FAO/IAEA CO-ORDINATED RESEARCH PROGRAMME

ON

THE USE OF IRRADIATED SEWAGE SLUDGE TO INCREASE SOIL FERTILITY, CROP YIELDS AND TO PRESERVE THE ENVIRONMENT

(D1-50.04)

INTRODUCTION TO THE PROGRAMME

WORKPLANS

EXPERIMENTAL GUIDELINES FOR PHASE 1

(UPDATED SEPTEMBER 1995)

PREPARED AT THE FIRST RCM HELD IN VIENNA
10 – 14 JULY 1995

Saliya Kumarasinghe
Scientific Secretary
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ON
THE USE OF IRRADIATED SEWAGE SLUDGE TO INCREASE SOIL FERTILITY,
CROP YIELDS AND TO PRESERVE THE ENVIRONMENT

1. BACKGROUND

Production of wastes and the practice of their disposal can be traced back to ancient times. However, the concept of systematic collection and treatment before disposal of solid and liquid wastes evolved only during the last century. Since then, rapid population expansion and urbanization brought with it many problems and the problem of utilization and disposal of sewage sludge is a serious one in many countries. One of the applications of sewage sludge is in the area of agriculture. Sewage sludge has essential nutrients for plant growth like N, P, K, micronutrients like Zn, Fe, Cu, Mn, other trace elements and considerable amounts of organic matter. However, they also contain pathogenic organisms, heavy metals and other toxic materials coming from industries. Because of possible health problems, sewage sludge should be disinfected of living organisms dangerous to human health and this can be achieved most efficiently through irradiation. Research has shown that irradiation is an effective means for reducing pathogens in sewage sludge to levels where sludge re-use in public areas meets the criteria for protection of public health. Two of the most efficient methods are gamma irradiation and electron beam irradiation. The first pilot scale sewage sludge gamma irradiation (using $^{60}$Co as a source) plant was established in Geiselbullach (near Munich) in Germany in 1973. This plant had the capacity to irradiate 150 m$^3$ of sewage sludge per day and the irradiated sewage sludge was used primarily for agricultural research. Several technical scale irradiation plants have been established in different countries (e.g. Argentina, India, Japan, Mexico and USA) since then and some using $^{137}$Cs as a source. In several industrialized countries, use of $^{137}$Cs for this purpose is an efficient means of reusing this radiisotope which is produced in substantial quantities as a by-product of reprocessing of defense and power nuclear fuel wastes. At present sufficient technical data is available on gamma treatment of sludges, permitting its application on the demonstration or commercial scale, but a few gaps in knowledge still exist especially for the practical application of electron beam technology and these are being addressed in the other Co-ordinated Research Programme implemented by the Division of Physical and Chemical Sciences.

Land application of municipal sewage sludge is being practiced throughout the world at different levels and their beneficial effects include increases in crop yields, soil organic matter, cation exchange capacity, water-holding capacity and soil fertility in general. High levels of nitrogen, phosphorus and micronutrients found in sewage sludge make it an excellent fertilizer. In addition, the high organic matter levels present can improve soil structure, particularly that of sandy soils in arid and semi-arid areas.

Sewage sludges applied to crop plants besides providing macro- and micro-nutrients also contain, unfortunately, heavy metals such as Cd, Cr, Ni, Pb, Co and Hg in amounts beyond those normally encountered in soils. Land applications have to be carefully made so that heavy metals do not accumulate to toxic levels in plants and enter into food chain or accumulate in soil and enter into groundwater. It is therefore important that appropriate measures are taken by the industries to ensure release of waste material with only environmentally acceptable levels of heavy metal contaminants.

At present, information on the availability of nutrients from sewage sludges to crops, its benefits as an organic amendment to soil, and the harmful effects of heavy metals on crop growth, is limited. Isotope and radiation techniques could provide a valuable tool in attempts to find answers to some of these questions. IAEA's involvement in studies of radiation processing of sewage sludge dates back to several years: A five-year Co-ordinated Research Programme on "Radiation Treatment of Sewage Sludge for Safe Reutilization" was completed in 1990. This programme involved participants from Canada, Germany, India, Indonesia, Italy, Japan, and the USA and forms a sound foundation for the present programmes. The present Co-ordinated Research Programme on "The Use of Irradiated Sewage Sludge to Increase Soil Fertility, Crop Yields and to Preserve the Environment" will deal with how isotope and radiation techniques could be used as a tool to find ways of utilizing sewage sludge to increase and sustain crop production and soil fertility in an environmentally sound manner. The world agriculture is at present urgently looking for ways by which integrated nutrient
management practices involving inorganic as well as organic fertilizers could be introduced into cropping systems so as to increase and sustain food production and soil fertility while at the same time minimizing the harmful effects on the environment. In this context, the initiation of this programme this year, is indeed very timely.

This is a joint programme conducted in collaboration with the Division of Physical and Chemical Sciences which is co-ordinating a related research programme on "Radiation Processing of Liquid Wastes and Water". In December last year, the two Divisions held two Consultants Meetings on irradiation of sewage sludge and liquid wastes, and their use in agriculture where the state-of-the-art of the subject was reviewed. The overall objectives of the Consultants Meeting conducted by the Soil Fertility, Irrigation and Crop Production Section was to review:

- The radiation processing of sewage sludge by gamma irradiation, electron beam radiation and other alternative methods.
- Applications of sewage sludge as a fertilizer for increasing soil fertility and crop production.
- Heavy metal (Cd, Cu, Ni, Pb and Zn) contamination of agricultural soils.
- Potential harmful effects of sewage sludge utilization as a fertilizer on the agricultural environment, i.e., soil, plant, food, groundwater, water streams, etc.

The Consultants recommended that the Joint FAO/IAEA Division co-ordinate a networked research programme with 12-13 contract holders from developing countries and 4-5 agreement holders from industrialized countries to assess the use of irradiated sewage sludge to increase soil fertility and crop yields in an environmentally sound manner. The overall objectives outlined for the recommended programme were:

- To assess sewage sludge, especially irradiated sludge, for its utility as a fertilizer and for increased crop production. Evaluate N and P uptake from the sludge under different soil and climatic conditions taking into account N and P losses, using \(^{15}\text{N}\) and \(^{32}\text{P}\) isotope techniques.
- To assess the role of sewage sludge as an organic matter amendment to improve soil fertility, using neutron probes and gamma density probes, and isotopes, such as \(^{14}\text{C}\), \(^{13}\text{C}\) and \(^{15}\text{N}\).
- To evaluate potential environmental contamination by:
  - Pathogens
  - Heavy metals
  - Organic pollutants
- The meeting recommended that the networked research programme should compare data across diverse soil and climatic conditions with researchers using the same experimental treatments. More general recommendations will therefore be able to be made on the type of sewage sludge to use for specific crops in developing countries.
- Close co-operation should be established between the Joint FAO/IAEA Division and other organizations having active roles in the irradiation and utilization of sewage sludge in agriculture.
- The findings of this programme should be published by the IAEA and made available as widely as possible.
- The IAEA laboratories at Seibersdorf, in collaboration with the Austrian Research Centre, should conduct supportive research and training in the use of \(^{15}\text{N}\), \(^{32}\text{P}\), and neutron probe in sewage sludge studies.
- Reference laboratories and standard materials must be identified for the analysis of nutrients, heavy metals and organic pollutants
- All of the investigations must take account not only the benefits of sewage sludge as an organic fertilizer but also the potential harmful effects on the environment: soil, plant, food quality, ground water and surface water quality. This is essential for the success of this programme and will be a contribution to the development of sustainable agricultural practices in an environmentally sound manner.
Based on the recommendations made, the Joint FAO/IAEA Division initiated this Co-ordinated Research Programme on "The Use of Irradiated Sewage Sludge to Increase Soil Fertility, Crop Yields and to Preserve the Environment". The first Research Co-ordination Meeting of this CRP was held at the Vienna International Centre in Vienna in which the 13 Contract Holders from Argentina, Bangladesh, China, Egypt, India, Indonesia, Malaysia, Mexico, Pakistan, Philippines, Portugal, Romania, Thailand and 4 Agreement Holders from Austria, Japan, United Kingdom and the United States of America, participated. Another Agreement Holder is likely to be selected in the near future, from Germany. At this RCM, the participants presented the country reports which highlighted the research activities in sewage sludge use in their own countries. These presentations were followed by the sessions on fine tuning the objectives of the programme, determining the scope, preparation of experimental guidelines and workplans.

2. **SCOPE OF THE PROGRAMME**

The scope of the programme includes studies on developing management practices for the efficient use of sewage sludge as an organic fertilizer for increasing and sustaining crop production and soil fertility in an environmentally friendly manner. In objectives will include in particular:

3. **OBJECTIVES**

This main objectives of the CRP are:

- To quantify the availability of N and P from sewage sludge to crops, using $^{15}$N and $^{32}$P tracer techniques.
- To assess increases in crop yields as a result of application of sewage sludge.
- To assess improvements in soil properties, particularly increases in organic matter content and water holding capacity.
- To estimate the pathogenic organism content in non-irradiated and irradiated sewage sludge.
- To assess the extent of contamination of soil by heavy metals by the use of sewage sludge.
- To determine the beneficial effects of micronutrients in terms of increasing crop yields and soil fertility.

4. **IMPLEMENTATION OF THE PROGRAMME**

The CRP will be implemented over a period of 5 years starting in 1995 and ending in 1999. For convenience, the programme may be divided into 3 phases, i.e. Phase I, Phase II and Phase III.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Date</th>
</tr>
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<tbody>
<tr>
<td>Phase I</td>
<td>January 1995 - November 1996</td>
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<tr>
<td>Phase II</td>
<td>December 1996 - May 1998</td>
</tr>
<tr>
<td>Phase III</td>
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</tr>
</tbody>
</table>

**Research Co-ordination Meetings (RCMs)**

<table>
<thead>
<tr>
<th>RCM</th>
<th>Date</th>
<th>Venue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st RCM</td>
<td>10-14 July, 1995</td>
<td>Vienna, Austria</td>
</tr>
<tr>
<td>2nd RCM</td>
<td>November 1996</td>
<td>Cairo, Egypt</td>
</tr>
<tr>
<td>3rd RCM</td>
<td>May 1998</td>
<td>Lisbon, Portugal</td>
</tr>
<tr>
<td>4th RCM</td>
<td>November 1999</td>
<td>Vienna, Austria</td>
</tr>
</tbody>
</table>
Main focus of activities in different phases

Phase I  -  Nitrogen ($^{15}$N)
Phase II  -  Phosphorus ($^{32}$P)
Phase III -- Water & organic matter (improvements in water holding capacity and organic matter content etc.) (Neutron probe)

5. EXPERIMENTAL GUIDELINES

(For Phase I experiments - January 1995 - November 1996)

In keeping with the special feature of a Co-ordinated Research Programme, a core experiment will be planned for each phase, leaving optional treatments to be included by the contractors depending on their research needs and capability to handle them.

5.1. FOCUS IN PHASE I - Nitrogen

5.2 Objectives

Primary
- To quantify the availability of N to crops from sewage sludge applied at different rates.
- To assess increases in crop yields as a result of application of sewage sludge.
- To assess the extent of decrease in contamination of soil by pathogenic organisms as a result of using irradiated sewage sludge compared to non-irradiated sewage sludge.

Secondary
- To assess improvements in soil properties, particularly increases in organic matter content and water holding capacity.
- To assess the extent of contamination of soil by heavy metals by the use of sewage sludge.

5.3 Experimental treatments

5.3.1. Crop species

The number of species to be used will be decided by each contractor. The amount of $^{15}$N available should be taken into consideration when increasing the size of the experiment. Maximum of 2 would be the best and easily manageable.

5.3.2. Sewage sludge treatment

- Non-irradiated
- Irradiated

Note:\n
a) If sludge is irradiated prior to drying, at a moisture content of >35 %, the minimum absorbed dose should be 3 kGy (300 kRad) if no simultaneous oxygenation occurs, and 2 kGy (200 kRad) if it does.
b) If the average, and not the minimum dose is measured routinely, and if the max. to min. dose ratio does not exceed 1.6, the corresponding average doses absorbed should be 4 kGy (400 kRad) and 2.7 kGy (270 kRad), respectively.
c) If the sludge can not be dried after irradiation (which is the preferred procedure), and the irradiation must be carried out on the air dried sludge, the minimum absorbed dose should be increased to 5 kGy (500 kRad), and the average absorbed dose - to 6.5 kGyO kRad), respectively.

1The participants are kindly requested to obtain the following information from the supplier of the irradiation service in their area:

i. Description of irradiator (lab, pilot or industrial scale), and in particular of the geometry of the irradiation chamber, in relation to source (plaque or cylindrical) and to product (whether packaged, in bulk, or in thin layer);
ii. The expected or measured dose rate distribution (iso-dose curves) in the sludge (whether packaged, in bulk or in thin layer);
iii. The irradiation procedure (batch or continuous, package, bulk or film, duration of treatment for above projected doses or residence time in irradiation chamber, oxygenation or other prior, concomitant or subsequent treatment, such as drying, heating, etc.);
iv. The dosimetric system to be used for monitoring the absorbed dose in the sludge and the frequency of monitoring.

The participants should convey this information, together with that on the physical characteristics (wet or dry, moisture content, if packaged - shape and dimensions, dried - or not -before or after irradiation, expected storage period before use, etc.) of the sludge they intend to use, to IAEA. This will enable a more detailed recommendation regarding the absorbed dose to be requested, so as to allow intercomparison of eventual effects on other major parameters in this study.

5.3.3 Treatments

CORE EXPERIMENT

Crop 1 - Cereal

1. Crop 1 + No sewage sludge + $^{15}$N labelled fertilizer - **locally recommended N rate**, 1 % a.e.

2. Crop 1 + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e. (reference for treatments 3-10)

3. Crop 1 + **Non-irradiated** sewage sludge, 50% of recommended rate irradiated sewage sludge, 100% of recommended N incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e. -

4. Crop 1 + **Non-irradiated** sewage sludge, 100% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.

5. Crop 1 + **Non-irradiated** sewage sludge, 150% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.

6. Crop 1 + **Non-irradiated** sewage sludge, 200% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.

7. Crop 1 + **Irradiated** sewage sludge, 50% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.

8. Crop 1 + **Irradiated** sewage sludge, 100% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.

9. Crop 1 + **Irradiated** sewage sludge, 150% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.

10. Crop 1 + **Irradiated** sewage sludge, 200% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
Crop 2 - vegetable (Same as above except that the crop is different)

1. Crop 2 + No sewage sludge + $^{15}$N labelled fertilizer - locally recommended N rate, 1 % a.e.
2. Crop 2 + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e. reference for treatments 3-10)
3. Crop 2 + Non-irradiated sewage sludge, 50% of recommended rate + Irradiated sewage sludge, 100% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
4. Crop 2 + Non-irradiated sewage sludge, 100% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
5. Crop 2 + Non-irradiated sewage sludge, 150% of recommended N rate, incorporated + $^{15}$N labelled fertilizers 20 kgN/ha, 10% a.e.
6. Crop 2 + Non-irradiated sewage sludge, 200% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
7. Crop 2 + Irradiated sewage sludge, 50% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
8. Crop 2 + Irradiated sewage sludge, 100% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
9. Crop 2 + Irradiated sewage sludge, 150% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
10. Crop 2 + Irradiated sewage sludge, 200% of recommended N rate, incorporated + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.

Optional Experiment

E.g., Estimation of BNF (Pastures and in crop rotation experiment)

If the objective is to assess BNF of legumes, each treatment should have two sets of plots, one for the fixing crop and the other for the non-fixing crop. $^{15}$N fertilizer can be added at the same rate of 20 kg N/ha at 20% atom excess to all treatments.

Fixing crop

1. Fixing crop + no sewage sludge + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
2. Fixing crop + Irradiated sewage sludge, 50% of recommended N rate + $^{15}$N labelled fert., 20 kgN/ha, 10% a.e.
3. Fixing crop + Irradiated sewage sludge, 100% of recommended N rate + $^{15}$N labelled fert., 20 kgN/ha, 10% a.e.

Reference crop

6. Ref. crop + no sewage sludge + $^{15}$N labelled fertilizer, 20 kgN/ha, 10% a.e.
7. Ref. crop + Irradiated sewage sludge, 50% of recommended N rate + $^{15}$N labelled fert., 20 kgN/ha, 10% a.e.
8. Ref. crop + Irradiated sewage sludge, 100% of recommended N rate + $^{15}$N labelled fert., 20 kgN/ha, 10% a.e.

Note:
You may use any other additional treatments or modified treatments depending on your particular requirements and circumstances.

Please retain all experimental plots for follow-up experiments throughout the entire five years

5.4. $^{15}$N fertilizer treatment

10% $^{15}$N atom excess ammonium sulphate to be applied as a uniform spray at the rate of 20 kgN/ha immediately (same day or the day after) after incorporation of the sewage sludge.
5.4.1. $^{15}$N fertilizer requirements

Enrichment to be used = 10 % atom excess
N rate = 20 kgN/ha = 2 gN/m$^2$
(Note: The following calculation is based on a sub plot size of 2.0 m x 1.4 m and an $^{15}$N area of 1.5 m x 1.0 m. The sub-plot size will vary depending on the row spacing and plant spacing which should give around 15 plants for harvesting)

For any one treatment:
1.5 m$^2$ (isotope area - one sub plot) x 4 reps = 6.0 m$^2$ x 2 (gN) = 12 gN = 12 x 100/21 = 57.14 g Ammonium Sulphate.
If you have for instance 10 treatments, then you need 571.40 g Ammonium Sulphate
(Note: When preparing the total $^{15}$N labelled fertilizer solution, prepare a little in excess (e.g. 100 ml or so) to take care of spillage etc and for the standard.

5.5. Other nutrients

P, K and other nutrients to be applied as recommended for the area.

5.6 Irrigation

Irrigate the field as necessary and ensure that the plants do not suffer from a water deficit.

5.7. Site selection

Select a field site that is flat and has not been in use for intensive cropping with high fertilizer inputs especially N in the recent past. The site should be as close as possible to the institute for easy supervision.

5.8. Experimental design

Complete randomized block design with 4 replicates (See Figs. 1 and 2)

5.9. Harvest

Harvest the plants in each sub-plot separately at physiological maturity. Cut the shoots about 5 cm above the ground. Separate into vegetative parts (leaves + stems + branches) and reproductive parts (pods/panicles). Weigh each part separately, chop into 2-3 cm pieces, sub sample and take about 250-300 grams. Record the weight of the sub sample accurately, dry in an oven at 70°C for 48 hours and grind to a powder. This will form your final sample. Follow instructions given in appendix II. Also include a sample of the fertilizer applied.

5.10. Sample analysis

Analyse the samples for total N and $^{15}$N enrichment.
5.11. Other parameters to be recorded at the beginning and at the end of the experiment

- pH (1:5 soil:CaCl₂)
- Total N (%)
- Organic C (%)
- Water holding capacity (See 5.12)
- Available P (Bray 2) or any other recommended method for the soil
- Exchangeable cations - Na, K, Mg, Al, etc.
- CEC
- Any other

5.12. Measurement of soil moisture

Those of you who have neutron probes and capability for measuring the soil water holding capacity, access tubes may be installed at the beginning of the experiment. The measurement should be carried out in the usual manner at regular intervals. Others may follow the instructions given in Appendix I D for determining the water holding capacity.

5.13. Monitoring heavy metal contaminants in the soil

Records should be made before applying the sewage sludge, after applying the sewage sludge and at the end of the experiment. (See Appendix I for Protocol - Provided by Dr. McGrath).

5.14. Monitoring pathogens in the soil

The contents of pathogenic organisms (mainly Coliforms and Ascaris ova) are to be determined in sewage sludge before and after irradiation. (See Appendix III for Coliforms – provided by Dr. Chang) Where suitable facilities and expertise are not available, it is advisable to seek assistance of a microbiologist or the Department of Public Health for obtaining this data.
<table>
<thead>
<tr>
<th>Country</th>
<th>Name of Chief Investigator</th>
<th>Crop species</th>
<th>$^{15}$N Fert. Req./No</th>
<th>Sample analysis</th>
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<tr>
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<td>Bangladesh</td>
<td>Ahmed</td>
<td>Rice/wheat</td>
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<tr>
<td>India</td>
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<td>Maize/mungbean</td>
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<td>Tomato/chillie</td>
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<td>AGREEMENT HOLDERS</td>
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<td>UK</td>
<td>McGrath</td>
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<tr>
<td>USA</td>
<td>Chang</td>
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</tbody>
</table>
Fig. 1 - Experimental field layout:

No SS 100 kg N/ha

Main plots

Non-irrad. SS

Irrad. SS

Sub-plots

4 hps

1 m

3 m

2.0 m
Row spacing = 0.25 m
Plant spacing = 0.20 m

\[ 15N \text{ area} = 1.50 \times 1.00 = 1.50 \text{ m}^2 \]

In treatments with sewage sludge it should be applied over the entire sub-plot.

Fig. 2 - Detailed diagram of a single sub-plot
Soil and Sewage Sludge digestion using Aqua Regia

(4:1 HCL/HN0₃ by volume)¹

1) Weight 0.5g of dry, ground soil into a boiling tube.

2) Add 5ml of aqua regia (4mls A.R. grade HCl first then 1 ml A.R. HN0₃) and leave to predigest overnight (or for at least 2 hours).

3) Digest in heating blocks using the following programme on an automatic timer/temperature controller. (Step 1 may be omitted if samples left in acid overnight.)

<table>
<thead>
<tr>
<th>Rise rate (secs °C/hrs)</th>
<th>Dwell (hrs)</th>
<th>Dwell Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>180</td>
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</table>

4) After cooling, add 5cm³ 20% HCL and reheat at 80°C for 30 minutes. Make nearly upto 20m1 mark with deionised water and allow to cool before making to volume. This will give final dilution of 5% HCl.

5) "Whirlimix" contents of the tube then filter through a Whatman no. 40 filter paper into a clean polyethylene vial for analysis.

6) Determine Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn by atomic absorption spectroscopy.

METHOD FOR AMMONIUM NITRATE EXTRACTABLE METALS

All materials/containers should be made of either polyethylene, polypropylene or Teflon/PTFE and acid washed before use.

1. Dry soil over night at 40 °C and sieve to < 2mm.
2. Weigh 20g soil in triplet into 3 separate containers.
3. Add 50ml of 1M NH₄NO₃ (AR grade) solution.
4. Shake suspension for 2hrs in a reciprocal shaker at 20 °C
5. After shaking, leave the soil suspension to stand for 5 minutes, then filter through a Whatman No. 42 filter paper into a clean bottle.
6. Acidify the filtrate to give 0.2% HNO₃, for analysis by ICP and/or GF-AAS.
Appendix I-C

Digestion of Plant Material using
Nitric/Perchloric Acids

*1 Weigh 0.250 - 0.500g dry, ground plant material into a boiling tube.
* if using 1g of sample then use 10ml mixed acids.

*2 Add 5ml mixed acids (85% HNO₃ s.g. 1.42 and 15% (60%) HCLO₄), whirlimix and leave to predigest for at least 4 hrs (overnight ideally) in a fume cupboard.

*3 Digest in heating block using the following programme (for new block).

<table>
<thead>
<tr>
<th>°C/Hr</th>
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<th>Hrs</th>
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<td>100</td>
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<td>190</td>
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<tr>
<td>Prog. 5</td>
<td>Step</td>
<td>25</td>
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</table>

*4 After cooling, add 5ml 20% HCl (to give final vol. of 5%), and rewarm at 80°C for 30 minutes, make nearly up to 20ml mark with deionised, distilled water and rewarm for a further 30 minutes.

*5 Whirlimix and allow to cool. Sample is now ready for analysis.
Determining Water Holding Capacity (WHC) of Soil

Accurately record all readings.

1. Air dry soil and sieve to < 2mm (moist soil can also be used).
2. Weigh Whatman No. 42 filter paper and record the weight. Use in step 3.
3. Weigh 50g of soil in triplicate onto 3 separate weighed Whatman No. 42 filter paper (15 cm dia).
4. Place filter paper with soil sample into a plastic funnel with a collecting container underneath.
5. Thoroughly soak the soil with dd water until there is approximately 2mm of water on the surface. The soil should at this point be fully saturated with water.
6. Cover the funnels with cling film to prevent evaporation from the soil surface.
7. Allow the soil to drain overnight –i.e., about 16 hrs.
8. Weigh the wet soil.
9. Place wet soils in an oven at 105 °C overnight or for 12 hrs to dry.
10. Weigh the dry soil again.

Calculations

\[
% \text{WHC of soil} = \frac{\text{Wt of water retained by soil} \times 100}{\text{Wt of oven dry soil}}
\]

\[
= \frac{(\text{Wt of wet soil + FP}) - (\text{Wt of oven dry soil + FP}) - (\text{Wt of water held by FP}) \times 100}{(\text{Wt of oven dry soil + FP}) - (\text{Wt of dry FP})}
\]

The % WHC is the amount of water held by the soil when it is 100% saturated.

E.g., If the %WHC of a soil is 40%. This means that 100g of saturated soil contains 40g water.
SOIL COLLECTION AND PREPARATION

1) Soil for microbiological studies is normally collected from the 10-23 cm depth for arable soils and 0-10 cm depth for grassland. Sampling is usually with an auger or soil corer, although an ordinary spade may sometimes be the best tool. If a large area of land is to be sampled, soil samples should be taken at intervals along the lines of a series of imaginary zigzags or “W”s across it. If the sampling area of a plot is restricted in some way, e.g. perhaps only the edges are permitted to be sampled, every effort should be made to ensure that the pooled soil samples give as representative a bulk soil sample as is possible.
NB: Always collect at least 20% more soil than you actually require; more if the soil is very stony.

2) It has been recommended that soils for use in experiments involving pesticide testing should not be collected when there has been no rainfall for 30 days (Anderson, 1987). Given that this is a fairly rare occurrence in the UK, this should not be a common problem. If a period of drought and a sampling date should coincide, the researcher may probably use his/her discretion as to whether or not to go ahead with the soil collection, depending on the nature of the experiment for which it is required. In any case, even if the soil is dry when sampled, it can normally be sieved somehow. Once sieved and carefully adjusted to about 40% WHC, the biomass can be reliably measured after about 7. d conditioning incubation. The measurements will be of biomass in the conditioned soil rather than in the soil as sampled. However, there are theoretical reasons and empirical observation which strongly indicates that the measurements may not be in serious error.

3) Once the soil has been collected and brought back to the Lab, it should be spread out on a piece of plastic sheeting, and large pieces of plant material, animals and stones, it should be stored at 4°C until preparation.

4) Most soils at field moisture content are usually too wet to be sieved immediately after collection, without smearing or rolling of the soil occurring. The soil must therefore be dried to a moisture content where sieving is possible without these problems occurring. The soil should be spread out as thinly as possible, while remaining as a continuous and even layer. This is particularly important around the edges of the soil. Larger lumps of soil should be carefully broken up by hand, so that the pieces of soil are more or less of the same size. If the soil does not break apart along natural fracture lines, but simply stretches or smears, then it must be left to dry intact, until the moisture content is such that the lumps can be broken apart like this. The ideal moisture content of a soil is that which allows it to crumble easily when A sieved, whilst still being moist enough to support the whole of its original microbial biomass.

5) While the soil is drying it MUST be turned (i.e. mixed) regularly, so that no part of the soil becomes too dry. The soil at the edges dries the quickest, and can become air-dry while the soil in the centre is still too wet to sieve. A piece of soil that is not in contact with others will dry quicker still, hence the importance of keeping the soil as a continuous layer at all times. If a few soil aggregates do become air-dry, they should be discarded. If, by accident, a significant proportion of the soil becomes air-dry, then it will be useless for experimental purposes, and a fresh sample of soil will have to be collected. If the soil is being dried in a glasshouse, it should be checked frequently on sunny days. Do not allow the soil to be in direct sunlight (make re the blinds are down). If the temperature of the glasshouse becomes very high (> 25°C or so), the soil will have to be removed to a cooler place, or returned to storage. If the soil takes longer than one day to reach the proper moisture consent (as is usually the cast), it should be returned to storage overnight, or, if it is very wet or in a permanently shady and cool position, it may be simply covered by another sheet of plastic.

6) Once the soil has reached the necessary moisture content, it can then be passed through a 2 mm mesh sieve. This is best achieved with the forgers or the back of a scrubbing brush. The entire soil sample must be sieved - do not discard any soil which will not pass readily through the sieve. It may be necessary to catty out the drying and sieving process several times.
NB: For soils of a very high clay content, it may not in fact be possible to pass mare than a small proportion of the soil through the sieve, whatever its moisture content. If this is the case, the remaining soil can be air-dried, then sieved using a mechanical roller mill. This soil should then be re-moistened with distilled water.
using a hand-held sprayer and then mixed thoroughly with the soil that was sieved by hand. The soil should then be incubated at 25°C for at least 7 days before continuing with the procedure, as described below. Alternatively, the researcher may consider whether the soil would better be broken up by hand as much as possible, and used in an unsieved slate (Ocio and Brookes, 1991). If this situation is encountered you are advised to seek a second opinion before proceeding with either option.

7) Once the soil has been sieved, it must then be cleaned of as much plans remains, animals, stones, etc., as is possible, which is best done with a pair of forceps, on a small sub-sample of soil at a time. It is advisable to keep a hand-held sprayer of distilled water to hand, to prevent the soil from drying out any further.

8) When the soil has been picked over, take sub-samples for determination of moisture content and water holding capacity (WHC), and store the soil at 4°C until use. It is advisable to use the soil as quickly as possible, and if storage is unavoidable, it should not normally exceed three months.

9) When the soil is to be used, spread it out thinly on some plastic sheeting, and using a hand-held sprayer, add enough water to adjust the soil to 40% WHC (the moisture content of soils previously stored at 4°C should be redetermined). This should be done gradually, by wetting the surface of the soil, mixing it gently but thoroughly, then applying more water, mixing, and so on until the required amount of water has been added. This is best determined by measuring the weight loss of the sprayer, rather than by filling the sprayer with the exact volume of water.

10) Cover the soil with a plastic sheet and leave for 1-2 hours. Put the soil in a plastic bag, insert a folded tissue in the top of the bag to permit air exchange, and tie loosely with a rubber band. Place the bag in an air-tight metal drum, together with a 100 ml jar of soda lime and several bottles containing distilled water. Incubate the soil at 25°C for 7-10 days. The soil is now ready for experimental use.

Post script

The above notes might seem, to some, a statement of the obvious. However, mishandling of soil is one of the largest sources of error in terms of reproducibility of results and comparisons with the work of others. Soil is our working material and the subject of our research. It is important to learn and apply the procedures involved in soil preparation, which should be regarded as an essential part of experimental procedure. Failure at this stage guarantees failure of the entire experiment.

References

# BCR SLUDGES

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<th>CRM 148</th>
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<td>Sewage sludge</td>
<td>Sewage sludge of industrial origin</td>
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<td>(4.1) µg/g</td>
<td>µg/g</td>
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Values in brackets are not certified.  
The certification report describes in detail the analytical procedure to obtain the aqua regia soluble content of the elements.  
The samples are in the form of powder in bottles containing 50g approximately.  

CRM 144 is recommended for sludges
## BCR SOILS

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<td>1 272 ± 30 µg/g</td>
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**Aqua regia soluble**: 1

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<td>(70) µg/g</td>
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**Matrix**

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**Loss at 900 °C**

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<td>0.2065</td>
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Values in brackets are not certified.

1 Details of the analytical procedure to obtain the aqua regia soluble content of the elements are given in the certification report.

The samples are provided in units of 50g in glass bottles.

**CRM 143 is recommended for soils.**
NIST Peach Leaves

Instructions for Drying: Samples of this SRM must be dried only by one of the following two procedures.

1. Drying in a desiccators at room temperature (approximately 22 °C) for 120 hours over fresh anhydrous magnesium perchlorate. The sample depth should not exceed one cm.

2. Freeze drying for 24 hours at a pressure of 13.3 Pa or lower and a shelf temperature of -5 °C or lower after having frozen the sample (not to exceed one cm in depth) at -40 °C or lower for at least one hour. At the end of the 24-hour period, samples are placed immediately in a desiccator with fresh anhydrous magnesium perchlorate. Samples are weighed after allowing a minimum of four hours to establish temperature equilibrium.

Note: Vacuum drying at room temperature and oven drying at elevated temperatures have resulted in excessive weight losses and therefore are not recommended.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1547 were tested for homogeneity by instrumental neutron activation analyses. No evidence of chemically significant inhomogeneity was observed.

Table 1 – Certified Concentrations of Constituent Elements

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<th>Concentration, µg/g</th>
<th>Concentration, wt. Percent</th>
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<td>Calcium</td>
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<tr>
<td>Magnesium</td>
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<tr>
<td>Nitrogen (Total)</td>
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<td>Potassium</td>
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<td>Boron</td>
<td>29 ± 2</td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>360 ± 19</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>3.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.87 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>98 ± 3</td>
<td></td>
</tr>
<tr>
<td>Mercury</td>
<td>0.031 ± 0.007</td>
<td></td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.060 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>0.69 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>0.120 ± 0.009</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>24 ± 2</td>
<td></td>
</tr>
<tr>
<td>Strontium</td>
<td>53 ± 4</td>
<td></td>
</tr>
<tr>
<td>Vanadium</td>
<td>0.37 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>17.9 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

Certified Concentrations and Uncertainties: The certified concentrations are equally weighted means of results from two or more analytical methods or the mean of results from a method of known accuracy. In the case of two or more methods, each uncertainty is the sum of a 95% confidence limit and an allowance for systematic error between the methods used. In the case of a method of known accuracy, each uncertainty is the sum of a 95% confidence limit and the known systematic error of the method.
## NIST Citrus Leaves

### Table 1. Certified Values of Constituent Elements

#### Major and Minor Constituents

<table>
<thead>
<tr>
<th>Element</th>
<th>Content$^1$ (Wt. Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>3.15 ± 0.10</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.82 ± 0.06</td>
</tr>
<tr>
<td>Sulphur</td>
<td>0.407 ± 0.009</td>
</tr>
</tbody>
</table>

#### Trace Constituents

<table>
<thead>
<tr>
<th>Element</th>
<th>Content$^1$, µg/g</th>
<th>Element</th>
<th>Content$^1$, µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>92 ± 15</td>
<td>Manganese</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Arsenic</td>
<td>3.1 ± 0.3</td>
<td>Mercury</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Barium</td>
<td>21 ± 3</td>
<td>Molybdenum</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.03 ± 0.01</td>
<td>Nickel</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.8 ± 0.2</td>
<td>Rubidium*</td>
<td>4.84 ± 0.06</td>
</tr>
<tr>
<td>Copper</td>
<td>16.5 ± 1.0</td>
<td>Sodium</td>
<td>160 ± 20</td>
</tr>
<tr>
<td>Iodine</td>
<td>1.84 ± 0.03</td>
<td>Strontium*</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Iron</td>
<td>90 ± 10</td>
<td>Zinc</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Lead*</td>
<td>13.3 ± 2.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$Based on dry weight: For drying instructions, see the section of this certificate on Instructions for Drying. The uncertainties are based on judgment and represent an evaluation of the combined effects of method imprecision, possible systematic errors among methods, and material variability for samples weighing 500 mg or more.

*For those elements determined by definitive methods, the uncertainties are given as 95% statistical tolerance intervals. See The Role of Standard Reference Materials in Measurement Systems, NBS Monograph 148, 1975 p. 14.
INSTRUCTIONS FOR PROPER PREPARATION AND IDENTIFICATION OF SAMPLES SENT TO THE IAEA LABORATORY FOR $^{14}\text{N}/^{15}\text{N}$ RATIO/TOTAL NITROGEN ANALYSES

I. PLANT SAMPLES

1. The samples have to be oven-dried and finely ground (particle size < 1 mm), approximate amount: 1 g.

2. The coding of the samples has clearly to indicate:
   a) the treatment number,
   b) the replicate number, and
   c) the origin of plant.
   e.g.: 2 R1 leaves
   or: 3 R2 stem
   treatment replicate

3. A list of treatments has to be attached to the samples, indicating e.g.:
   - the crop species,
   - the rate of N-15 fertilizer application,
   - the approx. N-15 enrichment of fertilizers,
   - the time of application,
   - the time of harvest(s), etc.

4. The N-15 labelled fertilizer(s) used for this specific experiment has(ve) to be sent together with the plant samples.
   In case of solid (homogeneous) fertilizer you must indicate:
   a) the chemical form, e.g. ammonium sulfate, urea, etc.
   b) the approximate at. % N-15 enrichment.
   A minimum of 100 mg material is needed.
   For fertilizer standards in the form of solutions, we need additionally the nitrogen concentration e.g.: 10 mg N/2 ml or 20 g urea/200 ml.
   The concentration should not be below 5 mg N/ml.
   A minimum of 1 ml is required.

5. Save an adequate amount of each sample in your laboratory for future analyses if needed.

II. SOIL SAMPLES

We can only accept, upon previous request and authorization through the Technical Officer of the project, soil samples (oven-dried and finely ground) for analyses provided that they contain at least 0.1% total Nitrogen
Coding instructions are the same as for plant samples.

III. EXTRACTS

Exceptionally, we may accept extracts, provided that they meet the following requirements:
   a) NO BORIC ACID EXTRACTS
   b) The N concentration has to be known and should be of at least 5 mg N/ml; approximate volume: 1 ml.
First Research Co-ordination Meeting
FAO/IAEA Co-ordinated Research Programme on
“The Use of Irradiated Sewage Sludge to Increase Soil Fertility, Crop Yields and to Preserve the Environment”

Vienna International Centre, Vienna, Austria
10 – 14 July 1995

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