmental Health and Safety.

2. Dispose of chemical wastes by approved methods only. Unwanted or no-longer-useful chemicals become chemical wastes.
3. Reagent bottles should be thoroughly cleaned up of any hazardous material prior to disposal.

1.2.7 Special Safety Requirements for Microbiological Laboratories

1. Do not ingest microorganisms. **Never** use your mouth to fill pipettes. Always use a bulb or other pipetting device to fill pipettes.
2. Do not eat, drink or smoke while doing lab activity.
3. Keep work area clean and clear. Use disinfectants to clean work areas.
4. Wash your hands thoroughly with soap after working with microorganisms.
5. Handle unknown bacteria with caution. Keep plates closed while observing growth from unknown samples. Transfer liquid cultures carefully.
6. Cover spilled cultures or broken tubes containing cultures with paper towels and soak the paper towels with disinfectant. After about 15 minutes discard the towels.
7. Discard all cultures, plates, toothpicks and swabs into a container that can be sterilized before disposal.
8. Bunsen burners are a hazard. Do not place flammable liquids (such as alcohol) near the flame. Keep loose sleeves and long hair away from the flame. Know the location of the nearest fire extinguisher.
9. Know the location of the nearest sink with running water and the eye wash station. Spills of chemicals on the skin or eyes should be flushed immediately with cool water.
10. Hot plates and objects heated with a Bunsen burner (such as an inoculating loop or a microscope slide) can cause burns. Be careful not to touch hot surfaces. Treat minor burns with ice.
11. Do not place contaminated loops, swabs, pipettes or other instruments on the lab bench. These items should be sterilized with a Bunsen burner or placed in disinfectant solution (swabs or pipettes) to kill microorganisms.

1.3 Quality Control in Testing Laboratories

One of the greatest issues facing calibration and testing laboratories nowadays is the increasing pressure to demonstrate competence in performing calibrations and testing. A "quality assurance system" has to be put in service to insure quality for clients, regulatory bodies and users of laboratories data, and accreditation bodies who will issue a certificate for laboratories as a proof of lab's ability to do certain types of analysis.

ISO definition of the term ***Quality*** states that it is the totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs. Translated to analytical service, it can be shown that the quality of the analytical result can be expressed as a confidence interval and that the real value is expected to lie within (since the measured value is an estimate of the real value) this interval. Therefore, when this level of confidence (uncertainty) is consistent with the analytical requirement, it can be said that it is of a good quality (fit for purpose). Otherwise, if the level of confidence is larger than the analytical requirements, it can be said that the quality is inadequate. To guarantee a stated level of confidence a quality assurance program shall be applied.

The following terminologies and their definitions are important in the subject of quality control (Elizabeth and Prichard, 2001):

Quality system is a set of procedures and responsibilities, which a company or organization has in place to ensure that staff have the facilities and resources to carry out measurements that will satisfy their clients. The quality system is a combination of quality management, quality assurance and quality control.

Quality assurance is the planned and systematic control, implemented in the quality system, which provides confidence that the analytical service and the data generated provide the quality required by the client.

Quality control is applied to procedures used to provide evidence of quality and confidence in the analytical results.

There are many "quality systems" for laboratories to choose, ISO 9001:2000 can be applied as a management system but involves no technical requirements. ISO 17025:2005, which is the choice for laboratories, applying the requirements of this standard both managerial and

technical guarantees an effective implementation of policies and procedures which give an evidence of "good quality data". Moreover, there are many quality systems, which can be applied in chemical analysis laboratories such as GLP (a mandatory applied in laboratories working on toxicity of new chemicals for safety purposes).

This chapter will concentrate mainly on quality control measures, data quality and statistical treatment of analytical data.

1.3.1 ISO 17025:2005 Standard

Requirements of this standard are divided into two types: management and technical.

- Management requirements:
  - Organization.
  - Management system.
  - Document control.
  - Review of requests, tenders and contracts.
  - Subcontracting of tests and calibration.
  - Purchasing services and supplies.
  - Service to the customer.
  - Complaints.
  - Control of nonconforming testing and/or calibration work.
  - Improvement.
  - Corrective action.
  - Preventive action.
  - Control of records.
  - Internal audits.
  - Management review.
- Technical requirements:
  - General.
  - Personnel.
  - Accommodation and environmental conditions.
  - Tests and calibration methods and method validation.
  - Equipment.
  - Measurement traceability.
  - Sampling.
  - Handling of test and calibration items.
  - Assuring the quality of test and calibration results.

- Reporting the results.

1.3.2 Trueness, Bias, Precision and Accuracy

It is necessary to discuss the concepts of accuracy, trueness, bias and precision before proceeding to the quality control tools.

-Trueness is defined as the closeness of agreement between the average value obtained from a large set of test results and an accepted reference value.

-Precision is the closeness of agreement between the repeated observations. It is usually expressed as a standard deviation.

-Accuracy is the closeness or agreement between a test result and the accepted reference value *.i.e.* it's a qualitative concept and involves a combination of random component (precision) and a common systematic error or bias component.

-Bias is the difference between the observed mean (of replicate measured values) and the reference value, Figure (1.1). A reference value may be the concentration of a certified reference material or a result of analysis obtained by application of a standard reference method of analysis.

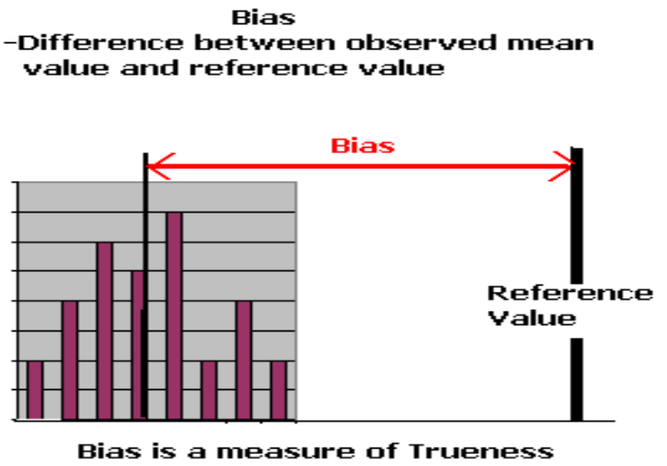

Figure (1.1): Bias as a measure of trueness.

1.3.3 Quality Control Tools

Many external and internal tools, which can be used by laboratories to confirm data quality and are within requirements stated by clients; these include but not limited to:

blanks, calibration, standardization, check samples, spiked samples, quality control samples, replicate analysis, control charting, frequent analysis of Certified Reference Materials (CRMs), and participation in a suitable Proficiency Testing (PT) program.

- **Reagent blank:** a solution prepared by using all of the analytical reagents used in the testing procedure; by using analyte free reagent used for sample preparation. Therefore, this blank does not contain either the matrix or the analyte. The advantage of having it is to compensate for the effect of reagents on the analytical procedure.

- **Laboratory fortified blank:** a reagent water volume to which a known amount of the analyte of interest has been added. This will help to evaluate laboratory performance on the analysis of this analyte calculated as a recovery percentage (here recovery is another way of expressing trueness of analysis).

$$\text{Recovery} = \frac{a}{b} \times 100\%$$

a: the concentration of the target analyte as measured by the laboratory in the fortified blank.

b: the concentration of the target analyte in the blank calculated gravimetrically.

- **Laboratory fortified matrix:** a real sample for which a known amount of the analyte of interest has been added before sample preparation, recovery is calculated as follows:

$$\text{Recovery} = \frac{a - c}{b} \times 100\%$$

a: the concentration of the target analyte as measured by the laboratory in the fortified sample.

b: the concentration of the target analyte in the blank calculated gravimetrically.

c: the concentration of the target analyte as measured by the laboratory in the sample without fortification.

- **Quality Control Sample (sometimes referred to as "check sample"):** a sample which resembles in terms of matrix and analyte the routinely analyzed samples. This sample is analyzed each time the analysis is performed. Results of analysis are used to confirm that the analysis process is under statistical control (i.e. analysis results are distributed normally and within certain limits)**.**

- **Replicate analysis**: repeated measurement on the same sample including all the preparation and analysis steps. The smallest number of replicate is to duplicate (2

analyses), the difference between the two results (range) can be statistically analyzed using the control chart.

- **Instrument calibration:** it's the introduction of a suitable mathematical model that describes the relationship between instrument signal and a group of solutions of the analyte of interest.

- **Participation in a suitable Proficiency Testing (PT) scheme:** Proficiency Testing is a widely-used quality tool for laboratories to compare the values of their measurements with peer laboratories. Proficiency tests are usually conducted by means of inter-laboratory comparisons, where similar samples are sent to all participating laboratories. Proficiency Testing is complementary to other quality control tools for laboratories as it monitors a laboratory's outputs. Organizer usually calculates both mean and standard deviation of participants after removing outliers by applying a suitable statistical techniques, a **z score,** which is given for each participant and is calculated as follows:

  $$\mathbf{Z = (x_i - X) / \sigma}$$

  in which:

  x_i is the result of lab i for that specific test.

  X is the assigned value, an estimate for the 'true value'.

  σ: target standard deviation.

  If:

  $|z| < 2$: satisfactory

  $2 < |z| < 3$: questionable

  $|z| > 3$: unsatisfactory

- **Analysis of certified reference materials (CRMs):** a CRM is a reference material, accompanied by a certificate, one of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence (ISO definition). CRMs can be used for many purposes in analytical chemistry, which is one of its uses is checking accuracy of routine analysis.

Control chart: is a graphical record of the results of the analysis of a quality control sample using a particular method. A Reference Material (RM) is used to construct a control chart by analyzing it many times (at least 7 but preferably 20), on different days, by different analysts, employing different calibrations, instruments, etc.

The RM should be analysed frequently (e.g. every day, every batch of analysis or every 10-20 samples, depending on the cost, importance of the test, and time available), and its result plotted on the control chart. The results should be statistically analysed on a regular basis (e.g. monthly) using special statistical tests (F-test for comparison of the spread, Student-t for the two means and Grubbs for outliers).

1.3.4 Validation of Analytical Methods

It is well known that there are at least three components for reliable data to be produced by a laboratory: one of which is to use validated methods, or to validate other methods if they are not readily available.

Why Is Method Validation Necessary ?

- Important element of quality control.
- Demonstrate whether the method is fit for a particular analytical purpose.
- In some areas, it is a regulatory requirement.
- Its data provide information, which enables the comparability of results from samples analyzed in different laboratories and using different methods to be assessed.
- Enable suitable quality control procedures.
- Support uncertainty estimation.
- Support traceability of measurements.

What are the tools of method validation?

-Blanks.
-Samples / test materials.
-Media (Reference Media).
-Fortified materials / solutions .
-Spiked materials.
-Independently characterized materials.
-(Measurement) Standards.
-Reference materials and Certified Reference Materials.
-Statistics.
-Replication.

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Chapter Two: Sampling, Handling and Preservation of Bio-solids

2.1 Background

Sludge quality data are essential to determine whether bio-solids reuse or sludge disposal may pose a threat to public health or the environment. Thus, as a general rule, sludge or bio-solids samples should be drawn from an appropriate sampling point and in such a manner that the sample represents, as well as possible, the quality of the sludge/bio-solids as it will be disposed of or reused.

In addition, sludge sampling and analyses are needed to assess compliance with the current requirements, Jordanian Standard No. (1145/2006). This standard contains guidelines that must be met for bio-solids produced in wastewater treatment plants in order to be used to improve soil fertility, as organic fertilizer for agricultural purposes, or to be disposed of in landfills as stated by the guidelines of this standard. For the purpose of this standard, bio-solids are classified into three types: I, II and III. These types depend on the measured chemical and microbiological parameters, which will be discussed later in this manual.

Focusing on sludge permitting places increased emphasis on the need to assess sewage bio-solids quality. The quantity and composition of sludge vary with the characteristics of the wastewater from which it is concentrated and with the concentration process used. The sludge to be sampled might be in the form of a liquid dewatered cake, compost product, or dried powder.

In order to sample a sludge stream effectively, it is necessary for sampling personnel to be aware of the physical characteristics of the sludge stream(s) at intended sampling locations. Two important physical characteristics of sludge with respect to sampling and analysis are viscosity and solids content. Solids content is the percent, by weight, of solid material in a given volume of sludge. Sludge has much higher solids content than most wastewaters. Solids content and solids settling characteristics determine whether a given sludge will separate into different fractions which increases the potential of obtaining a non-representative sample. Viscosity is the degree to which a fluid resists flow under an applied force. The viscosity of sludge is only somewhat proportional to solids content. This

property affects the ability to automatically sample a liquid, since friction through pipes is proportional to liquid viscosity.

2.2 Sampling Points Selection

In view of the variability of sludge quality; appropriate procedures must be followed to collect and analyze samples that accurately represent their quality. When selecting a specific sample point, the following two factors should be carefully considered:

1. Does the sample point represent the entire sludge stream?
2. Is the sludge stream flow or mass flux data available?

Three concerns that need to be addressed to ensure that the sample points selected will provide representative samples of the entire sludge stream: obtaining samples that are representative of the cross-section of the entire flow; obtaining well mixed samples; and obtaining samples of multiple sludge streams. Table (2.1) summarizes the sampling points of bio-solids.

Table (2.1): Sludge and Bio-solids Sampling Points.

Processed Sludge Type	Sampling Point
Anaerobically digested	Sample from taps on the discharge side of pumps.
Aerobically digested	Sample from taps on the discharge lines of pumps. If batch digestion is used, sample directly from the digester. Two cautions are concerning this practice: (1) If aerated during sampling, air entrains in the sample, volatile organic compounds may purge with escaping air. (2) When aeration is shut off, solids separate rapidly in well digested sludge.
Thickened "sludge"	Sample from taps on the discharge side of pumps.
Dewatered Processed "Sludge"	
Belt Filter Press, Centrifuge, Vacuum Filter Press	Sample from sludge discharge chute.
Sludge Press	Sample from the storage bin; select four points within the storage bin, collect equal amount of sample from each point and combine.
Drying Beds	Divide bed into quarters; grab equal amounts of sample from the center of each quarter and combine to form a grab sample of the total bed. Each grab sample should include the entire depth of the sludge (down to the sand).
Compost piles	Sample directly from the front-end loader as the sludge is being loaded into trucks to be hauled away.

2.3 Sample Collection

The following objectives should be considered during sample collection:

- The grab and composite samples should be as representative as possible.
- Effort must be made to minimize the possibility of sample contamination.
- The selected sampling method should be safe, convenient and efficient.

The following sections describe important considerations for selecting appropriate bio-solids sampling methods such as sampling equipments and proper sampling practices.

2.3.1 Sampling Equipments

In general, automatic samplers do not work well for sludge streams because of the solids content and viscosity of bio-solids. Therefore, it is preferable to sample liquid bio-solids streams manually. Sampling equipment must be made of materials, which will not contaminate or react with the bio-solids. The best material choices are Teflon, glass and stainless steel because they are considered to be relatively inert.

2.3.2 Proper Sampling Practices

- All sampling equipments should be cleaned between each sample period to prevent cross-contamination.
- Samples should be composted directly into sample containers.
- Sample collection procedures should be adequately documented.

  - **When sampling liquid sludge:**

- Sufficient time should be allowed, following pump start up, to clear line of stagnant sludge.
- Sludge should be allowed to flow for several seconds from tap prior to sampling in order to flush out stagnant sludge and solids accumulated in the tap.
- Before drawing a sludge sample, each piece of sampling equipment is to be rinsed three times with the sample to reduce the possibility of contamination from the previous grab.
- To prevent solids separation in the sample, glass, Teflon-coated stirring rods, or stainless steel spoons are used to mix the sample before splitting or transferring any portion of it to another container.

- **When sampling solid sludge:**

- For dewatered cakes, dried powder or compost product; combine equal amounts collected at various locations/depths for each grab sample to obtain a more representative sample.
- To produce a sample from multiple sample locations, grab samples are combined from each location (equal amounts or weighted based on flow or solids flux data) in a plastic or stainless steel pail and the sample is thoroughly mixed (with a scoop or spoon), then transferred to sample containers. This is not appropriate for microbial samples.
- When sampling drying beds, each bed is divided into quarters. From the center of each quarter, a single core sample is to be collected through the entire depth of the bio-solids using a coring device and avoiding large amounts of sand. Samples are then combined, thoroughly mixed in plastic or stainless steel pail and transferred to sample containers.

2.4 Sampling Frequency

As bio-solids quality is directly related to wastewater influent quality, which can vary from day to day and hour to hour, the sampling program should adequately address random and cyclic variation within the system and the potential for human exposure to bio-solids once it is disposed of or used. The following factors determine the frequency of a sampling program:

- Anticipated cyclical variation in pollutant loadings, although they are difficult to accurately predict. Anticipated cycles include daily industrial production cycles, weekly industrial production cycles, and other known or suspected production cycles, particularly those associated with intermittent batch discharges by significant industries. Longer-term production cycles, including seasonal and annual/multi-year production cycles (e.g., business cycles) do not need to be considered in determining monitoring frequency unless they are known to affect short-term variation in bio-solids quality.

- Risk of environmental exposures - as the risk of environmental exposure from bio-solids use or disposal increases, sampling frequency should be increased to provide

- Size - As influent flow increases, day-to-day bio-solids variability increases, as does outflow volume. Thus, where high volumes exist, the risk of adverse exposure is higher. Since variability and potential impact are major considerations, many sampling programs are based on size alone.

- Percentage of industrial flow - while bio-solids quality variability is directly related to the individual characteristics of the system, systems with little or no commercial/industrial contributors can expect relatively small variation in bio-solids quality.

- Treatment plant characteristics - as either detention time or mixing increases within a treatment plant, sampling frequency can be reduced since treatment processes will effectively composite sludge to a greater degree. For example, high rate digestion and storage/blending facilities will provide mechanical mixing of bio-solids; other plant technologies such as anaerobic digestion, aerobic digestion and storage provide longer bio-solids detention times, such processes require lower sampling frequency.

For the purposes of land application of bio-solids, the Jordanian Standard No. (1145/2006) states the minimum sampling frequencies required. These frequencies are listed in Table (2.2). The standard contains guidelines that must be met for bio-solids produced in wastewater treatment plants in order to be reused to improve soil fertility, as organic fertilizer for agricultural purposes, or to be disposed of in landfills as stated by the guidelines of the above mentioned standard. This standard includes general conditions of sampling, safety and transportation of bio-solids and sludge, as well as recommended frequencies of sampling rate of application as fertilizers, types of bio-solids, technical conditions and quality control procedures associated with sampling and analysis of bio-solids.

Table (2.2): Frequency of Sampling and Analysis Based on the Produced Bio-solids Amounts

BIO-SOLIDS PRODUCED AMOUNT (TON/YEAR)	FREQUENCY OF ANALYSIS (ONCE A YEAR)
Less than 300	once every one year
300-1500	once every 3months
1500-15000	once every 2 months
more than 15000	once a month

2.5 Sample Preparation and Preservation

To avoid potential errors while sampling, the following should be considered:

- Sample container material.
- Sample container preparation.
- Sample preservation.
- Holding time prior for analysis.

2.5.1 Sample Container Material

The requirements for sample containers are method-specific, but containers are usually made of Teflon, glass or polyethylene. Sample containers should be wide-mouthed for bio-solids sampling, particularly for solids (cake) sampling. Glass containers are frequently supplied with caps, which can cause sample contamination (phenol, phthalate compounds), for organic parameters. These glass container caps should be fitted with Teflon liners; aluminum liners could be used but they must be fitted precisely within the circumference of the cap to prevent tearing and possible sample leakage.

2.5.2 Sample Preservation

The following are specific recommendations regarding sample preservation:

- In instances where it is desirable to split one composite sample into several fractions, each having incompatible preservation requirements, it is acceptable to chill the entire sample to 4°C during compositing. Following the sample period, the composite is then cautiously mixed and split into various fractions, each of which is appropriately preserved.
- If processing of microbial samples cannot occur within one hour of collection, iced coolers should be used for storage during transport to the lab. Samples should be

held below 10°C during the maximum transport time of 6 hours. Note: these samples must be immediately refrigerated and processed at the lab within 2 hours of receipt.

- Whenever possible, sample containers should be pre-preserved. Thus, grab samples are preserved upon sampling and composite samples are preserved during compositing. However, this is not appropriate when sampling for metals or pathogens.
- In general, all samples should be chilled (4°C) during compositing and holding.
- For solid sludge samples (cake), adding chemical preservative is generally not useful since the preservative usually does not penetrate the sludge matrix. Preservation consists of chilling to 4°C.

Table (2.3) lists recommended preservation methods and maximum holding times for the analysis of bio-solids samples.

Table (2.3): Preservation Methods and Maximum Holding Times for the Analysis of Bio-solids samples.

Parameter	Sample preservation	Maximum holding time	Sample volume (L)	Sample mass (g)
Coliforms, fecal & total	Ice to 4^{o}C	6 hours	1-4	1
Salmonella	Ice to 4^{o}C	24 hours	1-4	4
Helminthes	Ice to 4^{o}C	2 weeks	1-4	4
Enteric viruses	Ice and to 4^{o}C or Freeze at 00C	24 hours	1-4	4
Kjeldal and organic nitrogen	Ice to 4^{o}C	28 days	0.3-1	-
Mercury	Ice to 4^{o}C	28 days	0.3-1	30-50
Metals (except (Hg), solid and semisolid	Ice to 4^{o}C	6 months	0.2-0.5	30-50
Ammonia & Phosphorus	Ice to 4^{o}C	48 hours	0.1-0.5	10-25

2.6 Documentation

Adequate documentation of bio-solids sampling activities is important for general program quality assurance/quality control, and is required by most monitoring regulations. Proper sampling activity documentation includes proper sample labeling, chain-of-custody procedures and a log book of sampling.

2.6.1 Sample Labeling

The following information should be written on the sample container using a self adhesive sticker (waterproof ink) or using a waterproof marker:

Figure (2.1): Labeling of samples.

Organization name:

Sample location:

Sample no.:

Sample type:

Date & time of sampling:

Collector:

2.6.2 Chain-of-Custody

Each sample shipment requires a chain-of-custody record. A chain-of-custody document provides a record of sample transfer from person to person. This document helps protect the integrity of the sample by ensuring that only authorized persons have custody of the sample. Following is an example of the chain of custody:

Table (2.4): Chain of Custody Record.

CHAIN-OF-CUSTODY RECORD			**Laboratory Name:**		
PROJECT NAME:					
SAMPLER(s): **Signature:**					
Date	Time	Location	Sample type	No. of container	Remarks
Relinquished by: Signature: Date & Time:			Received by: Signature: Date & Time:		
Relinquished by: Signature: Date & Time:			Received by: Signature: Date & Time:		
Relinquished by: Signature: Date & Time:			Received by: Signature: Date & Time:		

A sample-chain-of custody document and a sample label are provided in **Annex** (**2**).

2.6.3 Sampling Log Book

All sampling activities should also be documented in a bound log book. This book duplicates all information recommended for the chain-of-custody document, and notes all relevant observations regarding sample stream conditions.

References

- POW Sludge Sampling and Analysis Guidance Document (1989). EPA 833-B-89-100.
- Handbook for Sampling and Sample Preservation of Water and Wastewater (1992). EPA 600/4-82-029.

Chapter three: Physical, Chemical and Microbiological Analysis Laboratory Procedures

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This chapter covers the analytical procedures applied at the Environmental Research Center (ERC) for the physical, chemical and microbiological analysis of sludge and bio-solids samples.

3.1 Determination of Heavy Metals in Sludge and Bio-solids Samples Using Atomic Absorption Spectrophotometer (AAS)

3.1.1 Scope

This procedure describes the method used at the Environmental Inorganic Analysis Laboratory of the Environmental Research Center (ERC) for preparing sludge and bio-solids samples prior to analysis of the following metals (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Se, V, Zn) by AAS.

3.1.2 Reagents

1. Acids (mainly Nitric and Hydrochloric), all have been and must be periodically tested to insure low blank values.
2. Deionized water used to prepare blanks, standards and other solutions.
3. 1000-ppm stock standards of individual target metals, from E. Merck (Germancy) or Unicam (United Kingdom).
4. Working solutions of individual or mixtures of metals prepared by serial dilution of the above stock standards. Routinely, calibration involves a blank and at least three concentration levels per metal.

3.1.3 Apparatus

1. Atomic Absorption Spectrophotometer system employed consists of the following:
    - M6 SOLAAR AAS system with flame atomizer, computer and operating software,
    - Unicam VP – 90 cold vapor generating system, Hydride Generating System
    - Graphite Furnace.
2. Heating Plate.

3.1.4 Procedure

1- An exact weight of the pre-homogenized sample (about 1g) is to be transferred into a beaker and about 50 ml of aqua-regia mixture (Nitric acid: Hydrochloric acid 1:3) is added gradually.

2- The beaker is then covered with a watch glass, put on a hot plate, and heated at 100^{o}C for about 2 hours (temperature must not exceed 95^{o}C if Hg is one of the tested metals). The watch glass is then removed, washed and the walls of the beaker with deionized water. Some more water is added, and the temperature is increased from 100 to 200^{o}C. Heating the sample is continued until all acid fumes are evaporated, making sure that the sample never gets dry.

3- When evaporation of fumes stops, sample is cooled then the beaker walls and watch glass are washed with deionized water. Sample is to be filtered through a filter paper into a 50 ml volumetric flask to remove insoluble materials, and filtrate is to be completed up to volume with deionized water.

4- Sample is applied to Atomic Absorption Spectrophotometer.

3.1.4.1 Precautions

1. Avoid looking at the naked flame unless wearing UV-safety eyeglasses.
2. Certain metals (Ag, Au, Cu) can combine with acetylene to form unstable acetylides, which increase the risk of explosion or flashback in the spray chamber. It is important, therefore, to observe the following precautions:
    a) For samples containing these metals, concentrations being aspirated into the flame must be reduced to a minimum.

b) Water must be aspirated through the system at all times, from the very first instant the flame is ignited until after the flame is extinguished, because dry acetylides of Ag, Au and Cu can explode spontaneously. The spray chamber and burner system must be flushed after each sample by aspirating distilled water for 60 seconds, and at the end of each analytical session by aspirating water for 15 minutes.

c) Make sure that the burner and spray chamber are kept clean at all times, inside and outside. In particular, do not allow residue to build up on the burner slot.

3.1.5. Validation Records

Sensitivity and detection levels vary with the equipment, the element determined, the complexity of the matrix, and the technique selected. The optimum concentration range usually starts from the concentration of several times the detection level and extends to the concentration at which the calibration curve starts to flatten. Table (3.1) provides the detection limits obtainable by direct aspiration technique for M6 SOLAAR- AAS for each metal.

Table (3.1): Atomic Absorption Flame Instrumental Detection Limits for Solar M6 AAS and Recommended Wavelengths.

Metal	Wavelength (nm)	Direct Aspiration Detection Limits (mg/L)
Ag	328.1	0.1
Al	309.3	0.7
Ba	553.6	0.2
Cd	326.1	0.005
Co	345.4	0.03
Cr	425.4	0.05
Cu	324.8	0.02
Fe	372.0	0.04
Li	670.8	0.01
Mn	403.0	0.017
Mo	390.3	0.1
Ni	341.5	0.04
Pb	405.8	0.09
V	437.9	0.4
Zn	213.9	0.016

Some typical data of the precision and bias obtainable with the method of analysis, discussed previously, are presented in Table (3.2). These data are obtained from the analysis of dried sewage sludge sample. This sample is one of the Proficiency Testing samples from Aquacheck, LGC Standards Proficiency Testing. Sample's details: Distribution number 343, Group number 13. Sample preparation method used was wet/acid digestion using aqua regia mixture.

To one sample out of every ten (or one sample from each group of samples if less than ten are being analyzed) addition of a known amount of the metal of interest is required to confirm recovery. The amount of metal added should be approximately equal to the amount found. Recovery of added metal should be between 85 and 115%.

Table (3.2): Inter-Laboratory Precision and Bias Data for Atomic Absorption Methods-Direct Aspiration.

Parameter	Units	Laboratory Mean	No. of labs reporting	Overall Bias %	Robust RSD %	Median for all labs	Assigned value	Difference	% Difference	Z score
Cadmium	mg/kg	1.65	29	0	14.9	1.4	1.4	0.25	17.9	1
Chromium	mg/kg	123	31	0	11.1	122	122	1	0.820	0.08
Copper	mg/kg	296	32	0	8.06	304	304	-7.5	-2.47	-0.25
Lead	mg/kg	177	32	0	12.9	203	203	-25.7	-12.7	-1.27
Molybdenum	mg/kg	8	19	0	8.56	8.49	8.49	-0.49	-5.77	-0.33
Nickel	mg/kg	118	31	0	6.92	118	118	-0.400	-0.339	-0.03
Vanadium	mg/kg	25.7	15	0	13.3	25.7	25.7	0	0	0
Zinc	mg/kg	646	31	0	5.28	646	646	0	0	0
Cobalt	mg/kg	97	18	0	7.70	93.5	93.5	3.5	3.74	0.37
Iron	mg/kg	20300	25	0	7.99	19900	19900	447	2.25	0.22

Source: Aquacheck PT sample; dried sewage sludge, Distribution 343, Group 13.

References

- Kingston, H.M., I.L Barnes, T.J. Brady, T.C. Rains, and M.A. champ (1978). Analytical Chemistry, Vol. 50 (No. 14), pages 2064-2070 (for part IV).
- Operating Manual, M6 SOLAAR AA spectrometer, Thermo Elemental.

3.2 Determination of Total Kjeldahl Nitrogen (TKN) in Bio-solids Samples

3.2.1 Scope

This procedure authorizes the determination of Total Kjeldahl Nitrogen (TKN) in bio-solids samples.

3.2.2 Reagents

- Ammonia, free water grade (Nanopure water).
- Sodium Hydroxide (6N): dissolve 240 gm of sodium hydroxide in 800 ml of ammonia free water, cooling and shaking, complete the volume to 1L.
- Boric Acid: dissolve 20gm of boric acid in one liter ammonia free water.
- Potassium Sulfate, extra pure (K_2SO_4), Copper Sulfate 5 hydrate, extra pure ($CuSO_4.5H_2O$).
- Sulfuric acid, concentrated (not less than 96%).
- Sodium Carbonate (0.1N): weigh 0.6 – 0.7 gm of highly pure sodium carbonate, and dry at 250 ^oC for 4 hours, dry and cool in a desiccator then weigh 0.5 gm to the nearest 0.1 mg and dissolve in 100 ml ammonia free water (don't keep for longer than one week).
- Standard HCl or H_2SO_4 0.1 N (standardize by potentiometeric titration of 5 ml of 0.1 N standard sodium carbonate, and calculate the normality according to :

$$N = \frac{AXB}{53.00XC}$$

A = grams sodium carbonate weighed into 100 ml

B = ml of carbonate solution taken for titration

C = ml of acid used

3.2.3 Apparatus

-Digestion Apparatus: Use Kjeldahl flasks with a capacity of 100 ml in a semi-micro-kjeldahl digestion apparatus equipped with heating elements to accommodate kjeldahl flasks and suction outlet to vent fumes. The heating elements should provide the temperature range of 375 to 385 ° C for effective digestion.

- Steam Distillation Kjeldahl Apparatus connected to Auto titrator.

3.2.4 Procedure

- Weight of sample is to be chosen, depending on the water content, according to the following table:

	Dewatered	slurry	semi liquid
Weight (g)	0.5	5	50

-Complete the volume to 50 ml for dewater and slurry in digestion tube.

- Add 6.7 g Potassium Sulfate, 0.365 g of Copper Sulfate and 10 ml sulfuric acid.

- The mixture is to be digested for 5 hours in the digestion apparatus.

- After completing the digestion process, the mixture is allowed to cool.

- Distillation: the distillation apparatus is programmed to add about 100 ml 6N NaOH to the sample to make the solution basic, distillation duration is about 8 minute with 100% steam distillation, also 50 ml boric acid is to be added to the receiving beaker.

- After distillation the sample is automatically titrated by autotitrater with 0.1 N HCl and results are recorded.

- Blank: blank is to be carried through all steps of the procedure and the necessary correction are applied to the results.

- Calculation:

$$mg(TKN)/Kg = \frac{(A-B)*N*14000}{dryweigh(gm)}$$

Where:

A= Volume of HCl titrated with Sample, ml.

B= Volume of HCl titrated with Blank, ml.

N= Normality of HCl.

- Detection limit for TKN is 4.5 mg/L.

3.2.5 Validation

A regularly analyzed type dry sludge sample (Wadi Hassan April 2004), was grinded, and sieved at 2 mm. The sample was analyzed for TKN content, number of replicates = 8, and one analyst - date of analysis 8/8/2004.

Table (3.3): Analysis Data for Dry Sludge Sample from Wadi Hassan.

Trial number	Result (% TKN w/w)
1	4.94
2	4.97
3	4.88
4	4.92
5	4.94
6	4.91
7	4.93
8	4.88
Average	4.92
Standard deviation	0.0308

This sample was reanalyzed after one year by another analyst: number of replicates = 8, one analyst - date of analysis 8/8/2004.

Table (3.4): Analysis Data for Dry Sludge Sample from Wadi Hassan after One Year.

Trial number	Result (% TKN w/w)
1	4.86
2	4.83
3	4.83
4	4.83
5	4.78
6	4.91
7	4.83
8	4.85
9	4.83
10	4.88
Average	4.84
Standard deviation	0.0336

Applying: F test indicates that there is no difference between the spread of the two groups, student t test indicates no differences between the two averages which indicates that this sample is stable at least for one year of bottling.

Pooled standard deviation = 0.0324

References

- Methods of Soil Analysis, Part 3, Chemical Methods, Sparks and others, Soil Science Society of America, and American Society of Agronomy1996.

3.3 Determination of Ammonia-Nitrogen (NH_3-N) in Bio-solids Samples

3.3.1 Scope

This procedure authorizes the determination of ammonia-nitrogen in bio-solids samples.

3.3.2 Reagents

- Ammonia, free water grade (Nanopure water).
- Sodium Hydroxide (6N): dissolve 240 gm of sodium hydroxide in 800 ml of ammonia free water, cooling and shaking, complete the volume to 1L.
- Borate Buffer: add 88 ml of 0.1 N sodium hydroxide solution to 500ml approximately 0.025 M sodium tetraborate ($Na_2B_4O_7$) solution (9.5 gm $Na_2B_4O_7.10H_2O$/L) and dilute to 1 liter.
- Boric Acid: dissolve 20gm of boric acid in one liter ammonia free water.
- Sodium Carbonate (0.1 N): weigh 0.6-0.7 gm of highly pure sodium carbonate, and dry at 250^{o}C for 4 hours, dry and cool in a desiccator then weigh 0.5 gm to the nearest 0.1 mg and dissolve in 100 ml ammonia free water (don't keep for longer than one week)
- Standard HCl or H_2SO_4 0.1 N (standardize by potentiometric titration of 5 ml of 0.1 N standard sodium carbonate, and calculate the normality according to :

$$N = \frac{AXB}{53.00XC}$$

A = grams sodium carbonate weighed into 100 ml

B = ml of carbonate solution taken for titration

C = ml of acid used

3.3.3 Apparatus

Steam Distillation Kheldahl Apparatus connected to Auto titrator.

3.3.4 Procedure

- The weight of sample is chosen, depending on the water content, according to the following table:

Weight (g)	Dewatered	slurry	semi liquid
	2-5	15-20	50

- Complete the volume to 50 ml for dewatered and slurry.
- Transfer to distillation flask, add 2 ml borate buffer and adjust PH to 9.5 with 6N sodium hydroxide.
- Distill Sample for a period 8 min using 100% steam, add 50 ml boric acid to receiver.
- After sample distillation, titrate by autotitrater with 0.1 N HCl and record the result.
- Blank: Carry a blank through all steps of the procedure and apply the necessary correction to the results.

- Calculations

$$mgNH3-N/Kg = \frac{(A-B)*N*14000}{dryweigh(gm)}$$

Where:

A= Volume of HCl titrated with Sample, ml.

B= Volume of HCl titrated with Blank, ml.

N= Normality of HCl.

- Detection limit for ammonia is 3.7 mg/L.

3.3.5 Validation

A regularly analyzed sample (dry Sludge from Wadi Hassan) collected in April 2004 was analyzed by single analyst number of replicates 10 times the average of replicate = 175mg/Kg, s=6.61 mg/Kg, CV= 3.77%.

References

- Methods of Soil Analysis, Part 3, Chemical Methods, Sparks and others, Soil Science Society of America, and American Society of Agronomy1996.

3.4 Determination of Total Phosphorous in Bio-solids Samples

3.4.1 Scope

This procedure authorizes the determination of total phosphorous in bio-solids samples.

3.4.2 Reagents

- Distilled water or water of at least equivalent purity.
- Vanadate-Molybdate solution :

-Solution A: Dissolve 25 gm of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$ in 300ml distilled water.

-Solution B: Dissolve 1.25 gm metavanadate, NH_4VO_3, by heating to boiling in 300ml distilled water. Cool and add 330ml concentrated HCl.

- Cool solution B to room temperature, pour solution A into solution B, mix, and dilute to 1 L.
- Standard phosphate solution: dissolve 219.5 mg anhydrous KH_2PO_4 in distilled water and dilute to 1000ml; 1.00ml=50.0µg PO_4^{-3}-P, or use a stock phosphate solution (1000 mg/L). It is necessary for the solution to be traceable to NIST and uncertainty and expiry date are both specified.
- Sodium hydroxide solution: 6N: dissolve 240 gm (cautiously) of sodium hydroxide in 800 ml of ammonia free water, cool and mix completely, adjust the volume to 1L.

3.4.3 Apparatus

- Digestion Apparatus: COD digester equipped with 175 ml tall reaction vessels.
- Spectrophotometer, has a cell length of 20 mm.

3.4.4 Procedure

- Prepare calibration curve of standard phosphate 50 ml solution (as 0; 1; 2; 3; 4; 5 mg/l (PO_4-P) for the range measured at 400nm (low concentration) and as 0; 4; 8; 12; 16; 20 mg/l (PO4-P) for the range measured at 470nm .

- Weigh 0.05 gm of dry sludge and transfer it to a COD digestion tube.
- Add 10ml concentrated nitric acid and 2ml concentrated sulfuric acid , a reagent blank is analyzed each batch .
- The mixture is digested to 2ml volume.
- The samples are cooled, and 2-3 drops of phenolphthalein indicator are added to the samples. Neutralize with 6N sodium hydroxide solution.
- The volume is diluted to 50ml; the volume is divided into two equal portions. 25 ml for sample and the other 25ml as a reference or any dilution volume.
- Dilute each part of sample to 50ml in a graduated cylinder.
- One portion is measured as a reference sample after adding 10 ml of 33% HCl on spectrophotometer, record results.
- Add 10ml of Vanadate-Molybdate solution into the sample portion and measure absorbance after 30 min using the spectrophotometer, if this is the case the dilution factor will be 2, otherwise if more dilution is needed, the same volume is taken for both the colored portion and the reference, dilution factor equals 50 divided by this volume.

- Calculation

$$mg/L([PO4-P]) = \frac{\langle absorbance(sample-reference)\rangle + Intercept}{Slope} * D.F.$$

- DF: Dilution factor
- *Intercept* and *Slope* from the calibration curve.

$$mg/Kg([PO4-P]) = \frac{mg/L([PO4-P]) * 50}{Wt}$$

$$\%P = mg/Kg([PO4-P])/10000$$

(To convert from mg/kg to % divide by 10000)

Where:

Wt = weight of dry sludge (g)

3.4.5 Validation

- A dry sludge sample (Wadi Hassan April 2004), was grinded, and sieved at 2 mm. The sample was analyzed for TP content.
- number of replicates = 7, one analyst - date of analysis August 2004

Table (3.5): Analysis of Total Phosphorous.

Trial number	Result (% TP w/w)
1	1.2527
2	1.1668
3	1.2515
4	1.2955
5	1.1033
6	1.2551
7	1.2512
Average	1.2512
Standard deviation	0.066119

- A CRM sample was analyzed twice by the same analyst, same situations except weight (CRM details: TOC/TKN/Total-P/NH_3-N in Sludge produced by ERA, cat no. 545, Lot No. 1204).

Table (3.6): Analysis of CRM Sample for the Analysis of Total Phosphorous.

Trial number	Result-1- (% TP w/w) 0.05 g	Result-2- (% TP w/w) 0.075 g
1	2.8036	2.4616
2	2.3876	2.6161
3	2.7624	2.4489
4	2.5842	2.8132
5	3.0207	2.8816
6	2.8919	2.3603
7	2.9811	2.6398
Average	2.7759	2.6031
Standard deviation	0.22508	0.194067
Certified value ± performance acceptance limits	2.87 ± 0.89	

- Equipment Calibration (example):

The U.V. visible spectrophotometer is calibrated at 470nm for "Phosphate" analysis in aqueous solutions; utilizing of vanadomolybdophosphoric acid visible colorimetric method, using 2 mm cell and 4.0, 8.0, 12.0, 16.0 and 20.0 mg P/L solutions as follows:

Table (3.7): Analysis of Total Phosphorous Using Spectrophotometer.

Conc. of solution (mg P/L)	Absorbance
4.0	0.173
8.0	0.340
12.0	0.501
16.0	0.648
20.0	0.772

These data can be "correlated" using the least square regression method to build a linear relationship (calibration curve) between the equipment signal and concentration, using Microsoft Excel .

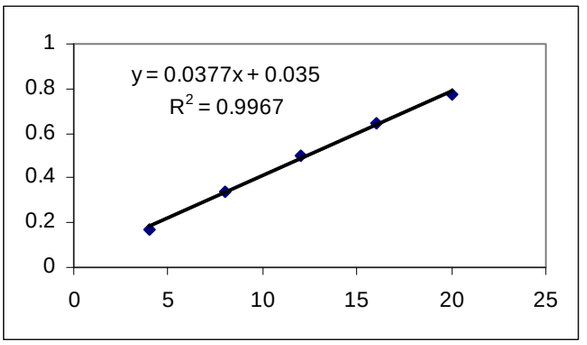


Figure (3.1): Calibration curve for Total Phosphorous analysis.

The calibration curve shall be inspected visually.

References

- Methods of Soil Analysis, Part 3, Chemical Methods, Sparks and others, Soil Science Society of America, and American Society of Agronomy1996.

3.5 Determination of Total, Fixed, and Volatile Solids in Bio-solids Samples

3.5.1 Scope

This procedure authorizes the determination of total, fixed, and volatile solids in bio-solids samples.

3.5.2 Apparatus

-Evaporating Dishes: Dishes of 100-ml capacity made of the following materials:

- Porcelain, 90-mm diam.
- Platinum-generally satisfactory for all purpose.
- High – silica glass.

-Muffle furnace for operation at 550 °C.

-Desiccators, provided with a desiccant containing a color indicator of moisture concentration or an instrumental indicator.

-Drying oven, for operation at 103 - 105 °C.

- Analytical Balance, capable of weighing to 10 mg.

-Graduated cylinder.

- Low-form beaker.

3.5.3 Procedure

3.5.3.1 Total solids:

- Preparation of evaporating dish: if volatile solids are to be measured, ignite a clean evaporating dish at 550°C for 2-5 hour in a muffle furnace. If only total solids are to be measured, heat dish at 103 to 105°C for 8-24 hour in an oven. Cool in desiccators, weigh, and store in desiccators.

- Sample analysis:

  -Fluid samples:

  1. If the sample contains enough moisture to flow more or less readily, stir to homogenize.

2. Place 25 to 50g in a prepared evaporating dish, and weigh, evaporate to dryness in an oven, dry at 103 to 105°C for approximately 48 hours.
3. Analyze at least 10% of all samples in duplicate.

-Solid samples:

1. Place 25 to 50g in a prepared evaporating dish and weigh.
2. Place in an oven at 103 to 105°C overnight.
3. Cool to balance temperature in a desiccator and weigh.
4. Analyze at least 10% of all samples in duplicate.

3.5.3.2 Fixed and volatile solids:

2. Transfer the dried residue to a cool muffle furnace.
3. Heat furnace to 550°C, and ignite for 4 hours.
4. Cool in desiccator to balance temperature and weigh.
5. Analyze at least 10% of all samples in duplicate.

- Calculations

$$\%\,Total\quad Solids = \frac{(A-B)\ \times\ 100}{C-\ B}$$

$$\%\,Volatile\quad Solids = \frac{(A-D)\ \times\ 100}{A-\ B}$$

$$\%\,Fixed\quad Solids = \frac{(D-B)\ \times\ 100}{A-\ B}$$

Where:

A = weight of dried residue + dish, mg.

B = weight of dish.

C = weight of wet sample + dish, mg.

D = weight of residue + dish after ignition, mg.

3.5.4 Validation

A normally analyzed sample was analyzed, 8 times, under repeatability conditions. It gave the following results:

Table (3.8): Analysis Data for Total, Fixed and Volatile Solids.

	TS	TVS	TFS
Average	92.6	35.5	57.1
Standard deviation	0.2	2.6	2.5
coefficient of variation	0.2	7.4	4.5

References

- APHA/AWWA/WEF (1998). Standard Method for the Examination of Water and Wastewater, 20th Edition, (For all other parts).

3.6 Determination of Organic Carbon in Bio-solids Samples

3.6.1 Scope

This procedure authorizes the determination of organic carbon in bio-solids samples.

3.6.2 Reagents

- Standard potassium dichromate solution ($K_2Cr_2O_7$), 0.083 M: Dry some $K_2Cr_2O_7$, primary standard grade in an oven at 105°C for 2hours; dissolve 24.518g in water and dilute to 1L. this solution is stable indefinitely.
- Standard ferrous ammonium sulfate solution, 0.2 M: dissolve 78.39g ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 ml concentrated H_2SO_4, cool and dilute to 1L with distilled water.
- Sulfuric acid reagent: Add 25-g Ag_2SO_4 reagent to concentrated H_2SO_4. Let stand 1 to 2 days to dissolve. Mix.
- Potassium hydrogen phthalate (KHP) standard ($HOOCC_6H_4COOK$): dry KHP to constant weight at 110°C. Dissolve 0.425 g in distilled water and dilute to 1000 ml. KHP is worked with a 20 ml batch to give COD 500 mg O_2/L, monthly preparation usually is satisfactory.

3.6.3 Apparatus

- Reflux apparatus consisting of 200 ml vessel, condenser, and hot plate.
- Operate block heater at *160±2*℃, with holes to accommodate digestion vessels.
- Titroprocessor apparatus, headspace, printer, and dosimate, for titrate the sample with ferrous ammonium sulfate solution.

3.6.4 Procedure

- Weigh 0.5g of fluid or solid Sludge. Place in a digestion vessels.
- Add 10 ml of the standard $K_2Cr_2O_7$ solution and swirl to mix.
- Add, carefully, *30* ml concentrated H_2SO_4.
- Dispense the acid a little at a time since it generates heat, and swirl gently to mix.
- Connect the vessel to the condenser.
- Place tubes in block digester preheated to 160°C and reflux for 2 hr.
- Cool to room temperature and place vessels in test tube rack.
- Rinse down the condenser with distilled water.
- Collecting the vessel's sample in a test tube.

- Put the tubes on the headspace of the titration processor and turn on the apparatus.
- Record the volume for each sample.
- Analyze a blank consisting of the entire same reagent but without the bio-solids

- Calculation

Take the printed results from the titration processor or take the excess volume of dichromate and calculate the results:

$$\text{Organic Carbon (mg/kg)} = \frac{18 \times C \times V \times 1000}{M} \times (1 - V_1/V_2) \quad \text{……...(1)}$$

Where:

C = concentration in mol L^{-1} of the dichromate solution(0.166M).

V = volume of dichromate solution used (10 ml).

V_1= volume of titrant used up in the sample determination (ml).

V_2= volume of titrant used up in the blank determination (ml).

M = weight of sample used (g).

Organic Carbon (%) = Organic Carbon (mg/kg) / 10000…..….(2)

References

Radojevic M., Bashkin V.N., Practical Environmental Analysis, RSC (1999).

3.7 Enumeration of Fecal Coliforms in Bio-solids (Three Tubes MPN Method)

3.7.1 Scope

This procedure is applied to liquid and dry sludge To estimate the most probable number for fecal coliforms in bio-solids.

3.7.2 Media and Materials

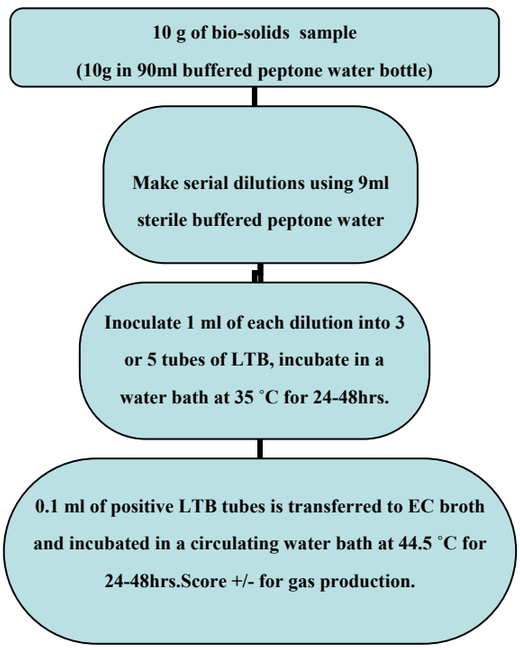
The following media are available in dehydrated form and prepared according to manufacturer instructions:

1- Buffered peptone water, 90 ml in 250 ml capacity bottles.
2- Buffered peptone water, 9 ml tubes for dilution.
3- Lauryl tryptose broth.
4- EC broth.
5- Incubator 35.0 ± 0.5 ˚C.
6- Water bath 44.5 ± 0.5 ˚C.

3.7.3 Analysis of Samples

- Add 10g of bio-solids sample to 250 ml bottles containing 90 ml of sterile buffered peptone water. Shake or stir for at least 10 minutes. This is the 10^{-1} dilution.
- Make 10 fold dilutions by adding 1ml of the 10^{-1} sample to a test tube containing 9ml of buffered peptone water. Serial dilute to 10^{-8} and 10^{-9}.
- Add 1 ml of each dilution to tubes containing 10 ml of Lauryl tryptose broth (LTB) and durham tubes. Prepare 3 or 5 replicates for each dilution depending on the MPN method.
- Incubate samples tubes in an incubator at 35 ˚C for 24 to 48 hrs.
- Score +/- for gas production. There will be air present in durham tubes and the broth will be turbid. Bubbles may also rise in the tube when shaken, these tubes are positive for coliforms.
- Add 0.1 ml from each positive LTB tube to tubes containing 10 ml EC broth and a durham tube. Incubate in a circulating water bath at 44.5 ˚C for 24 to 48 hrs.
- Score tubes +/- for gas production. These tubes are positive for fecal coliforms.
- Refer to the MPN table to determine MPN/gram for fecal coliforms.

3.7.4 Procedure: As in flow sheet



Reference

EPA (2006): EPA Method 1680: Fecal Coliforms in Sewage Sludge (bio-solids) by Multiple Tube Fermentation using lauryl Tryptose Broth (LTB) and EC Medium, July 2006.

3.8 Enumeration of Salmonella spp. in Bio-solids, MPN Method.

3.8.1 Scope

This procedure is applied to sewage sludge and dry bio-solids to estimate the most probable number of *Salmonella* spp. in bio-solids.

3.8.2 Media and Materials.

– The following media are available in dehydrated form and prepared according to manufacturer instructions:

7- Buffered peptone water, 90 ml in 250 ml capacity bottles.
8- Rappaport–Vassiliadis broth (RV).
9- Novobiocin.
10- Heckton Enteric (HE) Agar.
11- Tryptic Soy Agar (TSA).
12- Lysine Iron Agar (LIA)
13- Triple Sugar Iron (TSI).
14- *Salmonella* O antiserum Polyvalent Groups A-I & Vi, *Salmonella* H antiserum.
15- Oxoid Latex Test Kit for *Salmonella*.
16- Petri dishes.
17- Test tubes.
18- Straight and rounded-end loops.

3.8.3 Preparation and Analysis of Bio-solids Samples

- Add 10g of sludge sample to each one of the four bottles containing 90 ml of sterile buffered peptone water and shake or stir for at least 10 minutes. Each bottle may be labeled as A, B, C and D.
- Add 10 ml of the mixture from bottle D to each one of three empty sterile test tubes. Take 1 ml of the mixture of the same bottle D and add to each one of three test tubes containing 9 ml of sterile buffered peptone water. Vortex the tubes.

- Discard the bottle D after taking the samples. In this step, it can be observed that the bottles are inoculated with 10 gm of bio-solids, three test tubes with 1 g of bio-solids and three other test tubes with 0.1g of bio-solids. A triplicate dilution and nine solutions are created.

- Incubate bottles A, B and C and all test tubes at 35 ˚C incubator for 24 hours .
- Without mixing the dilutions, take 0.1 ml of each bottle and test tube and add it to a tube containing 10 ml of RV broth. Vortex and incubate at 42 ˚C for 24 hours in an Incubator. RV media preparation includes addition of novobiocin, a compound that inhibits gram-positive bacteria and increases *Salmonella* population.
- After 24 hrs, take one droplet with an inoculation loop and streak it in a (HE) agar plates. Incubate at 37 ˚C incubator for 24 hours. *Salmonella* colonies grow on HE plates as separate black and round dots with a colorless halo (indicating mobility). Results of possible *Salmonella* should be recorded.

3.8.4 Biochemical Analysis for Suspected *Salmonella* Colonies

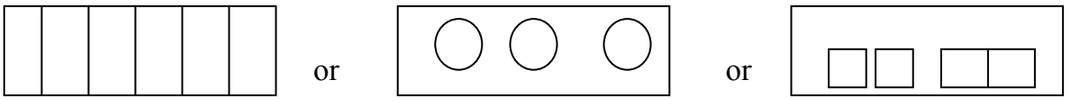
- Take two typical and separate suspected *Salmonella* colonies and streak them separately in the same TSA plates. Incubate at 37 ˚C for 24 hours. In this step, pure colonies are grown in plates.
- Using a sterile needle, pick the center of a well-isolated colony from TSA plates.
- Inoculate by stabbing to the base of the butt and streaking the slants of LIA and TSI tubes. TSA plates need to be saved for a later confirmation step.
- Cap the tubes loosely to ensure aerobic conditions, incubate at 35 ˚C for 18 to 24 hours.
- For TSI tubes, read for acid production in base of the slants, which will turn yellow. Also, inspect for alkaline slants that will turn red. Hydrogen sulfide reaction may be present.
- For LIA, examine at 18 to 24 and 40 to 48 hours for alkaline production in the base of the slant (purple color). Blackening at the apex of the slants will reveal hydrogen sulfide production.
- Record tubes as positive for *Salmonella* spp., if TSI and LIA conditions of *Salmonella* presence are observed. Otherwise, confirmation of results should be made using *Salmonella* O & H antisera.

- Serological test:

Check with polyvalent O and polyvalent H antisera

Slide preparation:

- Clean a glass microslide (Alternatively, use an agglutination slide).
- Divide the slide face into sections or compartments with a wax pencil.



10 mm 10 mm 10 mm

- Bacterial suspension*

- Place 0.5 ml of saline in a 13X100 mm test tube.
- Remove an isolated colony from the TSA plates with a bacteriological loop.
- Emulsify the growth in the saline. (The mixture should be milky with no large particles).

- Agglutination Test

- Place drops of selected antisera in each section on the slide.
- Add a loopful of bacterial suspension to the first antiserum drop, and mix well.
- Clean, flame, and cool the loop.
- Repeat steps 2 and 3 for each drop of antiserum.
- Rock the slide back and forth for 1 – 2 minutes.
  - Observe the mixed drops for clumping (agglutination). *Reactions taking longer than 2 minutes should be called "negative".*

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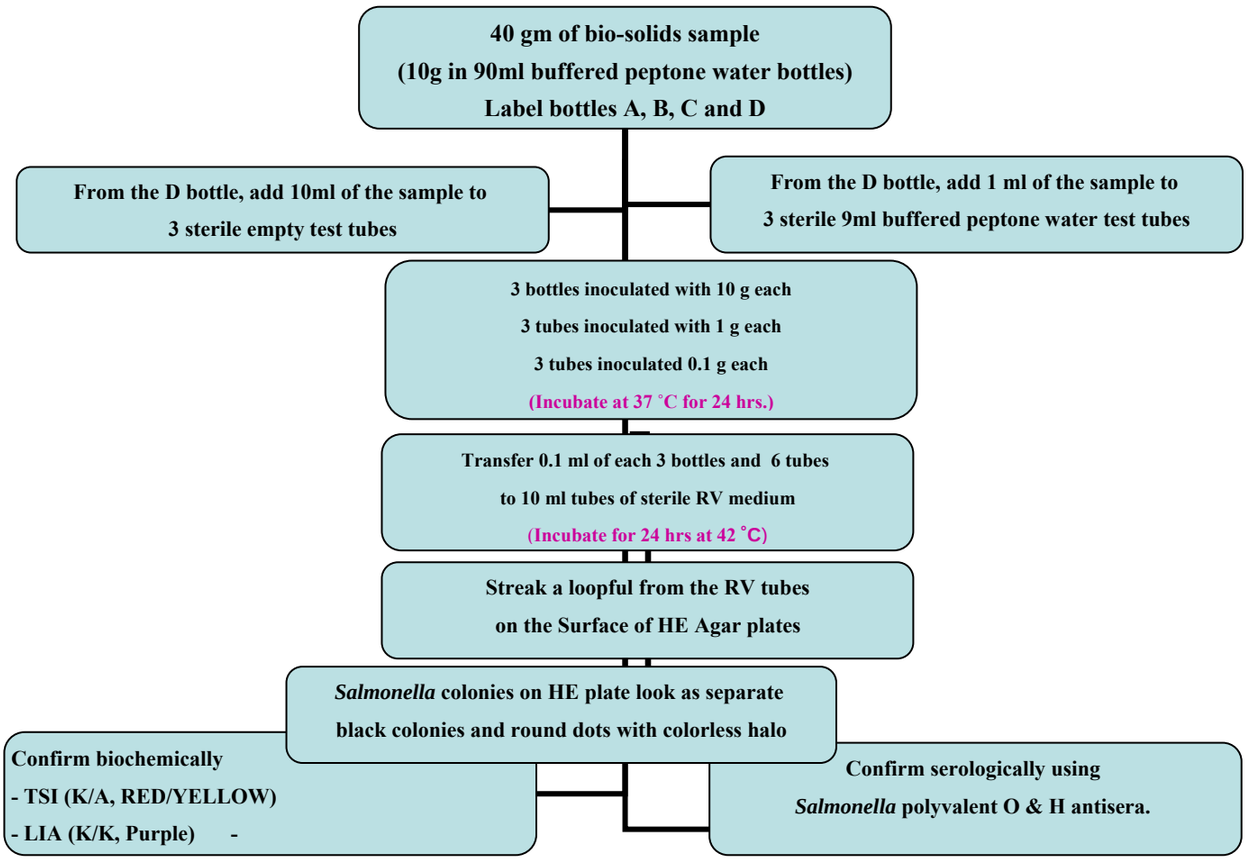
* These are live pathogenic bacteria: handle with care

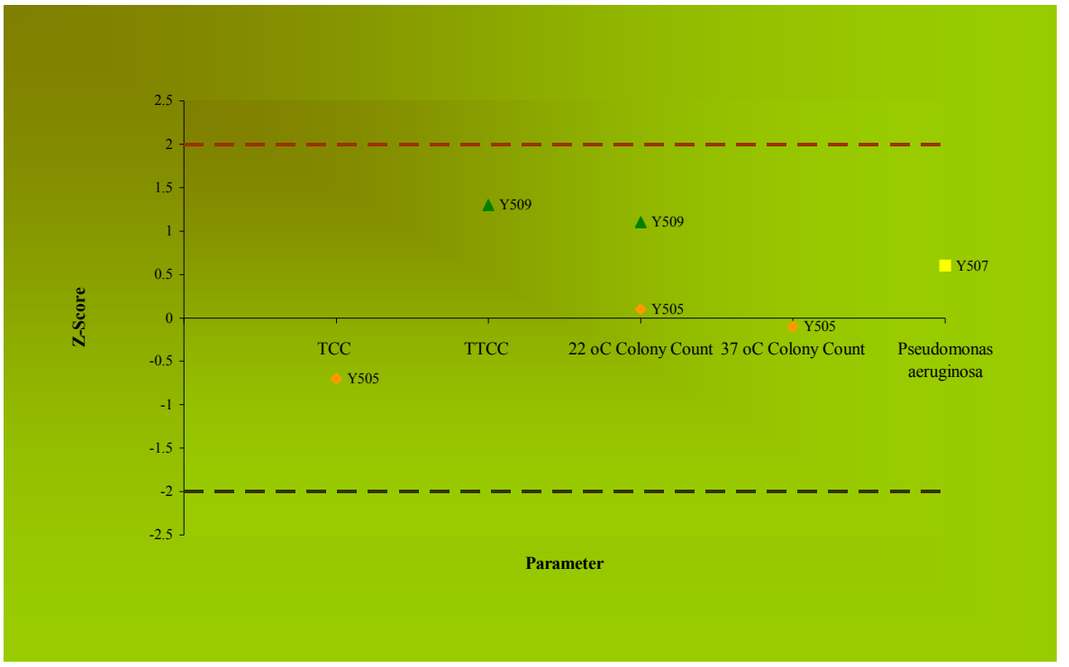
Procedure: As in flow sheet



3.8.5 Results Recording

Based on the number of positive tubes in each dilution, determine the MPN of *Salmonella*/4g of dry weight bio-solids.

Example: the following graph shows the Z-score for some microbiological analyses carried out at microbiological analysis lab at ERC / RSS.



Reference

EPA (2006) : EPA Method 1682: *Salmonella* in Sewage Sludge (Bio-solids) by Modified Semisolid Rappaport Vassiliadis (MSRV) Medium, July 2006.

3.9 Detection and Enumeration of Ascaris Ova in Sludge and Bio-solids

3.9.1 Scope

This procedure is applied to liquid and dry bio-solids to detect and enumerate *Ascaris* ova in sewage sludge and bio-solids.

3.9.2 Materials and Reagents

1. Stir bars.
2. 1 or 2 Liter graduate plastic beaker.
3. Blender.
4. 50 mesh sieve.
5. 400 mesh sieve.
6. 50 ml centrifuge tubes.
7. 15 ml tubes.
8. 1% 7X": 1L=999 ml phosphate buffered water + 1 ml of 7x detergent (TWEEN 80); adjust pH to 7.2 ±0.1 with 1 N NaOH.
9. Magnesium sulfate (sp. gr 1.20: 1L= 215g $MgSO_4$).
10. Phosphate buffered water: 1L=34.0 g KH_2PO_4, pH adjusted to 7.2 ±0.5 with 1 N NaOH.

3.9.3 Preparation and Analysis of Bio-solids Samples

- Dried or thickened samples: weigh about 150 g and place in about 250 ml phosphate buffered water in a beaker. Let it soak overnight at 4 ˚C., transfer to a blender and blend at high speed for one minute.

- Liquid samples: measure 500 ml or more (estimated to contain at least 25 g of dry solid) of liquid sample. Place half of the sample in a blender and add 100ml phosphate buffered water. Blend at high speed for one minutes. Transfer to a beaker and repeat the procedure for other half of the sample.

- Pour the homogenized sample into 1000 ml tall beaker rinsing the blender into it. Add 1%7x to reach 450 ml final volume.

- Stir the sample with a wooden applicator to ensure that the material floating on the surface settles. Additional 1%7x can be added and stirred if necessary. Let the sample settle for four hours or overnight at 4 ˚C.

- After settling vacuum aspirate just above the layer of bio-solids. Centrifuge the sample if not settled. Transfer the sediment into a blender and add phosphate buffered water to 250 ml, blend again for one minute at high speed.

- Transfer to a beaker, rinse blender and add 1%7x to reach 450 ml. Allow to settle for 2 hours at 4 ˚C if not settled centrifuge the sample.

- Add 150 ml 1%7x and stir for 5 minutes on a magnetic stirrer.

- Strain the homogenized sample through 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.

- Add 1%7x to 450ml final volume and allow to settle for 2 hours at 4 ˚C.

- Vacuum aspirate just above the layer of bio-solids. Mix sediments and distribute evenly to 50 ml graduated conical centrifuge tubes. Thoroughly wash any sediment from the beaker into the tubes using water from a wash bottle. Bring volume in tube to 25ml with water.

- Centrifuge for 10 minutes at 1,000 G (2,120 RPM). Vacuum aspirate supernatant from each tube down to just above the level of sediment (packed sediment should not exceed 5 ml; if so distribute evenly to among additional tubes, repeat centrifugation and aspiration of sample).

- Add 5 to 10 ml $MgSO_4$ solution (specific gravity 1.20) to each tube with a cap and mix for 15 to 20 seconds on a vortex mixer.

- Add additional $MgSO_4$ solution (specific gravity 1.20) to each tube to bring volume to 25 ml. Centrifuge for 5 to 10 minutes at 800 to 1000 G (1,890-2,120 RPM); do not use breaks.

- Allow the centrifuge to stop without a break. Pour the top 20-25 ml of supernatant from each tube through 400-mesh sieve supported in a funnel over a tall beaker, using a water spray bottle, wash excessive flotation fluid and fine particle through sieve.

- Rinse the sediment collected on the sieve into a 100ml beaker by directing the stream of water onto the upper surface of the sieve.

- After thoroughly washing the sediment from the sieve, transfer the suspension to the required number of 15 ml centrifuge tube, taking care to rinse the beaker into the tubes.

- Centrifuge the tubes for 3 minutes at 800 G (1890 RPM), and then discard the supernatant. If more than one tube has been used, transfer the sediment to a single tube, fill it with water and repeat centrifugation.

- Discard supernatant, add one drop of Lugols' Iodine (Iodine/Potassium Iodine solution) and add distilled water to a final volume of 1ml. Examine microscopically to enumerate the detected ova.

Reference

EPA/625/R-92/013, 1992: Environmental Regulations and Technology, Control of Pathogens and Vector Attraction in Sewage Sludge.

ANNEXES

ANNEX (A):

Bio-solids Reuse and Disposal

JS 1145/2006

Field of Application:

This standard contains guidelines that must be met for bio-solids produced in wastewater treatment plants in order to be used to improve soil fertility or as organic fertilizer for agricultural purposes or to be disposed of in landfills as stated by the guidelines of this standard.

1. Definitions:

1.1. Sludge : wet or dry solid material produced by wastewater treatment processes before treatment of this material.

1.2. Liquid Sludge : wet by-product of solid material from wastewater treatment processes that has a total solid content of 50-89%.

1.3. Dewatered Sludge: Dry by-product of solid material from wastewater treatment processes that has a total solid content not less than 10%.

1.4. Treated bio-solids: bio-solids treated with one or more of the treatment processes stated by this standard.

1.5. Wastewater: the disposed water produced by domestic uses that may contain liquid industrial wastes that was permitted to be disposed in the network as conditioned by formal parties.

1.6. Sludge dry weight: weight of solid material after drying at a temperature (103-105) ^{o}C or till a constant weight reached.

1.7. Producer: the party responsible for wastewater treatment and bio-solids production.

1.8. Distributor: the party responsible for distribution and transport of bio-solids to user.

1.9. User: the party that uses the bio-solids.

1.10. Collection: the process of gathering bio-solids after treatment and before use.

1.11. Bio-solids addition to soil: distribution on soil surface or injection beneath soil surface or mixing with soil.

1.12. Fruit Trees: trees produce fruits for human use.

1.13. Field crops: cereals and fodders that harvested once or more in a year.

1.14. Licensing committee: the governmental party that supplies the license to use bio-solids (Ministry of Environment).

1.15. Supervising parties: ministries and official institutions that supervise environmental and health conditions in accordance with current laws and guidelines.

1.16. Landfills: Defined sites by the licensing parties for disposal of solid and liquid wastes.

1.17. First class of bio-solids: Bio-solids that can be used in all fields stated by these standards. The quality and physical, chemical and microbial

1.18. Second class of bio-solids: Bio-solids that can be used for soil modification or to be disposed of in landfills. The quality and physical, chemical and microbial constitution of such bio-solids fits into the concentrations stated in table (1) of the standard.

1.19. Third class of bio-solids: Bio-solids that are permitted to be disposed of in landfills. The quality and physical, chemical and microbial constitution of such bio-solids fits into the concentrations stated in table (1) of the standard.

1.20. Pastures: Lands used for forage crops or trees plantation.

Abbreviations

The following abbreviations are applied for the purpose of this standard:

Abbreviation	Symbol
Arsenic	As
Cadmium	Cd
Chromium	Cr
Copper	Cu
Mercury	Hg
Molybdenum	Mo
Nickel	Ni
Lead	Pb
Selenium	Se
Total Fecal Coliform Count	TFCC
Zinc	Zn

2. General Conditions:

2.1 It is prohibited to use the untreated sludge.

2.2 It is not permitted for any party or person to use bio-solids unless the guidelines of these standards are met.

2.3 Bio-solids laboratory tests requested by the licensing committee or supervising parties should be carried out by an accredited party.

2.4 The user should get the official licenses from licensing committee at the Ministry of Environment and should supply all needed information including bio-solid addition site, nearby water resources, soil quality and crops to be planted. The licenses should be modified every two years and the supervising parties have the right to modify or cancel any license in the case of infringements.

2.5 The producer shall keep a special register for a period not less than five years, the register shall contain information about the quality and quantity of produced bio-solids and the treatment processes to be displayed when needed.

2.6 The user should provide guiding signs at the bio-solids reuse site.

2.7 If required, bio-solids shall be gathered and stored on covered land with lined bottom and sides and in a way that its quality will not be affected and for a period not more than three years.

2.8 It's prohibited to store bio-solids near wadis, areas exposed to floods, irrigation channels, water bodies and sites diversely affect surface water and groundwater.

2.9 Bio-solids are added during January and December for productive trees and during September and October for field crops and pastures, the addition process shall take place within one week before planting for irrigated areas.

2.10 It is not permitted to add bio-solids for lands planted with vegetables, also for parks, house gardens, green flats, and near residential areas. Also it is prohibited to add bio-solids to areas planted with root crops such as carrot, potato, radish or any other crops eaten cooked or uncooked.

2.11 The safety, health, and environmental conditions shall be met for bio-solids transport process:

- Transport vehicles shall hold obvious signs.
- Leakage from Transport vehicles is prohibited.
- It shall be kept clean.
- Transported bio-solids should be covered.

2.12 The addition process should be homogenous; within the amounts needed and the existed elements and nutrients covered by this standard.

- Addition process for irrigated land is carried out along planting lines, bio-solids is mixed with surface soil (10-20cm), there are no specific addition period but the addition process shall be carried out within a week before planting.

- For rainy land bio-solids is added before precipitation period (starting October) and mixed with surface soil, land slope shall be less than 5%.

- For pastures -as above- but cultivation shall be in contours, bio-solids is mixed with surface soil.

2.13 Bio-solids addition rate depends on nutrients in soil and other elements covered in this standards:

- The maximum nutrients and elements concentrations stated by this standard shall not be exceeded.
- The user shall investigate the nutrient concentrations in soil and crops to determine what is needed before application.

2.14 Supervising parties have the right to carry out needed laboratory tests for crops produced with bio-solids addition to insure safety of the product and general health and environmental conditions.

2.15 The producer shall provide a management plan for the supervising parties.

2.16 It's prohibited to dispose bio-solids in water bodies, wadis, groundwater recharge areas, wastewater networks with the exception of wastewater treatment plants receiving wastes from septic tanks.

2.17 For bio-solids filled in buckets for trading purposes, the approval from supervising parties is needed; in this case (US EPA) guidelines or any other guidelines are helpful.

2.18 In order to use bio-solids for other purposes other than that stated by this standard, technical studies and licenses are needed.

3. Health and Environmental Guidelines:

3.1 Its prohibited to use bio-solids in such a way that this will diversely affect the quality of groundwater or surface water including streams, water harvesting projects, and areas exposed to floods.

3.2 Supervising parties have the right to state other restrictions other than that stated by these standards in special cases in order to conserve the environment and to keep human safe.

3.3 Health and safety conditions should be kept for persons who are exposed to work with bio-solids.

3.4 The user and producer comply with health requirements, periodic inoculation for workers and providing this for supervising parties when requested.

3.5 Vector attraction shall be taken into consideration while gathering and using bio-solids.

3.6 Bio-solids shall be mixed with soil in a period not more than two days after addition.

3.7 Bio-solids shall not be added to lands with a slope more than 15%.

3.8 In the case of sludge addition near to residential areas, the addition sites should be 250 m away.

3.9 It is prohibited to consume fallen fruits in lands exposed to bio-solids addition.

3.10 It is prohibited to use lands with bio-solids addition for pasturing purposes before two months of addition.

3.11 It is requested to use warning signs for sights with bio-solids application in order to warn people from entering these sites.

3.12 Mechanical methods may be used in bio-solids addition whenever possible.

4. Technical Conditions:

4.1 Sludge must be treated before use with treatment methods stated by this standard.

4.2 Bio-solids to be disposed at landfills shall comply with the guidelines of this standard.

4.3 Soil characteristics should be identified before the first time of bio-solids addition.

4.4 For the purposes of this standard, bio-solids are classified into three classes, first, second and third class.

- First class bio-solids are used for agricultural purposes in modifying soil characteristics.
- Second class bio-solids are used to modify soil characteristics only.
- Third class bio-solids are permitted to be disposed of in landfills.

4.5 It is prohibited to add bio-solids to soil at a rate beyond 6 ton/ha per a year.

4.6 Tables (1) and (2) show the maximum permitted concentrations in bio-solids in addition to maximum annual rates and accumulation limits for elements

Table(A.1): Maximum Concentrations of Trace Metals in Soil.

Parameter	Unit	Concentration/ Bio-solids Class		
		First Class	Second Class	Third Class
As	**mg/kg Dry Weight**	41	75	75
Cd	**mg/kg Dry Weight**	40	40	85
Cr	**mg/kg Dry Weight**	900	900	3000
Cu	**mg/kg Dry Weight**	1500	3000	4300
Hg	**mg/kg Dry Weight**	17	57	57
Mo	**mg/kg Dry Weight**	75	75	75
Ni	**mg/kg Dry Weight**	300	400	420
Se	**mg/kg Dry Weight**	100	100	100
Pb	**mg/kg Dry Weight**	300	840	840
Zn	**mg/kg Dry Weight**	2800	4000	7500
TFCC	**MPN/g or CFU/g**	1000	2,000,000	-
Salmonella	**MPN/4g Dry Weight**	3	-	-
Helminthes	**Eggs/4g Dry Weight**	1	-	-

Table(A.2): Maximum Annual Rates and Accumulation.

Parameter	Annual Addition Rate (kg/ha/365 days)	Maximum Accumulation Limits (kg/ha)
As	1	20
Cd	1	20
Cr	25	500
Cu	35	700

Hg	0.85	17
Mo	0.9	18
Ni	5	100
Se	2	40
Pb	11	220
Zn	50	1000

5. Quality Control:

5.1 Collected bio-solids samples must be representative for the quantity to be used.

5.2 Sampling devices must be clean and made from none polluting material, for biology and microbiology tests sampling and incubation devices must be sterilized.

5.3 Sampling and samples incubation, transfer and analysis shall be carried out as stated by standard methods for the examination of water and wastewater reference (United states Health Association & the Federal American Society of Water Research) or any other accredited analysis methods for example the USEPA Bio-solids sampling and analysis guide.

5.4 Needed laboratory analyses for bio-solids samples must be carried out in accredited technical laboratories and approved by supervising parties.

5.5 Supervising parties have the right to analyze soil, bio-solids and crops as often as they want it done.

5.6 The frequency of sampling and chemical, biological and microbiological Analysis are as shown in table (3) below.

Table(A.3): Frequency of Analysis Based on Produced Bio-solids Amounts.

BIO-SOLIDS PRODUCED AMOUNT (TON/YEAR)	FREQUENCY OF ANALYSIS (ONCE A YEAR)
Less than 300	once every one year

300-1500	once every 3months
1500-15000	once every 2 months
more than 15000	once a month

6. Quality Control

Bio-solids user, through qualified laboratories, is requested to collect three samples of bio-solids and analyze them to insure their quality and that they are in compliance with the standards. The average of the three samples is used for classification purposes.

7. Bio-solids Treatment Levels:

First Level:

- Fermentation: sludge is stored in containers or landfills at a temperature of 20^{o}C for 60 days or at 40^{o}C for 15 days.

- Thermal drying: sludge is dried by direct or indirect contact with hot gases to reach moisture content of 15% or less and a temperature of 80^{o}C.

- Air drying: sludge is left to filtrate or to dry in sand basins so that the depth of sludge is not more than 25 cm, sludge must be kept in the basins for at least 45 days during April to October, solids content must reach at least 85%.

- Thermal aerobic digestion: sludge is mixed in an aerobic environment for 10 days at (55-60) ^{o}C solid content must reach at least 38%.

- Other methods used must be able to reach treatment levels such as the above methods.

Second Level:

- Aerobic digestion: sludge is mixed in an aerobic environment for a period of 40 days at 20 ^{o}C, solids content must reach 38% at least.

- Air drying: sludge is left to filtrate or to dry in sand basins so that the depth of sludge is not more than 25 cm, sludge must be kept in the basins for at least 30 days, solids content must reach 60% at least.

- Anaerobic digestion: this method is carried out by incubation of sludge in an anaerobic environment for 15 days at 35-55^{o}C or for 60 days at 20^{o}C, solids content must reach 38% at least.

- Other methods must be able to reach treatment levels such as the above methods.

Third Level:

- Sludge thickening to a solid content of 3%.
- Other methods must be able to reach treatment levels such as the above methods.

References:

-Syrian Standard Guidelines for Bio-solids Reuse, 2002.

-USEPA Guidelines part (503).

-Australian Standard Guidelines for Bio-solids Reuse , 1997.

-Obreza T, Conner G. The Basics of Bio-solids Application to Land in Florida. University of Florida & Institute of Food and Agricultural Sciences. 2003.

-Stehouwer R. Land Application of Sewage Sludge in Pennsylvania. Pennsylvania State University.1999.

-Indiana Administrative Code (IAC) No. 327. Land Application of Bio-solids, Industrial Wastes Product and Pollutant Bearing Water. Indiana Department of Environment and Management & Indiana Water Pollution Control Association. 2003.

-The Code of Virginia 12 VAC 5-585-10. Virginia Department of Health . 1997.

-Guidelines for the Utilization of Bio-solids and Other Wastes on Agricultural Land. Ministry of Agriculture, Food and Rural Affairs (OMAFRA). Ontario, 1996.

-Usage of Sludge Regulations, Israel Ministry of Environment . 2004.

ANNEX (B):
Chain of Custody Form

Following are sample labels and a chain-of-custody record used by RSS/ERC Laboratories for a soil sample collected on June 11th, 2008 labeled as R.1:

Figure (B.1): Labeling of a soil sample.

Organization name: RSS/ERC

Sample location: Madaba

Sample no.: R.1

Sample type: Soil

Date & time of sampling: June 11th, 2008

Collector: Nasir Budeir

Table (A.4): Chain of Custody Record.

CHAIN-OF-CUSTODY RECORD			Laboratory Name: Chemical Testing Unit		
PROJECT NAME: Bio-solids					
SAMPLER(s): R.1 Signature: Eng. Asma					
Date	Time	Location	Sample type	No. of container	Remarks
11/6/2008	13:00 PM	**Madaba**	**Soil**	**2**	
Relinquished by: Eng. Asma Signature: Eng. Asma Date & Time:			Received by: Eman Ta'an Signature: Eman Ta'an Date & Time:		
Relinquished by: Signature: Date & Time:			Received by: Signature: Date & Time:		
Relinquished by: Signature: Date & Time:			Received by: Signature: Date & Time:		