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Original article

The effect of applied nanozeolite and some plant residues on organic carbon changes in density and soluble fractions - incubation study

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ABSTRACT

The present paper studies organic carbon changes in different fractions in a soil treated with different levels of nanozeolite, alfalfa and wheat residue and incubated for 90 days. The results showed that the amounts of light fraction (LF) and heavy fraction (HF) increased with increasing the percentage of nanozeolite and plant residues in the soil. The highest amounts of LF (7.54 g LF/Kg Soil) and HF (11.10 g C/Kg Soil) were found when 30% nanozeolite, 5% wheat and alfalfa straws were added to the soil. Accordingly wheat straw and alfalfa straw were effective for increasing the LF and HF respectively. However they decreased with decreasing the soil organic matter (SOM) from the 1st day of experiment until the 90th day of experiment. In addition to that, soluble organic carbon fraction plays an important role in soil and many ecosystem processes. Soluble organic carbon in hot (2.22 g C/Kgsoil) and cool (1.54 g C/Kgsoil) water fractions increased with the addition of 30% nanozeolite and 5% plant residues particularly alfalfa straw in comparison with control. Although they increased after 30 days of incubation and then they decreased in the continuation of the experiment. In fact, organic carbon (OC) contents in density and soluble fractions increased with application and addition of 30% nanozeolite and 5% plant residues into the soil, However they decreased with decreasing the SOM after 30 days of incubation. This study revealed that, application of nanozeolite and plant residues improve carbon pools in density and soluble fractions and carbon

sequestration increase by increasing the OC contents in soil.

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1. Introduction

1.1. Density fractionation

Changes in total soil organic carbon (SOC) with changes in land use and management can be partly explained by the way C is allocated in different fractions of soil organic matter (SOM) (Tan et al., 2007). These fractions exhibit different rates of biochemical and microbial degradation (Stevenson, 1994) as well as different accessibility and interactions (Sollins et al., 1999).

The dynamics of SOC are usually described by dividing SOM into two or more fractions. Physical fractionation of SOM is useful for distinguishing specific C pools responsive to management, identifying the physical control of SOM (Cambardella and Elliott, 1993a; Collins et al., 1997), and characterizing the relationship between SOM and size distribution of aggregates (Feller et al., 1996).

Density fractionation is a laboratory procedure that physically separates soil into light and heavy fractions (Wander and Traina, 1996; Sollins et al., 1999). The procedure is useful for assessing labile pools of SOM that are more sensitive to cropping practice than is the total SOC pool in temperate soils (Janzen et al., 1992). Among liquids for density fractionation, sodium polytungstate (SPT) solution of 1.85 g .mL⁻¹ is often used (Magid et al., 1996; Six et al., 1998, 2002). Light fraction is commonly referred to a plant-like and less stable fraction with high C concentration (Golchin et al., 1994; Gregorich et al., 1996). Heavy fraction is a more stable and high-density organo-mineral fraction having lower C concentrations (Golchin et al., 1995a, b).

Light fraction of SOM is not only sensitive to changes in management practices (Cambardella and Elliott, 1992; Bremer et al., 1994) but also correlates well with the rate of N mineralization (Hassink, 1995; Barrios et al., 1996). By incubating bulk soil and density fractions, Alvarez and Alvarez (2000) observed that light fraction was the driving factor in soil respiration.

Light fraction supposedly represents an intermediate pool between undecomposed residues and humified SOM (Gregorich and Janzen, 1996). In contrast, the heavy fraction contains more processed SOM (Hassink, 1995; Wander and Traina, 1996) and can be a major sink for C storage in soil because it has a little mineralizable C (Barrios et al., 1996; Whalen et al., 2000) however is demonstrated by poor relationships with soil respiration (Alvarez and Alvarez, 2000).

The importance of light fraction (including free and occluded organic C within aggregates) is widely recognized for its role in formation and stability of soil structure, especially in stabilization of soil macroaggregates (>250 μ m) (Miller and Jastrow, 1990; Kay, 1998). Janzen et al. (1992) reported that light fraction of surface soil (0–7.5 cm) accounted for 2–17% of SOC, depending largely on cropping systems. However, there are few direct data quantifying these two fractions and their contributions to total SOC storage as related to changes in land use and tillage practices.

1.2. Water soluble organic carbon

Soluble organic matter in soils plays an important role in many ecosystem processes. For example, the size of this pool of organic matter and its availability as a substrate are critical to nutrient fluxes in agricultural systems. The amount and biodegradation of soluble organic matter in soil also have implication for different parts of the ecosystem, from the atmosphere (e.g., Production of greenhouse gases) to the hydrosphere (e.g., Water quality) (Gregorich et al, 2003).

Plant residue and humus are the most significant sources of soluble organic matter in soil (Kalbitz et al., 2000). In studies using $\delta^{13}\text{C}$ natural abundance technique to identify the source of carbon in labile organic matter fractions, Gregorich et al. (2000) observed that C isotope signature of water-soluble C was similar to that of humus, whereas for the microbial biomass C, it was similar to recent maize residue. They hypothesized that, although the water-soluble C pool was small, it had a high turnover and was in equilibrium with soil humus.

Results of Boyer and Groffman. (1996) suggested that, difference in water-soluble and biodegradable C between agricultural soils and forest soils (values were higher in agricultural soils) was due to increases in soluble humic materials in agricultural soils. Therefore, humus is likely the major source of water-soluble C because of the relatively large amount of humus present in soil relative to that contributed by the microbial biomass or recently deposited plant residues. Soluble organic matter is an important substrate for microorganisms (Marschner and Bredow, 2002) and is quickly depleted during incubation.

Laboratory studies have shown that microorganisms can decompose different amounts of the water-soluble organic matter fraction (Boissier and Fontvieille, 1993; Nelson et al., 1994; Boyer and Groffman, 1996). These studies, which ranged in duration from hours to months, indicated that 10–40% of the water-soluble organic C was decomposable under laboratory conditions. Boyer and Groffman (1996) reported that land use and soil depth had significant effects on the proportion of soluble organic C that was readily biodegradable C (i.e., Labile C). In order to fully understand the dynamics of soluble organic C, it is useful to have some knowledge of the biological nature of soluble organic N. But in contrast to soluble organic C, little work has been done to characterize soluble organic N.

The quantity and the biological nature of dissolved organic matter are affected by the extraction procedure used. Extraction procedures involving higher temperatures extract a greater amount of soluble organic matter than extractions carried out at room temperature. High temperature is known to hydrolyze organic structures, lyse cells, and dissociate organic materials from inorganic colloids (Nelson et al., 1994).

Davidson et al. (1987) found strong correlations between organic C extracted with hot water and mineralizable C, but noted that the extent to which heterotrophs could decompose the extracted C was uncertain. Nonmicrobial pools contribute to the hot water-extractable C (Sparling et al., 1998), and this material may represent a pool of organic matter involved in the formation of stable aggregates (Haynes and Swift, 1990).

During last years, great strides have been made in a number of research topics including characterizing the spatial and temporal variations in the concentration and flux of dissolved organic carbon (DOC) reviewed by Kalbitz et al., 2000 and Aitkenhead-Peterson et al., 2003), quantifying its role in soil chemistry and pedogenesis (e.g., Kaiser and Zech, 1998; Jansen et al., 2003; Cances et al., 2003), describing the chemical composition of DOM (Guggenberger and Zech, 1994; Kaiser et al., 2001; Strobel et al., 2001), and quantifying the availability of DOC to soil microflora (Zsolnay and Grolitz, 1994; Yano et al., 2000; Kalbitz et al., 2003; Marschner and Kalbitz, 2003). Thus the objective of the present study was to determine OC in water soluble C and density fractions of carbon in soil treated with different levels of nanozeolite and some plant residues in field capacity for 90 days.

2. Materials and methods

2.1. Study area

This study was conducted in Hamedan province, western Iran. This area was located between longitudes 47° 42' and 48° 45' E and latitudes 33° 28' and 34° 29' N. The climate of the region is semiarid with a mean annual precipitation of 300 mm and a mean annual temperature of 10 °C. Agriculture is a major industry and principal land use in Hamadan. Major crops grown in Hamadan are winter wheat (*Triticum aestivum* L.), Alfalfa (*Medicago sativa*), potato (*Solanum tuberosum*) and garlic (*Allium sativum*). The soil of the area is mostly classified as Aridisol.

2.2. Sampling, treatment and analysis of soil

The soil samples were collected from the top layer of soil (30cm) in an agricultural land. Soil, wheat straw, alfalfa straw and nanozeolite sampling and analysis. Wheat straw, alfalfa straw and nanozeolite pH and electrical conductivity (EC) were measured in a 1:10 Soil, plant residues and nanozeolite separately: water extract after shaking for 2 h. Their organic carbon (OC) was determined by wet oxidation (Walkley and Black, 1934). Total phosphorous was determined in acid (HCl) solution of ash of plant residues spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Peperzak et al. 1959). Total nitrogen content was determined by the Kjeldahl method (Hinds and Lowe 1980). Cation exchange capacity (CEC) of the soils were determined by the 1 M NaOAc, pH 8.2 method (Pratt 1965). Then the soil was then air-dried and mixed with different amounts of (<2 mm) wheat straw and alfalfa straw (0 and 5% w/w) separately. Samples were mixed with different amounts of nanozeolite (0, 10 and 30% w/w) and they were kept in field capacity moisture content

and lab condition. Untreated soils also incubated as controls. The treated soils, wheat straw, alfalfa straw and nanozeolite were uniformly wetted with a spray to a water content near field capacity. The approximation of field capacity of each mixture of soil, wheat straw, alfalfa straw and nanozeolite was determined gravimetrically the volume of water required to bring each of the soils to the approximated field capacity was determined by weighing daily. The treated and moistened soils were incubated in lab condition (20-25 °C) for 90 days. After 1, 5, 10, 20, 30, 45, 60, 75 and 90 days of incubation, a portion of each soil was taken for the study of organic carbon in density (light and heavy) and soluble (hot water and cool water) fractions.

2.3. Density fractionation

About 10 g dried sample was transferred to a 20 ml graduated centrifuge tube, and 50 ml of NaI solution ($d = 1.3 \text{ g. cm}^{-3}$) was added. Suspensions were immediately centrifuged at 3000rpm for 10 min. The supernatant containing the light fraction (LF) was decanted onto Whatman no. 50 filters (2.7- μm retention) and vacuum-filtered. The heavy fraction (HF) residue was resuspended twice in fresh NaI solution and the LFs were combined. Light and heavy fractions were then washed four times into preweighed tins with deionized water, afterwards dried at 55°C for 24 h in the oven, and weighed (Strickland and Sollins., 1987). Then organic carbon content in heavy fraction was determined by Walkley and Black, (1934) method.

2.4. Soluble water organic carbon fraction

The soluble water organic carbon in whole soil and the three aggregate fractions were extracted using cold water followed by hot water. Cold water-soluble organic matter was extracted from soils by adding 150 ml of distilled/deionized water to a tube containing 15g of air-dried whole soil or aggregate fraction. The soil:water suspension was shaken for 30 min and centrifuged at 4500 rpm for 20 min. The supernatant solutions were decanted and passed through a 0.45- μm cellulose nitrate filter. The weight of extraction tubes with remaining wet soil was recorded in order to calculate the amount of cold water extract remaining. Hot water-soluble organic matter was extracted from these soils by adding water to the wet soil remaining in each tube to return the water volume to 150 ml, then by placing the tubes in a water bath at 80 °C for 16 h. After this period of time, the samples were centrifuged, decanted, and filtered as above. Filtered solutions were stored in a refrigerator (4 °C) prior to incubation (Gregorich et al., 2003). Then organic carbon content in heavy fraction was determined by Walkley and Black, (1934) method.

2.5. Statistical data analysis

The experiment was a completely randomized factorial design with three replicates. The factors applied were alfalfa straw (0 and 5%), wheat straw (0 and 5%), Nanozeolite (0, 10 and 30%) and incubation time (1, 5, 10, 20, 30, 45, 60, 75 and 90 days). Experimental data of Light fraction, Heavy fraction and soluble fraction in hot water and cool water of organic carbon were subjected to analysis of variance and the means compared with the Duncan's new multiple range test by SAS Ver.9.2.

3. Results and discussion

3.1. Some of soil, plant residues and nanozeolite properties

Table 1 shows the sand, silt and clay contents were 69, 19 and 12%, respectively in the soil corresponding to a loamy sand texture. The soil was not saline (EC 1.1 dS.m⁻¹); equivalent calcium carbonate and pH values were 1.79% and 7.2 respectively, with low cation exchange capacity (CEC 4.80 cmolc.kg⁻¹), organic matter (OC 3.41 g.kg⁻¹).

Table 1

Some chemical and physical properties of soil used in this study.

EC (dS/m)	pH	% CCE	Total Organic C (g/Kg)	CEC (Cmolc/Kg soil)	Soil Texture		
					%Sand	%Clay	%Silt
1.1	7.2	1.79	3.41	4.80	69	12	19

Table 2 presents some properties of applied plant residues. Alfalfa and wheat straw had neutral pH (6 and 7.97), high OC (511 and 532 g.kg⁻¹) values and C/N (23.30 and 90.75) and C/P (85.20 and 123.50) ratios respectively. Some of applied nanozeolite properties present in (Table 3), also Nanozeolite compositions and their weight percentage are shown in (Table 4); according to this table, SiO₂ and Al₂O₃ (69.44 and 11.87 %) respectively had higher portion than other compositions.

Table 2

Some properties of applied plant residues in this study.

	pH	EC (dS/m)	Total organic C (g/Kg)	Total Nitrogen (g/Kg)	Total Phosphorous (g/Kg)	C/N	C/P
Alfalfa straw	6	9.5	511	22	5.98	23.30	85.20
Wheat Straw	7.97	4.3	532	7	4.31	90.75	123.50

Table 3

Some properties of applied Nanozeolite.

EC (dS/m)	pH	Organic C (g/Kg)	CEC (Cmolc/Kg soil)
0.98	7.17	1.03	400.39

3.2. The effect of nanozeolite and plant residues on oc in density fraction

Table 5 shows the analysis of variance of the effects of nanozeolite, plant residues application, incubation time, and their interaction on light fraction (LF), heavy fraction (HF), Soluble OC in Cool water and hot water fractions in soil. Also this table shows that the significant ($p < 0.01$) effects of application of nanozeolite, alfalfa and wheat straws, their interaction and incubation time on LF and HF, Soluble OC in Cool water and hot water fractions in soil. However, the interactions between nanozeolite and incubation time, plant residues and incubation time and nanozeolite, plant residues and incubation time did not have significant effects on mentioned organic carbon fractions in the soil.

Table 4

Nanozeolite compositions and their %Wt.

Composition	%Wt
SiO ₂	69.44
Al ₂ O ₃	11.87
Fe ₂ O ₃	1.30
Ti ₂ O	0.18
K ₂ O	1.31
Na ₂ O	0.68
CaO	3.28
MgO	0.99

Table 5

Analysis of variance (mean square) of the effects of zeolite, plant residues application, incubation time and their interaction on light (LF) and heavy fraction (HF), Soluble OC in Cool water and hot water fractions in soil.

Source	DF	Organic Carbon (gr/Kg soil)			
		LF (g LF/Kg Soil)	OC in HF (gC/Kg Soil)	Soluble OC in Cool water (g C/Kg soil)	Soluble OC in Hot water (g C/Kg soil)
Zeolite	2	524.45 **	1144.33 **	1.54 **	1.43 **
Plant Residues	3	54 **	201.44 **	0.19 **	0.80 **
Time	8	65.45 **	353.87 **	5 **	11.59 **
Zeolite*Time	16	60.48 ns	1.97 ns	1.66 ns	3.67 ns
Plant Residues*Time	24	19.59 ns	0.98 ns	1.16 ns	2.72 ns
Zeolite* Plant Residues	6	111.15 **	3.90 **	0.34 **	0.49 **
Zeolite*Plant Residues*Time	48	16.32 ns	0.54 ns	0.41 ns	0.92 ns
Error	216	0.02	0.05	0.1	0.1

**) Mean square of the treatment is significant at the 0.01 level.

ns) Mean square of the treatment is not significant.

Table 6 reveals the OC content in LF and HF increased by addition of nanozeolite and plant residues ($P < 0.01$). LF value in 30% nanozeolite plus 5% wheat straw (B compound) treatment was higher than the other treatments, its value 7.54 (g LF/Kgsoil) increased comparison with the control, because C:N ratio in wheat straw is higher than alfalfa straw thus subsequently wheat straw has lower stage of biodegradation by microorganisms in soil. This fraction of organic carbon decreased from the 1th day (20.61 g LF/Kg Soil) to the 90th day (16.99 g LF/Kg Soil) in during soil incubation (Fig. 1).

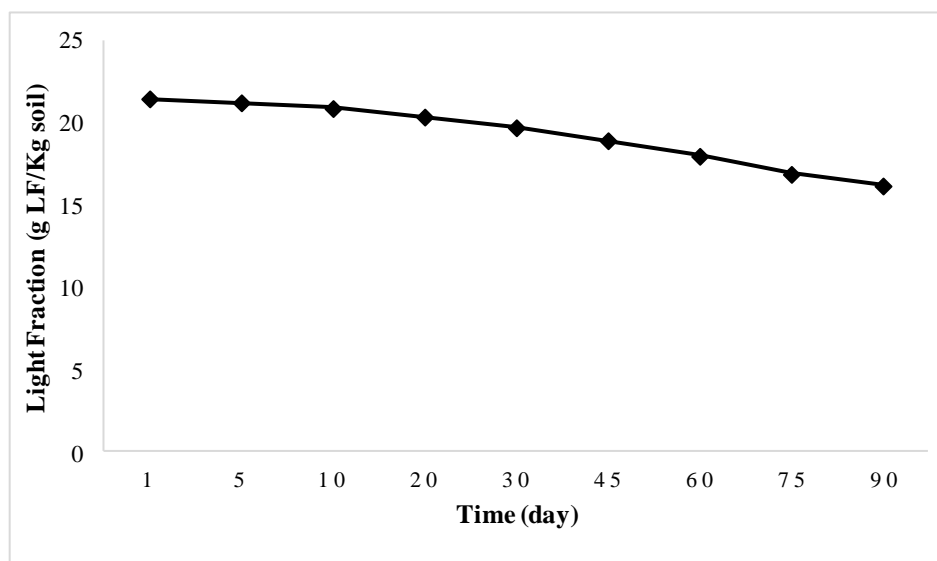


Fig.1. Light Fraction changes with the passage of time.

The heavy fraction value in 30% nanozeolite plus 5% alfalfa straw (A compound) was significantly increased (11.1 g C/Kg Soil) in comparison with the control (Table 6). Also this table investigates that A compound increased heavy fraction in comparison with the 10% nanozeolite plus 5% alfalfa straw and 0% nanozeolite plus 5% alfalfa straw compounds (3 g C/Kg Soil) and (8.2 g C/Kg Soil) respectively.

It is known that the alfalfa straw was more efficiency due to increasing organic carbon in heavy fraction than wheat straw in all of the treatments with the similarity percentage of nanozeolite (Table 6). Soil organic carbon (SOC) in the light fraction plays important role in retaining of cellulase molecule from washing out and nutrition of

soil microorganisms and subsequently humus production. Thus soil organic matter quality is an important factor in its disintegration rate (Schmidt et al, 2002; Beheshtiet al, 2012).

According to the Fig. 2, OC in heavy fraction had a distinct downward trend from the 1st day (27 g C/Kg Soil) until the 90th day (18.4 g C/Kg Soil). The recent research on organic carbon decay dynamics showed that LF and HF were decreased during of soil incubation (Hassink et al, 1995; Creamer et al, 2012). The results of Rovira and Vallejo's studies (2003) were in line with those of the present study.

Table 6

Light fraction and Heavy fraction content in all of treatment.

Treatment	LF (g LF/Kg Soil)	OC in HF (gC/Kg Soil)
Control	16.32±1.30a	17.60±3.00
0% nanozeolite+ 5% alfalfa straw	17.08±1.27	20.50±3.01
0% nanozeolite+ 5% wheat straw	18.04±1.28	19.70±3.02
10% nanozeolite+ 0% straw	16.50±2.15	17.98±3.02
10% nanozeolite+ 5% alfalfa straw	19.20±2.13	25.70±3.01
10% nanozeolite+ 5% wheat straw	19.52±2.14	24.60±3.02
30% nanozeolite+ 0% straw	16.63±2.10	18.10±3.00
30% nanozeolite+ 5% alfalfa straw (A compound)	21.78±2.12	28.70±3.02
30% nanozeolite+5% wheat straw (B compound)	23.86±2.11	27.00±3.01

a. Mean ± Standard deviation

The same letters are not significantly different at $p < 0.01$ using Duncan's LSD.

3.3. The effect of nanozeolite and plant residues on water soluble organic carbon fraction

As shown in Table 7, soluble organic carbon contents in hot water and cool water increased by addition of nanozeolite and plant residues especially alfalfa straw. The results showed that soluble OC in hot water in A compound was greater 2.22 (g C/Kg soil), 1.36 (g C/Kg Soil) and 2.06 (g C/Kg Soil) than control, (10% nanozeolite plus 5% alfalfa straw) and (0% nanozeolite plus 5% alfalfa straw) treatments respectively (Table 7). Work on the chemical composition of dissolved organic carbon (DOC), suggested that most DOM is an end product of microbial metabolism (Guggenberger and Zech, 1994); However short-term experimental manipulations of organic matter sources showed that fresh litter also contributes significantly to the production of DOC (Park et al., 2002).

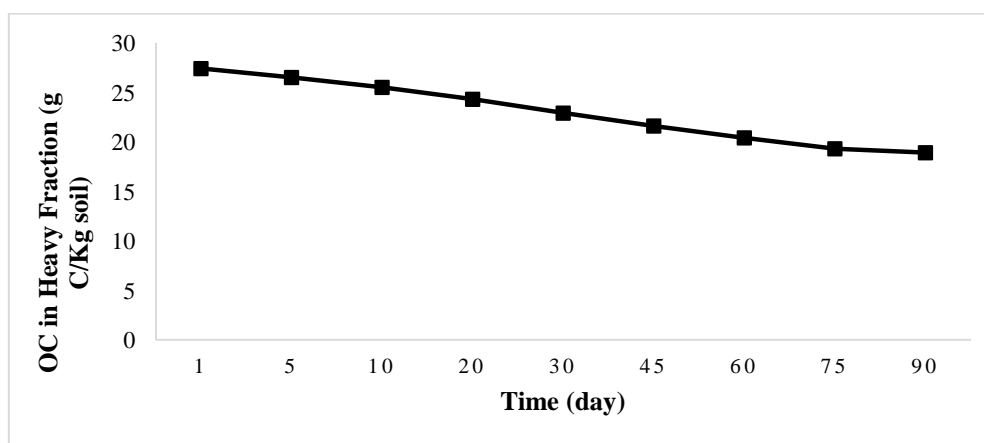


Fig. 1. Organic Carbon changes in heavy fraction with the passage of time.

These two views are not necessarily mutually exclusive, but they do point out the considerable difficulty in determining the influence of substrate (litter, soil organic matter), microbial community composition (Muller et al., 1999), and abiotic factors such as temperature and water flux on DOC production and flux (Brooks et al., 1999).

Based on Table 7, the same results were achieved similar the results of hot water to cool water, accordingly soluble OC in cool water increased with the greater percentage of Nanozeolite and plant residues particularly alfalfa straw. Soluble OC in cool water value increased 1.54 (g C/Kg soil) and 0.58 (g C/Kg soil) from control into the

(A compound) and (B compound) respectively. Thus (A compound) more effective to increasing soluble OC in cool water than (B compound).

Table 7

Soluble organic carbon in Hot and Cool water content in all of treatments.

Treatment	Soluble OC in Cool water (g C/Kg soil)	Soluble OC in Hot water (g C/Kg soil)
Control	1.93±0.358a	2.34±0.552
0% nanozeolite+ 5% alfalfa straw	2.01±0.360	2.50±0.546
0% nanozeolite+ 5% wheat straw	1.94±0.357	2.42±0.561
10% nanozeolite+ 0% straw	1.98±0.255	2.52±0.454
10% nanozeolite+ 5% alfalfa straw	2.33±0.251	3.20±0.461
10% nanozeolite+ 5% wheat straw	2.29±0.248	3.10±0.477
30% nanozeolite+ 0% straw	2.00±0.255	2.63±0.480
30% nanozeolite+ 5% alfalfa straw (A compound)	3.47±0.251	4.56±0.491
30% nanozeolite+5% wheat straw (B compound)	2.51±0.247	3.44±0.473

a. Mean ± Standard deviation

The same letters are not significantly different at $p < 0.01$ using Duncan's LSD.

Soluble OC content in hot water was higher than soluble OC content in cool water (Table 7), because hot water has greater ability to extract of lysis microbial cells and extractable soluble organic matter may be adsorbed to clay or complexed with other organic material produced by plants or decomposing organic matter than cool water (Guggenberger and Zech, 1994; Muller et al., 1999).

The plant residues with lower C:N ratio are a readily decomposable substrate for microorganisms and they have additional soluble OC content than plant residues with higher C:N ratio (Gregorich et al., 2003). Also these researchers found that the quantity of biodegradable soluble organic matter was related to the extraction procedure and the quantity of organic matter present in the soil.

Fig. 3 indicates that soluble OC in both hot water and cool water increased with over time from 1st day until the 30th day of incubation period but then decreased by the end of the experiment. Accordingly, the soluble OC in cool water increased from 2.17 (g C/Kg soil) in the 1st day to 2.68 (g C/Kg soil) in the 30th day and then it decreased by the end of experiment 1.92 (g C/Kg soil).

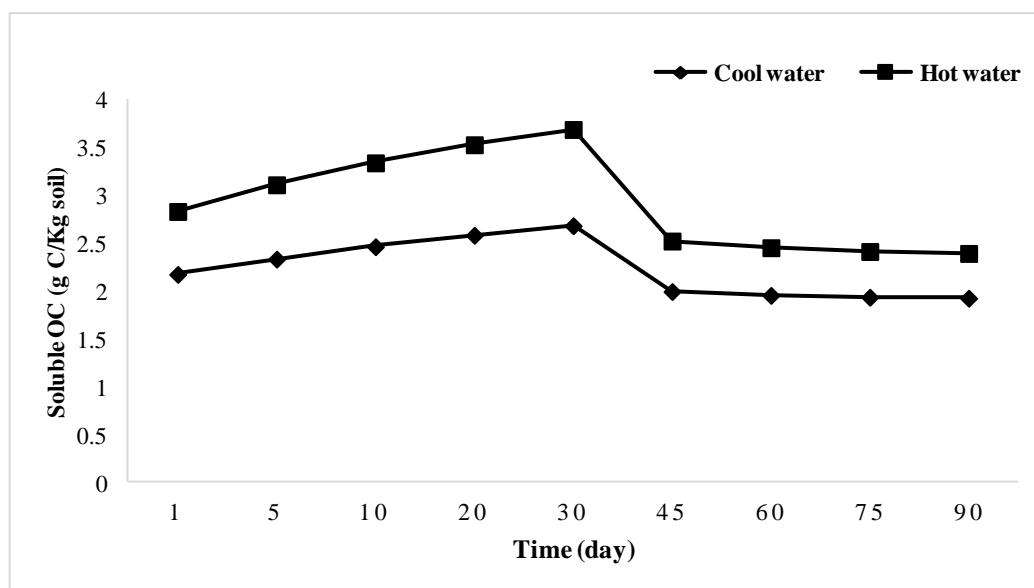


Fig.2. Soluble OC changes in hot and cool water with the passage of time.

As shown in Fig. 3, Soluble OC in hot water value increased from 2.83 (g C/Kg soil) in the 1th day to 3.69 (g C/Kg soil) in the 30th day and finally it decreased by the 90th day 2.39 (g C/Kg soil). Since soluble organic carbon was increased with the development and promoting plant residues biodegradation in the initial 30 days and when the growth and development of microbial communities were increased with the over time and subsequently soluble organic carbon decreased with the passage of time.

Kalbitz et al. (2003) observed that soluble OC increased with the passage of time, but in another study soluble OC decreased by over the time (Gregorich et al., 2003). Alfalfa straw has higher Soluble organic carbon than wheat straw, thus its degradation rate and OC content decreasing done by higher rate in this fraction (Swanston et al, 2002; Preston and Schmidt, 2006;). It is known in recent reviews that the organic matter quality is particularly important for SOC stabilization (Amelung et al., 2008; Schmidt et al., 2011).

4. Conclusion

The results of this study showed a light fraction, heavy fraction, water Soluble organic carbon were increased by addition of greater percentage of nanozeolite and plant residues into the soil. The results of this study show that light fraction was maximum in (B compound) than the other treatments. But OC in heavy fraction, Soluble organic carbon in hot water and cool water were maximum in (A compound) than the other treatments. LF and HF decreased with the passage of time from 1st day until the 90th day. Soluble organic carbon in hot water and cool water increased from 1st day until the 30th day and then they decreased by the end of the experiment. Indeed organic carbon content increased by application and addition of nanozeolite and plant residues into the soil, but these pools decreased with the passage of time. Finally can be said the application of nanozeolite and plant residues improve carbon sequestration process in soil.

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