# Evaluation of humus quality of forest soils with two extraction methods

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**Abstract:** Extraction method as one of the most important steps of the study of humic substances quality can affect the results. In this study three types of forests with different ages and vegetation were chosen to study the different extraction methods on the quality of extracted humic substances. A virgin forest, Bald Cypress forest and Eastern Cottonwood forest in the north of Iran were chosen. Some physical and chemical properties of soil samples were measured. The soil samples were extracted with NaHCO3 and Hot Water Percolation method. The quality of organic carbon in both kinds of extracts was studied using spectroscopy method. The E2:E3, E4:E6 ratios and URI were estimated. There were some differences in physical and chemical properties between different samples. Bald Cypress forest had the maximum amount of clay fraction in its texture. Also this samples contained the highest value of soil organic carbon. Virgin forest showed the maximum amount of total soil nitrogen which is probably related to the species of its vegetation. The minimum C:N ratio as a humification factor was observed in virgin forest sample. The organic compounds which extracted with NaHCO3 and Hot Water were different. The results indicated the presence of humic acid (HA) in all of samples which were extracted with NaHCO3 solution, however, Hot Water extracts contain compounds with fulvic acid (FA) properties. According to our results Hot Water Percolation is an appropriate method for extracting organic compound with more activity, simple structure, less carbon and low molecule weight (MW).

**Keyword**: Soil, Hot Water, Organic carbon, UV VIS, Fulvic acid, Humic acid.

## Introduction

Different vegetation types produce different soil properties and any changes in the ecosystem can damage soil properties (Islam & Weil, 2000; Lapola et al., 2010). Willis et al. (2003) found that the amount of carbon sequestration depends on forest management, yield class, trees type and regime. Humic substances (HS) as an important fraction of soil organic matter are determined as the dark colored amorphous polymers which are synthesized from biomass constituents or their metabolites biochemically and/or chemically in the environment (Baglieri et al., 2007). Fulvic acid (FA) is a mixture of weak aliphatic and aromatic organic acids and its composition and shape is quite variable (Beznosikov & Lodygin, 2009; Shin et al., 1996). Humic acid (HA) is another kind of HS with less activity than FA. HAs are termed polydisperse because of their variable chemical features (Lavrik et al., 2004; Schulten et al., 1991; Zavarzina et al., 2008). Different methods were used for different research with different aims. Soil scientists introduced different extraction solution to extract soil organic matter. Dilute NaOH solution was used to extract humic substances by different scientists (Schnitzer & Khan, 1978). However, NaF solution have been used for this reason (Schnitzer & Khan, 1978). Smith and Lorimer (1964) also reported HA extraction with dilute $Na_4P_2O_7$ from peat soils which was similar to dilute NaOH solution. Diluted $NaHCO_3$ was used as a adequate soluble extract in order to extract humic substances from soils (Adani et al., 1998; Chen et al., 1977; Makarov et al., 2002; Olk et al., 1995). Hot water extraction was introduced by Keeney (1966) to determine easily available N fraction. Using of hot water in order to soil extraction is recommended as a simple method to estimate total C and N fractions which have conventional potential to minerals in arable soils (Chodak et al., 2003). Füleky and Czinkota (1993) used another technique of hot water extraction that uses heat energy and pressure together which called Hot Water Percolation (HWP). According to them this method has several advantages such as: being easy to use, being fast and its ability to measure several parameters from the same solution.

Characterization of HS can be carried out using UV VIS spectroscopy. Soil scientists have used the ratio of optical densities or absorbance of dilute, aqueous humic and fulvic acid solutions at 254, 365 nm ($E_2$:$E_3$) and 465, 665 nm ($E_4$:$E_6$) in order to characterize these materials (Chen et al., 1977; Helms et al., 2008; McDonald et al., 2004; Yang & Xing, 2009). A low $E_2$:$E_3$ ratio reflects a high average MW. Higher MW and higher degree of condensation of the aromatic rings will be indicated by lower value of $E_4$:$E_6$ ratio. HAs have a lower $E_4$:$E_6$ ratio, more humification or ageing in comparison to FAs (Chen et al., 1977; Piccolo et al., 1992). The UV Absorbance Ratio Index (URI) values ($UVA_{210}/UVA_{254}$) provides the information on the relative proportions between UV-absorbing functional groups and unsaturated compounds. A higher density of functional groups corresponds to a higher absorption at 210 nm and producing a higher URI (Her et al., 2008). The aim of this study was to evaluate two kinds of extraction method on humic substances quality.

## Material and methods

Soil sampling was carried out from Safrabasteh area near Astaneashrafieh city in Gilan province, Iran. The sampling sites were located in the longitude of 49′ 57″ east and 37′19″ north with 15 m height above the sea level. The composite sampling was done from A horizon (0-20 cm) of a virgin forest which was mostly covered with Caucasian Alder (*Aluns subcordata*), Wych Elm (*Ulmus glabra*) and Caucasian Persimmon (*Populus caspica*); 40 years old Bald Cypress forest (*Taxodium distichum*) and a 15 years old Eastern Cottonwood (*Populus deltoids*).
The soil samples were air dried and ground to pass through a 2-mm sieve for further analysis. Soil texture was determined using pipette method. The EC and pH were measured using standard methods. The percentage of total organic carbon (TOC) and nitrogen (N) were measured according to wet oxidation and Kjeldahl method, respectively. In order to study the quality of organic carbon four grams of soil samples were extracted with 20 ml of 0.05 M $NaHCO_3$ and 30 g using Hot Water Percolation method (Füleky & Czinkota, 1993). Wet oxidation method was used to measure the amount of dissolved organic carbon (DOC) in Hot Water extracts (Buzás, 1988). The absorbance of UV VIS spectra was determined at 200-700 nm on both kinds of extracts by Spectrophotometer (JENWAY 6405/Vis.). The $E_2$:$E_3$, $E_4$:$E_6$ ratios and URI were estimated using the absorbance values at 210, 254, 365, 465 and 665 nm.

**Result and discussion**

The silty loam was the soil texture which estimated for all the samples (Tab. 1). The range of EC and pH was showed in Table 1. The most value of TOC and minimum amounts of it were found in Bald Cypress forest and Eastern Cottonwood forest , respectively (Tab. 1). The percentage of clay fraction in the samples of Bald Cypress forest and Eastern Cottonwood forest were maximum and minimum, respectively. A positive relationship was indicated between the percentage of clay fraction and organic matter in the soil (Anderson et al., 1981; Schnitzer & Khan, 1989). This positive relationship was observed in our results, Bald Cypress forest, because clay particles in the soil are able to stabilize some part of organic matter through binding process with them. Clay minerals have a high specific surface area and carry a charge which enables them to bind with organic matter and chemically stabilize them. In addition micro pores in clay aggregates protect organic matter physically (Wattel-Koekkoek et al., 2001). The maximum value of DOC was found in Bald Cypress forest and Eastern Cottonwood forest contained minimum value of it (Tab. 1). It was a logical result since the TOC value of Bald Cypress and Eastern Cottonwood forests were the highest and lowest (Tab. 1).

**Table 4:** The physico-chemical properties of samples.

| Forest type | VF | BCF | ECF |
|---|---|---|---|
| Sand (%) | 20.3 | 4.4 | 15.7 |
| Silt (%) | 57.3 | 65.5 | 64.0 |
| Clay (%) | 22.4 | 30.1 | 20.2 |
| Soil Texture | Silty loam | silty clay lome | Silty loam |
| EC ($\mu scm^{-1}$) | 2.01 | 2.80 | 0.84 |
| pH | 7.39 | 7.45 | 7.52 |
| TOC (%) | 3.28 | 4.901 | 1.92 |
| DOC(%) | 0.016 | 0.019 | 0.010 |
| N (%) | 0.127 | 0.091 | 0.054 |
| C:N | 26.2 | 53.9 | 35.9 |

VF: virgin forest, BCF: Bald Cypress forest, ECF: Eastern Cottonwood forest

The quantity of N in the sample of virgin forest was the maximum (Tab. 1). *Alnus* spp. can fix the nitrogen ($N_2$) and increase it in the soil by symbiosis with genus *Frankia* (Ekblad & HUSS□DANELL, 1995; Ribeiro et al., 2011; Roggy et al., 2004). Whereas one of the species in virgin forest was *Aluns subcordata,* it probably led to increase the amount of N in this sample. The range of C:N ratio between samples was 26.2 to 53.9, the samples of virgin forest and Bald Cypress forest had the minimum and maximum values, respectively (Tab. 1). C:N ratio is a factor of soil organic matter humification. Low value of C:N ratio demonstrates high degree of humification in the soil organic matter. The high value of N in the sample of virgin forest helps to provide the nitrogen needed by microorganisms and subsequent organic carbon decomposition. Both the reduction of organic carbon and increasing of N in the soil sample of virgin forest resulted to decrease the C:N ratio of this sample.

The UV-VIS spectra followed a decrease with increasing the wavelength in all of the samples and both extraction methods (A and B) (Figure 1). The spectra absorbance value showed a positive relationship with the amount of organic compounds in the solutions. The result indicated that the spectra absorbance value of Eastern Cottonwood forest extracts was less than the others (Tab. 2). It is obvious that the amount of dissolved organic matter and subsequently DOC of Eastern Cottonwood forest in both kinds of extracts ($NaHCO_3$ and Hot Water) were lower than the others (Tab. 1). The absorbance values were decreased with increasing wavelength (Tab. 2).
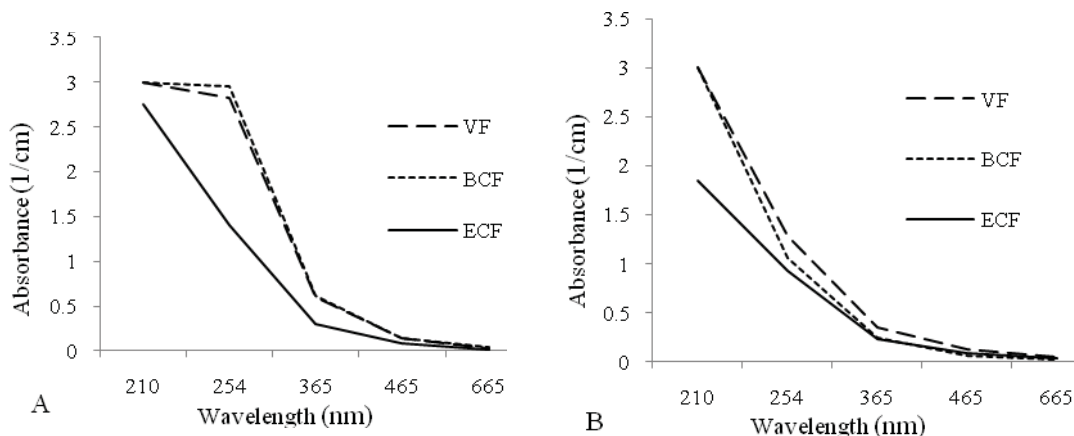


**Figure 8:** The relationship between the absorbance and wavelength in the different samples (VF: virgin forest, BCF: Bald Cypress forest, ECF: Eastern Cottonwood forest) , A) $NaHCO_3$ extracts, B) Hot Water extracts.

The absorbance value at the same wavelength of Hot Water extracts was lower than the $NaHCO_3$ ones (Tab. 2). It shows the presence of more organic compounds with larger structure, higher MW and more aromatic rings in the $NaHCO_3$ extracts. Helms et al. (2008) confirmed that the absorption at longer wavelengths is higher by the components with high molecular weight.

The values of $E_2:E_3$ and $E_4:E_6$ ratios of samples were showed in Table 3. The $E_2:E_3$ ratio was approximately similar in different samples which extracted with $NaHCO_3$ solution (Tab. 3). The sample of virgin forest extracted with $NaHCO_3$ solution had the maximum value of $E_4:E_6$ which has shown the presence of organic compound with lower MW and C:N ratio (Tab.1). It was determined that there is a better correlation between $E_4:E_6$ ratio and molecular size, carboxyl content, total acidity, O:C and C:N ratios than the aromaticity (Chen et al., 1977; Senesi et al., 1989). The range of $E_4:E_6$ value in some literature was estimated between 3.3 to 5.9 (Fong & Mohamed, 2007; Tan, 2000). The result of this study was between 3.838 to 4.966, so the $E_4:E_6$ ratio of all the samples which extracted with $NaHCO_3$ showed, presence of HA in their extracts.

HA contain a mixture of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids (Lavrik et al., 2004; Schulten et al., 1991; Zavarzina et al., 2008).

**Table 2:** The absorbance of sample extracts with $NaHCO_3$ and Hot Water at different wavelength.

| Forest type | | $NaHCO_3$ | | | Hot Water | | |
|---|---|---|---|---|---|---|---|
| | | VF | BCF | ECF | VF | BCF | ECF |
| Wave length (nm) | 210 | 3.000 | 3.000 | 3.000 | 3.000 | 3.000 | 2.452 |
| | 254 | 2.828 | 2.950 | 1.892 | 1.279 | 1.058 | 1.232 |
| | 365 | 0.602 | 0.610 | 0.409 | 0.348 | 0.250 | 0.323 |
| | 465 | 0.144 | 0.142 | 0.106 | 0.122 | 0.058 | 0.124 |
| | 665 | 0.029 | 0.037 | 0.026 | 0.053 | 0.020 | 0.053 |

VF: virgin forest, BCF: Bald Cypress Forest, ECF: Eastern Cottonwood Forest

**Table 3:** The optical parameters of the extracts.

| Extracted solution | Forest type | VF | BCF | ECF |
|---|---|---|---|---|
| $NaHCO_3$ | $E_2$:$E_3$ | 4.698 | 4.836 | 4.626 |
| | $E_4$:$E_6$ | 4.966 | 3.838 | 4.077 |
| | URI | 1.061 | 1.017 | 1.586 |
| Hot Water | $E_2$:$E_3$ | 3.675 | 4.232 | 3.814 |
| | $E_4$:$E_6$ | 2.302 | 2.900 | 2.340 |
| | URI | 2.346 | 2.836 | 1.990 |

VF: virgin forest, BCF: Bald Cypress Forest, ECF: Eastern Cottonwood Forest

The values of $E_2$:$E_3$ and $E_4$:$E_6$ ratios of Hot Water samples were differed from $NaHCO_3$ samples (Tab. 3). The range of $E_4$:$E_6$ for FA in the water solution and temperate area was estimated between 1.9-17.6, and for HA between 2.4-8.3 (Grøn et al., 1996). Our results of $E_2$:$E_3$ and $E_4$:$E_6$ of Hot Water extracts were placed in both FA and HA ranges but recent study of Takács and Füleky (2010) indicated that the dissolved organic matter of the HWP extracts is a mixture of organic materials which have some characteristics similar to the soil FA fractions and natural organic matter, so the DOC of Hot Water extracts in this study probably are placed in FA range.

The URI values were shown in Table 3. Her et al. (2004) indicated that the URI value of humic acids is the lowest (1.59), it is medium for fulvic acids (1.88) and the highest (13.50) for unsaturated compounds (e.g., proteins) with lowest aromaticity. The higher value of URI indicates unsaturated component in the extracts (Her et al., 2004). The URI value of all samples in $NaHCO_3$ solution were placed in the HA range (Tab. 3). Unsaturated compounds absorb UV spectra at both 254 and 210 nm wavelengths and functional groups absorb UV spectra more effectively at 210 than 254 nm. Therefore, a higher density of functional groups result in a higher absorption at 210 nm and produces a higher URI (Shon et al., 2006). The URI value for all of the samples which extracted with Hot Water were more than 1.88 which was estimated by Her et al. (2004) and showed the presence of FA in the HWP extracts (Tab. 3). The result of value demonstrated the presence of FA in Hot Water extracts. All of the optical factors ($E_2$:$E_3$, $E_4$:$E_6$ ratios and URI) showed the properties of FA in Hot Water extracts.

It has been indicated that $NaHCO_3$ solution is able to extract HA and FA compounds from soil organic matter (Baes & Bloom, 1990; McKnight et al., 1994; Nissenbaum & Kaplan, 1972). However, fulvic acid, a mixture of organic materials which have some characteristics similar to FA and natural organic matter were extracted with Hot Water (Takács & Füleky, 2010).

**Conclusion**

There were obvious differences between the types of organic compounds which extracted with $NaHCO_3$ and Hot Water Percolation method. The $NaHCO_3$ solution is able to extract both FA and HA fractions from soil samples. However, most of organic compounds which extracted with Hot Water Percolation method contained simpler structure, more activity, less MW, less carbon and aromacity that belongs to FAs properties. It seems that HWP method is suitable for extracting small and more soluble organic matter of the soil.

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