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Fertilizers and soil conditioners — Determination of humic and hydrophobic fulvic acids concentrations in fertilizer materials

Engrais et amendements minéraux basique - Détermination des acides humique et fulvique

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 134, *Fertilizers and soil conditioners*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Humic substances are present in all ecosystems: oceans, rivers, lakes, and top soils. Quantifying the amount of humic material present in these systems is essential for academic research and commercial applications, specifically agricultural soil and plant management.

The increased use of humic substances in agriculture has generated intense interest among producers, consumers, and regulators for a reliable method for quantification of the active ingredients in raw humic ores and commercial fertilizer products; specifically humic and fulvic acids. As both commercial trade and regulation of humic products are based on percentage (%) of the humic and fulvic acids in commercial humic products, use of % units instead of SI units is warranted, therefore incorporated into this standard.

This document establishes a method for the determination of humic acids (HA) and acidic hydrophobic fulvic acids (HFA). The method is based on an existing preparative procedure use by the International Humic Substances Society (IHSS) for extracting high purity HA and HFA from soil samples[1], which is a modified form of the "classical" technique described in detail by Stevenson[2]. The "classical" methods and the IHSS method were developed as preparative methods for the fractionation of soil organic matter; they were not intended to be used as quantitative analytical methods. The classical method of extracting humic acids and *fulvic acids* from soil humus utilize a "strong base" to extract the alkaline soluble materials, and then the alkaline extract solution is acidified to flocculate the humic acids, which appear to precipitate out of solution. The remaining substances in solution after alkaline and acid treatment were called *fulvic acids*.

This method modifies the "classical" technique in a number of ways:

— it determines the quantity of humic substances on an "ash free" basis (mineral salts excluded);

— the alkali extraction is done under anoxic conditions to reduce oxidation of the analytical sample during extraction;

— it defines the materials that are soluble in both alkali and acid as the Fulvic Fraction;

— it can differentiate products containing certain non-humic materials that some manufactures claim to contain humic substances;

— it further defines HFA as materials of low sulfur content[3] that bind to a hydrophobic resin at pH 1[4][5][6], instead the classical, and perhaps more common definition, for *fulvic acids* as materials that are defined as soluble in both acid and alkali solution. This stricter definition is necessary to distinguish HFA from mineral salts, polysaccharides, amino sugars, amino acids, proteins, acids, and carbohydrates that are extracted along with humic substances when using the "classical" method[1][4].

See Annex B for information on ISO/CD 19822 interlaboratory study.

Fertilizers and soil conditioners — Determination of humic and hydrophobic fulvic acids concentrations in fertilizer materials

1 Scope

This document specifies the procedure for the analysis of humic acids and hydrophobic fulvic acids which is applicable to dry and liquid materials used as ingredients in commercial fertilizers, soil amendments, and geological deposits.

2 Normative References

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
hydrophobic fulvic acids
HFA
materials composed of less than 0,75 % elemental sulfur (S) that are soluble in aqueous alkaline and acid solution and are adsorbed at pH 1 onto a polymeric adsorbent resin of moderate polarity. The resin is of a type designed for adsorption of amphiphilic compounds having molecular weights typical of fulvic acids

3.2
fulvic fraction
alkali extracted portions of humic substances that are soluble in both alkali and acid aqueous solutions

3.3
humic acids
HA
alkali extracted humic substances that are insoluble in strongly acidic solution and will precipitate from the alkali extract in acid solutions of pH 1

3.4
humic substance
major organic constituent of natural organic matter consisting of complex heterogeneous mixtures of carbon-based substances formed by biochemical reactions during the decay and transformation of plant and microbial remains

3.5
lignosulfonates
amorphous light to dark brown powder or liquid derived from the sulfite pulping of softwoods. The lignin framework is a sulfonated random polymer of three aromatic alcohols: coniferyl alcohol, p-coumaryl alcohol, and sinapyl alcohol, of which coniferyl alcohol is the principle unit

4 Principles

4.1 This method determines ash-free quantities of HA and HFA gravimetrically after separation from their matrix.

4.2 The method of extracting HA and HFA utilizes a strong base to extract the alkaline-soluble materials, and then, after removal of non-soluble components, the alkaline solution is acidified to flocculate the HA.

4.3 The liquid supernatant remaining after the removal of the HA is called the Fulvic Fraction. The Fulvic Fraction, which can contain Hydrophobic Fulvic Acids (HFA), is treated to determine the quantity of HFA in the Fulvic Fraction by selective adsorption onto a methacrylic-ester resin designed to separate the HFA from non-humic compounds. The material retained by the hydrophobic resin is referred to in the literature as the hydrophobic acid fraction of soluble organic matter[3].

5 Warnings

5.1 Requirements

5.1.1 Good laboratory practices

Related standards (e.g. ISO/IEC 17025) should be followed at all times in regards to personal protective equipment (safety glasses, handling strong acids, hydrochloric acid) and alkali (sodium hydroxide).

5.1.2 Moisture control

Humic and fulvic acids are hygroscopic materials; it is critical to prevent absorption of moisture during the handling of dried materials.

5.3 Lignosulfonates

Lignosulfonates will damage the hydrophobic resin. This analytical method cannot differentiate between hydrophobic fulvic acids and lignosulfonates, therefore pre-screening for the presence of lignosulfonates is recommended for liquid products of unknown origin.

See Annex A.

5.4 Temperature control

Do not exceed 65 °C when drying the humic and fulvic analytes. The analytes are subject to decomposition at higher temperatures.

6 Reagents

6.1 Sodium hydroxide solution, 0,1 M, dilute 3,99 g of 99,99 % purity NaOH in 1 l of deionized water.

6.2 Sodium hydroxide solution, 0,5 M, dilute 19,99 g of 99,99 % purity NaOH in 1 l of deionized water.

6.3 Hydrochloric acid solution, 6 M, dilute 12 M HCl with an equal part of deionized water.

6.4 Hydrochloric acid solution, 1 M, dilute 83,3 ml of 12 M HCl with 1 l of deionized water.

6.5 Hydrochloric acid solution, 0,1 M, perform a 1:10 dilution using 1 M HCl prepared in 6.4 with final volume of 1 l using deionized water.

6.6 Nitrogen gas (UN1066) 99,9 % purity.

6.7 Methacrylic-ester resin, 40–60 mesh, approximately 0,79 ml·g^{-1} pore volume, 225 Å mean pore size, 160 m^2·g^{-1} surface area, for adsorption of materials up to 150 000 MW, e.g. Supelite DAX-8 Resin, or any other available resin meeting equivalent properties.

6.8 Amberlite IR-120 strong cation exchange resin, hydrogen form.

6.9 Deionized water[8].

6.10 Acetone.

7 Apparatus

7.1 Analytical balance with draft guard: capacity 210 g, with readability to ±0,000 1 g.

7.2 Drying oven, capable of 120 °C, precision ±3 °C.

7.3 Centrifuge, minimum relative centrifugal force 1 500 × g, capable of 3 900 × g.

7.4 4 ml to 50 ml or 250 ml polyethylene or HDPE centrifuge tubes, or heavy duty high temperature resistant centrifuge tubes capable of 600 °C (for example; Kimble-Chase, catalog number 45212-50 KIMAX).

7.5 4 ml to 100 ml wide-form crucibles (for example: Fisher Scientific catalog number FB-965-M).

7.6 Rotary evaporator 400 ml capacity.

7.7 Magnetic stir plates and 5 cm to 7 cm long magnetic stir bars.

7.8 pH meter and electrode.

7.9 Electrical conductivity meter with probe having a calibrated cell constant of approximately one, as determined using standard protocols.

7.10 Spectrophotometer, capable of measuring ±0,005 absorbance units at 350 nm.

7.11 Peristaltic pump with a minimum flow rate of 1,2 ml·min^{-1} and tubing.

7.12 Muffle furnace.

7.13 Rotating shaking mixer.

7.14 Desiccator with silica gel (or its equivalent) as desiccant.

7.15 Erlenmeyer flask, 1 000 ml.

7.16 Beaker, 4 l.

7.17 Graduated cylinder, 1 000 ml.

7.18 Glass chromatography column, 4 cm × 25 cm for DAX-8 resin.

7.19 Glass chromatography column, 5 cm × 60 cm for IR120 H+ exchange resin.

7.20 Ceramic mortar and pestle.

7.21 Sieve, 74 µm (#200 US mesh).

7.22 Parafilm®[1)].

8 Preparing crucibles, drying, and weighing samples

8.1 Preparing crucibles

8.1.1 If using new crucibles, first wash them with acetone and then dry them in an oven at 105 °C for 2 hours.

8.1.2 Prepare previously used crucibles by washing in acetone, then firing them in a muffle furnace at 500 °C for 2 hours. Cool the crucibles in a desiccator to room temperature. Remove from desiccator when cool, record weight of the crucibles to four decimal places.

8.2 Drying and weighing solid analytical samples

8.2.1 If the analytical sample is a solid material, crush and screen approximately 5 g of the analytical sample to ≤75 µm making sure that the powder becomes well homogenized.

8.2.2 Transfer approximately 5 g analytical sample to a 100 ml crucible prepared according to 8.1.

8.2.3 Place the analytical sample in a drying oven for 24 h at a temperature of 62 ± 3 °C. (do not exceed 65 °C). If any clumping of the sample occurs during drying, break up the clumps with a glass rod. Continue drying until the sample is dried to a constant weight. This can take up to 24 h;

8.2.4 After achieving constant weight, remove the analytical sample from the drying oven and immediately place in a desiccator to cool.

NOTE Both humic and fulvic acids are hygroscopic materials, it is critical to prevent absorption of moisture during the handling of these materials.

8.2.5 Weigh out approximately 2,5 g test portion from the dried analytical sample in a pre-weighed crucible prepared according to 8.1, taking precautions to prevent moisture adsorption while handling. Record the test portion + crucible weight to four decimal places. Proceed to 9.1 immediately or return the crucible with test portion to the desiccator.

8.2.6 Determine the weight of the test portion by subtracting the weight of the crucible from the test portion + crucible; record the result as the Test Portion Dried Weight.

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1) Parafilm® is a trademark of Bemis NA, Neenah, Wisconsin, USA. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results. It is commonly used for sealing or protecting vessels. It is a ductile, malleable, waterproof, odorless, translucent and cohesive thermoplastic.

8.3 Drying and weighing liquid samples

8.3.1 For liquid analytical samples, thoroughly homogenize the analytical sample by shaking the sample for one minute in the container in which the liquid was delivered. Weigh out approximately 5 g test portion from the liquid analytical sample to four decimal places. Record this as the Liquid Test Portion Weight.

For liquid analytical samples with an expected HFA content <1 %, use a test portion weight of 10 g.

8.3.2 Follow steps 8.2.2 to 8.2.6 for drying the sample and weighing the test portion of the sample.

9 Extraction procedure

9.1 From this point on, the method is the same for both solid and liquid samples.

Transfer the prepared test portion of the analytical sample to a 1 l Erlenmeyer flask containing a 5 cm to 7 cm long magnetic stir bar. Add 0,1 M NaOH, constantly stirring on a stir plate, to a final volume of 1 l. Evacuate the head space with N_2 and cover with parafilm (or seal the flask with a similar material). Then mix vigorously on a stir plate (e.g. 300 rpm to 400 rpm). Stir liquid samples for 1 hour; stir the solid samples for 16 hours to 18 hours.

NOTE For solid samples, it can be convenient to perform this step late in the day so the solid samples can be stirred overnight.

9.1.1 After stirring, remove the flask from the stir plate, transfer to suitable centrifuge tubes and centrifuge the entire volume at 3 900 × g for 30 minutes to separate any insoluble material from the dissolved materials in the alkaline extract. Carefully transfer the alkaline extract to a clean 1 l Erlenmeyer flask containing a magnetic stir bar. Discard the insoluble materials.

9.1.2 While gently stirring the solution, adjust the pH of the alkaline extract solution to flocculate the HA from the acid soluble materials by adding 6 M HCl (1:1) drop-wise to the alkaline extract, until 1,0 ± 0,1 pH is reached.

9.1.3 Cover the flask with parafilm and mix for 1 h. After 1 hour, check the pH and readjust to pH 1,0 ± 0,05 with additional 6 M HCl, if necessary. If the pH falls below 0,95, adjust the pH back to 1,0 ± 0,05 with 0,5 M NaOH solution. Continue mixing the acidified extract until it stabilizes at pH 1,0 ± 0,05 for exactly 5 minutes. Do not let the acidified extract sit for longer than 5 minutes after the pH stabilizes. Remove the pH electrode.

9.2 Separation of HA

9.2.1 Once the pH is stable, remove the flask from the mixer, and cover the flask with parafilm. Allow the pH-adjusted extract to sit undisturbed for 4 hours ±5 minutes (do not exceed 4 hours). This stage is critical to prevent further partitioning of the HA and HFA constituents. The flocculated HA will drop out of solution.

9.2.2 Immediately centrifuge the extract solution at 3 900 × g for 30 minutes using pre-weighed 50 ml centrifuge tubes to recover the flocculated humic acids portion. Decant the supernatant (Fulvic Fraction), being careful not to include any of the flocculated HA. Typically, about 500 ml of the clarified extract can be decanted without disturbing the flocculated HA. If hydrophobic fulvic acids (HFA) analysis is to be performed, decant the fulvic fraction into a clean 1 l Erlenmeyer flask. [Alternatively: using heavy duty high temperature centrifuge tubes instead of plastic centrifuge tubes eliminates the need to transfer the flocculated HA to a crucible, decreasing labor and increasing precision.]