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Humic Acid Determination in some Compost and Fertilizer Samples

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Abstract

Seven fruit-based fertilizer samples obtained from the Ministry of Agriculture in Gaza include (Pow Humus, Humigan, Eptic, Liquid Potasium, Liquid H.A. Organic corrector Mix, Agriful and Agriful Antisal), have been analyzed for humic acid percentage content using sodium pyrophosphate. Zero humic acid content were found in the samples of Eptic, Liquid Potassium, Agriful and Agriful Antisal, which were in agreement with the reported values in the samples. Also, Pow Humus, Mix and Humigan fertilizer samples and two compost samples from the Agriculture school in Beit Hanoon (Gaza Strip) have been determined for their Humic acid contents using International Humic Substances Society (IHSS) method, modified UV-Vis and modified HPLC methods. It was observed that there is no accurate and precise method for humic acid determination. The best recommended method was the IHSS method as giving highest reasonable results but it is time wasting. The HPLC method is the second recommended method with comparing to IHSS method, despite its costs. UV-Vis method gives good results with just the Pow Humus, Humigan and Mix samples. It was found that different humic acid structures resulting from different sources, in addition NaOH solution modified the humic acid structure. These structures have been illustrated using FTIR after IHSS method for humic acid extraction in the fertilizer samples of Pow Humus, Mix and Humigan. Finally, pyrophosphate solution showed higher selectivity and milder than NaOH solution, in contrast it extracted just 30% of humic acid in the samples and the Redox titration of this method was of high errors.

Keywords:
Humic acids,
Fertilizers,
Compost,
Humic acid extraction.

1. Introduction:

Humic substances (HS) are major components of the natural organic matter (NOM) in soil and water as well as in geological organic deposits such as lake sediments, peats, brown coals and shales (Kiiski et al., 2009). They make up much of the characteristic brown color of decaying plant debris and contribute to the brown or black color in surface soils (Moore, 2001; Mendyk et al., 2016). They are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification).

Humic substances in soils and sediments can be divided into three main fractions: humic acids (HA or HAs), fulvic acids (FA or FAs) and humin (Mendyk et al., 2016). Humic substances have variable concentrations of humic and fulvic acids (Moore, 2001). Humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups with the average chemical formula $C_{187}H_{186}O_{89}N_9S_1$. The hypothetical structure for humic acid, contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and COOH groups variously placed on aromatic rings. The molecular weight of the compound is between 5,000 and 10,000 Daltons. It contains 33-36% of oxygen and 4% of nitrogen (Stevenson, 1994). Humic acids have long been used as plant growth enhancers and as soil fertilizers and play an important role in human health and animal husbandry (Enviromate, 2002; Asing, Wong, & Lau, 2009). The various functional groups in humic acid structure improve the physical and the chemical properties of the soil (Yang, Wang, & Xian, 2010). Many factors affect the amount of humic acid extracted include its chemical composition, types of organic material from which HA extracted, temperature, grain size, frequency of extraction, the extraction agent and its strength and drying procedure (Sasaki, & Oyamada, 1966; Asing, Wong, & Lau, 2009). A variety of methods for the isolation of humic acids from humic substances can be employed depending upon the nature of the material of humic substances. It is shown that NaOH is so far the best solvent of extraction available as it shows an efficiency of up to 80% extraction of humic substances (Swift, et al., 1996). Some methods for determination of humic substances (humic and fulvic acids) include determination of carbon in humic compounds, centrifuge method of (acid precipitation method), International Humic Substances Society (IHSS) method, UV-Vis spectrophotometric method, and HPLC method. In our study, Humic acid in different source fertilizer samples and composts have been determined based on carbon in humic compounds using pyrophosphate reagent for extraction, acid precipitation method depending on IHSS method, UV-Vis spectroscopic method at wavelength of 450 nm, and HPLC quantitative method. Also, a developed quantitative method depending on standard curve construction has been used in the present study.

2. Experimental:

2.1 Sample Collection and Storage:

A seven fruit-based fertilizer samples was obtained from the Ministry of Agriculture in Gaza-Palestine, include Pow Humus, Humigan, Eptic, Liquid Potassium, Liquid H. A. Organic corrector Mix, Agriful and Agriful Antisal, in which all liquids except for the Pow Humus sample which is solid. Liquid samples were stored in glass bottles, while the solid one was stored in plastic bottle, all at 4 °C. Two other compost samples were made at the Secondary School of Agriculture in Beit Hanon- Gaza.

2.2 Chemicals and Reagents:

Solutions of 0.1N pyrophosphate, saturated sodium sulphate,1N of sulfuric acid, 0.1N sulfuric acid, 1N potassium dichromate, diphenyl amine indicator, 0.5 N NaOH solution, 1% NaOH solution, and concentrated HCl solution were prepared from analytical pure substances that purchased from (Merck, Germany). Sodium pyrophosphate was prepared by the molecular dehydration of dibasic sodium phosphate, Na_2HPO_4 at 450 °C for 4 hrs.

2.3 Equipments:

The optical absorption of samples were obtained by using (a single beam GENESYS 10 UV Scanning Spectrophotometer) in the range (190 – 1100 nm) of automatically rotation. Centrifugation was conducted by (Combi-514R with Max. speed of 15000 rpm). Samples were shaken with ELEIA-Multi mechanical Shaker. The HPLC method was achieved by (Chrom Tech HPLC with diode array detector, using isocratic elution and Machery Nagel RP-C 18 column, 15 cm length).

2.4 Determination of Humic Acid:

2.4.1 Determination of Carbon in Humic Compounds:

Extraction of humic substances was done by weighing 5 g of the fertilizer sample in 250ml-cylinder, then 100 ml of the pyrophosphate solution was added on the sample, that was closed by a parafilm to isolate CO_2 and the air from the sample then left for 18 hrs. Saturated sodium sulphate (25 ml) was added, then the mixture was mixed and left for 15min. This mixture was filtered with discarding the first drops of the filtrate. To determine the humic acid content, 5ml of 1N sulfuric acid solution was added to 25ml of the filtrate pipetted in a conical flask, then the pH of the solution was adjusted to 1.3-1.5 using the 1.0 N sulfuric acid solution. The acidified solution was heated in a water bath at 70-80 °C until humic acid begin to precipitate in a jelly-like shape. The solution was allowed to cool down for 1 hr for completion of the humic acid precipitation, which was then filtered in another conical flask, the precipitate on the filter paper was washed three times with the 0.1N sulfuric acid solution to get rid of the remaining fulvic acids. The precipitate on the filter paper was dissolved by successive washing with the warm 0.1N NaOH solution (40-50 °C) to return the precipitate to the filtrate (the total valume of the NaOH solution was 50 ml). The basic solution was allowed to cool and transferred to 100ml- volumetric flask and completed to the mark with distilled water. 10 ml of the previous solution was piptted in a conical flask . Potassium dichromate solution (2.5 ml of the 1.0 N) and 5 ml of the concentrated sulfuric solution were added to the conical flask and was left for 15 min. If the solution is dark oily, then it has a high organic acid content and needs doubled amounts of the dichromate and sulfuric acid solutions to oxidize the organic acids. The conical flask was sealed tightly and left for 30 min. Then 50 ml of distilled water was added to the former solution and 5 ml of phosphoric acid solution (80%) to clearly show the end point (the solution turned from dark-purple into green-colored solution) upon addition of 0.5ml diphenylamine indicator solution. The basic solution was titrated against the 1.0 N ferrous sulphate solution. The volume was recorded which is the sample volume (S). The blank solution was a mixture of 2.5 ml of the dichromate solution, 5 ml of the concentrated sulfuric acid solution and 5 ml of the 80% phosphoric acid solution in a conical flask. Distilled water (50 ml) was then added and the mixture was allowed to cool down, then 0.5 ml of the diphenylamine indicator was added. The solution was titrated against the 1.0 N ferrous sulphate solution and the volume was recorded as (B). The humic acid percentage was calculated as was reported previously in determination of carbon in humic compounds- determination (Walkley, & Black, 1934; Jackson, 2005). This procedure was repeated three times.

2.4.2 Acid Precipitation Method (IHSS):

The 7 previous mentioned fertilizer samples were used for the analysis. The amount of the fertilizer sample was weighed and recorded as to give approximately 500 mg of dry humic acid precipitate according to the provided value recorded on the sample. 50 ml of the 0.5N NaOH solution was added on the weighed sample in a predried and weighed centrifuge bottle, which was capped and sealed tightly. This mixture was shaken on a mechanical shaker for 1.5 hrs for solid samples or 30 min for liquid samples. After shaking, the cap was rinsed with 1% NaOH solution, then the sample mixture was centrifuged for 20 min at 2000 rpm. The supernatant was decanted into a second, previously weighed centrifuge bottle. NaOH solution (1%, 5-10 ml) was added to the first bottle and shaken vigorously and centrifuged as previously mentioned. The second supernatant was combined with the first by decanting into the second centrifuge bottle. The first bottle was discarded with the precipitate. To the combined extracts in the second bottle, the pH of the extract was lowered to pH ≤ 1 using the concentrated HCl solution. The sample was centrifuged for 20 min at 2000 rpm. The liquid was carefully decanted and discarded. A 25 ml of distilled water previously adjusted to pH ≤ 1 (with HCl) was added to the bottle, capped and shaken vigorously to free all precipitate from the bottom and was centrifuged. The liquid was decanted and discarded, and the last step was repeated. The precipitate was transferred to a previously weighed crucible with the acidified water and was dried overnight (nearly for 6 hrs) at 100-110 °C, which was then cooled in a desiccator and weighed. The percentage of the Humic acid was calculated by division of the weight of the dry Humic acid by the sample's weight (Page, 1982; Klute, 1986).The volume capacity of the centrifuge bottle was nearly 45 ml, so the sample was half-divided into two bottles, and the amounts of the solutions was also half-divided. The IR for the humic acid extracted from Pow Humus, Mix and Humigan samples were obtained after this procedure (duplicate acidified water-wash). A modification was applied on this procedure, as the latter steps were performed, but washing with the acidified water was just for one time. Two compost samples were analyzed as previously mentioned procedure. The weight of the sample was taken to be 10 g. A well characterized Humic sample was the Pow Humus sample (reported 85% of Humic acid), was used as a reference material. Each procedure was repeated three times.

2.4.3 UV-Vis Spectroscopy Modified Method:

The Pow Humus fertilizer solid sample was taken as a reference material (accurately humic acid determination from the centrifuge method to be 64.6% W/W). For calibration curve construction, 5 g of Pow

Humas was weighed, then diluted into 50 ml by 0.5M NaOH solution (100g Pow Humas sample has 64.6 g humic acid (based on the average of results of centrifuge method), so 5g of the sample will have 3.23g humic acid, and the percentage will be 6.46% W/V). The solution was transferred in two falcon tubes and shaken for 1.5 hrs, then centrifuged at 2000 rpm for 20 min. Each solution was decanted in another tube, and 5ml of 1% NaOH solution was pipetted on the precipitate in the original tube and centrifuged. The first supernatant was decanted on the original one for each tube. (0, 0.1, 0.2, 0.4, 0.6, 0.8) % W/V solutions from the above solution in 50ml volumetric flask was prepared supposing the reference material has 6.46% W/V (sample dilution was by distilled water). Then a dilution of 1:100 was prepared for each of the above solutions. The absorbance was measured at 450 nm. As a result of no humic acid content in Eptic, Liquid Potasium, Agriful and Agriful Antisal, just for the Humigan, Mix and Powhumus samples, 5 g from the sample was weighed and diluted in 50 ml volumetric flask by 0.5 M NaOH solution, then the above mentioned procedure was followed. The blank solution was 10 ml of the 0.5 M NaOH solution and 1ml of 1.0 % NaOH solution diluted in 100 ml volumetric flask by the distilled water. For the two compost samples, 10 g of each sample was weighed; 50ml of 0.5M NaOH solution was added by a pipette. The solution was transferred in two falcon tubes and shaken for 1.5 hrs, then centrifuged at 2000 rpm for 20 min. Each solution was decanted and filtered by filter papers, then transferred in a 100ml-volumetric flask. 5 ml of 1% NaOH solution was pipetted on the precipitate in the original tube, centrifuged, decanted and filtered, then transferred to the 100 ml-V.F. The solution was diluted to 100 ml by distilled water. Another 1:100 dilution was performed. Absorbance was taken at 450 nm (De Nobili, Bragato, Alcaniz, Puigbo, & Comellas, 1990). This procedure was repeated three times.

HPLC Quantitative Method:

Pow Humus sample (5.0 g) was weighed and dissolved in 50 ml- V.F. with 0.5 M NaOH (which is 64600 ppm**)**. This solution was transferred to polyethylene falcon tubes of 45 ml capacity. The solution was shaken for 1.5 hr using a mechanical shaker and centrifuged at 2000 rpm for 20 min. Each solution was decanted in another tube, 5ml of 1% NaOH solution was added on the precipitate in the original tube and the steps from the second one was repeated. The above solution (1 ml) was diluted by distilled water in 100 ml volumetric flask to give 646 ppm. 300, 250, 200, 150, 100 ppm were prepared by pipetting (11.60, 9.67, 7.74, 5.80, 3.87ml) from the stock solution and dilutes in 25 ml distilled water. For Humigan and Mix fertilizer samples, 5.0 g was weighed and diluted in 50 ml- V.F. with 0.5 M NaOH solution and the above steps for centrifugation were repeated. The fertilizer solution (1.5 ml) was diluted in 50ml volumetric flask with distilled water after centrifugation. Using HPLC method with a mobile phase of (90% ACN and 10% H_2O) and isocratic flow with a rate of 1ml/min, the peak areas and the retention times were measured for both the standard solutions and the fertilizer sample solutions in the UV-Vis range at $\lambda = 254$ nm. The standard curve was constructed and the humic acid percentage in the fertilizer samples were determined. For the two compost samples, two 5.0 g of each compost sample were weighed in two falcon tubes and 50 ml of 0.5M NaOH was divided onto the tubes. The tubes were shaken for 1.5 hr. The solutions were filtered in 50 ml V.F., then transferred into two falcon tubes and centrifuged at 2000 rpm for 20 min, the solutions were decanted into another falcon tubes and 5 ml of 1% NaOH soln. was added onto each precipitate and centrifuged. Each supernatant was decanted on the first one. The supernatant for each compost samples (3 ml) was diluted into 50 ml volumetric flask with the distilled water, in which the latter were measured for their retention time and peak area using the HPLC system (Hori, & Okuda, 1961). This procedure was repeated three times.

3. Results and Discussion:

3.1 Determination of Carbon in Humic Compounds:

As given in Table 1, the samples, Eptic, Liquid Potassium, Agriful and Agriful Antisal, has a zero humic acid content which is consistent with the reported values. In which the percentage of the total organic content could be fulvic acid, non-humic substances include identifiable, high-molecular-weight organic materials such as polysaccharides and proteins and simpler substances such as sugars, amino acids, and other small molecules.

Table 1 *Humic acid percentage extraction in via pyrophosphate carbon determination of humic compounds*

No. of sample	Name of sample	Wt. taken, g	Reported %(w/w) of H.A.	Average % (w/w) of H.A.	Relative error	RSD
1	**Pow Humus**	5.0560	85%	9.290%	-89.00%	10.96%
2	**Humigan**	5.1770	12.5%	3.075%	-75.40%	37.48%
3	**Eptic**	5.1990	16%(organic acid)	0.000%	--------	--------
4	**Liquid Potasium**	5.0174	28.76%(organic acid)	0.000%	--------	--------
5	**Liquid H.A.,mix**	5.0650	12%	7.010%	42.00%	47.21%
6	**Agriful**	5.1034	25%(total F.A.)	0.000%	--------	--------
7	**Agriful Antisal**	5.0156	0%	0.000%	--------	--------

The result of Pow Humus (solid sample), has a relative error of -89% as a result of the average value of 9.290% w/w of H.A., while the reported one is 85% w/w. The Humigan sample has an average value of 3.075% w/w of H.A with a relative error of -75.4% based on the 12.5% reported value of H.A. The Mix sample has a relative error of 42% with the average and reported value of 7.010% and 12%, respectively. Also, the precision between the replicates was very low with a relative standard deviations (RSD) of (10.96, 37.48, and 47.21)% for the samples 1, 2 and 5, respectively. So, this procedure has a very low accuracy. The relative error for solid sample is higher than for a liquid sample. In addition; liquid samples makes more homogenous mixture with pyrophosphate solution than solid samples, which leads to more extraction of the organic acids. Also, from previously reported results it was shown that 30% of the humic acid can be extracted by the pyrophosphate reagent as given in Table 2 (Tien, & Kirk, 1983).

Table 2 *Reagents used for the extraction of humic substances*

Extractant	% Humic substances extracted
NaOH	80
$Na_4P_2O_7$	30
Organic Chelates: acetylacetone Cupferron, hydroxyquinoline	30
Formic acid	50

3.2 Acid Precipitation Method:

In this method, the zero humic acid content for the samples 3, 4, 6 and 7 is also confirmed. However, the accuracy and the precision are far higher than the first mentioned method by pyrophosphate extraction. Table 3 shows the results of this method with relative errors by (24.31, 28.96 and 16.92)%, and with RSD values of (4.95, 3.09 and 5.21)%, for samples 1, 2 and 5, respectively.

Table 3 *Humic acid percentage for fertilizer and compost samples, determined by the precipitation method*

No. of sample	Name of sample	Reported %(w/w) of H.A.	Average % (w/w)	Relative error	RSD
1	**Pow Humus**	85%	64.34%	24.31%	4.95%
2	**Humigan**	12.5%	8.88%	28.96%	3.09%
3	**Eptic**	16%(organic acid)	0.00%	--------	--------
4	**Liquid Potasium**	28.76%(organic acid)	0.00%	--------	--------
5	**Liquid H.A., mix**	12%	9.97%	16.92%	5.21%
6	**Agriful**	25%(total F.A.)	0.00%	--------	--------
7	**Agriful Antisal**	0%	0.00%	--------	--------
8	**Compost 1**	--------	%1.63	--------	--------
9	**Compost 2**	--------	%2.42	--------	--------

These results are reasonable with results reported previously which showed that 80% of humic acid is extracted by NaOH reagent (Table 2) (Tien, & Kirk, 1983). Also, it was noticed that the average results upon just a single washing with the acidified distilled water (pH<1), are 84.80% for sample 1, 12.00% for sample 2, and 11.66% for sample 5. Relative errors for the former samples are 0.24, 4.00 and 2.83%, respectively, which indicates the samples may be analyzed by the centrifuge method with single acidified water-wash. The second wash with the acidified water leads to the removal of heavy metals, ashes and sodium metal from NaOH solution. By referring to previously reported results which given in Table 4, the humic/fulvic acid percentage for composts ranges from 5-25 % (Mayhew, 2004), and the average results for the first and second composts are 1.63% and 2.42% with relatively low RSD values of 5.22% and 8.88%, giving a confidence limit at 95% confidence level ($t_{95\%}$=4.303 at degrees of freedom of 2) of 1.63% ± 0.21 and 2.42% ± 0.53, respectively. The results are quite consistent with the value range in Table 4, but it is low as the range reported is for both humic and fulvic contents. Also, composts are solids which do not make homogeneous solution with 0.5M NaOH solution, and some of humic acid will be lost. The FTIR spectra for Pow Humus, Humigan and Mix extracts showed that the common functional groups: O-H stretching, aliphatic and aromatic C-H stretching, C=C stretching in benzene rings, symmetrical and assymetrical C-H deformation, at around ν = 3600, 3020, 2900, 1700, 1450, and 1380 cm^{-1} respectively were detected. These results are in agreement with several studies reported previously as given in Table 5 (Elvidge, 1988). There is a little bit difference between the FTIR spectral results obtained and that of results reported of the different sources of the humic acid, due to different conditions and processing and may due to the effect of the NaOH base used for the extraction. This method is supposed to be the most accurate and precise method, according to the results of this study and the previous studies of some researchers (Sawhney, Isaacson, & Stevenson, 1982; Kasim, Ahmed, Majid, Muhamad, & Yusop, 2007; Kasim, Ahmed, Majid, Muhamad, & Yusop, 2008).

Table 4 *Different sources of humic substances in varying concentrations**

Natural sources	% Humic/Fulvic acid
Leonardite	25 to 90
Compost	5 to 25
Peat	5 to 20
Peat Moss	5 to 20
Lignite	5 to 15
Manure	1 to 3
Soft coal	2 to 5
Hard coal	0 to 1

Source: (Mayhew, 2004)

Table 5 *Important IR absorption bands of humic substances of lignin origin**

Position cm^{-1}	Band origin
3450-3400	OH stretching
2940-2820	OH stretching in methyl and methylene group
1715-1710	C=O stretching nonconjugated to the aromatic ring
1675-1660	C=O stretching in conjugation to the aromatic ring
1605-600	Aromatic ring vibrations
1515-1505	Aromatic ring vibrations
1470-1460	C-H deformations (asymmetric)
1430-1425	Aromatic ring vibrations
1370-1365	C-H deformations (symmetric)
1330-1325	Syringyl ring breathing
1270-1275	Guaiacyl ring breathing
1085-1030	C-H, C-O deformations

Source: (Elvidge, 1988)

3.3 UV-Vis Spectroscopy Modified Method:

This is a new proposed method, which is a combination between the acid precipitation (centrifuge) and UV-Vis techniques. The three replicates give a square of the correlation factors R^2 around 0.9996 for the calibration curve, Figure 1 is for the first replicate's calibration curve. The results are given in Table 6, Taking into account that the extracted H.A. from the Pow Humus was supposed as a standard material with the percentage of H.A. of 64.60% (w/w), according to the results of the acid precipitation method. The average percentage of H.A. in Pow Humus, Humigan and Mix samples are 64.58, 8.66 and 9.62 with RSD values of 0.0558, 0.653 and 1.76 and the relative errors according to the reported values are -24.02, -30.72 and -19.83 respectively. This indicates a good precision of the method and weak accuracy when referring to the reported value. However, previous studies show that there was no universal and accurate method for determination of H.A. There is no statistical difference between the results of the UV-Vis method and acid precipitation method for determination of the H.A. in fertilizer samples, as the $t_{calculated}$ (0.5636) < $t_{tabulated}$ (4.303), at 95% confidence level and two degrees of freedom. However, for the compost samples, the results with referring to the acid precipitation method have relative errors of 65% and 81% for the first and the second compost, respectively. These results indicate failure of the UV-Vis method for determination of humic acid content in compost samples. This may due to the effectiveness of NaOH solution in extraction which reaches 70%, with reference to Table 2, in addition to the incomplete solubility of the compost samples and filtration needed, in contrast with fertilizer samples.

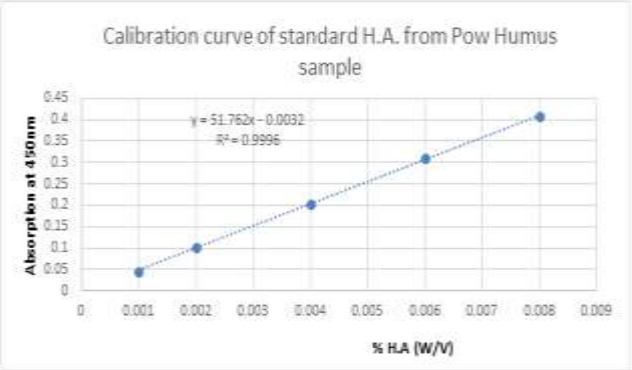

Figure 1 *The first replicate's calibration curve of standard H.A. from Pow Humus sample*

Table 6 *The results of UV-Vis method for H.A.%*

Sample	Reported %H.A.	Average % H.A.	Relative error	RSD
Mix	12	9.62	-19.83%	1.760%
Humigan	12.5	8.66	-30.72%	0.653%
powhumus	85	64.58	-24.02%	0.0558%
Compost 1	…..	0.44	…..	…..
Compost 2	…..	0.45	…..	…..

3.4 HPLC Quantitative Method:

Table 7, shows the results of fertilizer samples Mix and Humigan with average percentages of 9.73% and 9.18, respectively. The RSD values were 0.71% and 0.23%, which relates good precision. The relative errors according to the reported values were -18.92% and -26.56%. These values may be due to the washing with acidified water for just one time using acid precipitation method. However, the $t_{calculated}$ (1.97) < $t_{tabulated}$(12.706), at 95% confidence level and a degree of freedom. In addition, the $t_{calculated}$ (2.041) < $t_{tabulated}$ (12.706), at 95% confidence level and a degree of freedom, so there was no statistical difference between the results of the HPLC method and acid precipitation method for determination of the H.A. in fertilizer samples and composts. Higher humic acid contents in compost samples are obtained by acid precipitation method than HPLC method. This could be as a result of ash content in the first mentioned method, also the adsorption of humic acid on the C_{18} column of HPLC system and the sensitivity limit of the detector may lead to lower values of humic acid. The retention times and peak areas with the standard curve for the first trial are illustrated in Table 8 and Figure 2.

Table 7 *The results of HPLC method for H.A.%*

Sample	Reported %H.A.	Ave %	Relative error	RSD
Mix	12	9.73	-18.92%	71.00%
Humigan	12.5	9.18	-26.56%	0.23%
Compost 1	…..	1.24	…..	…..
Compost 2	…..	1.28	…..	…..

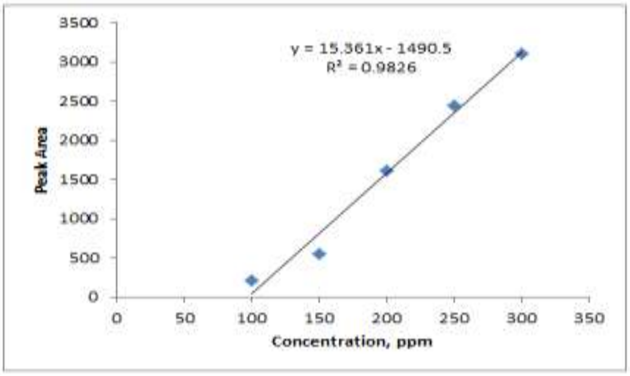

Figure 2 *Standard curve for the first trial of humic acid determination via HPLC method*

Table 8 *Peak areas and retention times for the standard solutions at the first trial of HPLC determination method of humic acid*

Concentration, ppm	Peak Area	Retention time (min)
100	205.47	0.86
150	550.23	0.84
200	1612.07	1.06
250	2440.03	1.14
300	3100.89	1.14

Conclusion:

Actually, according to the present study and the previous studies, there is no recommended standard method to give accurate and reproducible results for humic acid content. This is due to the absence of proper solvent to extract completely the humic acids and the different sources of humic acid which implies different structures. It is concluded that the best method for humic acid determination in fertilizer and compost samples is the acid precipitation method (IHSS method). The IHSS method is time consuming, to solve this problem, it is advisable to follow the modified method of UV-Vis determination of humic acid just for fertilizer samples which makes homogeneous solution with NaOH solution. The HPLC method gives acceptable results for humic acid percentage in both fertilizer and compost samples. To arrange them according to time wasting, they would be: IHHS method, HPLC method, UV-Vis method. While arranging them according to the increasing costs and equipment needed, IHSS method will be the cheapest, then UV-Vis method and finally, HPLC method. However, fulvic acid can be determined using HPLC method. Further researches and studies could be performed for seeking of fulvic acid determination methods and modified methods for accurate humic acid content determination.

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تحديد نسبة حمض الهيوميك في بعض عينات الأسمدة

تم تقدير النسبة المئوية لمحتوى حمض الهيوميك في سبع عينات أسمدة نباتية والتي تم الحصول عليها من وزارة الزراعة في غزة وهي: (Pow Humus, Humigan, Eptic, Liquid Potasium, Liquid H.A.Organic corrector Mix, Agriful and Agriful Antisal) وذلك باستخدام بيروفوسفات الصوديوم. وتم الحصول على محتوى صفري لحمض الهيوميك في عينات Eptic وLiquid Potassiu وAgriful وAgriful Antisa، وهذه النتيجة متوافقة مع القيم المكتوبة على العينات. كما تم تقدير النسبة المئوية لمحتوى حمض الهيوميك في عينات أسمدة Pow Humus وMix وHumigan وعينتي سماد إضافيتين تم الحصول عليها من المدرسة الزراعة في بيت حانون (قطاع غزة) باستخدام طريقة International Humic Substances Society (IHSS) وطريقة الاشعة فوق البنفسجية المعدلة، وكذلك طريقة كروماتوجرافي السائل عالي الأداء (HPLC). وقد لوحظ أنه لا توجد طريقة صحيحة ودقيقة لتحديد حمض الهيوميك في عينات الأسمدة المختلفة.

وقد كانت أفضل طريقة يمكن أن يوصى بها هي طريقة IHSS حيث أعطت أعلى نتائج معقولة ولكنها أخذت وقتا أطول من غيرها من الطرق. وتأتي طريقة HPLC في المرتبة الثانية بالمقارنة مع طريقة IHSS، بالرغم من تكلفتها العالية. أما طريقة الأشعة فوق البنفسجية فهي تعطي نتائج جيدة مع عينات أسمدة Pow Humus وHumigan وMix. وقد تبين أن التركيب البنائي للأحماض الهيومية يختلف حسب مصادرها المختلفة، كما أن هيدروكسيد الصوديوم المستخدم في تحليل العينات له دور في تغيير التركيب البنائي. وتم توضيح التركيب البنائي لحمض الهيوميك المستخلص من عينات Pow Humus وMix وHumigan باستخدام جهاز FTIRبعد الاستخلاص باستخدام طريقة IHSS.

وأخيراً، أظهر محلول البيروفوسفات أعلى انتقائية وأكثر اعتدالا من محلول هيدروكسيد الصوديوم، وعلى النقيض فإنه استخلص فقط 30٪ من حمض الهيوميك في العينات كما أن معايرة الأكسدة والاختزال باستخدام هذه الطريقة أعطت أخطاءً عالية.

كلمات مفتاحية:
حمض الهيوميك،
الأسمدة،
الدبال،
استخلاص الحمض الهيومي.