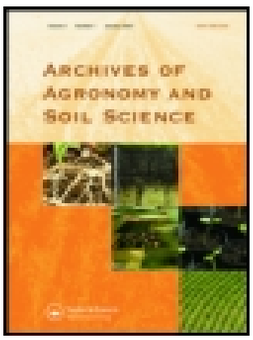

Archives of Agronomy and Soil Science

ISSN: 0365-0340 (Print) 1476-3567 (Online) Journal homepage: https://www.tandfonline.com/loi/gags20

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To cite this article: Karim Shahbazi, Mostafa Marzi & Shida Tabbakhian (2019): The Comparative Evaluation of Humic Acid determining Methods in Humic-Based Commercial Fertilizers, Archives of Agronomy and Soil Science, DOI: 10.1080/03650340.2019.1575511

To link to this article: https://doi.org/10.1080/03650340.2019.1575511

Accepted author version posted online: 26 Jan 2019.

Publisher: Taylor & Francis

Journal: *Archives of Agronomy and Soil Science*

DOI: 10.1080/03650340.2019.1575511

The Comparative Evaluation of Humic Acid determining Methods in Humic-Based Commercial Fertilizers

Karim Shahbazi, Mostafa Marzi, Shida Tabbakhian

Education and Extension Organization (AREEO), Tehran, Iran. Kshahbazi@swri.ir.
Department of soil science, University of Tehran, Tehran, Iran. Mostafamarzi@ut.ac.ir

Education and Extension Organization (AREEO), Tehran, Iran. Shidatabbakhian@yahoo.com.

Abstract

The increased use of humic acid (HA) as a plant-growth stimulant in recent years has led to an intense interest in finding an accurate and reliable method to quantify it in an acceptable manner among producers and consumers. Today, there are four common laboratory methods for the determination of HA, including the CDFA, Colorimetric Method, ISO 5073, and NSM. To date, there has been no comparison among these methods to evaluate the quantity of HA across a wide range of commercial fertilizers containing various additives. In the present study, the four aforementioned methods were used to determine the HA content in 22 samples containing a wide range of physical and chemical properties. According to NSM's principles the method and their consistency with the classical method, it was used as a reference method to make comparison among the other methods. Compared to the NSM, CDFA, and ISO 5073 methods that underestimate the HA content (13.8 and 1.5%, respectively), however, it has been demonstrated that the colorimetric method overestimated the HA content by 64.2%. The low ratio of extractant to sample and the presence of soluble organics are the main reasons for under and over estimation in CDFA and colorimetric method respectively.

Keywords: colorimetric method, gravimetric methods, humic acid, titrimetric method.

Introduction

Humic substances (HSs) are defined as complex poly-disperse, macro-molecule, and heterogeneous mixtures that are produced by the biodegradation of plant and animal residues. They are abundantly found in nature. The different fractions of HSs (humic acid (HA), fulvic acid (FA), and humin) have significant effects on plant growth and soil fertility, and they have recently been produced in large quantities and utilized as fertilizers and growth stimulants in order to increase crop production. Therefore, these substances are very important in crop production. Regarding to the complexity and heterogeneity of HSs, especially humin, human knowledge is limited with respect to its chemical structure (Nebbioso et al. 2015; Tadini et al. 2015). These substances include different parts that are based on a motif of aromatic structures, possibly due to the presence of black carbon or coal residues as well as aliphatic structures, protein substances, polysaccharides, and lipids (Mao et al. 2011; DiDonato et al. 2016). Humic substances have been practically defined by specific fractionation methods (Stevenson 1994). This operational definition inevitably leads to the fact that HA and FA content is dependent on the type of extraction, rather than their intrinsic HSs properties. Thus, the specific conditions in any isolation method (which affect the chemical behavior of humic materials) may lead to changes in the classification of these substances, in terms of HA and FA (Stevenson 1994).

Humic substances are divided into three major fractions, according to their solubility in alkaline and acidic solutions including; 1- FA (soluble in both alkaline and acidic mediums, with molecular weights of less than 2000 Dalton (Da); the mean length and diameter of the macromolecules are 60 and 2 nm, respectively), 2- HA (soluble in alkali and insoluble in an acidic medium, with molecular weights approximately ranging from 2000–5000 Da; its chemical structure often contains phenols and long carboxylic fatty acids, which is reported to be more hydrophobic than FA), and humin (insoluble in both

acid and alkali media; the major part of humin is made up of tar, which mixed with fatty acids and HA)(Allard 2006; Giovanela et al. 2010; Sutzkover-Gutman et al. 2010; Katsumi et al. 2016). Humin and HA have been considered as the main components of HSs and they have the same structural and decomposition properties (Schulten and Schnitzer 1997). Humic substances vary in terms of their composition, structure, molecular weight, and position of functional groups, which depends on their origin and age. Naturally, the elemental composition of HSs contain 40–60% carbon, 30–50% oxygen, 4–5% nitrogen, 1–2% sulfur, and 0–3% phosphorus (Sutzkover-Gutman et al. 2010).

Humic substances extraction is carried out by different extractants, i.e., alkaline solutions (NaOH, sodium pyrophosphate ($Na_4P_2O_7$), sodium fluoride (NaF), sodium hexametaphosphate $(NaPO_3)_6$, sodium orthophosphate (Na_3PO_4), sodium borate ($Na_2B_4O_7$), NaCl, NaBr, and NaI) and cation exchange resins (Aiken 1985; Shirshova et al. 2006). The extraction of humic materials with alkaline solutions, which is known as the classic method, results in the highest amount of HSs. Although the alkaline extraction of HSs may stimulate oxidation, hydrolysis, and cleavage reactions of the humic structure depending on the source, conditions, and duration of extraction (Shirshova et al. 2006), when it is carried out under the N_2 atmosphere, the damage caused by oxidation is reduced. At the same time, it was observed that mild extractants, such as sodium pyrophosphate, cation exchange resin, etc., significantly extract less HSs than alkaline solutions (Hayes 1985; Shirshova et al. 2006). Stevenson (1982) has stated that the ideal extraction method of HSs must meet the following four criteria: 1- the method should not alter the natural characteristics of the isolated material, 2- the extracted HSs must be free of inorganic contaminants, such as clay particles and polyvalent cations, 3- the extraction is ideal, if the acquired fractions of HSs represent

the entire molecular mass series, and 4- the extraction method must be applicable in different HSs throughout the world (Stevenson 1982).

As HA does not has a clear definition and chemical structure, no accurate analytical technique known to quantify it. Currently, four methods have been used in various laboratories to estimate the HA content; these methods include a colorimetric method based on a procedure developed by Mehlich (1984), a volumetric method (ISO 5073) (ISO 2013), and two weighting methods—one developed by the California Department of Food and Agriculture (CDFA) (CDFA 2009) and the other one is the New Standardized Method (NSM), which was developed by Lamar et al. (2014) and was based on a modification of the procedure detailed by Swift (1996).

The basis of all the four methods are based on HA solubility in dilute alkaline solution, but their main difference relies on the extraction and determination of HA in the extracted content. In the colorimetric method, the quantity of HSs is determined by comparing the intensity of the color of the alkaline extract of the sample to the intensity of the color produced by the extract of a standard amount of Aldrich HA. In the CDFA method, after extraction, the pH of the extract is acidified, and then, the precipitated HA is removed from the acidified extraction solution by centrifugation (containing FA and other soluble substances that may be present in the prototype). After that the HA is washed with acid distilled water, dried in an oven, and then weighed (Lamar and Talbot 2009). To determine the HA content, based on the volumetric method (ISO 5073), carbon in the HA extracts is oxidized with potassium dichromate, which is followed by the titration of excess dichromate with ammonium ferrous sulfate standard solution. To evaluate the HA content, the amount of carbon is multiplied by the ratio of HA to organic carbon (this coefficient must be determined by the appropriate method).

The NSM is intended to quantify HA and FA in solid and liquid commercial humic products, peat, soil, and humate-containing geological deposits. This method is based on a procedure for extracting HA and FA from natural materials. Similar to the Swift method, NSM is a modified form of the "classical" technique, which is described in detail by Stevenson (1982). The classical method of extracting HA and FA from soil humus utilizes a strong base to extract the alkaline-soluble materials; then, after the removal of the insoluble components, the alkaline solution is acidified to precipitate HA. The remaining substances in the solution after alkaline and acid treatment are called "FA" (Lamar et al. 2014). In the NSM, contrary to the classic method of initial extraction which is carried out under the N_2 atmosphere, HSs is determined based on the removal of ash (Lamar et al. 2014). Based on the presented definitions, the sources of error appear to be minimal in this method. Hence, in the present study, the NSM method is used to compare the other ones (CDFA, ISO 5073 and colorimetric method). The purpose of this study was to compare the different methods of measuring HA content in various commercial samples and, with different HA, in order to achieve a reliable method as well as to identify and analyze the sources of error among the methods.

Materials and Methods

Sampling

In this study, 22 samples containing HA were chosen from different sources that have high variety in terms of HA concentration as well as other compounds and chemical elements; then, they were subjected to testing for HA concentration by using four methods in two replicates.

Sample Preparation

Solid and dry samples were grinded into a fine powder, which was much easier to transfer through a 60-mesh sieve. For liquid samples, prior to weighing, they are

thoroughly mixed for one minute with a glass rod to ensure that all the particles that are probably at the bottom of the container are also mixed with the sample.

Analytical Methods

In the selected samples, organic carbon was determined by wet oxidation by using a potassium dichromate oxidizer (Walkley and Black 1934), electrical conductivity and pH were determined in a ratio of 1:10 in the extracted solutions. After burning at 500 °C, the samples were diluted by 6 N of nitric acid. The Ca, Mg, Na, K, Fe, and Si concentration were measured by using inductively coupled plasma emission spectroscopy (ICP-OES Perkin Elmer Optima 2100 DV).

CDFA Method

The specific weight of the sample (containing approximately 0.5 g of HA) was transferred to a 100 mL centrifuge tube and then 50 mL of 0.5 N NaOH was added. The cap was tightly closed and shaken in a mechanical shaker for 1.5 hrs for the solid and 30 minutes for the liquid samples. The suspension was then centrifuged at 2,000 RPM for 20 minutes and the supernatant was transferred to another pre-weighed centrifuge tube. In the next step, 10 mL of 1% (0.25 M) NaOH was added to the first tube and then the mixture was vigorously shaken and centrifuged. The supernatant was then added to the second tube and acidified by adding a sufficient amount of concentrated HCl (pH value $\leq$ 1). The tube was then centrifuged at 2,000 RPM for 20 minutes. The clear yellow solution was carefully decanted and discarded. To remove excess salts, the precipitate (i.e., the HA) was washed by the addition of 25 mL of distilled water, which was previously adjusted to a pH value $\leq$1 (with concentrated HCl), vigorously shaken to resuspend the precipitate, and then centrifuged. The process was repeated once again and finally a centrifuge tube containing HA was placed in the oven at 110 °C overnight

(if required, more time was acceptable to dry the sample). The tube was then cooled in a desiccator and then weighed. Then, by using the initial weight of the sample and the final HA, the percentage of HA was calculated (CDFA 2009).

The Method of Lamar et al. (2014) or NSM Method

A portion of the sample (which contains 2.5 g of HA for solids and 0.6–0.2 g for liquid samples) is weighed and transferred to a one-liter Erlenmeyer flask, which results in a final volume of 1 L by 0.1 N of NaOH. The upper atmosphere of the Erlenmeyer flask, which was covered with a parafilm, was replaced with N_2 gas. The solution was sharply stirred by a magnetic stirrer for 16–18 hrs for solid samples and for 1 hour for liquid samples. The suspension was then centrifuged at 2800 RPM for 10 minutes to separate the insoluble materials and the supernatant was transferred to another Erlenmeyer flask and the pH value was adjusted with 6 M HCl on 1 ± 0.05. The Erlenmeyer flask cap was covered by parafilm and shaken for 1 hour and the solution pH value was rechecked and, if necessary, adjusted at 1 ± 0.05 by concentrated HCl or 0.1 N NaOH to ensure pH stability. The electrode was kept inside the solution for five minutes. After that, the suspension was left to stand for 4 hrs to precipitate the HA. The supernatant was decanted and the remaining mixture was transferred to pre-weighed 50 mL centrifuge tubes and centrifuged for half an hour at 2800 RPM to separate the precipitated HA (if required, greater speed and time can be used). The supernatant was carefully decanted and the centrifuge tubes containing HA were placed in an oven at 90 °C for drying the HA to reach a constant weight (usually in 24 hrs). Then, the centrifuge tubes were cooled down and weighed and the weight of the HA extracted was measured by considering the difference between tube weight, and tube weight + precipitate. In the case of solid samples, the percentage of the moisture content was determined according

to the standard method; by regarding the effect of the moisture content on the initial weight, the dry weight of the sample was calculated (Lamar et al. 2014).

To determine the amount of ash, dried HA was transferred to a pre-weighed ceramic container, and the weight of the ceramic container and HA were taken into consideration. Then, the container was placed in a muffle furnace for 6 hrs at 500 °C. Finally, with respect to the residual ash weight and the initial HA, the ash percentage was calculated.

To measure the amount of impurity, insoluble substances in the alkaline solution were placed in the oven for a period of adequate time (usually 24 hrs) for drying; at last, the impurity percentage was calculated by dividing the dry weight of the remaining residue on the initial weight of the sample (Lamar et al. 2014).

Colorimetric Method

To determination of HA by colorimetric method, around 0.5–2 g of the sample (depending on the amount of HA) was transferred to a 200-mL volumetric flask and 100 mL of the extractant solution (0.2M NaOH, 0.003M DTPA, and 0.02M ethyl alcohol) was added and completely stirred; this was left to stand for 16–18 hrs. Then, without any stirring, 10 mL of the solution was transferred to a 50-mL volumetric flask and was made to fill the volume by distilled water (Lamar and Talbot 2009). The standard HA used in this study was obtained from the purification of the sodium humate salt that is produced by Aldrich Company (CAS 68131-04-4). To provide pure standards and by removing the soluble compounds that may have absorbance at the HA-measuring wavelength, the sodium humate salt was purified. Purification was carried out according to the classical method. In the classical method, 1 g of the HA sample was placed in a 1 L graduated cylinder and was filled to 1 L with 0.1 N NaOH. After mixing and partially dissolving the HA, the alkaline mixture was completely transferred to a 1-liter

Erlenmeyer flask and the sample was fully mixed by a magnetic stirrer. After mixing for 1–2 hrs, the alkaline extract was centrifuged to remove insoluble mineral and organic components. Both of these procedures were performed after evacuating the headspaces of the cylinder and the Erlenmeyer flask with N_2 and the cap was sealed by parafilm. The pH value of the alkaline extract was then acidified to reach a pH value = 2.0 with concentrated HCl; it was then left to stand for 24 hrs. The HA precipitate was removed from FA by centrifugation in 50-mL tubes. The aforementioned HA extract was purified by repeatedly washing with HCl/HF solution (5 mL of concentrated HCl and 5 mL of 52% HF in 990 mL of distilled water) to minimize the its ash content (Schnitzer, 1982). The amount of residual ash in the sample was determined by the dry combustion method. In order to prepare the standards, 1 g of Aldrich-purified salt, including its ash content, was transferred to a 200-mL volumetric flask; 100 mL of the extraction solution was added and then the other steps were performed, such as the sample on the standard and the sample left to stand for 16–18 h. By using this standard solution, dilute standards were prepared in 50-mL volumetric flasks as follows. In the first flask, 10 mL of the standard solution was added; in the second balloon, 5 mL of standard solution and 5 mL of extractant solution were added; in the third flask, 10 mL of the extracting solution was transferred by a pipette and was measured, in terms of volume, with distilled water. These flasks contained concentrations of 1000, 500, and 0 mg L^{-1} of HA, respectively. The spectrophotometer was adjusted at a wavelength of 650 nm and calibrated by standard solutions; the concentration of HA was measured (Lamar and Talbot 2009).

The ISO 5073 Method

Determination of HA by ISO 5073 method was performed by weighing of 0.2 g of the sample and transferring it to an Erlenmeyer flask. After that 150 mL of the extracting

solution (alkaline sodium pyrophosphate solution for measuring total HA and sodium hydroxide solution for measuring free HA) was added to Erlenmeyer flask and mixed. Then, a glass funnel was placed on the flask and heated in a boiling water bath for 2 hrs; it was frequently shaken during this time. After cooling at room temperature, the extract was transferred to a 200-mL volumetric flask. Then, the solution was filtered and 5 mL of the filtered solution transferred to the 250-mL Erlenmeyer flask; 5 mL of 0.4 M potassium dichromate solution was added thereafter. After that, 15 mL of concentrated sulfuric acid was added to the Erlenmeyer flask and heated in a boiling water bath for 30 minutes, and then, after cooling, it was made to reach a volume of 100 mL by deionized water. It was then titrated with 0.1% ammonium ferrous sulfate solution (which was standardized by a 0.1 molar potassium dichromate solution) in the presence of 1–10 *phenanthroline* as an indicator to reach a brick red. The aforementioned steps were also carried out for the blank (non-sample) solution (ISO 2013).

Data Analysis

For analyzing the data obtained from the different used methods, the method of Lamar et al. (2014) was considered as a reference method and the accuracy of the other methods were evaluated based on that. The DSAASTAT, an excel add-ins, was used to calculate the Pearson correlation between the results obtained from different methods. The ANOVA was conducted for the statistical analysis and differences of obtained values from each method were evaluated using Duncan's Multiple Range Test at 5% level of significance by the SAS software.

Result and Discussion

General properties of samples

The data given in Table 1 shows a wide variety of samples in the used commercial fertilizers. These fertilizers were physically categorized as liquid and solid as well as in

terms of chemical compositions containing different amounts of nutritional elements. As shown, the range of carbon changes in the samples is very high (from 1.5–51.7%), which can indicate a wide range of HA. Since the fertilizers that exist in Iranian markets are produced by different internal and foreign companies and the samples were collected from various markets, it is hoped that the origin of these HAs are different and come from distinct regions. The total soluble salts were almost high and have been shown in the electrical conductivity of the samples. The pH values of the samples also varied from 3.52–11.7. A high pH value is referred to in liquid samples as HA is soluble in a high pH value. The nutritional element concentration changes were in the following ranges: Ca, Mg, K, Na, Fe, and Si; the respective ranges were 0.07–7.2, 0.01–1.2, 0.14–19.2, 0.25–11.6, 0.02–5.4, and 0.00–2.9%. The concentration of these elements is observable in HA measurement. If these cations are present in a dissolved form, it may lead to the formation of a complex with HA and flocculation may occur when the thickness of the diffuse double layer is reduced by increasing the concentrations of the salts (Aiken 1985; Hong and Elimelech 1997). No significant relationship was observed among the general properties of the samples, such as EC, pH value, the concentration of the elements, and extracted HA in different methods (data not reported), which is probably due to the high proportion of the extracting solution to the sample weight in the HA extraction process, which may lead to the affectless of these parameters in HA determination. In addition, since the extraction process is carried out by alkaline solutions with high ionic strength, the ineffectiveness of pH and the inherent electrical conductivity of the sample are possible.

Comparison between four methods for determination of HA

As shown in Table 2, there was a significant difference among the four methods. The values obtained using the CDFA method was 13.8% lower than the NSM method.

Owing to the same principles of extraction of the two CDFA and NSM methods, the same measurements of HA values was expected as in both the methods, the HA was extracted by an alkaline solution (NaOH), and then, the extracted HA was acidified and, finally, the HA precipitate was measured. The results showed that there is a significant difference between the measured HA in these two methods and the HA values that are obtained by using the CDFA method were greater in the nine samples and smaller in thirteen samples in comparison to the NSM method. Statistically, only in 40% of the samples, no significant difference was observed. The obtained results were different from those reported by Lamar and Tolbot (2009). They reported that the CDFA method experienced overestimation (52%) in determining HA in eight samples. They argued that in the CDFA method, impure HA was measured (the ash was not removed); so, the HA was overestimated. However, unlike Lamar and Tolbot (2009), in this study, the HA value measured by the CDFA method is significantly lower than the NSM method. To investigate this hypothesis, the amount of ash was measured in the CDFA method; then, by subtracting it from the impure amount, pure HA was calculated (Table 5). As shown in the results, in the samples, the HA value is estimated by using a CDFA method greater than the NSM method and the presence of ash can be considered as one of the main reasons for this difference—this was, however, not true for the other samples. The differences in the measured values by the two methods can be attributed to the difference in the extraction process such that although the principal of the extraction is same, the details of the two methods are different (e.g., the ratio of sample to solution, shaking time, and the use of nitrogen gas for oxygen depletion—these factors can influence the extracting ability of HA). The HA that is measured by the ISO 5073 method is the closest to the NSM method (just 1.5% underestimated), but the results obtained by this method in certain samples, such as the numbers 4, 6, and 10, indicated

a significant difference with the NSM method (Table 3). Sample Number 6 did not produce a clear supernatant in the acidification step when the HA was measured by the CDFA method, thus indicating the presence of alkaline soluble substances in this sample. Certain other samples with such a property were selected and their HA content was measured. The results showed that in these samples, there is a great difference between the results obtained by the ISO 5073 method and other methods; thus, the results obtained by this method are, on average, 4.7 times the values measured by the NSM method (Table 4). The samples that have such properties are mostly liquid and their HA content is low. Therefore, this method cannot be applied to samples that have such properties. In addition to HA, FA, and other water and alkaline-soluble compounds (including amino acids, proteins, sugars, and fatty acids) are extracted, and the values obtained by this method are more than an estimation of humic content. As the values of these compounds are greater in the samples, it leads to greater HA overestimation. In the ISO 5073, the carbon content of the extract is oxidized with potassium dichromate and followed by the titration of the excess dichromate by an ammonium ferrous sulfate solution. To measure the HA content, after measuring the carbon, the amount of carbon is multiplied by the ratio of HA to organic carbon. In this study, the ratio of HA to organic carbon was considered as 1.72. These samples were liquid and, according to the given reasons, could overestimate the amount of HA. The values for HA were estimated in Sample Number 15 by using the ISO 5073 method, which was significantly lower than the values obtained using the NSM method, which is unlikely to be expected (Table 3). Sample number 15 has a high value of HA and is likely to be added to potassium dichromate in these samples, which will be not able to oxidize all the existing carbon. The extraction step in the ISO 5073 method was carried out by the alkali sodium pyrophosphate (total HA) or sodium hydroxide (free HA) and its carbon was

determined. No significant difference was found between the average amount of total and free HA, which can be indicative of the same power of these two extractants in HA extraction. It should be noted that the scope of this method is limited to brown coal and lignite, and it is not applicable to other commercial products that may contain a diverse soluble organic compounds, such as amino acids, proteins, sugars, and fatty acids (Lamar and Talbot 2009).

The colorimetric method produced significantly greater HA values (64.2% higher) than the NSM method (Table 2). As previously mentioned, the standard used in this method was obtained from the purification of Aldrich HA by using the classical method. In almost all the samples, the measured HA values were estimated as greater than or equal to the values measured by the NSM method. The differences of obtained values between the colorimetric and NSM method increased as the amount of HA increased in the samples and in some cases the estimated HA was greater than 100% i.e. Sample 15 and Sample 22. The colorimetric method produced significantly greater HA values than the NSM method in the selected samples as well (Table 4). In the colorimetric method, the HA content was estimated by comparing the intensity of the color produced by the extract in the standard solutions. In this method, FA and other water–alkali-soluble compounds, including amino acids, proteins, sugars, and fatty acids, will be extracted (Hayes and Graham 2000). Hence, in the colorimetric method, the HA will not be solely estimated, and aside from this, FA and other alkaline-soluble compounds that are active in a wavelength of 650 nm will be measured as well (Lamar and Talbot 2009). For equivalent concentrations, the absorbance of HSs increases with an increase in the molecular weight, carbon percentage (C), degree of condensation, and carbon ratio in aromatic rings to carbon in aliphatic chains (Stevenson 1982). Thus, if all the humic compounds had a specific molecular weight and structure, the colorimetric method

could be accurately used to estimate the concentration of HSs. However, the HA extracted from different sources or even the same source can vary widely in terms of the distribution of molecular weight, degree of condensation, carbon content, and the degree of aromaticity to aliphaticity (Stevenson 1982). In addition, the standard used in the HA colorimetric method was Aldrich Humate Sodium salt and it is likely that the HA of Aldrich was not same from one batch to another (e.g., in terms of molecular weight distribution) and cannot be considered as a good representative of the HAs extracted from different deposits. Thus, it was considered a weak standard (Lamar and Talbot 2009). Nevertheless, this method is used not only to determine the composition of humic acids but also composition of samples. By removing the results of the two samples, Sample 15 and Sample 17, the average values obtained by the colorimetric method (43.1%) will be close to the values obtained using the NSM method (28.8%).

As shown, using different methods for measuring HA in solid and liquid samples lead to different results (Table 5). The values obtained by using the CDFA method were less than the NSM method in different fertilizers and ash removal may is led to an increase in the differences. The difference between the HA measured by ISO 5073 and the NSM method in liquid fertilizers was higher than in the solid fertilizers, thus, the HA measured by ISO 5073 in liquid and solid fertilizers were 103.1 and 97.8% of NSM method, respectively. However, in general, the difference was minor. For example in normal samples that obtained from humic substances mines, lignite or leonardite, the method will have a same result with NSM but the ISO 5073 will have a great overestimation if the samples have a different additives like all of alkali soluble organics and also some nutrients such as iron. As shown in Table 4, the ISO 5073 method has a high overestimation in selected samples.

The colorimetric method produced significantly greater HA values than the NSM method in solid (65.8%) and liquid (56%) fertilizers. The colorimetric overestimation may have different reasons but the main reason is attributed to the heterogeneity of the texture of the samples. The results of this study showed that when the used standard material is not purified, the making standard was performed based on the HA content of humate sodium salts, whose obtained values were significantly closer to the values obtained from the NSM method (Figure 1). Interference material in humate sodium salts can have an absorbance in the wavelength of 650 nm; when the non-purified standard is used, the samples and standards have a greater matching background and it can have less error in HA estimation by the colorimetric method.

Comparison of Ash and Impurity Content in the CDFA and NSM Methods

The comparison of the average amounts of ash and impurities between the CDFA and NSM methods are provided in Table 5. As observable, greater ash content is reported in the NSM method, which sounds like a logical process, because in CDFA, the mineral compounds are removed from the sample by washing. For this reason, the ash is not measured in the CDFA method. Although the comparison of results showed no significant difference between the ash content in the two methods at 5% of significance level, the ash content was high in many samples in the CDFA method, which may result in HA overestimation.

The comparison of the average amounts of impurities between the two methods showed the consistency in most samples and indicated no significant difference. In those samples, where a significant difference was shown, it was higher in the CDFA than the NSM method, which indicates the inability of the CDFA method in HA extraction. Therefore, in order to increase the extraction potency, the CDFA method should be modified. To increase the extraction ability of the CDFA, some changes for example:

the normality of extractant, extractant to sample ratio, and extracting time maybe is needed.

Correlation between the Measured HA by Different Methods (Comparison of all the Samples)

The correlation between the measured HA by CDFA, colorimetric, and ISO 5073 method with NSM was 89.9, 91.7, and 90.7%, respectively (Table 6). In certain samples with high HA, there were large differences between CDFA and NSM, and the values obtained by CDFA were lower than NSM. These results indicate that in many samples containing high amount of HA, CDFA method is not capable of HA extraction completely, and it needs to be modified. For example, in the present study, four samples have such behavior. By disregarding these exceptions, the positive correlation between the two methods increases to 98.6%. The existence of alkaline-soluble materials is the main source of the overestimation in ISO 5073 and colorimetric methods. By removing these samples, the correlation between these methods and NSM were increased by 97.5 and 96.6%, respectively.

In Figure 1, the measured HA by different methods was compared with the NSM method. In this figure, the data was sorted in an ascending order, according to the measured HA by NSM (the range of HA in the samples varied from 1.69–70.9%) and the graph of the measured values by different methods were drawn in comparison to NSM. The results showed that the CDFA method, in comparison to the NSM method, overestimated the HA in those samples in which the HA concentration ranges from 30–60% (particularly, those between 30–40%). Depending on the sample, the sample-to-extraction solution ratio (1:2.5 in CDFA in comparison to 1:400 in NSM), and shaking time (1.5 hrs in CDFA in comparison to 16–18 hrs in NSM) may be not sufficient to complete the extraction HA. In all the concentration ranges, the colorimetric method

overestimates the HA in comparison to NSM and it was more significant at higher concentrations. In comparison to the NSM method, the ISO 5073 method overestimates the HA at lower concentrations (less than 10%) and underestimates it at higher concentrations (more than 50%). As mentioned above, the existence of soluble organic materials, e.g., FA, amino acids, fatty acids, lipids, and lignosulfonates are the main sources of interferences in these methods. Thus, the ISO 5073 and the colorimetric methods cannot applicable for these samples. Other researchers also reported that the colorimetric and ISO 5073 methods overestimate the HA due to the presence of alkaline solutions, such as proteins, amino acids, lipids, and FA (Lamar and Talbot 2009).

Conclusion

The current study has investigated the four common laboratory methods for determination humic acid in commercial humic-based fertilizers. The obtained results showed that the CDFA method underestimates the HA in comparison to the NSM method (13.8%). The comparison of the average amounts of the impurity and ash between the CDFA and NSM methods indicated that in the CDFA method the impurity is higher but the amount of ash is lower than NSM. The colorimetric method overestimates the HA in many under-studied samples (64.2%), which can be due to the lack of a matching standard and sample backgrounds. Nearly the same results were obtained for the ISO 5073 and NSM methods (98.6%). However, in certain samples containing alkaline soluble materials (mostly liquid samples with relatively low HA concentration), the ISO 5073 method greatly overestimates the HA, which makes it inapplicable for these samples. The results of the correlation coefficients between the four methods indicated that the consistency among the results obtained by the CDFA, colorimetric, and ISO 5073 method along with the NSM method were 89.9, 91.7, and 90.7, respectively.

Acknowledgments

We thank the technical staff of laboratories department of Soil and Water Research Institute of Iran for the assistance during the experiments and Mrs. Elahe Malekzadeh, Mrs. Fatemeh Mansouri and Mrs. Narges Maboudi for their guidance to setup the experimental methods. This research was supported by the Soil and Water Research Institute of Iran with a project entitled "comparison of methods for determination of humic acid in fertilizer materials (project number: 12-10-10-069-96068-961638)".

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Table 1. Some Properties of the Studied Fertilizers Containing HA.

Sample No.	State	OC (%)	EC (dS/m)	pH	Ca (%)	Mg (%)	K (%)	Na (%)	Fe (%)	Si (%)
1	Solid	41.7	13.0	9.70	0.57	0.09	0.18	6.61	0.72	2.86
2	Solid	40.3	14.5	9.94	1.22	0.08	10.5	0.58	0.62	0.54
3	Solid	45.2	16.3	9.31	1.52	0.43	1.59	0.83	1.36	0.35
4	Liquid	8.15	5.91	9.69	0.78	0.15	3.26	0.33	0.27	0.09
5	Liquid	8.25	7.41	9.60	0.80	0.15	3.13	0.27	0.26	0.11
6	Solid	30.9	56.5	6.96	0.86	1.17	8.16	11.6	0.07	0.12
7	Solid	1.49	4.29	9.27	0.07	0.01	1.01	0.45	0.04	0.01
8	Solid	29.5	25.5	10.1	0.75	0.22	15.5	0.85	0.62	0.34
9	Solid	41.7	14.0	9.84	0.71	0.08	11.3	0.64	0.64	0.37
10	Liquid	10.7	6.13	10.2	0.16	0.01	2.66	0.40	0.02	0.00
11	Liquid	6.97	7.72	9.37	0.49	0.10	3.02	0.25	0.18	0.08
12	Liquid	30.9	6.66	7.38	7.20	0.42	0.22	0.51	0.96	0.11
13	Liquid	6.60	8.49	10.2	0.11	0.03	3.29	0.82	0.08	0.00
14	Solid	40.3	23.0	11.5	0.78	0.06	8.59	3.92	0.19	0.18
15	Solid	48.3	14.8	9.81	0.73	0.16	9.62	3.07	0.26	0.13
16	Solid	39.8	15.1	8.49	0.33	0.13	8.72	0.74	0.91	0.30
17	Solid	51.6	10.2	9.86	0.38	0.09	8.62	1.64	0.69	0.31
18	Solid	39.1	3.18	3.95	0.75	0.22	0.14	0.65	0.50	0.10
19	Solid	27.8	3.9	9.37	0.58	0.12	19.0	1.39	5.53	1.17
20	Solid	30.4	7.23	6.41	1.52	0.43	1.59	0.83	1.36	0.35
21	Liquid	13.7	13.4	10.9	0.49	0.14	8.55	0.48	0.29	0.09
22	Solid	50.5	5.61	3.52	1.84	0.66	1.80	1.09	0.69	0.02

Table 2. Measured HA Values by Four Different Methods in the Studied Samples. CDFA= California Department of Food and Agriculture, NSM= New Standardized Method, ISO 5073= Volumetric method, HA= Humic acid.

Method	HA (%)
CDFA	27.7
NSM	32.2
Colorimetric Method	52.8
ISO 5073	31.7

Table 3. The Comparative Results of Four Different Methods for the Determination of HA.

Sample No.	Sample State	*Methods			
		CDFA (%)	NSM (%)	Colorimetric Method (%)	ISO 5073 (%)
1	Solid	49.2b	49.6b	68.6a	45.5c
2	Solid	53.1c	55.7b	71.3a	48.2d
3	Solid	52.4b	51.7b	69.1a	50.5b
4	Liquid	5.7c	4.5c	11.0a	8.7b
5	Liquid	5.7c	5.5c	11.4a	8.7a
6	Solid	3.0bc	1.7d	4.57b	34.5a
7	Solid	1.7b	1.9b	4.1a	2.6ab
8	Solid	30.3c	36.0b	78.6a	34.4b
9	Solid	61.0b	56.0c	69.1a	45.5d
10	Liquid	8.6b	7.7b	13.6a	13.6a
11	Liquid	6.1b	6.3b	9.8a	7.5b
12	Liquid	20.2d	35.9b	49.1a	29.2c
13	Liquid	10.1a	7.5b	11.2a	7.27b
14	Solid	37.3d	43.6c	60.0a	46.0b
15	Solid	65.7c	70.9b	156a	47.8d
16	Solid	18.3d	29.7c	57.6a	31.2b
17	Solid	21.0c	60.7b	145a	59.9b
18	Solid	26.2d	39.6b	67.2a	36.9c
19	Solid	31.1b	30.4b	37.9a	35.6a
20	Solid	24.5d	38.0b	55.4a	30.9c
21	Liquid	58.6b	58.7b	90.2a	55.0b
22	Solid	19.6b	16.9d	21.6a	17.9c

* A mean comparison was carried out based on Duncan's Multiple Range Test (MRT) at 5% level of significance.

Table 4. The Comparative Results of Four Different Methods for the Determination of HA in Selected Samples.

Sample No.	Sample State	*Methods			
		CDFA (%)	NSM (%)	Colorimetric Method (%)	ISO 5073 (%)
1	Liquid	4.0b	2.9b	4.3b	26.5a
2	Liquid	4.1b	3.5b	4.5b	22.6a
3	Liquid	3.9b	3.1b	3.5b	23.5a
4	Liquid	3.5b	2.6b	3.4b	22. 8a
5	Liquid	3.6d	8.4c	16.1a	13.7b
Mean	-	3.8	4.4	6.9	20.6

*A mean comparison was carried out based on Duncan's MRT at 5% level of significance.

Table 5. Average Concentration of HA in Liquid and Solid Samples.

Method	HA (%)	
	liquid	solid
CDFA(pure)	16.1	31.6
CDFA(impure)	16.4	33.0
NSM	18.0	38.8
ISO 5073	18.6	37.8
Colorimetric Method	28.1	64.4

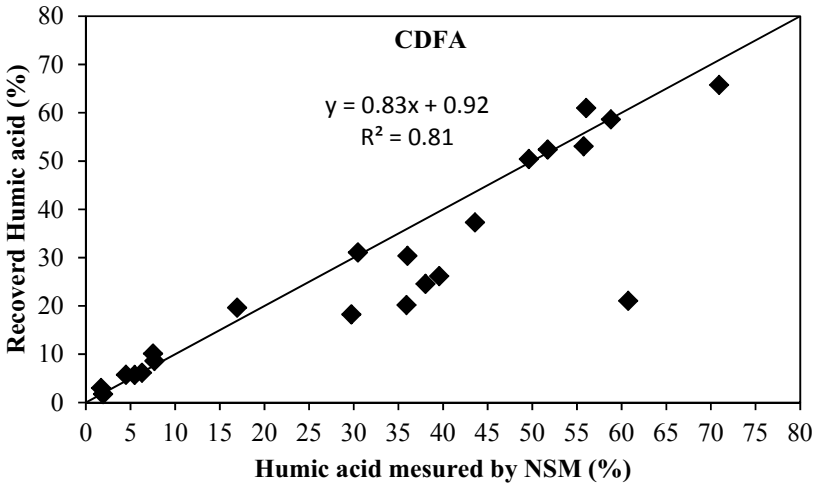
Table 6. Ash and Impurity Content Comparison in the CDFA and NSM Methods.

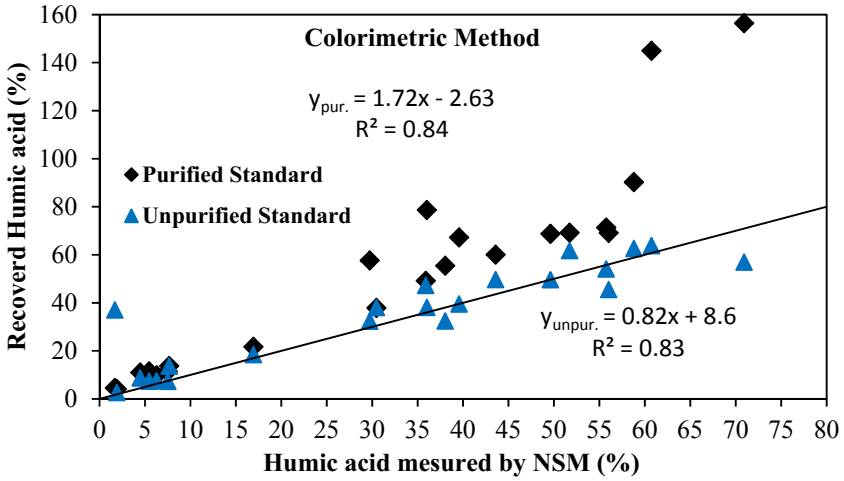
Sample No.	Sample State	Ash Content*		Impurity*	
		CDFA (%)	NSM (%)	CDFA (%)	NSM (%)
1	Solid	15.4b	27.8a	25.4a	25.4a
2	Solid	12.6a	16.9a	19.4a	18.2a
3	Solid	7.67a	11.2a	17.0a	11.7b
4	Liquid	8.15b	21.1a	14.6a	12.4b
5	Liquid	7.62b	20.8a	14.8a	12.4b
6	Solid	0.5b	5.8a	4.7a	2.1b
7	Solid	1.9b	15.8a	0.7a	0.57b
8	Solid	3.5b	16.6a	34.0a	22.0b
9	Solid	10.8b	16.8a	20.7a	19.9a
10	Liquid	1.9b	14.7a	0.7b	1.40a
11	Liquid	5.3b	16.8a	7.4a	6.3b
12	Liquid	5.6b	15.7a	79.1a	52.1b
13	Liquid	3.8b	16.4a	0.4a	0.6a
14	Solid	9.4b	15.6a	11.7b	13.3a
15	Solid	1.7b	13.2a	18.6a	13.3a
16	Solid	2.3b	17.2a	69.8a	39.8b
17	Solid	0.8b	14.9a	57.3a	14.2b
18	Solid	2.9b	16.0a	69.2a	40.7b
19	Solid	10.5b	23.7a	19.5a	12.9b
20	Solid	2.8b	19.2a	76.4a	13.2b
21	Liquid	2.1b	14.1a	34.8a	17.3b
22	Solid	13.4a	14.4a	2.6a	1.8b

*A mean comparison was carried out based on Duncan's MRT at 5% level of significance.

Table 7. Correlation among the Results of the Four Different Methods of HA Measurement.

	CDFA	NSM	Colorimetric Method	ISO 5073
CDFA	1.00	-	-	-
NSM	0.89	1.00	-	-
Colorimetric Method	0.72	0.91	1.00	-
ISO 5073	0.79	0.90	0.82	1.00





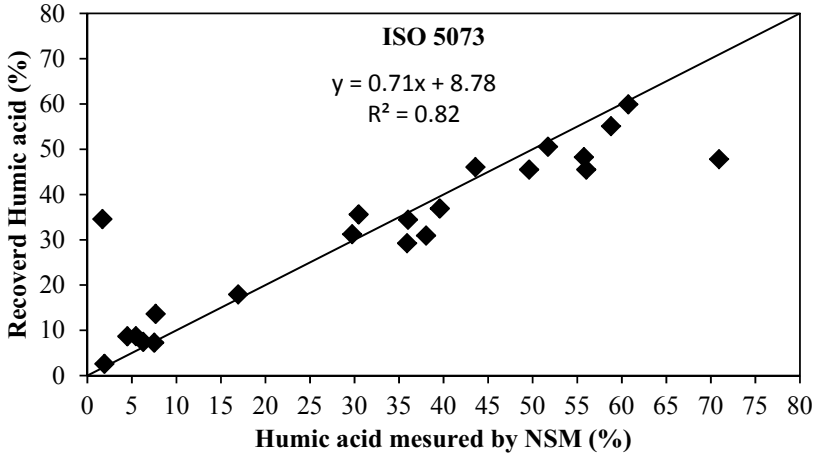


Figure 1. Comparison of the Values of HA measured in Different Methods with the NSM Method.