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Organic Carbon in Humic Fractions in Soil Influenced by Organic, Inorganic and Bio Nitrogen Fertilizers under Different Incubation Periods

Khalafalla, M.Y.

Soils & Water Dept., Fac. Agric., Al-Azhar Univ., Assiut, Egypt.

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Abstract

Humic substances can be subdivided into three major fractions Humin (Hu), Humic acid (HA) and Fulvic acid (FA). Humic substances are a good source of energy for beneficial soil organisms, plus its key fractions of soil organic matter, a major aspect controlling the flexibility of plant nutrition irrespective of land use system through different processes and mechanisms. The main objective of this study was to investigate changes in the content of humic, non-substances in soil treated with manure, inorganic and bio nitrogen fertilizers under different incubation periods. soil samples were collected after 14, 30 and 60 days from incubation to determined HA, FA, and Hu.

In the first period, results showed that the application of mineral fertilizers increased the contents of humic acid compared with fulvic acid in the soil. Thus, the application of rabbit manure resulted in the most significant increase in the content HA compared with control treatment, and vice versa with the FA. The humic substances in this study is characterized by higher content of FA than HA after the third period of incubation 60 days, significant differences between some treatments in terms of humin content than observed for control treatment. The overall trend was humin (36 to 47%) > HA (7 to 37%) > FA (10 to 34%). More FA than HA were observed in the second and third period, with the exception of the first period was less. After 60 days incubation period, the HA/FA ratio varied from 0.42 to 1.53 ppm for all treatments, except (FOB) treatment was 2.22 ppm. More FA than HA indicates low humification rates. The distribution of C among the different fractions changed during incubation periods, due to the rabbit manure and nitrogen fertilizer added to the soil.

Keywords: *Humic substances, soil organic matter, Nitrogen fertilizer.*

Introduction

The soil organic matter (SOM) dynamics in the semi-arid soils is not well known. Soil organic matter plays a key role in plant nutrition, maintaining the productivity of soil, providing energy and substrate for biological activity and modifying the physical and chemical characteristics. Silva *et al.* (2013) found that the application of organic composts increased SOM levels in the semi-arid region of northeastern Brazil. Organic carbon in the soil is located in discrete pools (Jones & Donnelly, 2004). Soil organic carbon (SOC) is divided into pools having different properties and rates of turnover depending on stabilization mechanisms: (1) easily decomposable – labile fraction with a short residence time of a few months to several years, (2) stabilized by physical–chemical interactions with soil mineral components – intermediary fraction with a residence time of decades, and (3) biochemically recalcitrant – stabile fraction with residence time of centuries to millennia (Lal, 2009; Bruun *et al.,* 2010).

Humic substances are the most widely distributed organic products of biosynthesis on the face of the earth (Tan, 2003), exceeding the amount of carbon contained in all living organisms by approximately one order of magnitude (Steinberg, 2003). Humic substances in soils are the dark brown, fully decomposed (humified) remains of plant or animal organic matter. It is the most chemically active compounds in soils with cation and anion exchange capacities far exceeding clays. They are long lasting critical components of natural soil systems, persisting for hundreds to thousands of years, which can be destroyed in less than fifty years by some agricultural practices (Tan, 2003).

Major portion of SOM mainly composed of humic substances/ humus (stable organic matter compounds, consists of decayed organic matter of plant, animal and microbial origin) and almost termed synonym with SOM (Schnitzer, 2000). The composition and characteristics of humic fractions are related to their functionality and reactivity in soil. Three fractions are obtained by extraction with alkaline solution (Schnitzer, 1999): Soil humic fraction is classified as humic acid (HA): soluble in bases that precipitate in acid solution, Fulvic acid (FA): soluble in both acid and alkali solution and humin (HN): that is insoluble and inert part. Structural formation, chemical composition and stability of these humic substances are influenced by many variables like climate, parent material, altitude, vegetation type and soil management (Katyal, 2000). Humic acid contains many functional chemical groups that help to physically modify and improve the chemical properties of the soil and biologically stimulate plant growth (Anon. 2003). Fulvic acid is found in different sources such as soil lignin, peat, compost and vermicompost (Hemati *et al.* 2012). The main objective of this study was to investigate changes in the content of humic, non-humic substances in soil treated with manure, inorganic and bio nitrogen fertilizers under different incubation periods.

Materials and Methods

Surface soil samples (0 – 30 cm) were collected from scatter points at The Experimental Farm, Fac. of Agric., Al-Azhar Univ., Assiut that located 375 km south Cairo, Egypt (27° 12ˉ 16.67˭ N latitude and 31° 09ˉ 36.86˭ E longitude). The soil samples were air dried, crashed and sieved through a 2 mm sieve. Then all soil samples were mixed well to form one composite sample. Some chemical and physical properties of the soil are presented in Table 1(a and b) measured according to Page *et al.* (1982) and Klute (1986).

Table 1. Some physical and chemical properties of the tested soil

a- Physical properties

Particles size percentage			Texture Class	Moisture content θv%		AW (%)	Bulk density (g/cm^3)
Sand	Silt	Clay		FC	WP		
40.50	31.00	28.50	Clay Loam	31.50	15.30	15.70	1.57

FC = field capacity WP = wilting point AW = available water

b- Chemical properties

O.M. (%)	$CaCO_3$ (%)	pH	ECe (dS/m)	Soluble ions (meq/L)							Available nutrients (ppm)		
				CO_3 + HCO_3	Cl^-	SO_4	Ca	Mg	Na	K	N	P	K
1.65	2.1	7.63	0.93	1.98	1.05	5.5	2.64	1.0	5.0	0.30	56.5	36.8	343

OM = organic matter pH= soil reaction ECe=electrical conductivity

Twenty kg of the soil sample was prepared to the following treatments:

1) Organic fertilizer + bio-fertilizer named as OB
2) Slow release nitrogen fertilizer + organic fertilizer named as SO
3) Fast release nitrogen fertilizer + organic fertilizer named as FO
4) Slow release nitrogen fertilizer + organic fertilizer + bio-fertilizer named as SOB
5) Fast release nitrogen fertilizer + organic fertilizer + bio-fertilizer named as FOB
6) Control (without any fertilization) named as C

Rabbit manure (Table 2) as an organic fertilizer at a rate of 3.5 ton/ fed was thoroughly mixed with 250 g soil sample (0.88 g rabbit manure/ 250 g soil sample). Liquid bio-fertilizer (10 ml) contains both nitrogen fixers (*Azotobacter chroococcum* & *Azospirillum lipoferum*) and phosphate dissolving bacteria (*Bacillus polymixa*) at a ratio 1:1 was sprayed on surface soil sample upon wetted soil to field capacity.

Table 2. Some chemical properties of rabbit manure

pH (1: 2.5)	EC (dS/m) (1:2.5)	N %	Organic carbon %	K_2O %	P_2O_5 %
7.10	8.40	1.00	10.60	1.00	1.38

Two mineral nitrogen fertilizers (Urea 46.5% N as a fast nitrogen fertilizer, 76 kg/ fed, and ureaform 40% N as a slow release nitrogen fertilizer, 87 kg/ fed) were separately mixed well with 250 g of soil sample (19 mg urea or 22 mg ureaform/ 250 g soil sample). Two hundred and fifty grams from each earlier mentioned treatment were placed into plastic containers. All treatments were subjected to three incubation periods (14, 30 and 60 days) under laboratory condition (25–30 ^{0}C). The treatments were organized in a randomized block design with three replications to form an aggregate of 54 exploratory units. The treatments were moistened to the field capacity and their moisture level was monitored and adjusted weekly.

Soil samples were taken at incubation periods after 14, 30 and 60 days to determine total organic carbon (TOC), fulvic acids (FA), humic acids (HA) and humines (Hu). The fraction of humic substances (HS) was obtained by the sum of C of the three fractions, and the fraction of non-humic substances (NHS) was calculated as the difference between TOC and humifed C (HS).

Total organic carbon (TOC).

Total organic carbon was determined by wet oxidation of the organic material using potassium dichromate according to Walkley & Black method (Nelson and Sommers, 1982), which measures the easily oxidized carbon, divided by 0.86 (Richter *et al*., 1973) to carry the total organic carbon. This is the official method for TOC in Argentina. Briefly, organic matter from the soil (1 g) was oxidized with $K_2Cr_2O_7$ 1 N (10 ml) in concentrated sulfuric acid for 30 min, followed by titration of excess $K_2Cr_2O_7$ with ferrous-ammonium sulfate 0.5 N and N-phenyl anthranilic acid to indicate the end point.

Extraction and purification of humic acid and fulvic acid

The procedure used for the extraction of the humic acid (HA) and fulvic acid (FA) fractions was the method developed by the International Humic Substances Society (Swift 1996).

Briefly, 2g of air-dried samples of soil and 20 ml of 0.1 N NaOH at a ratio (soil/solution) of 1/10 (w/v) were placed in 50.0 mL tubes. Before centrifuging, samples were shaked for 20 hours at 150 rpm, then, the supernatant was separated by centrifugation at 4,000 rpm and the supernatant was collected in a flask. The precipitate remaining in the tube corresponded to the humin fraction (HU), which was dried in a laboratory oven at 45°C. The alkalin extract (AE) collected in the flask consists of the humic acid (HA) and fulvic acid (FA) fractions; the pH was adjusted to the pH between 1 and 2 with Hydrochloric acid concentrate 36%. The solution was settled for another 24 h, the insoluble humic substances were precipitated. The precipitated (HA) and the supernatant (FA) were separated by centrifuging at 4000 rpm for 15 min. The precipitate was collected and the supernatant was again adjusted to pH between 4.5 and 5 using NaOH solution. The fulvic acid fraction got was settled after 24 h. Both the humic substances and fulvic acid fractions were washed with distilled water to remove impurities. Finally, Organic C was determined in each of the organic fractions by the method described above for TOC.

Statistical analysis

The soil data were the mean values of three replicates with their standard deviation. The differences between mean treatments were tested using the Duncan's test at the P = 0.05 level.

Results and Discussions

About 200 years ago, the names humic acid, fulvic acid and humin were used to describe what workers believed to be three distinct fractions of humic substances. The three fractions were separated from various materials by using “classical” extraction techniques with aqueous (water) solutions.

1- First period of incubation (14 days).

1.1- Humic fractions. Data in Fig. (1), show the effect of nitrogen fertilization on humic fractions. Humic acid (HA), fulvic acid (FA) and humin (Hu) amounts were affected significantly ($P<0.05$) by sources of nitrogen fertilization.

Based on the data in Fig (1) it is clear that all treatments were the same approximation in amount of humic acid (HA), ranging from 3.75 to 3.51 ppm, while organic manure + bio-fertilizer (OB) and control (C) treatments were less than 1.66 and 2.00 ppm respectively. The total content of Fulvic acid (FA) in more treatments soil was less than the total content of humic acid, values range between than 2.52 and 0.91 ppm, while the total content of Fulvic acid was high form humic acid for (OB) and (C) treatments ranged between 2.40 and 2.85 ppm respectively.

The application of mineral fertilizers increased the contents of humic acid compared with fulvic acid in the soil. Thus, the application of rabbit manure resulted in the most significant increase in the content (HA) compared with (C) treatment, and vice versa with the (FA).

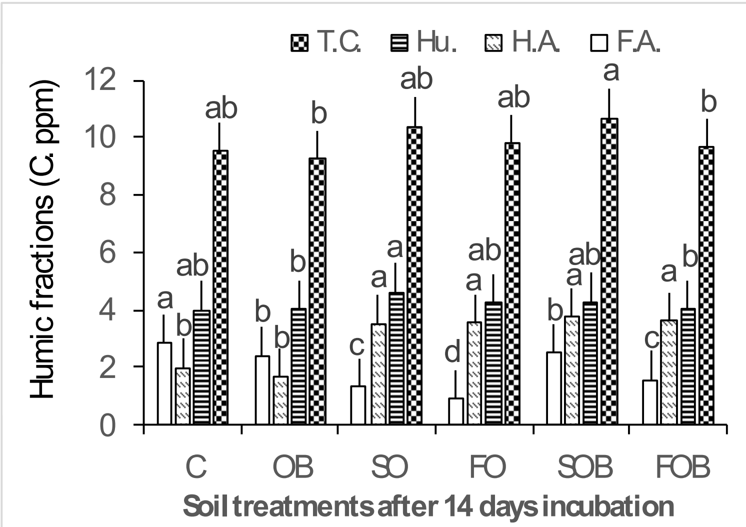

Fig. 1. Humic fractions in relation to different source of nitrogen fertilization after 14 days. (a, b, c, d) are statistically different at 0.05 levels.

Total organic carbon (TOC) contents were highest in the ureaform + organic manure + bio-fertilizer (SOB) and ureaform + organic manure (SO) treatments, differing from the control; the TOC levels were similar to the (C) treatment in all treatments. Total carbon accumulated in the soil resulting from the application of different organic composts is well documented in the literature. In a study by Damatto Júnior *et al.* (2006), fertilization with organic compost of sawdust and manure improved SOM, pH, P, Ca, sum of bases, CEC, and base saturation in the 0.00-0.20 m layer of soil under banana plants. The increase in TOC reported by the authors was attributed to the carbon derived from the aforementioned materials used in management of the areas. In the treatment

(SOB), humin (Hu) increased by 44.59% compared with the (C) treatment 35.03%. The amount of (Hu), values range between than 3.30 and 4.63 ppm.

1.2- Humic, non-humic substances (HS, NHS) and HA/FA ratio.

Humic substances are critical components of water and soil ecosystems, which are essential to soil genesis and the global cycling of carbon and nutrients. The interactions among microbes, clays and minerals are dependent upon humic substances. The vast agronomic and environmental importance of these materials is just beginning to be appreciated. Humic substances represent the compartment with the most stable soil organic matter (SOM) fraction.

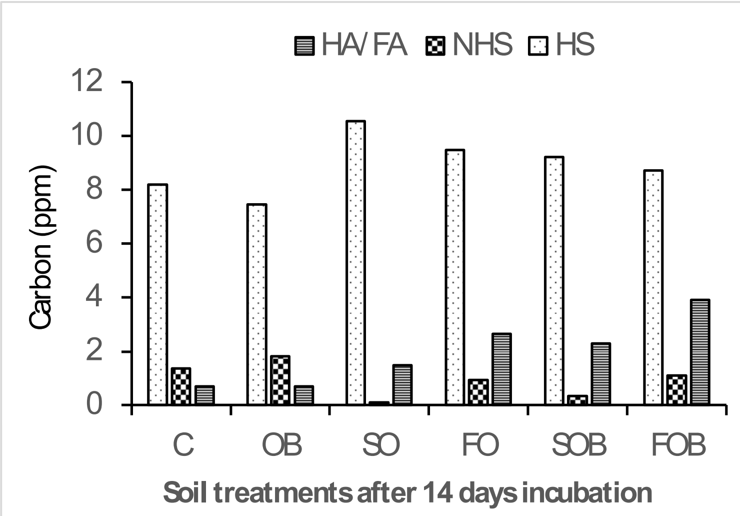

Fig. 2. Carbon in humic, non-humic substances (HS, NHS) and HA/FA ratio in relation to differences source of nitrogen fertilization after 14 days.

The increase in the humic substances (HS) observed in the all treatments occurred only in the (FA) and (Hu) fractions, ranged between 8.72 to 10.56 ppm, with no significant differences in the (HA) fraction (Fig 2) Compared with the (C) treatment 8.19 ppm. This increase improved chemical and physical soil properties, resulting in greater resistance to organic carbon (OC) loss. The carbon contents of the non-humifed SOM fraction (NHS) decreased significantly in all treatments, compared to the (C) treatment (Fig 2). However, only treatments with application of nitrogen fertilization resulted in higher levels of humic substances (HS) than in the (C) treatment. Although humic substances (HS) are more stable under changes resulting from management operations, accounted for approximately 88.6% 98.7% of TOC. Similar results of HS accumulation were reported by Adani et al. (2007) for soil treated with organic compost for four years. These substances are the result of chemical and biological transformations of plant residues and synthetic activity of soil microflora, representing the highest and most stable percentage of soil TOC. Humic substances accounted for approximately

70% to 80 % of SOM (Moreira and Siqueira, 2006).

The HA/FA ratio can be considered an index of humifcation of organic matter in the soil. The lower HA/FA ratio, the lower the humifcation rates of organic matter, which may be driven by edaphic processes, soil management and/or recent input of organic matter (Stevenson, 1994). Fulvic acids are the most reactive fractions, but with lower chemical stability. This indicates an unfavorable characteristic, which can facilitate cation leaching and illuviation of humifed clays in the form of organic complexes. Caetano *et al.* (2013) consider the FA and light organic matter more sensitive to changes caused by agricultural soil management than TOC. In tropical soils, the intense mineralization of organic matter and restrictions to soil biological activity reduce the HA/FA ratio (Benites *et al.,* 2003).

In this study, the HA/FA ratio in all treatments ranged from 1.49 to 3.91 ppm, except for (OB) and (C) treatment, HA/FA ratio lower than 1, ranged between 0.69 and 0.70 ppm respectively, thus indicating the predominance of FA over HA and low polymerization of humic substances as a result of low humifcation in (OB) and (C) treatment. Fontana *et al.* (2011) also found HA/FA ratios ranging from 0.6 to 0.9 in areas with different vegetation cover in the Atlantic forest region, with lowest values in the most disturbed areas.

2- Second period of incubation (30 days).

2.1- Humic fractions. The data in Fig. (3), show humic fractions as affected by the different source's nitrogen fertilization. The application of nitrogen to humic fractions significantly (P<0.05) affected on the decreased of the amount of humic acid from fulvic acid other than the initial incubation period. The amount of (HA) from by (SOB) and (SO) treatments were highest one during a short-term incubation while it was low with soil fertilized by urea with or without bio-fertilizers (FOB), (FO) treatments than in the (C) treatment.

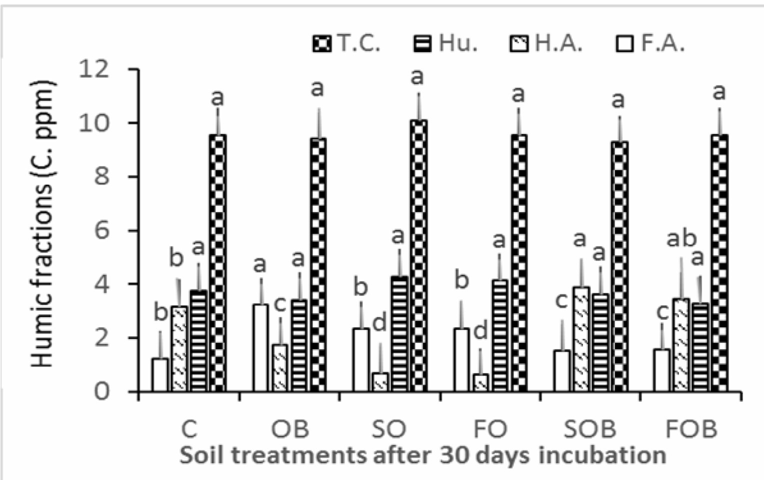

Fig. 3. Humic fractions in relation to different source of nitrogen fertilization after 30 days. (a, b, c, d) are statistically different at 0.05 levels.

Fulvic acid increased in all treatments compared to control treatment, (FA) ranged from 1.53 to 3.22 and 1.24 ppm respectively. As for humic forms (HA & FA), there was a difference between the treatments and some of them and the (C) treatment, while there were no significant changes in the most stable forms of humified soil carbon such as humin (Hu). Also, almost similar results are recognized after the second 30 days or the first 14 days and total organic carbon values range between 9.27 and 10.11 ppm. TOC may be not sensitive enough to detect the effect of management on carbon dynamics. Since the most abundant organic fractions in the soil are those of slower cycling, many years are needed to detect consistent differences in TOC (Gómez *et al.,* 2001). In this sense, more labile fractions of soil organic carbon (FA, HA) could be a more useful indicator of soil quality and an efficient tool for detecting the progress of soil degradation and loss of fertility.

2.2-Humic, non-humic substances (HS, NHS) and HA/FH ratio.

Humic substances are formed by the decomposition of plant and animal residues by microorganisms . Humic substances are the components of humus and as such are high molecular weight compounds that together form the brown to black hydrophilic, molecularly flexible, polyelectrolyte called humus. General, data in Fig. (4). indicated that the (HS) for all treatments after 30 day is less than that resulted from treatments after 14 day, except (OB) treatment. The (HS) ranged between 7.12 to 9.08 ppm, the amount of (HS) from (FOB) and (FO) treatments were lower one during a short-term incubation than the (C) treatment. The amount of (NHS) from (FOB) and (FO) treatments were high values range between 2.78 and 2.43 ppm respectively, then the (C) treatment 1.36 ppm.

The HA/FA ratio Fig. (4). in (OB), (FOB) and (FO) treatments ranged from 0.27 to 0.55 ppm, Compare with other treatments and (C) treatment. In generally, the trend for HA/FA ratio after 30 days incubation was a decrease in value in all treatments compared to the (C) treatment, this trend reverses after 14 days. This HA/FA ratio followed the descending order of C > SO > SOB > OB > FOB > FO treatment.

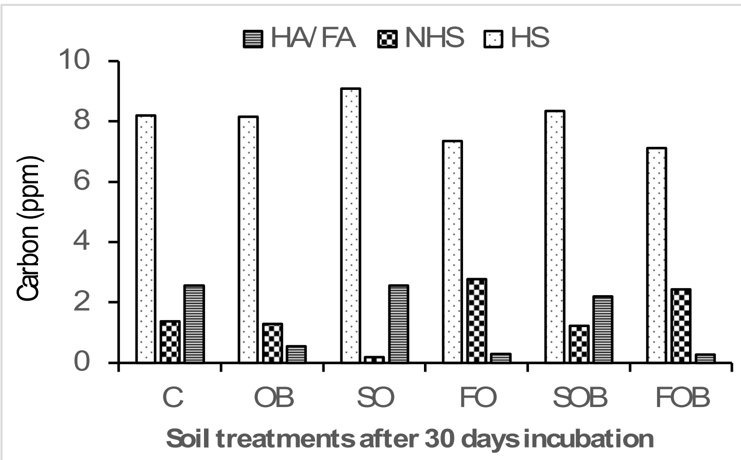

Fig. 4. Carbon in humic, non-humic substances (HS, NHS) and HA/FA ratio after 30 days.

3- Third period of incubation (60 days).

3.1- Humic fractions. Concentrations of carbon in fulvic acid (FA), humic acid (HA), and humin fractions in the all treatments are presented in Fig. 5. Fulvic acid (FA) and humic acid (HA) amounts affected significantly ($P<0.05$) by sources of nitrogen fertilization. Ranged values of (HA), (FA), and humin varied from 1.12 to 3.02 ppm, 1.36 to 3.18 ppm, and 3.85 to 4.59 ppm, respectively. As expected, humin contents were higher than those of the (HA) and (FA) fractions, for all treatments (Fig. 5). The humic substances in this study is characterized by higher content of (FA) than (HA) after the third period of incubation 60 days, significant differences between some treatments in terms of humin content than observed for control treatment. The humin fraction is less prone to change with management practices, because it is the most stable and recalcitrant fraction (Stevenson, 1994).

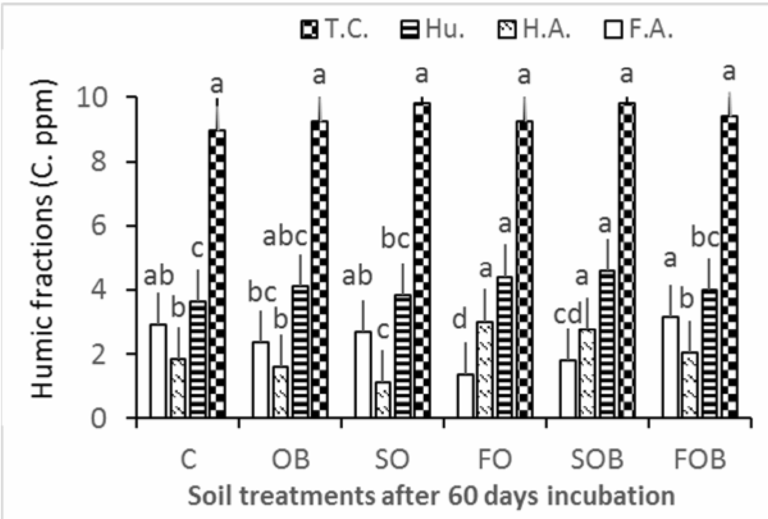

Fig. 5. Humic fractions in relation to different source of nitrogen fertilization after 60 days. (a, b, c, d) are statistically different at 0.05 levels.

A higher concentration of carbon in the humin fraction of the (SOB) and (SO) treatments in all periods of incubation could be related to intensive microbial activity and consequently a higher SOM decomposition rat. Furthermore, humin constitutes a major part of the total organic carbon (TOC), and has an intimate association with the soil mineral fraction (Moraes *et al.,* 2011). Since the concentrations of humic substances are influenced by the amounts of organic residue inputs and TOC, calculation was made of the proportion of each fraction (fulvic acids, humic acids, and humin) (Fig. 5). The overall trend was humin (36 to 47%) > HA (7 to 37%) > FA (10 to 34%). More (FA) than (HA) were observed in the second and third period, with the exception of the first period was less.

3.2- Humic, non-humic substances (HS, NHS) and HA/FA ratio.

Humic substances are key fractions of soil organic matter, a major aspect controlling the flexibility of plant nutrition irrespective of land use system through different processes and mechanisms. Land use system practices and its management is a factor governing the quantity and quality of organic matter in turn humic substances. Data presented in Fig. 6. Show the (HS) and (NHS). In general, the (HS) was highest in the third period compared with previous periods; the increase in the HS-C observed in (FOB), (FO), (SOB) and (SO) treatments, ranged values between 95 and 98% respectively. than (OB) treatment 83%. While (NHS) decreased for these treatments.

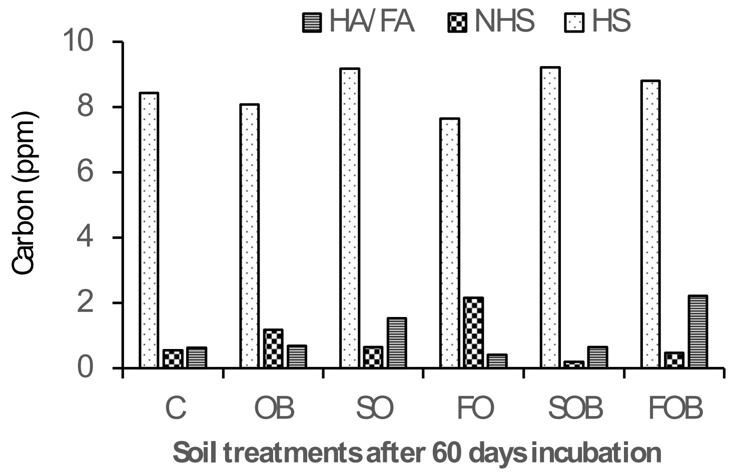

Fig. 6. Humic, non-humic substances (HS, NHS) and HA/FA ratio after 60 days.

The ratio between humic and fulvic acids (HA/FA) reflects the mobility of soil organic carbon. A HA/FA ratio near 1 is indicative of good quality organic material that could enhance soil physical properties and improve plant growth. HA/FA ratio >1 indicates loss of the more labile FA fraction, a situation very common in sandy soils. In this period, the HA/FA ratio varied from 0.42 to 1.53 ppm for all treatments, except (FOB) treatment was 2.22 ppm. More (FA) than (HA) indicates low humification rates.

4- Analogous among incubation periods

In the FO (fast release nitrogen + organic fertilizers), total organic carbon (TOC) content was significantly ($P<0.05$) as the incubation period increased compared to all treatments, Fig. (7). While humin no significantly in all treatments, compared to the SOB (slow release nitrogen + organic + bio fertilizers) treatment,

Fulvic acid (FA) amount affected significantly ($P<0.05$) by incubation period increased in the all treatments. While humic acid (FA) amount affected significantly ($P<0.05$) by incubation period increased in the of SOB (slow release nitrogen + organic + bio fertilizers), FO (fast release nitrogen + organic fertilizers), SO (slow release nitrogen + organic fertilizers), FOB treatment (fast release nitrogen + organic + bio fertilizers) and C control, except for OB treatment (organic + bio fertilizers).

Conclusions

The use of humic fractions to evaluate changes in soil carbon dynamics due to agricultural use was more effective than determination of total organic carbon. The distribution of carbon among the different fractions changed during incubation periods, due to the rabbit manure and nitrogen fertilizer added to the soil. Amongst this fractions, fulvic acid predominated, resulting in a HA/FA ratio < 1 with 30 and 60-days incubation. The predominance of a higher percentage of fulvic acids, as compared to humic acids, indicated a slow rate of SOM decomposition. (FA) was effective in discriminating changes in all incubation periods, due to its characteristic lability. On the other hand, the (Hu) fraction was stable, and Sustainable management practices should therefore be employed in order to achieve soil stability and biological productivity.

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الكربون العضوي في الصور الهيومية في التربة المتأثرة بالسماد النيتروجيني العضوي وغير العضوي والحيوي خلال فترات التحضين المختلفة

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قسم علوم الأراضى والمياه- كلية الزراعة- جامعة الأزهر- أسيوط- مصر

الملخص

المواد الدبالية تنقسم الي ثلاثة صور رئيسية، الهيومين (Hu) وحمض الهيوميك (HA) وحمض الفولفيك (FA). تعتبر المواد الهيومية مصدرًا جيدًا للطاقة للكائنات الحية المفيدة في التربة، بالإضافة إلى الصور الرئيسية للمواد العضوية في التربة، وهو جانب رئيسي يتحكم في مرونة تغذية النبات بغض النظر عن نظام استخدام الأراضي من خلال عمليات وآليات مختلفة. الهدف الرئيسي من هذه الدراسة هو معرفة التغيرات في محتوى المواد الدبالية وغير الدبالية في التربة المعاملة بالأسمدة النيتروجينية العضوية وغير العضوية والحيوية خلال فترات تحضين مختلفة. تم جمع عينات التربة بعد ١٤ و ٣٠ و ٦٠ يومًا من التحضين لتقدير الهيومين وحمض الهيوميك وحمض الفولفيك.

تأثرت كميات حمض الهيوميك (HA) وحمض الفولفيك (FA) بشكل معنوي (P <0.05) بمصادر التسميد النيتروجيني خلال فترات التحضين المختلفة. ففي الفترة الأولى، أظهرت النتائج أن استخدام الأسمدة المعدنية زاد من محتوي حمض الهيوميك مقارنة مع حمض الفولفيك في التربة. وبالتالي ، أدت إضافة سماد الأرانب الي الزيادة الأكثر أهمية في محتوى حمض الهيوميك (HA) مقارنا مع معاملة الكنترول، والعكس بالعكس مع حمض الفولفيك. تتميز المواد الهيومية في هذه الدراسة بمحتوى عالى من حمض الفولفيك (FA) أكثر من حمض الهيوميك (HA) بعد فترة التحضين الثالثة ٦٠ يومًا ، مع وجود فروق ذات دلالة إحصائية بين بعض المعاملات من حيث محتوى الهيومين من تلك الملاحظة لمعاملة الكنترول. وكان الاتجاه العام، الهيومين (٣٦% - ٤٧%) أكبر من حمض الهيوميك (٧% - ٣٧%) أكبر من حمض الفولفيك (١٠% - ٣٤%). وقد لوحظ أن نسبة حمض الفولفيك (FA) أكبر من نسبة حمض الهيوميك (HA) في الفترة الثانية والثالثة ، باستثناء الفترة الأولى. بعد فترة التحضين الثالثة ٦٠ يوما ، تراوحت نسبة HA / FA من ٠,٤٢ إلى ١,٥٣ جزء في المليون لجميع المعاملات، بإستثناء المعاملة (FOB) التي بلغت ٢,٢٢ جزء في المليون. زيادة حمض (FA) عن حمض الهيوميك (HA) يشير إلى انخفاض معدلات التدبل. تغير توزيع صور الكربون المختلفة خلال فترات الحضانة، بسبب سماد الأرانب والأسمدة النيتروجينية المضافة إلى التربة.