

# SZENT ISTVÁN UNIVERSITY

## DOCTORAL SCHOOL OF ENVIRONMENTAL SCIENCES

# CHARACTERIZATION OF SOIL HUMIC SUBSTANCES IN HUNGARIAN AND IRANIAN SOILS

Ph.D. DISSERTATION

MARIEH NADI

GÖDÖLLŐ 2012

# Title: Characterization of soil humic substances in Hungarian and Iranian soils

Discipline:Environmental SciencesName of Doctoral School:Environmental SciencesDirector:Prof. Dr. György Heltai

Supervisor:

Prof. Dr. György Füleky

Approval of School Leader

\_\_\_\_\_

Approval of Supervisor

\_\_\_\_\_

## LIST OF CONTENTS

1.	INT	FROE	DUCTION	1
	1.1.	The	most important aims	2
2.	LIT	ERA	TURE REVIEW	3
	2.1.	Natu	ral organic matter in the environment	3
	2.2.	Soil	organic matter	3
	2.2.	.1.	Soil organic matter beneficial	4
	2.2.	.2.	Sources and mechanisms of soil organic matter	7
	2.2.	.3.	The nature of soil organic matter contributors	8
	2.2.	.4.	Soil organic matter decomposition 1	0
	2.2.	.5.	Humification process 1	1
	2.2.	.6.	Humic substances fractions	3
	2.2.	.7.	Chemical structure of humic substances	4
	2.2.	.8.	Methods utilized for study of soil organic matter	6
	2	2.2.8.1	Degraded method to analysis humic substances	6
	2	2.2.8.2	2 None degraded methods to analysis humic substances 1	7
	2.2.	.9.	UV VIS spectroscopy 1	9
	2	2.2.9.1	Use of UV VIS in soil organic matter study	0
	2.2.	.10.	NMR	1
	2	2.2.10	.1 <sup>13</sup> C NMR	2
	2	2.2.10	.2 <sup>1</sup> H NMR	4
	2	2.2.10	.3 Use of NMR in soil organic matter study	4
	2.2.	.11.	Soil organic matter extraction	5

	2.2.1	1.1 Hot Water Percolation method	25		
	2.3. Veg	getation and its effects on soil organic carbon	26		
	2.3.1.	Reforestation	27		
	2.3.2.	Caucasian Alder	28		
	2.3.3.	Eastern cottonwood	29		
3.	MATER	IAL AND METHODS	31		
	3.1. Hur	ngarian soil	31		
	3.1.1.	Sampling	31		
	3.1.2.	Soil extraction	31		
	3.1.3.	Physical and chemical characteristics of soils	32		
	3.1.4.	UV VIS Spectroscopy	34		
	3.2. Iran	ian samples	35		
	3.2.1.	Site information	35		
	3.2.2.	Soil sampling	35		
	3.2.3.	Soil extraction	36		
	3.2.4.	Physical and chemical characteristics	36		
	3.2.5.	Soil fractionation	38		
	3.2.6.	UV VIS Spectroscopy	39		
	3.2.7.	NMR Spectroscopy	39		
	3.2.8.	Statistical analysis	39		
4.	RESUL	TS AND DISCUSSION	40		
4.1. Hungarian soils					
	4.1.1.	Physical and chemical properties	40		

	4.1.2.	Kinetics of dissolved organic carbon of HWP method	
	4.1.3.	UV VIS Spectroscopy	
	4.1.3	B.1 HWP extracts	
	4.1.3	8.2 NaHCO <sub>3</sub> extracts	46
	4.1.4.	Humic substance quality	47
4	.2. Irar	nian soils	49
	4.2.1.	Physical and chemical properties	49
	4.2.2.	UV VIS Spectroscopy	53
	4.2.2	2.1 HWP extracts	53
	4.2.2	2.2 NaHCO <sub>3</sub> extracts	55
	4.2.3.	Humic substance quality	57
	4.2.4.	NMR Spectroscopy	58
	4.2.4	<sup>13</sup> C and <sup>1</sup> H NMR of sand fraction	58
	4.2.4	<sup>13</sup> C and <sup>1</sup> H NMR of Silt fraction	63
	4.2.4	<sup>13</sup> C and <sup>1</sup> H NMR of Clay fraction	68
	4.2.4	1.4 The summary of NMR spectroscopy results	
5.	CONCL	LUSION	
5	5.1. Nev	w scientific results	83
6.	SUMM	ARY	
7.	RELAT	TED PUBLISHMENTS	87
8.	ACKNO	OWLEDGMENT	88
9.	USED F	REFERENCE	

#### 1. INTRODUCTION

Organic materials are an important constituent of all natural environments. Organic molecules are found in both soluble and insoluble forms in a variety of environments such as, atmospheric aerosols, soils, sediments and aquatic systems (Thurman 1985). Vegetation significantly influence the quantity and quality of organic material in the soil. Different vegetation produce different soil properties and any changes in the ecosystem will upset the balance of the soil (Islam and Weil 2000; Lapola et al. 2010).

One of the important fractions of soil organic matter are humic substances (HS) which 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). Components which are more easily decomposed will be lost from humic substances initially. Fulvic acid (FA) is a mixture of weak aliphatic and aromatic organic acids and its composition and shape is quite variable (Beznosikov and 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).

In order to study soil organic matter, different methods have been used. Extraction as an important step of soil organic matter study has been considered by different scientists. Dilute NaOH solution was used to extract humic substances by different scientists (Schnitzer and Khan 1978).

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).

Characterization of organic matter can be carried out using different method. Ultra Violet Visible (UV VIS) spectroscopy, Fourier transformed Infrared (FT IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and, etc. (Christensen et al. 1998). Soil scientists have used the ratio of optical densities or absorbance of dilute, aqueous humic and fulvic acid solutions at 465 and 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 and Xing 2009).

Today NMR spectroscopy has become probably the most important method in order to determine chemical composition of humic substances (Preston 1996; Preston 2001).

Two important properties that dictate the behavior of organic molecules in the environment are functional group chemistry and macromolecular structure (Schnitzer 1991). Evaluation of these two properties is complicated by the compositional and structural heterogeneity of the natural organic molecules, and their ability to form intramolecular and intermolecular hydrogen bonds, which further modify their structure and chemical reactivity (Myneni 2002).

Present study included two parts, in the first part we used HWP as a modern method to extract HSs of soil. The results of characterization of HSs which extracted with this method was one of the most important goals that could lead to an important evolution in soil organic matter extraction method. In the second part we have chosen a forest site which its native species (*Aluns subcordata*) replaced with different ratios of non native species (*Populus deltoids*). Some parts of virgin forests in studying area were clear cut in order to culture a rapid growth non native species and used for wood products, however, this species may seriously affect the ecosystem. We used NMR spectroscopy to characterize the quantity and quality of organic components in soil sample of virgin forest and different ratios of non native species. Since the amount and type of organic components in each fraction.

#### 1.1. The most important aims

- To study the optical properties of humic substances extracted with Hot Water Percolation method.
- The comparison of quantity and quality of soil humic substances in virgin and planted forests in the same climate and geographic area.
- To study the effect of different ratios of Alnus:Populus on soil organic matter.
- To study the differences of organic component quantity and quality in different soil fractions.

#### 2. LITERATURE REVIEW

#### 2.1. Natural organic matter in the environment

Organic material is an important constituent of all natural environments. Organic molecules are found in both soluble and insoluble forms in a variety of environments such as, atmospheric aerosols, soils, sediments and aquatic systems (Thurman 1985). Both living and non-living organic matter play important roles in the global biogeochemical cycles of carbon (C), nitrogen (N), phosphorus (P) and many other elements. Natural organic matter (NOM) bioavailability and composition affects soil fertility, carbon dioxide sequestration, nutrient cycling and metal speciation. NOM is produced by the activities of micro as well as macro fauna and flora and can undergo a wide variety of alteration processes such as physical degradation and aggregation, microbial remineralization, diagenesis, and photochemical reactions.

The large number of different primary sources, coupled with the various alteration processes, leads to large variations in NOM composition. Two important properties that dictate the behavior of organic molecules in the environment are functional group chemistry and macromolecular structure (Schnitzer 1991). Evaluation of these two properties is complicated by the compositional and structural heterogeneity of the natural organic molecules, and their ability to form intramolecular and intermolecular hydrogen bonds, which further modify their structure and chemical reactivity (Myneni 2002). One of the biggest challenges facing geochemists is to gain a better understanding of the relationship between chemical composition and structure, and the biogeochemical behavior of NOM (Myneni 2002).

#### 2.2. Soil organic matter

Organic material is anything that was alive and is now in or on the soil. For it to become organic matter, it must be decomposed into humus. Soils and sediments contain a large variety of organic materials that can be grouped into humic and non-humic substances. The latter include those whose physical and chemical characteristics are still recognizable, such as carbohydrates, proteins, peptides, amino acids, fats, waxes, low-molecular weight organic acids etc. Most of these compounds are

attacked relatively readily by microorganisms and have usually only a short life span in soils and sediments. Humic substances arise from the chemical and biological degradation of plant and animal residues and from synthetic activities of microorganisms (Schnitzer and Khan 1978). Soil organic matter consists of a variety of components. These include, in varying proportions and many intermediate stages, an active organic fraction including microorganisms (10-40%) and resistant or stable organic matter (40-60%) which referred to humus (Bot and Benites 2005). The chemical composition of the plant material is a major factor shaping, the properties of the humic substances produced and the course of humification process (Debska et al. 2007). The molecular features of the compounds are variable, depending on their age and origin. For this reason, humic substances are defined operationally, rather than structurally (Yates and von Wandruszka 1999). Humic substances are defined as dark-colored amorphous polymers synthesized from biomass constituents or their metabolites biochemically and/or chemically in the environment. It has been considered that humic substances initially lose the components that are relatively more easily decomposed, after which condensation-polymerization and oxidative reactions proceed, resulting in a more stable structure (Baglieri et al. 2007). The properties of humus depend on both the habitat factors such as: the soil type, plant cover, climatic conditions and anthropogenic factors (Debska et al. 2007).

### 2.2.1. Soil organic matter beneficial

Organic matter affects both the chemical and physical properties of the soil and its overall health. Properties influenced by organic matter include: soil structure, moisture holding capacity, diversity and activity of soil organisms, which are beneficial and harmful to crop production, cation and anion exchange, nutrient availability, and etc. It also influences the effects of chemical amendments, fertilizers, pesticides and herbicides (Bot and Benites 2005; Mackowiak et al. 2001). Some of the most important influences of humic substances on the soil and plants are:

 Humic substances are a good source of energy for beneficial soil organisms: humic substances and non-humic (organic) compounds provide the energy and many of the mineral requirements for soil microorganisms. Beneficial soil organisms lack the photosynthetic apparatus to capture energy from the sun thus must survive on residual carbon containing substances on or in the soil. Energy stored within the carbon bonds function to provide energy for various metabolic reactions within these organisms. Beneficial soil organisms perform lots of beneficial functions which influence soil fertility and plant health (Coates et al. 2002; Reeves 1997; Siuda and Chrost 2002).

- Humus functions improve the soil's water holding capacity: the most important function of humic substances within the soil is their ability to hold water. Humic substances help create a desirable soil structure that facilitates water infiltration and helps hold water within the root zone. Because of their large surface area and internal electrical charges, humic substances function as water sponges. These sponges like substances have the ability to hold seven times their volume in water, a greater water holding capacity than soil clays (Bot and Benites 2005; Carter 2002; Cooperband 2002; Tranvik 1992).
- Humic substances are key components of a friable soil structure: various carbon containing humic substances are key components of soil crumbs (aggregates). Complex carbohydrates synthesized by bacteria and humic substances function together with clay and silt to form soil aggregate. As the humic substances become intimately associated with the mineral fraction of the soil, colloidal complexes of humus-clay and humus-silt aggregates are formed. Soils with good crumb structure have improved tilth, and more porous openings (open spaces). These pores allow for gaseous interchange with the atmosphere, and for greater water infiltration (Carter 2002; Cooperband 2002; Piccolo and Mbagwu 1999).
- Degradation or inactivation of pesticides and toxic substances is mediated by humic substances: soil humic substances function to either stabilize or assist in the degradation of these substances. In the microbial degradation process not all of the carbon contained within these toxins is released as CO<sub>2</sub>. A portion of these toxic molecules, primarily the aromatic ring compounds are stabilized and integrated within the complex polymers of humic substances. Humic substances have electrically charged sites on their surfaces which function

to attract and inactivate pesticides and other toxic substances (Bot and Benites 2005; Farenhorst 2006; Wauchope et al. 2002).

- Humic substances buffer (neutralize) the soil pH and liberate carbon dioxide: humic substances function to buffer the hydrogen ion concentration of the soil. Once the soil is neutralized, and then many trace elements formerly bound in the soil and unavailable to plant roots, because of alkaline or acidic conditions, become available to the plant roots. Humic substances also liberate carbon dioxide from calcium carbonates present within the soil. The released CO<sub>2</sub> may be taken up by the plant or it may form carbonic acids. The carbonic acids act on soil minerals to release plant nutrients (Ritchie and Dolling 1985; Wright 1989).
- Soil temperatures and water evaporation rate are stabilized by humic substances: humic substances function to help stabilize soil temperatures and slow the rate of water evaporation. The insulating properties of humic substances help maintain a more uniform soil temperature, especially during periods of rapid climate changes, such as cold spell or heat waves. Because water is bound within the humic substances and humic substances reduce temperature fluctuations, soil moisture is less likely to be released into the atmosphere (Carter 2002; Devine et al. 2006).
- The electrical features of humic substances influence known chemical reactions: both groups of complex organic acids, humic acids and fulvic acids have been proven to be involved in three specific chemical reactions. These reactions are commonly termed, (1) electrostatic (columbic) attraction, (2) complex formation or chelating, and (Chen et al. 1977) water bridging. Chelating of plant nutrients such as iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), and calcium (Ca) reduces their toxicity as cations, prevents their leaching, and increases their uptake rate by plant roots (Bot and Benites 2005; Lavrik et al. 2004; Reeves 1997).
- Humic substances aid in the decomposition of soil minerals by forming metal-organic-clay complexes, a process termed soil genesis: soil formation (soil genesis) involves a complex of transition mineral elements, such as copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn)

from soil minerals with humic acids (HAs), fulvic acids (FAs) and clays (Provin et al. 2008; Tiessen et al. 1994).

Humic substances because of their molecular structure can provide numerous benefits to crop production. They help to break up clay and compacted soils, assists in transferring micronutrients from the soil to the plant (Reeves 1997). Plant growth is influenced indirectly and directly by humic substances. There is positive correlations between the humus content of the soil.

- Uptake of major plant nutrients is mediated by humic substances: one stimulative effect of humic substances on plant growth is enhanced uptake of major plant nutrients: nitrogen (N), phosphorus (P), and potassium (K). When adequate humic substances are present within the soil the requirement for N-P-K fertilizer applications is reduced. As the level of humic substances in soils become depleted the misleading demand for higher concentrations of N-P-K results (Bot and Benites 2005; Schnitzer and Khan 1978; Tiessen et al. 1994).
- The absorption of humic substances into seeds has a positive influence on seed germination and seedling development: The application of humic acids or fulvic acids to seeds will increase the seed germination; resulting in higher seed germination rates (Önemli 2004; Reeves 1997).
- Humic substances have a very pronounced influence on the growth of plant roots: when humic acids and/or fulvic acids are applied to soil, enhancement of root initiation and increased root growth are observed. Thus the common observation that humic acids and fulvic acids are root stimulators.

#### 2.2.2. Sources and mechanisms of soil organic matter

Different pools of soil organic matter are present in soils and they have different turn over time and sizes. Soil organic matter is composed of:

 Decomposing plant and animal residues that are usually still recognizable in appearance and composition as protein, carbohydrate, lipid, etc.

- Microbial products formed during decomposition; may or may not be recognizable as protein, etc.
- Microbial cells.
- Cells of multi cellular soil biota like worms, arthropods, etc.

Humic substances especially, humic acid, fulvic acid and humin. These are probably more resistant to degradation than the others. (Bot and Benites 2005; Doyle et al. 2004; Schnitzer and Khan 1978). The transformation and movement of materials within soil organic matter pools is a dynamic process influenced by climate, soil type, vegetation and soil organisms. The following five mechanisms were distinguished:

- A continuous soil cover of living plants which together with the soil architecture facilitate the capture and infiltration of rainwater and protects the soil.
- A litter layer of decomposing leaves or residues providing a continuous energy source for macro- and micro-organisms.
- The roots of different plants distributed throughout the soil at different depths permit an effective uptake of nutrients and an active interaction with microorganisms.
- The major period of nutrient release by micro-organisms coincides with the major period of nutrient demand by plants.
- Nutrients recycled by deep-rooting plants and soil macro fauna and micro fauna (Bot and Benites 2005).

#### 2.2.3. The nature of soil organic matter contributors

Human management, climate, soil type and structure influence the species compositions of most terrestrial habitats and ultimately the composition of soil organic matter (SOM). SOM, composed of above and below ground plant, animal and microbial residue is present in a continuum of plant and animal remains at different stages of humification. Complete separation of humic and non humic substances is generally not possible, therefore in order to better understanding the humification and soil processes, it is necessary to focus on the major soil contributors, the largest being fresh plant materials. The main components of plants are polysaccharides, lignin, aliphatic polymers (hydrolyzable and non-hydrolyzable), proteins, phenols and lipids. In contrast, SOM is much more complex providing evidence that there has been substantial chemical alteration of plant materials to form the SOM.

Approximately 70 to 75 percent of the dry weight from plants originates from polysaccharides. These major forms of carbohydrates are known to be important contributors to SOM (Stevenson 1994). Polysaccharides are known to function as storage material. Therefore, they serve as carbon and energy suppliers or as structural components (De Leeuw and Largeau 1993). Alkali-soluble polysaccharides are defined as hemicellulose (Cheshire 1979). Both cellulose and hemicellulose are important plant carbohydrates. Glucose, the most important building block of polysaccharides is present in cellulose, starch, glycogen, and other important structures. In addition to polysaccharides, monosaccharides from various plant sources also contribute to SOM (Cheshire 1979).

Next to polysaccharides, lignin is the second most abundant component of most plants that is known to contribute to SOM (Stevenson 1994). It makes up 15 to 30 percent of the total dry matter in woody plants, and 10 to 15% in other plant tissues. It exists in cell walls of vascular plants, but not in mosses, algae, and microorganisms (Dria 2004). Lignin is a heteropolymer composed of a combination of three building blocks, coniferyl alcohol, synapyl alcohol and coumaryl alcohol. It has a structural and protective function for the plant cell walls. Gymnosperm lignin is composed of predominantly polymerized coniferyl alcohol structures, referred to as guaiacyl lignin. Angiosperms have both guaiacyl lignin and syringyl lignin, composed of polymerized synapyl alcohol units. Grass lignin, on the other hand, is composed of all three monomers (Dria 2004).

Other contributors of SOM derived from plants are aliphatic polymers. They include at least two major types in plants, the hydrolyzable, and nonhydrolyzable aliphatic polymers. Hydrolyzable aliphatic polymers include cutin, which is a cross-linked polyester that coats in leaf cuticles serving as an aerial barrier, and suberin, a protective layer in roots serving as a barrier against unfavorable external influences. Both consist of long-chain aliphatic biopolyesters crosslinked by ether bridges (Kolattukudy and Espelie 1985). Non-hydrolyzable forms include cutan and suberan. The chemical

compositions of these compounds are poorly understood but have been found to be persistent in soils and produce pyrolysis products with alkane and alkene homologous pairs (Tegelaar et al. 1995).

Proteins, the other components of plants, are generally polymers composed of 20 amino acids. They are easily depolymerized and exist in multiple shapes due to their various functions. However, because of their relatively low concentrations in most terrestrial plants they are not considered to be a primary source of organic matter in forest or agricultural ecosystems (Dria 2004).

Phenolic compounds of low and high molecular weight can contribute to plant biomass. Traditionally, low molecular weight phenols include catechol, stilbenes and flavanoids (Harborne 1997). Polyphenols comprise mainly tannins. It is speculated that tannins present a high degree of resistance against degradation and thus are important contributors to SOM (De Leeuw and Largeau 1993; Wilson and Hatcher 1988).

Finally, lipids are defined as those plant compounds that are soluble in polar organic solvents (Solomons 1992). They do not present any structural uniformity and are easily decomposed. Therefore, they are not considered as a main organic matter component that can survive humification (De Leeuw and Largeau 1993).

#### 2.2.4. Soil organic matter decomposition

Decomposition is closely related to humification, mineralization, dissolved organic matter dynamics, and SOM stabilization. During the first phase of decomposition, rapid mineralization of labile components is the dominant process. In the second phase of decomposition, mineralization still occurs but is slowed down due to the accumulation of refractory molecules.

The composition of organic matter is changed during humification process which is stated in summary:

 Decrease of O-alkyl carbon during humification may be mainly attributed to the preferential mineralization of polysaccharides. The polysaccharide carbon determined with wet chemistry decreased from 25% to 8%, whereas O-alkyl carbon decreased only from 57% to 42%. Either fractions of O-alkyl carbon were not accessible to hydrolysis because of physico-chemical stabilization, or non-polysaccharide, yet not identified structures. Further, the O-alkyl-C fraction not accounted for by wet chemistry appeared to increase during humification.

- The increase of alkyl-carbon contents during humification can be explained by enrichment of extractable and bound lipids (Hempfling and Schulten 1989; Ziegler and Zech 1989) or plant-derived biopolyesters and non-saponifiable aliphatic biomacromolecules of plant and microbial origin (Tegelaar et al. 1989b). Using dipolar dephasing <sup>13</sup>C NMR spectroscopy (Kögel-Knabner 1992) were able to show that mobile alkyl components are degraded with soil depth, whereas the rigid ones appear to be selectively preserved, probably due to cross-linking of aliphatic polymers.
- Increase of the aromatic carbon contents generally is not very pronounced. In temperate forests, aromaticity has even been shown to decrease during litter decomposition and humification (Zech et al. 1992). It is indicated that in humus layers of tropical forests aryl-carbon contents are much lower than O-alkyl-carbon contents and usually lower than alkyl-carbon contents. A relative enrichment of non-lignin aromatic structures is indicated with progressive decomposition and humification. Less is known about the nature of these non-lignin aromatic structures. In general, there is a concurrent increase of C-substituted aryl carbon with decreasing O-aryl-C intensities (phenolics) in temperate forest A horizons.
- Increase of carboxyl-C contents may be partly related to the oxidative cleavage of lignin sidechains and of the aromatic ring itself during degradation. This is confirmed by the results of CuO oxidation, which indicate an increase of the acid-to-aldehyde ratio of the vanillyl units (ac/al) from non humified layer to humified layer. Additional processes like oxidation of polysaccharides and the accumulation of fatty acids may contribute to the increase of carboxyl groups during humification.

#### 2.2.5. Humification process

The condensation hypothesis has been described by several authors (Flaig et al. 1975; Kononova et al. 1961). This hypothesis is based on the principle that decomposition products of plants (carbohydrates,

quinones or polyphenols) can undergo condensation reactions with amino acids to form dark colored materials or humic substances. Condensation models vary in the nature of precursors proposed to undergo condensation, but may include the following compounds: amino acids and monosaccharides (Maillard 1913), polyunsaturated fatty acids reacting to form humic acids as proposed for marine organic matter (Harvey and Boran 1985), and phenols with themselves and/or with amino acids (Flaig 1988).

The hypotheses assume a total decomposition of plant remains into simple monomers (e.g., sugars, phenols, amino acids). These monomers then condense and polymerize to form increasingly complex macromolecules and eventually humic acid. In 1937, Waksman proposed a hypothesis that constitutes the basis for the degradation model, the alternate humification hypothesis. This model was further developed and discussed in literature by Orlov (1995) and references therein. The general concept for this hypothesis is that humic acids are formed by oxidative reactions from high molecular weight biopolymeric compounds, such as lignin and other refractory, difficult to degrade biopolymers.

Critical reviews of the models are available in numerous publications (Hatcher and Spiker 1988; Hedges 1988; Zech et al. 1997). Many investigators have inferred that soil humic substances are mostly composed of an aromatic core (Stevenson 1994). However, the application of solid-state <sup>13</sup>C NMR on whole soil and size-fractionated soil samples has drastically changed the view of the formation of humic substances (Baldock et al. 1997; Wilson and Hatcher 1988). Rather than the increase in aromaticity proposed for humification by others, the <sup>13</sup>C NMR data show that the decomposition of plant materials in soils leads to an accumulation of aliphatic structures, whereas the concentration of anomatic structures vary depending on the nature of the soil (Baldock et al. 1997). This accumulation of aliphatic structures has been partially attributed to aliphatic biopolymers derived from plants, originating from extractable lipids, cutin and suberin biopolyesters, and nonhydrolyzable biopolymers such as cutan and suberan (Kogel-Knabner 1997; Tegelaar et al. 1989a). Both lipids and biopolyesters (cutin and suberin) are considered to have a low preservation potential in soil when compared to cutan and suberan (De Leeuw and Largeau 1993). On the other hand, cutan, suberan and other aliphatic macromolecules can be preserved from degradation due to their recalcitrance. The preservation of these aliphatic substances is usually denoted by alkane/alkene doublets in pyrolysis products (Augris et al. 1998; Kögel-Knabner 1992; Tegelaar et al. 1989a).

Zech et al. (1997) indicated that with increasing humification the following alterations are detectable:

- weak increase of alkyl carbon;
- decrease of methoxyl C, O-alkyl C, and O-aryl C;
- increase of C-substituted aryl C and carboxyl C.

In the mineral soil profile, signals due to carboxyl carbon and C-substituted aryl carbon further increased, whereas signals induced by phenolic carbon, O-alkyl carbon, methoxyl carbon, and alkyl carbon decreased. Lignin structures are considerably modified during humification, mainly by demethylation, loss of phenolic moieties, side-chain oxidation, and increasing dominance of C-substituted recalcitrant aromatic structures. Using pyrolysis-field ionization-mass spectrometry (Hempfling and Schulten 1989) have shown that C-C linked lignin moieties are selectively preserved in forest soils of the temperate zones in comparison to the less resistant ether-linked structures.

#### 2.2.6. Humic substances fractions

The classical method of fractionation of humic substances is based on differences in solubility in aqueous solutions at widely differing pH levels, in alcohol and in the presence of different electrolyte concentrations (Schnitzer and Khan 1978). The fractions have been operationally defined as:

#### – Fulvic acid

The fraction soluble in water under all pH conditions (Baglieri et al. 2007; Schnitzer and Khan 1978; Yates and von Wandruszka 1999). Fulvic acids are a mixture of weak aliphatic and aromatic organic acids. Their composition and shape is quite variable. Fulvic acids are light yellow to yellow-brown in color (Beznosikov and Lodygin 2009; Shin et al. 1996).

#### – Humic acid

The humic acid substances fraction are not soluble in water under pH < 2 conditions, but is soluble at higher pH (Baglieri et al. 2007; Yates and von Wandruszka 1999). Humic acid comprise a mixture of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids. Humic acids are termed

polydisperse because of their variable chemical features (Lavrik et al. 2004; Schulten et al. 1991; Zavarzina et al. 2008). Humic acid is the major extractable component of soil humic substances. It is dark brown to black in color.

#### – Humin

The fraction which is not soluble in water at any pH value and black in color (Baglieri et al. 2007; Schnitzer and Khan 1978; Yates and von Wandruszka 1999). The chemical and physical properties of humins are only partially understood. Humin present within the soil is the most resistant to decomposition (slow to breakdown) of all the humic substances (Baglieri et al. 2007; Schnitzer and Khan 1978).

The qualitative and quantitative composition of humic substances can change depending on the origin and the degree of maturity (Debska et al. 2007). A related feature distinguishing FAs from HAs is that the former have a lower molecular weight and greater functional group density. The molecular weight range commonly quoted for FA is 500 to 5000 Da, while it is more than 5000 Da for HA and can extend to values more than 1000000 Da (Yates and von Wandruszka 1999).

#### 2.2.7. Chemical structure of humic substances

The elemental analysis of humic substances reveals that they are primarily composed of carbon, oxygen, hydrogen, nitrogen, and sulfur in complex carbon chains C-C-C-C and carbon rings with C-C, C-N, and C=O groupings (Baglieri et al. 2007; Beznosikov and Lodygin 2009; Schulten et al. 1991). These major elements are always present regardless their origin and country or continent. Besides elemental composition, groups composition is used to characterize humic substances as it gives information about the chemistry and structural properties of humic substances (Peña-Méndez et al. 2005).

Various three-dimensional flexible structure combinations of benzene, acetal, ketal, naphthalene, lactol, and furan ringed compounds, and aliphatic (carbon chains) compounds (Baglieri et al. 2007; Debska et al. 2007). The oxidative degradation of some humic substances produces aliphatic, phenolic, and benzene carboxylic acids in addition to n-alkanes and n-fatty acids (Yates and von

Wandruszka 1999). Fulvic and humic acids are complex mixtures of large molecules. Molecules of humic acids show a higher proportion of the fraction possessing hydrophobic properties as compared with the proportion of hydrophilic fraction. The degree of polydispersity of molecules of humic acids is related to their hydrophilic and hydrophobic properties (Debska et al. 2007).

The studies suggest that HAs may be aggregates of smaller heterogeneous organic molecules including sugars, organic acids, and other aliphatic and aromatic components likely having molecular weights of several hundred Daltons (Li et al. 2003). A comparative analysis of the molecular structure of preparations of HAs and FAs showed: FAs are enriched with oxygen-containing fragments, which explains their better solubility in water and migration ability (Beznosikov and Lodygin 2009), when fulvic acid was high aggregated still soluble in aqueous solution (Chen et al. 1977). The high content of carboxyl groups (up to 27.1%) causes acid aggressiveness of FAs relative to soil minerals and the ability to form complex compounds with cations of iron, aluminum, and other metals, converting them into soluble forms. As a consequence of an increased amount of carbohydrate fragments (up to 23.2%), FA molecules are subjected to greater degradation by soil microorganisms than HA molecules, which consist more than half of aliphatic chains and aromatic fragments (Beznosikov and Lodygin 2009).

Humic substances have spontaneous structure in some condition such as high pH. According to Yates and von Wandruszka (1999) the spontaneous aggregation of aqueous humic acids can be intramolecular (involving a single polymer chain) or intermolecular (involving multiple chains). The formation of both the intra- and intermolecular species depends on a range of solution conditions, including the concentration and nature of the HS, pH, temperature, the nature and concentration of cationic species present in solution. pH plays an important role in the condensation, molecule weight and composition of HAs and FAs. As viewed under the scanning electron microscope, FA at pH 2-3 occurs mainly as elongated fibers and bundles of fibers, forming a relatively open structure. With increase in pH, the fibers tend to mesh into a finely woven network to yield a sponge-like structure. Above pH 7, a distinct change in the structural arrangement and an improved orientation can be observed. At pH 8, the FA forms sheets which tend to thicken at pH 9. At pH 10, fine, homogeneous grains are visible. The effect of pH on the HA structure is similar to that observed on FA, except that

because of low solubility in water, the pH range had to be narrowed between 6 to 10, and the pH at which the major transitions occur is higher. Thus, there is a gradual transition from a fibrous structure at low pH to a more sheet-like one at higher pH. Simultaneously, the particles become smaller as the pH increases. The aggregation of FA particles at low pH can be explained as being due to hydrogenbonding, Van der Waal's interactions and interactions between  $\pi$  electron systems of adjacent molecules. As the pH increases, these forces become weaker, and because of increasing ionization of CO<sub>2</sub>H and phenolic OH groups, particles separate and begin to repel each other electro statically, so that the molecular arrangements become smaller and smaller but better oriented. Similar aggregation-dispersion phenomena can be observed for HA, although over a narrower range (Schnitzer and Khan 1978).

#### 2.2.8. Methods utilized for study of soil organic matter

Chemical changes observed between the samples of fresh plant materials and soil samples can be utilized to explain the humification processes (Zech et al. 1997). Over the past 100 years, numerous methods, both degradative and non-degradative, have been utilized to study humification and SOM (Schnitzer and Khan 1972).

#### 2.2.8.1 Degraded method to analysis humic substances

Degraded methods used for the characterization of humic materials include oxidations in alkaline and acidic media, reduction, hydrolysis, thermal, biological degradations etc (Schnitzer and Khan 1978). The traditional procedures used for studying humic substances structure include oxidation with KMnO<sub>4</sub>, alkaline CuO, alkaline nitrobenzene, hypochlorite, HNO<sub>3</sub>, and H<sub>2</sub>O<sub>2</sub>. Reduction using Zndust distillation and Na amalgam; base and acid hydrolysis and enzymatic degradation (Chefetz et al. 2002). The primary objective of these treatments is to recover simple extractable monomers or fragments that can easily be analyzed and are believed to be representative of the humic substances main macromolecular structure. Gas chromate graphic-mass spectrometric technique make possible the separation and the qualitative and quantitative identification of micro-amounts of organic compounds in complex mixtures, have greatly enhanced the efficacy of chemical and possibly also of biological degradation as structural tools (Schnitzer and Khan 1978). Pyrolysis-GC/MS is a powerful analytical tool for obtaining molecular-level information about SOM and humic substances. In the past, the data from this technique have been used to design two and three dimensional model structures of humic substances (Chefetz et al. 2002). Pyrolysis provides a method of analyzing complex, heterogeneous high molecular weight compounds that are difficult or even impossible to analyze in their polymeric form. Upon pyrolysis, a given sample is rapidly heated in an inert environment. Due to the large amount of energy received, the macromolecules are fragmented and the volatile products are swept into a GC/MS for separation and identification (Dria 2004).

A technique, which has been developed to overcome this limitation, is thermochemolysis with TMAH (tetrametyl ammonium hydroxide). This method selectively cleaves ester and certain ether linkage (such as  $\beta$ -O-4 aliphatic-aryl bonds) in macromolecular organic matter through a chemolytic procedure that hydrolyzes and methylates ester and ether linkages, and assists in the depolymerization and methylation of lignin (Chefetz et al. 2002). Information gained from each analytical technique or chemical modification is important because the individual techniques complement each other. When used together, they provide awareness against biases that may evolve because of structural selectivity that each technique harbors.

#### 2.2.8.2 None degraded methods to analysis humic substances

There are some non degraded methods in order to analysis humic substances:

#### – Ultraviolet absorption spectroscopy

Ultraviolet (UV) absorption spectroscopy is commonly used to study various properties of the dissolved organic matter, such as its aromaticity, hydrophobic content, and biodegradability (Beznosikov and Lodygin 2009).

The light absorption of humic substances appears to increase with increase in: (a) the degree of condensation of the aromatic rings that these substances contain, (b) the ratio of carbon in aromatic "nuclei" to carbon in aliphatic side chains, (c) total carbon content, and (d) molecular weight (Chen et al. 1977).

#### – Infrared spectroscopy

The Infrared (IR) spectra reflect the preponderance of oxygen-containing functional groups, that is,  $CO_2H$ , OH and C=O in the humic materials. While IR spectra of humic materials provide worthwhile information on the distribution of functional groups, they indicate little about the chemical structure of humic "nuclei"(Schnitzer and Khan 1978).

#### Nuclear Magnetic Resonance spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of sample (humic substances) as well as its molecular structure. Solid-state NMR offers a generic description of the major classes of C-containing groups in a sample and has contributed significantly to information regarding the chemical nature of humic substances (Chefetz et al. 2002). In order to achieve the desired results, a variety of NMR techniques are available.

#### High-performance liquid chromatography

The High-performance liquid chromatography (HPLC) technique can be used for the determination of the molecular weight and/or size of different organic compounds. However, the lack of a suitable standard with the same structure and properties makes difficult the evaluation of the results in the case of natural organic matter and humic substances. Although there are attempts to describe the molecular size distribution of the natural organic matter using HPLC size exclusion chromatography (Müller et al. 2000; Pelekani et al. 1999; Perminova et al. 1998; Peuravuori and Pihlaja 1997).

#### Fluorescence spectroscopy

Fluorescence spectroscopy is known as a sensitive, noninvasive and relatively simple tool for studying the chemical behavior of both simple and complex organic compounds and humic substances. It has been used to characterize the natural organic matter in surface waters (Coble 1996; De Souza Sierra et al. 1994). Emission, excitation and synchronous scan spectroscopy have been used to differentiate humic and fulvic acids, as well as humic substances from different origins (Senesi 1990; Senesi et al. 1991). Compared to the emission excitation or synchronous scan techniques, Total

Luminescence is time consuming but it provides the most information about the fluorophores in the samples.

#### – X-ray analysis

X-ray analysis has been used by several workers for elucidating the structure of soil humic substances. Diffraction patterns of HAs usually show broad bands near 3.5 A, whereas those for FAs exhibit halos in the 4.1-4.7 A region (Schnitzer and Khan 1978).

#### Electron microscopy and electron diffraction

This technique has been used for observing shapes and sizes of HA and FA particles (Schnitzer and Khan 1978).

#### - Viscosity measurements

Viscosity measurements can provide important information on particle shapes and sizes, particle weights and polyelectrolytic behavior of macromolecules in aqueous solutions (Schnitzer and Khan 1978).

Also another methods such as Molecular weight measurement, Electrometric titrations, Electron spin resonance spectrometry, Surface tension measurements, Vapor pressure osmometry, Gel filtration, Ultracentrifuge, and etc have been used in this case (Schnitzer and Khan 1978).

#### 2.2.9. UV VIS spectroscopy

Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. Molecules containing  $\pi$ -electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti bonding molecular orbitals. The more easily excited the electrons the higher the wavelength of light it can absorb.

UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Determination is usually carried out in solutions.

Solutions of transition metal ions can be colored (i.e., absorb visible light) because within the metal atoms can be excited from one electronic state to another. The color of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the color of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the color and changes the wavelength of maximum absorption.

Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water-soluble compounds, or ethanol for organic-soluble compounds. (Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy, ethanol absorbs very weakly at most wavelengths.) Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases. While charge transfer complexes also give rise to colors, the colors are often too intense to be used for quantitative measurement.

#### 2.2.9.1 Use of UV VIS in soil organic matter study

Characterization of humic substances can be carried out using UV VIS spectroscopy. Humic substances generally show strong absorbance in the UV–Vis range (from 190 to 800 nm), particularly in the UV region, because of the presence of aromatic chromophores and/or other organic compounds (Schnitzer and Khan 1972). In fact, the UV absorptivity at 260 nm is commonly used to determine the relative abundance of aromatic content of natural organic matter because transitions in substituted benzenes or polyphenols occur in this wavelength region. Accordingly, the UV/Vis spectra of the

three natural organic matter fractions showed a generally decreased absorptivity (or optical density) as the wavelength increased (Chen et al. 2002).

Soil scientists have used the ratio of optical densities or absorbance of dilute, aqueous humic and fulvic acid solutions at 465 and 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 and Xing 2009). Higher molecular weight and higher degree of condensation of the aromatic rings will be indicated by lower  $E_4/E_6$  ratio value. Humic acids have a lower  $E_4/E_6$  ratio, more humification or ageing. This ratio is independent of the concentration of the humic substances but is characteristic of different natural organic matter fractions or humic substances obtained from different sources. The  $E_4/E_6$  ratio for HAs is usually <5.0; the ratio for FAs ranges from 6.0 to 8.5 (Chen et al. 1977). They also determined that low  $E_2/E_3$  ratio reflects a high average molecular weight. A low  $E_4/E_6$  or  $E_2/E_3$  ratio for soil HA may be largely attributed to the absorption by aromatic C=C functional groups. Additionally, a high degree of condensation of the aromatic rings and the large molecular weight of soil HA are believed to contribute to its relatively high absorption in the visible range. Specific UV absorbance (SUVA) (L/mg m) which is the ratio of the UV absorbance at 254 nm (cm<sup>-1</sup>) with dissolved organic carbon (mg/L) is useful for dissolved organic carbon characterization.

The UV Absorbance Ratio Index values (URI) (UVA<sub>210</sub>/UVA<sub>254</sub>) 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).

#### 2.2.10. NMR

Nuclear magnetic resonance (NMR) spectroscopy is a technique based on the magnetic properties of atomic nuclei. It is used to distinguish between different functional groups/compound classes present in organic compounds and can be applied to liquid as well as solid samples. NMR spectroscopy as an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure. For example, NMR can quantitatively analyze

mixtures containing known compounds. For unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion. In order to achieve the desired results, a variety of NMR techniques are available.

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

#### 2.2.10.1 <sup>13</sup>C NMR

The 1D <sup>13</sup>Carbon NMR experiment is much less sensitive than Proton (<sup>1</sup>H) but has a much larger chemical shift range. Its low natural abundance (1.108%) and proton decoupling means that spin-spin couplings are seldom observed. This greatly simplifies the spectrum and makes it less crowded. <sup>13</sup>C is a low sensitivity nucleus that yields sharp signals and has a wide chemical shift range.



Figure 1: Example of the chemical shift of <sup>13</sup>C NMR

A typical analysis of a <sup>13</sup>C NMR spectrum consists of matching expected chemical shifts to the expected moieties. Each type of signal has a characteristic chemical shift range that can be used for assignment. The chemical shifts are used for the initial assignment of the spectrum. For ethyl benzene, we see that the signals at 15.6 and 28.9 ppm fall in the aliphatic region and therefore belong to the  $CH_2$  and  $CH_3$  carbons. Usually  $CH_3$  has a lower chemical shift than  $CH_2$  so can be provisionally assigned to 15.6 and 28.9 ppm, respectively. The remaining signals are in the aromatic region at 125.6, 127.8, 128.3 and 144.2. The carbon not attached to any protons is called 'quaternary' (four-fold) even though this is a misnomer for unsaturated carbons such as in our case where it is only attached to only three other carbons. Quaternary carbons usually give sharper signals than other carbons and usually give weaker signals under normal acquisition conditions – decoupling and relatively short repetition times. This is because of their slow relaxation. The chemical shifts of aromatic carbons not attached to protons (for ethyl benzene C1) are generally higher than for those attached (for ethyl benzene C2, C3 and C4). Therefore the signal at 144.2 ppm is provisionally assigned to C1.



Figure 2: <sup>13</sup>C NMR chemical shift ranges (on the ppm scale) and chemical structures of common C functional groups observed in natural samples (Malcolm 1989).

#### 2.2.10.2 <sup>1</sup>H NMR

The 1D <sup>1</sup>H (Proton) NMR experiment is the most common NMR experiment. The proton (<sup>1</sup>Hydrogen nucleus) is the most sensitive nucleus (apart from tritium) and usually yields sharp signals. Even though its chemical shift range is narrow, its sharp signals make proton NMR very useful. A typical analysis of a <sup>1</sup>H NMR spectrum may proceed as follows:

The number of protons of each type in the spectrum of a pure sample can be obtained directly from the integrals of each multiplet. This is only true if the multiplets are well separated and do not overlap the solvent or residual water signals and provided that the molecule is not undergoing slow conformational exchange. A routine NMR spectrum yields integrals with an accuracy of +/-10%. Accuracies of +/-1% can be achieved by increasing the relaxation delay to five times the longitudinal relaxation time (T<sub>1</sub>) of the signals of interest. Where multiplets overlap, the total integral of the spectral region may be used.

From the table of the proton chemical shifts one obtains information about each type of proton and can carry out a preliminary assignment.

Using chemical shifts, the peak at 1.2 ppm is in the expected range for  $CH_3$  (methyl) and at 3.7 and 3.9 ppm are compatible with  $CH_2$  (methylene). The chemical shift of OH is very dependent on solvent and other experimental conditions so cannot be assigned by chemical shift alone.

Using integration, we expect  $CH_3$  to have an integral of three,  $CH_2$  to have an integral of two and OH to have an integral of one. This is the case and so the assignment is complete.

#### 2.2.10.3 Use of NMR in soil organic matter study

The first attempt to use nuclear magnetic resonance spectroscopy for structural characterization of soil humic substances was reported by Barton and Schnitzer (1963) and Neyroud and Schnitzer (1972) (Kogel-Knabner 1997). NMR is one of the best techniques to characterize the structure and properties of organic matter. Today it has become probably the most important method in order to determine chemical composition of HS (Preston 1996; Preston 2001). NMR is a technique that exploits the quantized separation of nuclear spin energy levels for various NMR-active nuclei in a strong magnetic field. Variations in electron densities surrounding each nucleus in organic and inorganic molecules

allow us to obtain information about the nature of the bonds linking the nuclei. This provides chemical structural information for the compounds. The most common uses of this technique is the characterization of the functional groups of organic carbon such as: alkyl, O-alkyl, aryl, carboxyl etc.

#### 2.2.11. Soil organic matter extraction

Soil scientists introduced different extraction solution to extract soil organic matter. Dilute NaOH solution was used to extract humic substances by different scientists. However, NaF solution have been used for this reason (Schnitzer and Khan 1978). Smith and Lorimer (1964) also reported HA extraction with dilute Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> from peat soils which was similar to dilute NaOH solution. The concentration of the NaOH solution affects the yield of the humic material extracted as well as its ash content.

Ponomarova and Plotnikova (1968) and Levesque and Schnitzer (1966) found 0.1 N NaOH to be more efficient than higher NaOH concentrations (Schnitzer and Khan 1978). However, the most suitable extraction solution for isolating humic materials low in ash was either 0.4 N or 0.5 N NaOH solution (Schnitzer and Khan 1978). Neutral salts of mineral and organic acids have been used for the extraction of humic substances, but yields are usually low (Schnitzer and Khan 1978). NaP<sub>2</sub>O solution extracts not only humic substances but also organo-mineral complexes without destroying nonsilicate forms of sesquioxides. It has been shown that pyrophosphate was difficult to remove from humic materials during purification (Schnitzer and Khan 1978). Diluted NaHCO<sub>3</sub> 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).

#### 2.2.11.1 Hot Water Percolation method

For a long time there has been great requirement need to use water for routine soil extraction method. Water was tried in the determination of available nutrient content in soils since the middle of the last century, but the analytical measurement of nutrients was difficult because of its low solvent power. In the past decades several methods were introduced to increase the solvent power of water or to increase the speed of the process. At the beginning of this century, soil samples were autoclaved by König (1906) in water vapor at 400-500 kPa pressure (Füleky and Czinkota 1993), this method was good especially for potassium.

Hot water extraction was introduced by Keeney (1966) to determine easily available N fraction. Also hot water extraction was used in determination of boron, when the soil-water suspension was boiled for a few minutes (Berger and Truog 1944). Amer et al. (1955) used anion exchanger resin besides water to reach a total desorption of phosphorus. Paauw (1969) increased the efficiency of extraction by increasing the soil: water ratio to 1:50 in the case of phosphorus (Füleky and Czinkota 1993). 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). This method has several advantages such as: being easy and fast to use and its ability to measure several parameters from the same solution. Takács and Füleky (2010) used HWP method for isolation and characterization of dissolved organic matter from soils.

#### 2.3. Vegetation and its effects on soil organic carbon

Vegetation effects on soil chemical, physical and biological properties including: inorganic chemical elements, water content, soil temperature, soil organic matter, texture, structure, soil color, microbial biomass etc. Thus each change on vegetation will effect on soil properties and subsequently environment. Vegetation significantly affected the distribution of quantity and quality of the organic carbon in the soil. Different vegetation types produce different soil properties and any changes in the ecosystem can damage soil properties (Islam and Weil 2000; Lapola et al. 2010).

Forests provide economical, ecological, social and aesthetic services to natural systems and humans (Hassan et al. 2005). Refuges for biodiversity, hydrologic cycle, landscape, provision of food, medicinal, wood and protection of soil resources are the most benefits of forests. In addition, forests affect climate through carbon sequestration, energy, water, air pollution absorption and other chemical species with the atmosphere (Bonan 2008; Willis et al. 2003).

Clearing of forests for wood products and cultivation have altered climate. However, climate is influenced by world's forests through chemical, physical and biological processes which affects the hydrologic cycle, planetary energetic and atmospheric composition (Bonan 2008).

Kouki et al. (2001) indicated that the fragmentation of old-growth forests is regarded as one of the most important causes for the recent decline of several forest-dwelling species in Fennoscandia. Willis et al. (2003) found that the amount of carbon sequestration depends on forest management, yield class, trees type and regime. Native broadleaved forest in subtropical China has almost been extirpated by human activities, and replaced by coniferous plantation with short-rotation.

Some studies showed that introduction of broadleaved trees, such as Alnus cremastogyne (a N-fixing species) and Kalopanax septemlobus, into pure carbon. *Lanceolata* plantation forests enhanced litter production and carbon storage (Wang et al. 2007), and improved soil fertility (Chen and Wang 2004; Chen et al. 1990).

Some of the major problems in the tropics are the forest clear cutting, forest degradation and the deterioration of land productivity through human disturbance and inappropriate agricultural practices (Parrotta et al. 1997). Ayanaba (1976) indicated the effects of forest clearing and cropping on the C, N and S reserves of soils under secondary lowland rain forest. He found that the soils which had been cropped for two years contained less total C and N than the corresponding forest soils. The rapidity of declining was less when crop residues were returned to the soil. Conversion of tropical forest lands to agriculture affects the soil through its effect on soil organic matter and nutrients as well as on physical properties (Brown and Lugo 1990). It is indicated that site history and management may play more important role in the changing of soil C and N stocks than the soil type (Neill et al. 1997).

#### 2.3.1. Reforestation

Reforestation is the natural restocking of existing forests and woodlands that have been depleted, usually through deforestation. Reforestation can be used to improve the quality of human life by soaking up pollution and dust from the air, rebuild natural habitats and ecosystems, mitigate global warming since forests facilitate biosequestration of atmospheric carbon dioxide, and harvest for resources, particularly timber.

The term reforestation is similar to afforestation, the process of restoring and recreating areas of woodlands or forests that may have existed long ago but were deforested or otherwise removed at some point in the past. Sometimes the term re-afforestation is used to distinguish between the original forest cover and the later re-growth of forest to an area. Special tools, e.g. tree planting bar, are used to make planting of trees easier and faster.

Reforestation of large areas can be done through the use of measuring rope (for accurate plant spacing) and ribbeds, (or wheeled augers for planting the larger trees) for making the hole in which a seedling or plant can be inserted. Indigenous soil inoculants can optionally be used to increase survival rates in hardy environments.

A debatable issue in managed reforestation is whether or not the succeeding forest will have the same biodiversity as the original forest. If the forest is replaced with only one species of tree and all other vegetation is prevented from growing back, a monoculture forest similar to agricultural crops would be the result. However, most reforestation involves the planting of different feedlots of seedlings taken from the area often of multiple species. Another important factor is the natural regeneration of a wide variety of plant and animal species that can occur on a clear cut. In some areas the suppression of forest fires for hundreds of years has resulted in large single aged and single species forest stands. The logging of small clear cuts and or prescribed burning, actually increases the biodiversity in these areas by creating a greater variety of tree stand ages and species.

#### 2.3.2. Caucasian Alder

*Alnus subcordata*, Caucasian Alder, is a species in the family Betulaceae, native to temperate areas of Iran and the Caucasus. It is a deciduous tree growing to 15-25 m tall, closely related to the Italian Alder (*A. cordata*), with similar glossy green cordate leaves 5-15 cm long. The flowers are catkins, the male catkins very slender, 8-15 cm long, the female catkins small, maturing into a woody cone-

like fruit 2-3 cm long containing numerous small winged seeds. Its wood is employed in paper production. It has a calorific value of about 4.6 cal/g.

Species of Alnus have been planted for many decades for erosion control, soil improvements, reclaiming strip mine areas and revegetating and sustaining roadsides (Cervantes and Rodríguez-Barrueco 1992; Schwencke and Carú 2001). Alnus spp. can grow and improve soil fertility due to their ability to fix atmospheric nitrogen in natural and disturbed soils (Hibbs and Cromack Jr 1990; Wheeler and Miller 1990). The mycorrhizal Alnus roots may be advantageous in infertile areas by enhancing nutrient uptake in those environments (Monzón and Azcón 2001). Trees harbouring arbuscular mycorrhizal fungi and *Frankia* may have the advantage of high tolerance to environmental stresses (Sylvia and Williams 1992). A dual symbiosis located in the roots, between plants and microorganisms capable of fixing dinitrogen and improving phosphate absorption from the soil may represent a great advantage to Alnus spp. Such plants may develop well in unfavorable environments (Berliner and Torrey 1989).

#### 2.3.3. Eastern cottonwood

*Populus deltoides*, the eastern cottonwood, is a cottonwood poplar native to North America, growing throughout the eastern, central, and southwestern United States, the southernmost part of eastern Canada, and northeastern Mexico.

*Populus deltoides* is a large tree growing to 20–40 meters (66–130 feet) tall and with a trunk up to 1.8 meters (5.9 ft) diameter, one of the largest North American hardwood trees. The bark is silvery-white, smooth or lightly fissured when young, becoming dark gray and deeply fissured on old trees. The twigs are grayish-yellow, stout, with large triangular leaf scars. The winter buds are slender, pointed, 1–2 cm long (0.39–0.79 inches), yellowish brown, and resinous. The leaves are large, deltoid (triangular), 4–10 cm (1.6–3.9 inches) long and 4–11 cm (1.6–4.3 inches) broad with a truncated (flattened) base and a petiole 3–12 cm (1.2–4.7 inches) long. The leaf is very coarsely toothed, the teeth are curved and gland tipped, and the petiole is flat; they are dark green in the summer and turn yellow in the fall (but many cottonwoods in dry locations drop their leaves early from the

combination of drought and leaf rust, making their fall color dull or absent). Due to the flat stem of the leaf, the leaf has the tendency to shake from even the slightest breeze. This is one of the identifying characteristics. It is dioecious, with the flowers (catkins) produced on single-sex trees in early spring. The male (pollen) catkins are reddish-purple, 8-10 cm (2.1-3.9 inches) long; the female catkins are green, 7-13 cm (2.8-5.1 inches) long at pollination, maturing 15-20 cm (6.9-7.9 inches) long with several 6-15 mm (0.24-0.59 inches) seed capsules in early summer, which split open to release the numerous small seeds attached to cotton-like strands.

Poplars, with their high productivity and ease of vegetative reproduction, are particularly suitable for generating raw material with characteristics tailored to particular products and processes (Klasnja et al. 2003). Extensive research at the Poplar Research Institute has shown that, in the region of Yugoslavia, eastern cottonwood is the most productive and most promising forest species for supplying the chemical and semi-chemical pulping industries (Klasnja et al. 2003).

#### 3. MATERIAL AND METHODS

#### 3.1. Hungarian soil

#### 3.1.1. Sampling

16 soil samples were selected from Soil Bank of soil science laboratory of Szent Istvan University of Gödöllő. Soil samples including: Magyaróvár, Szarvas, Új-Szeged, Homokszentgyörgy, Gagyvendégi, Nagykanizsa, Nyíregyháza, Magyaregregy, Szilvásvárad, Etes, Putnok, Mezőhegyes, Kompolt, Nagyszentjános, Szeged-Öthalom, Kecskemét.

#### 3.1.2. Soil extraction

#### Hot Water Percolation method

The soil extraction was carried out using HWP method (Füleky and Czinkota 1993). 30 g of each soil sample was extracted with HWP instrument. The extraction was carried out continuously to 10 fractions which contained 100 ml solution and in order to find the kinetics of dissolved organic carbon (DOC) the volumes of all fractions were recorded.



Figure 3: The sample extracts of Hot Water Percolation methods.

#### NaHCO<sub>3</sub> solution

A suspension of four grams of soil samples and 20 ml of 0.05 M NaHCO<sub>3</sub> were prepared. Suspensions were shaken for two hours and centrifuged at 5000 rpm for 5 minutes. The Clear supernatant solution was filtered and used for next steps.
#### – NaOH solution

A suspension of two grams of soil samples and 20 ml NaOH 0.5% were prepared. Suspensions were rested one night, shaken for two hours and centrifuged at 5000 rpm for 5 minutes. The Clear supernatant solution was filtered and used for next steps.

### – NaF solution

A suspension of two grams of soil samples and 20 ml NaF 1% were prepared. Suspensions were rested one night, shaken for two hours and centrifuged at 5000 rpm for 5 minutes. The Clear supernatant solution was filtered and used for next steps.

# 3.1.3. Physical and chemical characteristics of soils

Some physical and chemical properties of Hungarian soils such as: the percentage of sand, silt and clay; pH, EC were determined using Hydrometer, pH meter and EC meter, respectively.

#### – Organic matter

0.3 g of soil samples was weighed and 10 ml 0.4M  $K_2Cr_2O_7$  was added to them. The mixture was heated in order to complete digestion process. In the next step, some distilled water was added to the mixture to 50 ml volume. The base of this analysis was titration, so 3 drops of indicator were added to the mixtures and they were titrated with 0.2 M Fe (NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> solution.

$$K_{2}Cr_{2}O_{7}+8H_{2}SO_{4} \longrightarrow 2K_{2}SO_{4}+2Cr_{2}(SO_{4})_{3}+H_{2}O+3O_{2}$$

$$3C+3O_{2} \longrightarrow 3CO_{2}$$

$$K_{2}Cr_{2}O_{7}+7H_{2}SO_{4}+6FeSO_{4} \longrightarrow K_{2}SO_{4}+Cr_{2}(SO_{4})_{3}+Fe(SO_{4})_{3}+7H_{2}O$$

$$2Cr^{6+}+6Fe^{2+} \longrightarrow 2Cr^{3+}+6Fe^{3+}$$

Using the following equation the organic matter amounts of each sample was calculated:

OM (%) = 
$$\frac{(a-b) f}{c}$$
 100

Where the items are:

OM: organic matter

a: the amount of blank titration.

b: the amount of sample titration.

f: blank factor.

c: soil weight.

### Dissolved organic carbon

Dissolved soil organic carbon was measured according to Turrin method (wet oxidation) Buzás (1988).

$$K_{2}Cr_{2}O_{7}+8H_{2}SO_{4} \longrightarrow 2K_{2}SO_{4}+2Cr_{2}(SO_{4})_{3}+H_{2}O+3O_{2}$$

$$3C+3O_{2} \longrightarrow 3CO_{2}$$

$$K_{2}Cr_{2}O_{7}+7H_{2}SO_{4}+6FeSO_{4} \longrightarrow K_{2}SO_{4}+Cr_{2}(SO_{4})_{3}+Fe(SO_{4})_{3}+7H_{2}O$$

$$2Cr^{6+}+6Fe^{2+} \longrightarrow 2Cr^{3+}+6Fe^{3+}$$

in this method:

10 ml 0.2 N  $K_2Cr_2O_7$  solution is added to 10 ml of HWP extracts and the mixture is taken in the 120 °C oven for 20 min.

In the next step the volume of mixture rises with adding 50 ml distilled water in each sample. The mixtures are titrated with 0.2 N (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>6H<sub>2</sub>O solution, 2 or 3 drops of Ferroin [Fe(C<sub>12</sub>H<sub>8</sub>N<sub>2</sub>)<sub>3</sub>] is used as the indicator.

Calculation:

DOC (mg) = [(b-a) f] 0.06

b = the amount of blank titration (ml).

a = the amount of sample titration (ml).

f = blank factor.

The percentage of DOC of HWP extracts was calculated:

DOC (%) = 100 DOC (mg)/3

#### 3.1.4. UV VIS Spectroscopy

#### HWP and NaHCO<sub>3</sub> extracts

The absorbance of UV VIS spectra of all sample extracts were measured at 200-700 nm by JENWAY 6405/Vis. Spectrophotometer.

The data of the most important absorbance wavelength including: 210, 254, 365, 465 and 665 nm were selected and the  $E_2/E_3$ ,  $E_4/E_6$ , UV Absorbance Ratio Index (URI) and Specific UV Absorbance (SUVA) were estimated:

 $E_2/E_3$ , the UV absorbance ratio of 254 to 365 nm.

 $E_4/E_6$ , the UV absorbance ratio of 465 to 665 nm.

URI, the UV absorbance ratio of 210 to 254 nm.

SUVA was estimated according to following equation:

(Absorbance value at 254 nm \*100)/ organic carbon (mg/L)

#### NaOH and NaF extracts

The absorbance of recent extracts were measured using VIS spectra at 400-700 nm.

#### – Determination of humus quality (K factor)

The basis of determination the quality of humus (Buzás 1988) is the chemical properties of NaF and NaOH solution which tend to dissolve different organic compounds from the various humus components of soils. The raw and acidic humus fractions tend to dissolve primarily in NaOH solution, while the high-quality humus fractions of larger molecules enter into solution more easily in the NaF solution. The studied soil samples were treated separately with both chemicals. The light absorbing capacity of the solutions was measured using VIS spectroscopy. A comparison of the gained values finally yields to humus stability number. When this is divided by the total humus content (H) the result will be the humus stability coefficient (K).

$$K = \frac{E \text{ NaF}}{E \text{NaOH} \cdot H}$$

where the items are:

 $E_{NaF}$  = the UV absorbance mean of NaF extracts at 400-700 nm.

 $E_{NaOH}$ = the UV absorbance mean of NaOH extracts at 400-700 nm.

H= the percentage of humus.

#### **3.2.** Iranian samples

### **3.2.1.** Site information

Soil sampling was carried out from Safrabasteh area near Astaneh Ashrafieh city in Guilan province, Iran. The sampling sites were located in the longitude of 49' 57" east and 37'19" north, near Sefidrood river with 15 m height above the Caspian sea. The annual mean temperature is 11.6 °C, the maximum 20.2 °C in July and the minimum 2.4 °C in January, the average annual rainfall is 1200 mm. According to morphological properties the site soil is classified to Inceptisol category. The original vegetation was converted to planted forest 50 years ago for research projects. The virgin forest of this area was mostly covered with Caucasian Alder (*Aluns subcordata*), Wych Elm (*Ulmus glabra*) and Caucasian Persimmon (*Populus caspica*).

Since the Eastern Cottonwood (*Populus deltoids*) is one of the most rapid growth species which is useful for wood product some parts of virgin forests in this area were clear cut in order to culture this species. But Eastern Cottonwood was not the native species and may seriously affects the ecosystem of this area, so it has been examined to use mixed culture plantation of Caucasian Alder and Eastern Cottonwood in order to prevent more damages.

#### 3.2.2. Soil sampling

A composite sampling was done from A horizon (0-20 cm) of a virgin forest (VF) and a 15 years old planted forest (PF) which was cultured with a mixture of Caucasian Alder and Eastern Cottonwood with the rate of 100:0, 70:30, 50:50, 30:70 and 0:100 (Alnus:Populus) in three replication and completely randomized.



Figure 4: Planted forest, a mixture of Caucasian Alder and Eastern Cottonwood.

# 3.2.3. Soil extraction

### Hot Water Percolation method

The soil extraction was carried out using HWP method (Füleky and Czinkota 1993). 30 g of each soil sample was extracted with HWP instrument.

# - NaHCO<sub>3</sub> solution

A suspension of four grams of soil samples and 20 ml of 0.05 M NaHCO<sub>3</sub> were prepared. Suspensions were shaken for two hours and centrifuged at 5000 rpm for 5 minutes. The Clear supernatant solution was filtered and used for next steps.

# 3.2.4. Physical and chemical characteristics

soil samples were air dried and ground to pass through a 2-mm sieve for further analysis. Soil texture carried out using pipette method. pH and EC were measured using standard methods, including pH meter and EC meter instruments.

#### - Total organic carbon:

Total organic carbon was measured according to following formula from organic matter as explained before, for each sample.

TOC (%) = 
$$\frac{0.0006 (a - b)f}{c}$$
 100

Where the items are:

TOC(%): total organic carbon percentage

a: the amount of blank titration.

b: the amount of sample titration.

f: blank factor.

c: soil weight.

# - "Total" nitrogen:

Total nitrogen measurement was carried out according to Buzás (1988). In this method at the first step (digestion), 0.3 - 0.5 g of soil sample is weighed, 2.5 ml 25% CrO<sub>3</sub> solution and 5ml 96% H<sub>2</sub>SO<sub>4</sub> are added to sample. The mixture should be warm on flame to green.

The digested mixture is moved to distiller and the pH is adjusted using 20 ml 50% NaOH solution. The output fluid which contain  $NH_3$  is collected in 3%  $H_3BO_3$  solution and titrated with 0.005 M  $H_2SO_4$ .

$$2(NH_4)BO_3 + 3H_2SO_4 \rightarrow 3(NH_4)_2SO_4 + 2H_3BO_3$$

Calculation:

N (mg) = [(a-b)0.14 c]

N % = 100 N (mg)/d

Where the items are:

a = the value of titrated solution (0.005 M  $H_2SO_4$ ) for sample.

b = the value of titrated solution (0.005 M  $H_2SO_4$ ) for blank.

c = the titrated solution factor.

d = sample weight.

#### Humic substances extraction

In order to extract soil humic substances 10 ml 0.5 N NaOH solution was added to 1g of each samples and the mixture was rested for one night. The mixture was centrifuged for 5 min, 5000 rpm and filtered to a clear solution. The humic substances were dried to soft powder using Dried freezing method.

# 3.2.5. Soil fractionation

The soil fractionation was carried out using sonication method. Ultra sonic instrument was calibrated for each soil sample individually. The sonication time and pals were different for samples (Tab. 1).

sample	Pals/Sec	Power %	Time (min)	Soil:Water					
VF	0.7	80	25	1:3					
PF 100:0	0.7	80	30	1:3					
PF 70:30	0.6	80	11	1:3					
PF 50:50	0.6	80	11	1:3					
PF 30:70	0.6	80	11	1:3					
PF 0:100	0.7	80	22	1:3					

Table 1: The different setting items of ultrasonic instrument for different samples.



Figure 5: Fractionation of soil sample using ultrasonic instrument.

After sonication, sand fraction was separated using sieve (0.5 mm or Mesh NO. 35) and the rest of mixture moved to the 1 L cylinder, every 8 h the top 10 cm of mixture which contain clay fraction

was moved to another container. Cylinder volume was brought to 1 liter, mixed and taken to rest for 8 h, the proceeding is repeated till there was no clay fraction in the cylinder. Fractions are dried in 40 °C oven.



Figure 6: Mixture of silt and clay fractions in the cylinders and clay fraction in the containers.

# 3.2.6. UV VIS Spectroscopy

The UV VIS Spectroscopy analyses carried out on HWP and NaHCO<sub>3</sub> extracts as before explanations. The  $E_2/E_3$ ,  $E_4/E_6$  ratios, URI and SUVA were estimated for each sample.

## 3.2.7. NMR Spectroscopy

20 mg of dried humic substances were dissolved in 0.6 mL, 0.1 M NaOD/D<sub>2</sub>O for liquid NMR experiment (Baglieri et al. 2007). <sup>1</sup>H and <sup>13</sup>C spectra were recorded using Bruker Avance 400 Ultrashield NMR spectrometer at 400.22 and 100.63 MHz, respectively, 8000-10000 scans were done over night (about 13 h) for each sample.

# 3.2.8. Statistical analysis

Data was analyzed in randomized design, using SAS statistical software, proc GLM and LSD Test was used to separate the means (P < 0.05).

### 4. RESULTS AND DISCUSSION

### 4.1. Hungarian soils

#### 4.1.1. Physical and chemical properties

The physical and chemical properties of samples are shown in Table 2. Different samples showed different texture since Magyaróvár, Új-Szeged, Gagyvendégi, Nagykanizsa, Nagyszentjános, Szilvásvárad samples showed silty loam; Szarvas, Magyaregregy, Putnok, Mezőhegyes, Szeged-Öthalom, silty clay loam; Nyíregyháza, Kecskemét, sand; Etes, Kompolt, silty clay and Homokszentgyörgy, sandy loam. The range of pH of samples was between 4.8-8.1 which related to Homokszentgyörgy, Putnok (both 4.8) and Kecskemét, respectively. The percentage of organic carbon (OC) in different samples was different, Kompolt and Kecskemét contained the maximum and minimum amounts of OC, respectively (Tab. 2).

Place	Texture	Salt%	pH <sub>H2O</sub>	OC%	Sand%	Silt%	Clay%
Magyaróvár	Silty loam	0.08	7.6	1.970	5.2	69	25
Szarvas	Silty clay loam	0.09	5.8	1.696	5.2	58	37
Új-Szeged	Silty loam	0.10	7.8	0.963	13	62	25
Homokszentgyörgy	Sandy loam	< 0.01	4.8	0.906	54	37	9.4
Gagyvendégi	Silty loam	0.04	6.0	1.331	3.6	69	27
Nagykanizsa	Silty loam	0.02	5.1	1.146	22	62	16
Nyíregyháza	Sand	< 0.01	4.9	0.841	88	4.8	7.4
Magyaregregy	Silty clay loam	0.07	6.6	0.602	10	54	36
Szilvásvárad	Silty loam	0.06	6.9	1.214	12	65	23
Etes	Silty clay	0.15	6.6	2.336	9.2	46	45
Putnok	Silty clay loam	0.03	4.8	1.382	12	57	31
Mezőhegyes	Silty clay loam	0.08	7.7	2.979	5.2	66	28
Kompolt	Silty clay	0.07	6.1	3.057	4.8	49	46
Nagyszentjános	Silty loam	0.06	7.7	1.84	16	62	21
Szeged-Öthalom	Silty clay loam	0.06	7.7	1.786	16	55	29
Kecskemét	Sand	< 0.01	8.1	0.319	95	2.8	2.5

Table 2: Physical and chemical properties of different samples.

#### 4.1.2. Kinetics of dissolved organic carbon of HWP method

Extraction of total DOC using HWP method has increased with time and in all of the samples the most increasing was at the first five minutes.

The process followed from the two terms order kinetics equation which is:

$$Y = A_1[1 - exp(-k_1x)] + A_2[1 - exp(-k_2x)]$$

Where "Y" is the measured DOC, "t" is the time, "A<sub>1</sub>" is the maximum amount of DOC in the first process, "A<sub>2</sub>" is the maximum amount of DOC in the second process, "K<sub>1</sub>" is the rate constant of the first process and "K<sub>2</sub>" is the rate constant of the second process. The highest amount of DOC was in the first fraction of extract, and the amount of HWP dissolved organic carbon in the fractions was decreased with increasing the time of extraction process (Fig. 7). After passing about 5-6 minutes of extraction process, there was a slow increasing in the amount of HWP dissolved organic carbon in the fractions in the fractions of soil samples which contained more clay in their texture e.g. Szarvas, Magyaregregy, Etes and Kompolt, but there was no increasing of HWP dissolved organic carbon in the fractions of some

ones with sand texture e.g. Kecskemét and Nyíregyháza (Fig. 7).



Figure 7: Diagrams of DOC kinetics of Magyaróvár sample.

The percentage of dissolved organic carbon which extracted with HWP method and total organic carbon in the samples was shown in the Figure 8. Dissolved organic carbon was a small fraction of

total organic carbon, however the maximum part of total organic carbon which extracted with HWP method (as dissolved organic carbon) was found in Nyíregyháza (9.85% of total organic carbon) and the minimum in Mezőhegyes (1.56% of total organic carbon). This result indicate that HWP method is able to extract a little amount of organic carbon of the soil (Fig. 8).



Figure 8: The differences between the percentage of total organic carbon (TOC) and dissolved organic carbon (DOC) in different samples.

# 4.1.3. UV VIS Spectroscopy

### 4.1.3.1 HWP extracts

The absorbance values at 210, 254, 365, 465 and 665 nm in the first fraction of HWP extracts were shown in Figure 9. In all the samples the UV-VIS spectra followed the featureless, monotonic increase with decreasing wavelength. The spectra absorbance value has a positive relationship with the amount of organic compounds in the solutions. Magyaregregy and Kecskemét samples contained the less amounts of dissolved organic matter in the first fraction (Appendix, Tab. 1) and showed the

less absorbance values at 210 nm. The maximum and minimum absorbance values at 254 nm were found in Kompolt and Kecskemét, respectively (Fig. 9), it is obvious that these samples contain the most and less dissolved organic matter in their first fraction (Appendix, Tab. 1).



Figure 9: UV VIS absorbance of HWP extracts.

The absorbance value at 254 nm was found a measurement point of the amount of organic carbon in different fractions of HWP extracts (Fig. 10). There was a good correlation between the absorbance at 254 nm and the amount of HWP dissolved organic carbon in the fractions (Fig. 10).



Figure 10: The correlation between HWP dissolved organic carbon (HWP–DOC) and absorbance value at 254 nm in different fractions (×: first fraction, +: second fraction and•: the last fraction) of Homokszentgyörgy sample.

There was also a good correlation between the absorbance at 254 nm and HWP dissolved organic carbon in the total first fractions of all 16 soil samples (Fig. 11). The increasing of HWP dissolved organic carbon lead to increasing the absorbance at 254 nm.



Figure 11: The correlation between HWP dissolved organic carbon (HWP–DOC) of the first fractions of all 16 soil samples and absorbance at 254 nm.

The results of  $E_2/E_3$  and  $E_4/E_6$  ratios were shown in Table 3. The  $E_2/E_3$  ratio is considered as a measurement of the aromaticity and is used widely to characterize the natural organic matter. The lower ratio indicates the higher aromaticity and MW (Chen et al. 1977). The  $E_2/E_3$  ratios in the samples were in a narrow range (3.29-4.84) and almost similar in different samples (Tab. 3). It indicates that the samples have similar level of humification, condensation of aromatic rings and molecular weight (Chen et al. 1977; McDonald et al. 2004).

The  $E_4/E_6$  ratios of samples were in a wide range (2.14-13.5), Nyíregyháza, Putnok, Nagyszentjános, Gagyvendégi, Kompolt, Kecskemét had the lowest  $E_4/E_6$  ratio, respectively (Tab. 3). These results indicated that theses samples are more humified than the others and subsequently the molecular weight of dissolved organic carbon of these samples is lower.

The range of  $E_4/E_6$  ratio of HA in water solution was estimated between 2.4 to 9.2 and for FA, 1.9 to 20.7 (Christensen et al. 1998; Fong and Mohamed 2007; Grøn et al. 1996). Present samples were

classified in both these groups (FA and HA) but recent study of HWP extracts (Takács and Füleky 2010), showed the  $E_2/E_3$  ratio of IHSS soil FA, HA and NOM were 4.10, 2.30 and 4.29, respectively, probably present samples contain organic components with FA and NOM properties (Tab. 3). It seems that the samples which extracted with HWP method contain simple compounds with low molecular weight and less aromaticity and they showed FAs properties.

<b>C</b> 1		F (F)	IDI	CT TT LA
Samples	$E_2/E_3$	$E_4/E_6$	URI	SUVA
Magyaróvár	4.31	9.00	1.87	1.69
Szarvas	4.58	8.00	2.14	1.33
Új-Szeged	4.76	7.25	3.30	1.41
Homokszentgyörgy	4.70	8.60	0.98	1.85
Gagyvendégi	4.67	2.97	1.19	1.71
Nagykanizsa	4.28	4.43	2.31	1.19
Nyíregyháza	3.29	2.14	1.35	2.28
Magyaregregy	4.84	8.00	2.69	0.99
Szilvásvárad	4.56	12.17	1.05	1.90
Etes	4.82	13.5	2.14	2.34
Putnok	4.68	2.75	1.24	0.95
Mezőhegyes	4.45	6.25	1.57	1.23
Kompolt	4.09	3.33	1.05	1.43
Nagyszentjános	4.04	2.90	1.76	2.07
Szeged-Öthalom	4.23	5.87	2.00	1.30
Kecskemét	3.98	3.71	3.32	1.35

Table 3: UV VIS absorbance parameters of HWP extracts

URI values provide 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). 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 (e.g., amino) groups result in a higher absorption at 210 nm and subsequently produces a higher URI (Shon et al. 2006). URI value for HA is the lowest (1.59) and it is medium for FA (1.88). The higher value of URI indicates unsaturated component in the extraction for example it is about 13.5 for a protein (Her et al. 2004). Most of samples in this study have shown URI value between 0.98-3.3 (Tab. 5). The URI values of Szilvásvárad, Homokszentgyörgy, Kompolt, Putnok, Gagyvendégi, Nyíregyháza and Mezőhegyes samples were in the range of humic acid while Nagyszentjános and Magyaróvár samples were classified in the range of FA, the other samples which had the URI value more than 2, probably, contain lower aromaticity and more unsaturated compounds (Tab. 3). These results showed presence of HA and FA with low molecular weight in the most HWP dissolved organic carbons.

SUVA, however, has a single relationship with molecular weight irrespective of HS sources. Hur and Kim (2009) indicated that SUVA may be more related to molecular weight of the HS than the chemical nature of it. Surampalli (2004) found samples with high SUVA value (i.e., >3 L/mg m) contain more humic-like organic carbon and higher molecular weight, where as lower SUVA value (<3 L/mg m) indicates the presence of organic carbon with lower molecular weight which are more fulvic-like in character. In this study, SUVA indicated humic substances with low molecular weight with great hydrophilic compounds in the HWP extracts (Tab. 3) which is in good agreement with Takács and Füleky (2010).

# 4.1.3.2 NaHCO<sub>3</sub> extracts

The value of  $E_4/E_6$  ratio of NaHCO<sub>3</sub> extracts which showed in Table 4 are in the range of 2.65 to 4.83 (Magyaregregy and Homokszentgyörgy, respectively). The range of  $E_4/E_6$  value of HA in some literature was estimated between 3.3 to 5.0 for cool area and it estimated about 9.0 for FA (Fong and Mohamed 2007; Schnitzer 1977). The result of  $E_4/E_6$  ratio of NaHCO<sub>3</sub> extracts indicated the presence of HA in the extracts of all the samples (Tab. 4).

There were obvious differences in the result of UV VIS in HWP and NaHCO<sub>3</sub> extracts and also between different parameter in HWP extracts (Tab. 4).

 $E_4/E_6$  ratio of NaHCO<sub>3</sub> extracts in most of the samples was lower than  $E_4/E_6$  ratio of HWP extracts. NaHCO<sub>3</sub> has been known as a good solution to extract humic substances from the soil and there was not a good agreement in  $E_4/E_6$  of HWP and NaHCO<sub>3</sub> extracts in the samples.

It seems that HWP method is able to extract the organic components with low molecular weight and aromatic structure, which are not in a high humification level.

Samples	$E_4/E_6$				
Sumples	NaHCO <sub>3</sub> extracts	HWP extracts			
Magyaróvár	3.64	9.0			
Szarvas	4.67	8.0			
Új-Szeged	3.5	7.25			
Homokszentgyörgy	4.83	8.6			
Gagyvendégi	3.92	2.966			
Nagykanizsa	3.51	4.435			
Nyíregyháza	2.98	2.145			
Magyaregregy	2.65	8.0			
Szilvásvárad	3.41	12.17			
Etes	4.00	13.5			
Putnok	3.39	2.75			
Mezőhegyes	3.22	6.25			
Kompolt	3.33	3.33			
Nagyszentjános	3.87	29.0			
Szeged-Öthalom	3.48	5.875			
Kecskemét	4.75	3.714			

Table 4: The  $E_4/E_6$  values of different extracts .

# 4.1.4. Humic substance quality

Different values of K factor of the samples collected in Table 5, according to K factor index there are five soil types (Buzás 1988):

- 1- Plant residues rich layers ( $<10^{-4}$ )
- 2- Humic level of peat and salty soil  $(10^{-3})$

- 3- Humic level of marsh, meadow–podzolic brown forest soil  $(10^{-2})$
- 4- Brown forest soils  $(10^{-1})$
- 5- Substances of chernozem soil (> $10^1$ ).

Samples	NaOH	, NaF	Soil class (Hargitay)
Sumples	Q	K	bon chubs (nurghuy)
Magyaróvár	11.29	3.327	4 (1.0)
Szarvas	1.373	0.47	3 (0.1)
Új-Szeged	8.079	4.865	4 (1.0)
Homokszentgyörgy	0.283	0.181	3 (0.1)
Gagyvendégi	O.627	0.273	3 (0.1)
Nagykanizsa	0.220	0.111	3 (0.1)
Nyíregyháza	0.280	0.193	3 (0.1)
Magyaregregy	0.934	0.899	3 (0.1)
Szilvásvárad	0.761	0.364	3 (0.1)
Etes	13.55	3.364	4 (1.0)
Putnok	0.300	0.126	3 (0.1)
Mezőhegyes	25.73	5.009	4 (1.0)
Kompolt	0.629	0.119	3 (0.1)
Nagyszentjános	6.778	2.137	4 (1.0)
Szeged-Öthalom	46.72	15.177	5 (10.0)
Kecskemét	0.655	1.191	4 (1.0)

Table 5: The soil quality factors and soil type of different samples.

Based on this index the samples of this study includes: Szarvas, Homokszentgyörgy, Kompolt, Nagykanizsa, Nyíregyháza, Magyaregregy, Szilvásvárad, Putnok and Gagyvendégi were placed in the third group (Marsh, meadow soils, podzolos brown forest soil humus levels), Magyaróvár, Új-Szeged, Etes, Kecskemét, Nagyszentjános and Mezőhegyes in the fourth (Brown forest soils) and Szeged-Öthalom fifth (Chernozem humic substances) (Tab. 5).

### 4.2. Iranian soils

## 4.2.1. Physical and chemical properties

The physical and chemical properties of soil samples are shown in the Table 6. The texture of soil samples was silty loam, the range of EC was between 1.26 to 2.29  $\mu$ s/cm and the pH was in the range of natural.

Tuble 6. The physical and enclinear properties of anterent son samples.								
Forest type*	Alnus:Populus%	Sand(%)	Silt(%)	Clay(%)	Soil Texture	EC (µs/cm)	$pH_{\rm H2O}$	
VF		20.29	57.26	22.44	Silty loam	2.39	7.42	
	100:0	17.77	63.09	19.14	Silty loam	1.61	7.53	
	70:30	19.10	62.08	18.82	Silty loam	1.74	7.48	
PF	50:50	18.49	63.15	18.36	Silty loam	1.50	7.50	
	30:70	8.575	72.92	18.51	Silty loam	2.15	7.54	
	0:100	15.72	64.04	20.24	Silty loam	1.02	7.54	

Table 6: The physical and chemical properties of different soil samples.

\* VF: virgin forest, PF: planted forest.

There was a significant differences (P<0.05) in the percentage of total organic carbon (TOC), total nitrogen (N) and C:N ratio of different samples (Appendix, Tab. 3). Virgin forest sample contained the maximum value of TOC, there was not a significant difference between 100:0 and 70:30 samples of planted forest (Fig. 12). The 0:100 contained minimum value of TOC. Virgin forest covered with a variety of old and native species, both the age and species kind influence the quantity of TOC in the soil.

The percentage of N for the virgin forest was the greatest and there was no significant difference between virgin forest and 100:0 of planted forest (Fig. 13). The 50:50, 30:70 and 0:100 samples of planted forest contained the minimum amount of N (Fig. 13).



Figure 12: The percentage of TOC in the samples.

*Alnus* spp. is able to fix the atmospheric nitrogen ( $N_2$ ) and increase it in the soil by symbiosis with genus *Frankia* (Ekblad and Huss-Danell 1995; Roggy et al. 2004). Since *A. subcordata* is the most native species of the sampling site, it was obvious that the amount of N were decreased with decreasing the ratio of Alnus:Populus in planted forest (Fig. 13).



Figure 13: The percentage of N in the samples.

The range of C:N ratio between samples estimated between 26.18 to 50.39, the lowest and highest values were found in virgin forest and 30:70 sample of planted forest, respectively (Appendix, Tab. 3).



Figure 14: The C/N ratio of samples

There was a significant difference (p <0.05) in the percentage of TOC, N and C:N ratio of soil fractions (Fig. 15 and Appendix Tab, 4). According to statistical analysis of the TOC, clay and silt fractions contained the maximum and minimum values of TOC, respectively (Fig. 15). The amount of N in the clay fraction was the highest and there was no significant difference between sand and silt fractions (Fig. 15). Sand fraction had the maximum value of C:N ratio and no significant difference was found between silt and clay fractions in this parameter (Fig. 15).



Figure 15: Significant differences of TOC, N and C:N ratio in soil fractions.

The clay fraction showed the maximum values of TOC, N and the less C:N ratio. There is a positive relationship between the percentage of clay fraction and organic matter in the soil (Anderson et al. 1981; Schnitzer and Khan 1978). 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 enable 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 highest value of C:N ratio was found in sand fraction which shows the presence of labile organic matter in the sand particle size. This kind of organic matter contains components which can decompose rapidly and turned to more complex compounds with less activity and more stability.

The relationship between organic carbon and N stabilization in soils contain clay and silt plus clay were reported in different studies (Feller and Beare 1997; Hassink 1997). As well as clay content, clay type influences the stabilization of organic carbon and N in the soil (Feller and Beare 1997).

The results of soil fractions and samples type interaction in the TOC, N and C:N ratio were shown in Table 7.

Clay fraction of virgin forest contained the maximum and silt fraction of 30:70 of planted forest showed the minimum values of TOC (Tab. 7). There was no significant difference between the amount of TOC in the silt fraction of 100:0, 70:30, 50:50, 30:70 and 0:100 of planted forest (Tab. 7). The highest and lowest values of N were found in the clay fraction of virgin forest and sand fraction of 0:100 of planted forest, respectively (Tab. 7). No significant difference was found in N content among sand and silt fractions in all the samples (Tab. 7).

Taleshi et al. (2009) studied the effect of Alder vegetation on the restoration of forest soil and indicated that's positive role on increasing the amount of soil nitrogen because of their capability of nitrogen fixation. It has shown that the mixed cultured are the most appropriate strategy to provide a broader range of environmental services, such as: protection, restoration and biodiversity conservation (Cusack and Montagnini 2004; Guariguata et al. 1995; Parrotta and Knowles 1999).

Between different ratios of Alnus:Populus in planted forest in aspect of quantitative parameters, the 100:0 and 70:30 samples showed the most similarity to the virgin forest, so the sample 70:30 is the acceptable ratios of mixed culture of planted forest in order to restoration the forests of studying site.

Forest	Almus Dopulus %	Î	FOC (%)	1	N (%) C:N				N (%) C:N		
type*	Amus.ropulus 70	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay	
VF		<sup>1</sup> g 2.33	hi 1.44	a 6.38	е 0.040	е 0.033	a 0.342	abc 132.37	cd 67.96	d 18.74	
	100:0	h 1.66	hij 1.19	b 5.25	е 0.023	е 0.025	b 0.243	bcd 94.46	cd 50.56	d 21.56	
PF	70:30	cd 4.21	ij 0.94	с 4.47	е 0.040	е 0.015	с 0.203	abcd 107.66	cd 80.51	d 22.16	
	50:50	f 2.96	j 0.69	de 3.66	е 0.022	е 0.017	cd 0.181	abc 132.44	cd 41.09	d 20.24	
	30:70	ef 3.4	j 0.62	е 3.57	е 0.020	е 0.018	d 0.161	ab 182.30	d 35.14	d 22.30	
	0:100	h 1.71	j 0.82	ef 3.41	е 0.008	е 0.017	cd 0.168	a 195.61	cd 48.62	d 20.31	
LSD			0.61	L		0.0384			96.221		

Table 7: Interaction of soil samples and soil fractions in TOC, N and C:N ratio.

1: Values followed by the same letter are not significantly different at P < 0.05.

\*: VF: virgin forest, PF: planted forest.

# 4.2.2. UV VIS Spectroscopy

# 4.2.2.1 HWP extracts

In all the samples the UV-VIS spectra followed a decrease with increasing the wavelength (Fig. 16). Virgin forest and 100:0 of planted forest contained higher values of DOC in their HWP extracts (Fig. 17). The positive relationship between the absorbance value and DOC is obvious (Fig. 16 and 17).

The UV-VIS parameter of samples are shown in Table 8, the range of  $E_4/E_6$  ratio in present samples (virgin and planted forests soil samples) was very narrow and between 2.20 to 2.75 in different samples.



Figure 16: The relationship between the absorbance and wavelength in HWP extracts.



Figure 17: The amount of dissolved organic carbon (DOC) in HWP extracts.

The range of  $E_4/E_6$  ratio 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), but the result of Takács and Füleky (2010) indicated that the dissolved organic matter of HWP extracts have some characteristics similar to soil FA fractions and natural organic matter, so DOC of Hot Water extracts in this study probably are classified in FA range.

Her et al (2004) estimated the URI value for different HS fractions, according to them, URI value for humic acids is the lowest (1.59), and it is medium for fulvic acids (1.88). The values of URI in HWP

extracts of these samples were in a good agreement with their  $E_4/E_6$  ratio and show the presence of FA in the sample extracts (Tab. 11).

Forest type*	Alnus:Populus %	$E_2/E_3$	$E_4/E_6$	URI
VF		3.68	2.30	2.35
	100:0	3.73	2.20	3.27
	70:30	3.69	2.24	2.49
PF	50:50	3.48	2.36	2.34
	30:70	4.02	2.75	2.13
	0:100	3.96	2.50	1.98

Table 8: The optical parameters of the qualitative properties of the HWP extracts.

\*: VF: virgin forest, PF: planted forest.

# 4.2.2.2 NaHCO<sub>3</sub> extracts

In all the samples the UV-VIS spectra followed a decrease with increasing the wavelength (Fig. 18). Helms et al. (2008) confirmed that the absorption at longer wavelengths is higher by the components with high molecular weight. Our result showed the spectra absorbance value of virgin forest extracts was higher than the others (Fig. 18). It is obvious that the amount of total organic carbon in virgin forest was higher than the others (Fig. 12). A reduction of total organic carbon is observed from virgin forest to 0:100 of planted forest, this decreasing of dissolved organic carbon is also observable by their spectra absorbance value (Fig. 18).



······g-·· (-----)

Figure 18: The relationship between the absorbance and wavelength in different samples.

There was a good correlation between the percentage of TOC of samples and the absorbance at 254 and 365 nm, but the 365 nm showed a better regression (Fig. 19).



Figure 19: The relationship between the percentage of TOC and the absorbance at 254 and 365 nm.

The range of  $E_2/E_3$  ratio of NaHCO<sub>3</sub> extracts of the samples was between 4.61 to 4.76, and it was approximately similar in different samples (Tab. 9). The value of  $E_4/E_6$  ratio was ranged from 3.51 to 4.97 (Tab. 9). The sample of virgin forest had the maximum value of  $E_4/E_6$  which indicate the presence of organic compounds with lower MW and C:N ratio.

Forest type*	Alnus:Populus %	$E_2/E_3$	$E_{4}/E_{6}$	URI
VF		4.70	4.97	1.06
	100:0	4.71	3.51	1.18
	70:30	4.76	3.78	1.41
PF	50:50	4.65	4.75	1.67
	30:70	4.63	4.19	1.82
	0:100	4.61	4.16	1.96

Table 9: The optical parameters of the qualitative properties of NaHCO<sub>3</sub> extracts of samples.

\*: VF: virgin forest, PF: planted forest.

According to the range of  $E_4/E_6$  ratio in temperate area which estimated between 3.3 to 5.9 for HA (Fong and Mohamed 2007; Schmitt et al. 1997; Schnitzer and Khan 1978; Tan 2000), optical parameter of NaHCO<sub>3</sub> extracts showed the presence of HA (Tab. 9). 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). The highest value of  $E_4/E_6$  ratio was found in virgin forest (Tab. 9) that indicates less MW and higher humification level of organic component in this sample.

The URI value of most of samples including, virgin forest, 100:0, 70:30, 50:50 and 30:70 of planted forest (Tab. 9) were classified in the HA range (Her et al. 2004) and contained organic matter with more humification. These results indicated that 0:100 of planted forest are not so humified and include a mixture of organic materials which have characteristics similar to the soil FA fractions (Tab. 9).

# 4.2.3. Humic substance quality

According to K factor classification there is 5 class ((Buzás 1988)

- 1- Plant residues rich layers ( $<10^{-4}$ )
- 2- Humic level of peat and salty soil  $(10^{-3})$
- 3- Humic level of marsh, meadow-podzolic brown forest soil  $(10^{-2})$
- 4- Brown forest soils  $(10^{-1})$
- 5- Substances of chernozem soil (> $10^1$ ).

soil type of all samples (virgin and planted forests) were classified in Brown forest soils (Tab. 10).

5. The son quarty factors and son type of anterent samples of virgin and planted to						
Forest type*	Alnus:Populus %	Q	K	Soil type		
VF		3.34	0.58	3 (0.1)		
	100:0	1.35	0.29	3 (0.1)		
	70:30	1.03	0.26	3 (0.1)		
PF	50:50	0.72	0.22	3 (0.1)		
	30:70	0.67	0.19	3 (0.1)		
	0:100	0.61	0.19	3 (0.1)		

Table 3: The soil quality factors and soil type of different samples of virgin and planted forests.

\*: VF: virgin forest, PF: planted forest.

### 4.2.4. NMR Spectroscopy

# 4.2.4.1 <sup>13</sup>C and <sup>1</sup>H NMR of sand fraction

Virgin forest contained variety of all types of carbon in different shifts (Tab. 11). Highest proportions of organic carbon is in alkyl (0-45 ppm), aldehyd and ketone (190-220 ppm), respectively (Tab. 11, Fig. 20).

The saturated organic carbon which are present in aliphatic carbon were classified in lipids, cetin, suberin, proteins, aliphatic biopolymers and unknown compounds (Zech et al. 1992) that are the main components of plants.



Figure 20: <sup>13</sup>C NMR of virgin forest sand fraction.

<sup>1</sup>H NMR of virgin forest indicated H forms linked to aliphatic groups, carbohydrate structure and aromatic molecules in the regions of 0.5-3, 3-4.2 and 6-8 ppm (Baglieri et al. 2007), respectively, high proportions of aliphatic groups and carbohydrate structure are the results of humification process of labile organic matter in this sample (Fig. 21).





The <sup>13</sup>C NMR of 100:0 planted forest (Tab.11) showed high proportion of O–alkyl carbons in the chemical shifts of 45-110 ppm and carbonyl, carboxyl, ester, amid functional groups in the range of 165-190 ppm (Fig. 22).



Figure 22: <sup>13</sup>C NMR of sand fraction of 100:0 planted forest.

<sup>1</sup>H NMR result of this sample shows H forms linked to aliphatic groups (0.5-3 ppm), carbohydrate structures (3-4.2 ppm) and very small peaks of aromatic molecules (6-8 ppm), the aliphatic H shows the higher peaks and this organic group are the main component in this sample (Fig. 23).



Figure 23: <sup>1</sup>H NMR of sand fraction of 100:0 planted forest.

~ .		Cnemical shifts							
Sample	Fraction	0-45 <sup>a</sup>	45–110 <sup>b</sup>	110-165 <sup>c</sup>	165-190 <sup>d</sup>	190- 220 <sup>e</sup>			
				0/-					
				70					
VF	Sand	67.57	0.68	0.68	0.68	30.41			
100:0	Sand	0.07	59.11	0.07	40.68	0.07			
70:30	Sand	38.17	40.08	8.01	13.36	0.38			
50:50	Sand	0.18	6.13	63.75	0.18	29.77			
30:70	Sand	15.7	31.55	22.13	27.47	3.14			
0:100	Sand	26.45	45.50	15.34	12.43	0.26			

Table 4: The proportion of different types of carbon and chemical shifts of them in sand fraction.

a: Alkyl carbons (aliphatic –CH, –CH, and –CH groups); b: O-alkyl carbons (C of amino groups as well as of O–CH, C–O groups of carbohydrates, alcohols, and ethers); c: Aromatic and olefin carbons; d: Carboxyl, carbonyl, ester and amid carbon; e: Aldehyd and ketone carbon.

High proportions of alkyl and O–alkyl carbons are presented in 70:30 sample of planted forest (Tab. 11). Figure 24 also shows the presence of carbonyl, carboxyl, esters and amid functional groups in the range of 165-190 ppm in this sample. The amount of these compounds are more than virgin forest and less than 100:0 of planted forest (Tab. 11) which is obvious in shorter peaks of this sample (Fig. 24).



Figure 24: <sup>13</sup>C NMR of sand fraction of 70:30 planted forest.

The result of <sup>1</sup>H NMR of 70:30 planted forest indicated a variety of alkyl and O–alkyl carbons in addition to a wide range of carbonyl, carboxyl, esters and etc. (Fig. 25). There are high proportions of H forms linked to aliphatic group (0.5-3 ppm), carbohydrate structures (3-4.2 ppm) and small for aromatic molecules, the main components in this sample are aliphatic groups (6-8 ppm),



Figure 25: <sup>1</sup>H NMR of sand fraction of 70:30 planted forest.

In the 50:50 planted forest the highest proportion of organic carbon were aromatic and olefin in the chemical shift of 110-165 ppm (Fig. 26 and Tab. 11). Aldehyd and ketone carbons in the chemical shift of 190-220 ppm also have high proportion after aromatic and olefin in this sample (Tab. 11).



Figure 26: <sup>13</sup>C NMR of sand fraction of 50:50 planted forest.

The result of <sup>1</sup>H NMR of sample 50:50 is very similar to 70:30 but the peaks are higher in the regions 0.5-3 and 3-4.2 ppm in 70:30 and shows much more H in the same structure position in this sample (Fig. 27).



Figure 27: <sup>1</sup>H NMR of sand fraction of 50:50 planted forest.

The result of 30:70 planted forest showed a variety of alkyl carbons in chemical shift of 0-45 ppm, and O–alkyl carbons in 45-110 ppm. There were some aromatic and olefin carbon in 110-165 ppm in addition to carbonyl, carboxyl and amid functional groups in 165-190 ppm, also there were aldehyd and ketone carbons in the lowest proportion (Fig. 28, Tab. 11).



Figure 28: <sup>13</sup>C NMR of sand fraction of 30:70 planted forest.

H forms in this sample are similar to the sample 50:50, but a reduction in the height of peaks in all regions specially in the region 0.5-3 ppm is obvious, and shows lower proportion of aliphatic H than the later sample (Fig. 29).



Figure 29: <sup>1</sup>H NMR of sand fraction of 30:70 planted forest.

In the sample 0:100 of planted forest higher proportions of O–alkyl and alkyl carbons were found in chemical shift of 0-110 ppm. Aromatic and olefin carbons (110-165 ppm), carboxyl, carbonyl and amid carbons in chemical shift of 165-190 ppm also were found in different values, the lowest value belongs to aldehyd and ketone carbons in 190-220 ppm (Tab. 11).



Figure 30: <sup>13</sup>C NMR of sand fraction of 0:100 planted forest.

The result of <sup>1</sup>H NMR of this sample showed structures of alkyl and O–alkyl carbons, the structure of carbonyl, carboxyl and amid carbons, the aliphatic H (0.5-3 ppm) and carbohydrate H (3-4.2 ppm) in high proportions and low aromatic H are shown (Fig. 31).



Figure 31: <sup>1</sup>H NMR of sand fraction of 0:100 planted forest.

# 4.2.4.2 <sup>13</sup>C and <sup>1</sup>H NMR of Silt fraction

The <sup>13</sup>C NMR of silt fraction of virgin forest is shown in Figure 32. A diversity of different types of carbon, alkyl, O–alkyl, aromatic and olefin, carbonyl, carboxyl, ester, amid, aldehyd and ketone carbons were found in this sample (Tab. 12).

Chemical shifts						
Sample Fi	Fraction	0-45 <sup>a</sup>	45–110 <sup>b</sup>	110-165 <sup>c</sup>	165-190 <sup>d</sup>	190- 220 <sup>e</sup>
				%		
VF	Silt	16.92	29.27	20.81	27.24	5.75
100:0	Silt	68.03	0.68	2.72	0.68	27.89
70:30	Silt	33.33	22.67	0.33	43.33	0.33
50:50	Silt	21.88	16.63	0.22	61.05	0.22
30:70	Silt	0.94	22.21	26.50	37.87	12.48
0:100	Silt	24.27	34.22	15.05	22.813	3.64

Table 5: The proportion of different types of carbon and chemical shifts of them in silt fraction.





Figure 32: <sup>13</sup>C NMR of silt fraction of virgin forest.

The result of <sup>1</sup>H NMR shows different H forms linked to aliphatic groups (0.5-3 ppm), carbohydrate structures (3-4.2 ppm) and aromatic molecules (6-8 ppm), the proportion of aliphatic H was more than carbohydrate structures and there were very small peaks related to aromatic H (Fig. 33).



Figure 33: <sup>1</sup>H NMR of silt fraction of virgin forest.

The result of <sup>13</sup>C NMR of 100:0 sample of planted forest showed higher proportion of alkyl (0-45 ppm), aldehyd and ketone (190-220 ppm) and small values of carbonyl, carboxyl, amid, O–alkyl and aromatic carbons (Tab. 12).



Figure 34: <sup>13</sup>C NMR of silt fraction of 100:0 of planted forest.

According to <sup>1</sup>H NMR the components of this sample are a mixture of all three mentioned organic components, high proportion of aliphatic groups (0.5-3 ppm) and low proportion of aromatic (6-8 ppm) is obvious (Fig. 35).



Figure 35: <sup>1</sup>H NMR of silt fraction of 100:0 of planted forest.

Alkyl, O–alkyl, carbonyl, carboxyl, ester and amid carbons are the highest proportions of carbons which found in the 70:30 sample of planted forest (Fig. 36, Tab. 12).



Figure 36: <sup>13</sup>C NMR of silt fraction of 70:30 of planted forest.

<sup>1</sup>H NMR result of the sample 70:30 was very similar to the sample 100:0 (Fig. 35 and 37).



Figure 37: <sup>1</sup>H NMR of silt fraction of 70:30 of planted forest.

Different types of carbon were found in the 50:50 sample of planted forest. The proportion of carboxyl, carbonyl, ester and amid carbon (165-190 ppm) was the most. Alkyl and O–alkyl carbons



(0-110 ppm) indicated higher proportion after carboxyl, carbonyl, ester and amid carbon (Fig. 38,

ppm Figure 38: <sup>13</sup>C NMR of silt fraction of 50:50 of planted forest. A variety of different types of organic carbon were found in <sup>1</sup>H NMR of 50:50 sample, the diversity

of aliphatic groups with separate and sharp peaks in the range of 0.5-3 ppm which are the main compounds in this sample is obvious. The values of carbohydrate structure in the range of 3-4.2 ppm and aromatic molecules (6-8 ppm) also are more than later samples (Fig. 39).



Figure 39: <sup>1</sup>H NMR of silt fraction of 50:50 of planted forest.

In the 30:70 of planted forest, carbonyl, carboxyl, ester and amid carbons (165-190 ppm) showed the highest proportion of organic carbon, furthermore aromatic and olefin carbons (165-190 ppm), O–alkyl carbon (45-110 ppm) indicated high proportion (Fig. 40, Tab. 12).



Figure 40: <sup>13</sup>C NMR of silt fraction of 30:70 of planted forest.

<sup>1</sup>H NMR of 30:70 sample of planted forest showed the highest diversity and proportions of aliphatic and carbohydrate H in this sample in the regions 0.5-3 and 3-4.2 ppm, respectively, aromatic H in this sample also is higher than the other sample (Fig. 41).



Figure 41: <sup>1</sup>H NMR of silt fraction of 30:70 of planted forest.
The result of <sup>13</sup>C NMR of 0:100 planted forest indicated different proportion of alkyl, O–alkyl, carbonyl, carboxyl, ester, amid, aromatic and olefin, aldehyd and ketone carbons (Fig. 42, Tab. 12).



Figure 42: <sup>13</sup>C NMR of silt fraction of 0:100 of planted forest.

There is very small and little peaks of aliphatic H in the sample 0:100 (Fig. 43).



Figure 43: <sup>1</sup>H NMR of silt fraction of 0:100 of planted forest.

# 4.2.4.3 <sup>13</sup>C and <sup>1</sup>H NMR of Clay fraction

There were different types of carbon in clay fraction of virgin forest. The highest proportion of organic carbon belongs to alkyl, O–alkyl and carbonyl, carboxyl, ester and amid carbons were in higher proportion than aromatic, olefin, aldehyd and ketone carbons (Fig. 44, Tab.13).



Figure 44: <sup>13</sup>C NMR of clay fraction of virgin forest.

The H forms linked to aliphatic, carbohydrate and aromatic structures is shown in Figure 45 and in the regions 0.5-3, 3-4.2 and 6-8 ppm, respectively. The highest proportion was aliphatic and the lowest aromatic H (Fig.45).



Figure 45: <sup>1</sup>H NMR of clay fraction of virgin forest.

In the sample 100:0 of planted forest, the maximum proportion was found in alkyl and O–alkyl carbon in chemical shift of 0-110 ppm (Fig. 46, Tab. 13). Some carbonyl, carboxyl, ester and amid carbons (165-190 ppm), and aromatic, olefin, aldehyd and ketone carbons were found in lowest proportions (Tab. 13).

		Chemical shifts							
Sample	Fraction	0-45 <sup>a</sup>	45–110 <sup>b</sup>	110-165 <sup>c</sup>	165-190 <sup>d</sup>	190- 220 <sup>e</sup>			
				%					
VF	Clay	39.06	39.84	4.3	16.41	0.39			
100:0	Clay	44.05	39.21	0.44	15.86	0.44			
70:30	Clay	40.32	32.26	0.40	26.61	0.40			
50:50	Clay	52.36	27.75	0.52	18.85	0.52			
30:70	Clay	29.07	14.53	24.71	31.39	0.29			
0:100	Clay	3.19	25.51	27.87	25.22	18.21			

Table 6: The proportion of different types of carbon and chemical shifts of them in clay fraction.

a: Alkyl carbons (aliphatic –CH, –CH, and –CH groups); b: O-alkyl carbons (C of amino groups as well as of O–CH, C–O groups of carbohydrates, alcohols, and ethers); c: Aromatic and olefin carbons; d: Carboxyl, carbonyl, ester and amid carbon; e: Aldehyd and ketone carbon.



Figure 46: <sup>13</sup>C NMR of clay fraction of 100:0 planted forest.

The result of <sup>1</sup>H NMR indicated presence of huge amounts of aliphatic and carbohydrate H (0.5-3 and 3-4.2 ppm) that are the main organic structures in the sample 100:0, it also contains aromatic H (6-8 ppm) in small proportion (Fig. 47).



Figure 47: <sup>1</sup>H NMR of clay fraction of 100:0 planted forest.

Organic carbons were found in the sample 70:30 of planted forest includes, alkyl (0-45 ppm), O–alkyl (45-110 ppm), and carbonyl, carboxyl, ester and amid carbons (165-190 ppm) had the most proportions, respectively (Tab. 13, Fig. 48).



Figure 48: <sup>13</sup>C NMR of clay fraction of 70:30 planted forest.

<sup>1</sup>H NMR result of 70:30 sample is shown in Figure 49, this sample also contains high values of aliphatic and carbohydrate H (0.5-3 and 3-4.2 ppm) as the main organic structures as well as aromatic molecules in low proportion (Fig. 49).



Figure 49: <sup>1</sup>H NMR of clay fraction of 70:30 planted forest.

Alkyl carbon (0-45 ppm) was the highest proportion of organic carbon in 50:50 sample of planted forest (Fig. 50, Tab. 13). O–alkyl (45-110 ppm) and carbonyl, carboxyl, ester and amid carbons (165-190 ppm) were the other high organic carbon after alkyl (Tab. 13).



Figure 50: <sup>13</sup>C NMR of clay fraction of 50:50 planted forest.

The result of <sup>1</sup>H NMR showed a variety of aliphatic and carbohydrate H (0.5-3 and 3-4.2 ppm) as the main organic H and aromatic H (6-8 ppm) in low proportion (Fig. 51).



Figure 51: <sup>1</sup>H NMR of clay fraction of 50:50 planted forest.

In the sample 30:70 of planted forest there were all types of carbon including alkyl (0-45 ppm), O–alkyl (45-110 ppm), olefin and aromatic (110-165 ppm), carbonyl, carboxyl, ester and amid carbons (165-190 ppm) and their proportions were approximately similar (Tab. 13, Fig. 52).



Figure 52: <sup>13</sup>C NMR of clay fraction of 30:70 planted forest.

All types of H are found in this sample but their values are less than later samples and the peaks are shorter (Fig. 53).



Figure 53: <sup>1</sup>H NMR of clay fraction of 30:70 planted forest.

In sample 0:100 of planted forest the proportion of O–alkyl (45-110 ppm), olefin and aromatic (110-165 ppm), carbonyl, carboxyl, ester and amid carbons (165-190 ppm), aldehyd and ketone were approximately similar and higher than alkyl carbons (Tab. 13, Fig. 54).



Figure 54: <sup>1</sup>H NMR of clay fraction of 0:100 planted forest.

According to <sup>1</sup>H NMR the sample 0:100 have the highest diversity in H forms in different regions, the main organic compounds contain aliphatic groups (Fig. 55).



Figure 55: <sup>1</sup>H NMR of clay fraction of 0:100 planted forest.

### 4.2.4.4 The summary of NMR spectroscopy results

<sup>1</sup>H and <sup>13</sup>C NMR spectroscopy analysis carried out on three soil fractions (sand, silt and clay). The result of <sup>13</sup>C NMR showed different proportion of alkyl; O-alkyl; aromatic and olefin; carboxyl, carbonyl, ester and amid; aldehyd and ketone carbons in different samples (Tab. 14). This may be due to the fact that the soil organic matter in top soils is dominated by high proportions of plant residues with a relatively uniform composition.

Organic compounds in sand particle size contained high proportion of oxygenated aliphatic groups (Tab. 14) that can be assigned methoxyl groups, O-alkyl groups and dioxygenated alkyl groups (Mao et al. 2001; Wilson 1987).

In the sand fraction of sample 100:0, the highest proportion of organic carbon was found in chemical shift of 45-110 ppm (oxygenated aliphatic groups). Most of the O-alkyl carbon found in the soils is hydrolyzable (Kogel-Knabner 2000). In some arable soils that investigated by Kiem et al (2000), the contents of carbohydrate carbon were significantly correlated with the signal intensities of O-alkyl carbon.

There were oxygenated aliphatic and aliphatic groups (0-110 ppm) as the highest proportions of organic carbon in the sand fraction of sample 70:30 (Tab. 14). Kogel-Knabner (2000) reported sand-sized fractions are dominated by high proportions of O-alkyl carbon, followed by alkyl carbon and indicating the plant fragment origin of this fraction.

In the sample 50:50, the main proportion of organic carbon in sand particle size was found in aromatic carbon region (110-165 ppm). Oxygenated aliphatic carbons (45-110 ppm) was the highest in the 30:70 and 0:100 samples (Tab. 14).

The proportion of aromatic carbon in sand fraction of different samples was different and it was lower than aliphatic structure (except 50:50 sample) (Tab. 14). Zech et al (1992) indicated that the aromatic and olefin structures are found in lignin and tannins compounds. With humification progress, these compounds degrade to simple organic material with more aliphatic structures. During humification, lignin derived structures are further transformed to aromatic humic-like structures (Deshmukh et al. 2002). It can be concluded that higher and lower proportions of aliphatic and aromatic carbon in sand

fraction show the lowest humification in the sand particle size organic matter. But the average aromaticity of sand fraction in different samples was the highest in comparison to silt and clay fractions which contain organic components in higher humification level (Tab. 14).

			aromaticity				
Sample	Fraction	0-45 <sup>a</sup>	45–110 <sup>b</sup>	110-165 <sup>c</sup>	165-190 <sup>d</sup>	190- 220 <sup>e</sup>	
			<u> </u>				
	Sand	67.57	0.68	0.68	0.68	30.41	0.9
VF	Silt	16.92	29.27	20.81	27.24	5.75	31.06
	Clay	39.06	39.84	4.3	16.41	0.39	5.16
	Sand	0.07	59.11	0.07	40.68	0.07	0.11
100:0	Silt	68.03	0.68	2.72	0.68	27.89	3.81
	Clay	44.05	39.21	0.44	15.86	0.44	0.53
	Sand	38.17	40.08	8.01	13.36	0.38	9.29
70:30	Silt	33.33	22.67	0.33	43.33	0.33	0.59
	Clay	38.46	19.23	0.38	41.53	0.38	0.65
	Sand	0.18	6.13	63.75	0.18	29.77	90.99
50:50	Silt	21.88	16.63	0.22	61.05	0.22	0.56
	Clay	52.36	27.75	0.52	18.85	0.52	0.65
	Sand	15.7	31.55	22.13	27.47	3.14	31.9
30:70	Silt	0.94	22.21	26.50	37.87	12.48	53.39
	Clay	29.07	14.53	24.71	31.39	0.29	36.17
	Sand	26.45	45.50	15.34	12.43	0.26	17.58
0:100	Silt	24.27	34.22	15.05	22.813	3.64	20.46
	Clay	3.19	25.51	27.87	25.22	18.21	49.27

Table 7: The proportion of chemical shifts and different types of carbon in different fractions.

a: Alkyl carbons (aliphatic –CH, –CH, and –CH groups); b: O-alkyl carbons (C of amino groups as well as of O–CH, C–O groups of carbohydrates, alcohols, and ethers); c: Aromatic and olefin carbons; d: Carboxyl, carbonyl, ester and amid carbon; e: Aldehyd and ketone carbon.

The maximum proportions of aliphatic carbon in sand fraction of different samples were found in the 70:30 and 0:100 samples (Tab. 14). The highest and lowest aromaticity in sand fraction were in the 50:50 and 100:0 samples, respectively (Tab. 17).

The result of <sup>1</sup>H NMR of sand fractions indicated the presence of different H forms linked to aliphatic groups, carbohydrate structure and aromatic molecules in the shifts 0.5-3, 3-4.2 and 6-8 ppm, respectively.

Figure 56 shows <sup>1</sup>H NMR of sand fraction, for all samples, virgin forest contains all three mentioned H forms in different proportions. Aliphatic H was the main organic group and aromatic the lowest. In the sample 100:0 the proportions of all aliphatic, carbohydrate and aromatic H were lower than virgin forest (Fig. 56).



Figure 56:<sup>1</sup>H NMR of sand fraction of samples

In the sample 70:30, the proportions of aliphatic and carbohydrate H were lower and higher than virgin forest and 100:0, respectively, but aromatic H was higher than both virgin forest and the sample 100:0 (Fig. 56). The samples 50:50 and 30:70 shows similar results while the peaks in some regions was higher in the sample 50:50 (Fig. 56). In the sample 0:100, high proportions of aliphatic and carbohydrate H in the shifts 0.5-3 and 3-4.2 ppm is obvious, the aromatic H (6-8 ppm) was lower than two other H forms. Overall the most important difference in the sand fraction between virgin and planted forest was the aromatic H that was in virgin forest lower than planted forest.

<sup>13</sup> C NMR showed that organic compounds of silt fraction contained high proportion of carbonyl and carboxyl carbon in chemical shift of 165-190 ppm (Tab. 14). Schnitzer and Khan (1978) indicated that the major oxygen-containing functional groups in humic substances are carboxyl, hydroxyls and carbonyls. Zech et al. (1992) found the increasing of carboxyl groups from non humified to humified layer in the forests soil. They explained that not only oxidation of lignin side-chain but also additional processes may contribute increasing of carboxyl groups. Kogel-Knabner (2000) found that aldehyd, ketone and carboxylic acid are the result of lignin degradation.

In the 100:0 sample the highest proportion was found in chemical shifts of 0-45 ppm and alkyl carbon region (Tab. 14). The presence of high proportion of alkyl carbon in this sample is probably the result of O-alkyl compounds degradation. The most aromaticity in silt fraction was found in 30:70 sample (Tab. 14).

<sup>1</sup>H NMR result of silt fractions indicated the presence of aliphatic, carbohydrate and aromatic H in the shifts of 0.5-3, 3-4.2 and 6-8 ppm in different proportion (Fig. 57). All H forms in silt fraction of virgin forest sample had smaller peaks in comparison to sand fraction of this sample, specially aromatic molecules.

The result of <sup>1</sup>H NMR of silt fraction in planted forest showed higher aliphatic and carbohydrate H in comparison to sand fraction of this samples (except 0:100), the proportion of aromatic H in the silt fraction of the sample 30:70 also was greater in sand fraction. Overall the proportions of aliphatic and carbohydrate H of planted forest were higher than virgin forest in this faction (Fig. 57).

The clay fraction mostly contained highest proportions of aliphatic which followed by oxygenated aliphatic groups in chemical shift of 0-45 and 45-110 ppm, respectively (Tab. 14). The fine fractions

of soils are more different from the bulk soil in their organic matter composition than the coarse fractions. Clay-size fractions generally show a higher content of alkyl carbon than the whole soils, as revealed by <sup>13</sup>C NMR spectroscopy (Mahieu et al. 1999).



Figure 57: <sup>1</sup>H NMR of silt fraction of samples

The most biological stabilization of organic carbon was found in soils is alkyl carbon (Paul and Van Veen 1978). There is an increasing of alkyl carbon during humification which can be explained by enrichment of extractable and bound lipids (Hempfling and Schulten 1989; Ziegler and Zech 1989). According to Li et al (2003) the content of aliphatic carbons increases in more humified humic substances and the types of the carbons become more diverse in this components.

The clay fraction of 0:100 and 100:0 samples contained the highest and lowest proportion of aromaticity (Tab. 14). However the difference between 100:0 and 70:30 clay fraction in aromaticity was negligible. Lignin decomposing process changes the chemical structure to residual lignin polymer and a reduction in the quantity of aromatic carbon (Baldock and Skjemstad 2000). Lignin is the second most abundant component of most plants that is known to contribute to the soil organic matter (Stevenson 1994). Lignin structures are considerably modified during humification, mainly by demethylation, loss of phenolic moieties, side-chain oxidation, and increasing dominance of carbon substituted recalcitrant aromatic structures (Zech et al. 1997).

The result of <sup>1</sup>H NMR of clay fraction is shown in Figure 58, all H forms in the clay fraction of the virgin forest sample showed higher and lower peaks in comparison to silt and sand fractions, respectively. In the planted forest samples the proportions of aliphatic and carbohydrate H were higher than sand and silt (Fig. 58).

The aromatic H in the clay fraction of 0:100 sample was higher than sand and silt, the result also showed more diversity in aliphatic and carbohydrate H in clay fraction of this sample (Fig.58).



Figure 58: <sup>1</sup>H NMR of clay fraction of samples

#### 5. CONCLUSION

The result of Hungarian soil showed that there is a good correlation between the value of HWP dissolved organic carbon and the absorbance at 254 nm. In all the samples the UV-VIS spectra followed a decrease with increasing wavelength. URI and SUVA showed humic and fulvic acids with low molecular weight in the HWP extracts. The optical parameters indicated that most of humic substances in HWP extracts contain more small compounds with low molecular weight and less aromaticity. It seems that HWP method is suitable to extract small and more soluble fraction of fulvic acids which has low degree of polymerization, low MW, less carbon and more oxygen content. A few amount of humic acid contain a mixture of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids was extracted with this method.

The result of second part of study (Iranian soil) showed that virgin forest sample contained the maximum values of total organic carbon and nitrogen. The C:N ratio of virgin forest sample was lower and indicated more humification degree of soil organic matter in this sample.

The organic carbon and nitrogen quantity of planted forests with the higher ratio of *A. subcordata* which was the most native species of the sampling site was similar to virgin forest. Clay fraction contained the maximum amounts of total organic carbon, nitrogen and less C:N ratio. The most humified organic matter was observed in clay fraction.

Virgin forest and 100:0 of planted forest contained more values of dissolved organic carbon in their HWP extracts. There was a positive relationship between the absorbance value and dissolved organic carbon.

The results of UV VIS spectroscopy determined that FA components are the most dissolved organic carbon in Hot Water extracts. Furthermore, URI values of HWP extracts were in a good agreement with  $E_4/E_6$  ratio and showed the presence of FA in these extraction.

The organic compounds in all of samples except 0:100 (Alnus:Populus) of planted forest which extracted with NaHCO<sub>3</sub> showed HA properties which includes components with more carbon and molecular weight, less oxygen and solubility in comparison to FA and subsequently more stable organic fraction in the soil. <sup>1</sup>H and <sup>13</sup>C NMR indicated different types of organic carbon with different varieties which are the results of humification process. Virgin forest had a wide range of aliphatic

carbon that confirms more humification degree in this sample. Also the presence of different kind of organic carbon in different proportions indicated a balance of input and degraded organic matter during long term humification process.

Sample 0:100 of planted forest contained the most proportion of aromatic and olefin carbons which show less degree of humification in this sample. Organic compounds structure of 70:30 of planted forest was more similar to virgin forest. Overall the soil organic carbon quantity and quality were differed between virgin and planted forests and the best ratios of mixed culture to restoration and preservation of forests and environment besides wood production in the studying site, was 70:30 of Alnus:Populus species.

### 5.1. New scientific results

- The E<sub>2</sub>/E<sub>3</sub>, E<sub>4</sub>/E<sub>6</sub> and URI parameters indicated that the most humic substances extracted with Hot Water Percolation method contain fulvic acid compounds.
- There is a good correlation between the value of HWP dissolved organic carbon and the absorbance at 254 nm.
- The organic carbon quantity of planted forest with the higher ratio of Alnus which was the most native species of the sampling site was more similar to virgin forest.
- In case of soil organic carbon quality and quantity, 70:30 was found the best ratio of Alnus:Populus species.
- NMR study showed that the most of organic compounds in sand particle size contained high proportion of oxygenated aliphatic groups while in silt they were carbonyl and carboxyl carbons and for clay fraction both aliphatic, oxygenated aliphatic as well as carbonyl and carboxyl carbons.

#### 6. SUMMARY

Soil organic matter as one of the most important fraction in the soil affects both chemical and physical properties of the soil including: soil structure, moisture holding capacity, diversity and activity of soil organisms, which are beneficial and harmful to crop production, cation and anion exchange, nutrient availability, and etc.

Vegetation significantly influence the quantity and quality of organic material in the soil. Different vegetation produce different soil properties and any changes in the ecosystem will upset the balance of the soil.

One of the most important fractions of soil organic matter are humic substances which determined as the dark colored amorphous polymers which are synthesized from biomass constituents or their metabolites biochemically and/or chemically in the environment.

Fulvic acid is a mixture of weak aliphatic and aromatic organic acids and its composition and shape is quite variable. Humic acid is another kind of humic substances with less activity than fulvic acid. Humic acids are termed polydisperse because of their variable chemical features.

In order to study soil organic matter, different methods have been used. Hot Water Percolation was introduced as a technique which uses heat energy and pressure to perform water extractions.

Characterization of humic substances 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 465 and 665nm ( $E_4/E_6$ ) in order to characterize these materials. Higher molecular weight and higher degree of condensation of the aromatic rings will be indicated by lower  $E_4/E_6$  ratio value. Furthermore low  $E_2/E_3$  ratio (254 to 365 nm) reflects a high average molecular weight. URI values provides good information of the relative proportions between UV-absorbing functional groups and unsaturated compounds. SUVA, has strongly correlated with percentage of aromatic rings.

In this study which included two parts, we selected 16 soil samples from Hungary. Soil samples were extracted with HWP method. Each extraction was including 10 fractions and each fraction contained 100 ml solution. In order to measure the kinetics of dissolved organic carbon, the volumes of all fractions which extracted from samples were recorded and the amount of HWP dissolved organic carbon in each fraction was measured. The absorbance of UV VIS spectra was measured at 200-700

nm.  $E_4/E_6$  and  $E_2/E_3$  ratios were determined, URI, SUVA was estimated in each sample. Soil samples were extracted with NaHCO<sub>3</sub> and their  $E_4/E_6$  ratio was determined. The extracting of soil samples were performed using NaOH and NaF. The absorbance capacity of recent extracts were measured using VIS spectra at 400-700 nm and the humus quality factor (K) was estimated.

In the second part soil sampling was carried out from both virgin and planted forests of Guilan province, Iran. Virgin forest of this area was mostly covered with Caucasian Alder. Since the Eastern Cottonwood is one of the most rapid growth species which is useful for wood products, some parts of virgin forests in this area were clear cut in order to culture this species. A composite sampling was done from A horizon (0-20 cm) of a virgin forest and a 15 years old planted forest which was cultured with a mixture of Caucasian Alder and Eastern Cottonwood with the rate of 100:0, 70:30, 50:50, 30:70 and 0:100 (Alnus:Populus) in three replication and completely randomized.

Some physical and chemical properties of soil samples were measured. Soil fractionation carried out using sonication method. The percentage of total organic carbon and nitrogen and C:N ratio were measured in soil samples and fractions. Data were analyzed using SAS statistical.

In order to study the quality of organic carbon, soil samples were extracted with HWP method and NaHCO<sub>3</sub> solution. The absorbance value of UV VIS spectra was determined at 200-700 nm. The  $E_2/E_3$ ,  $E_4/E_6$  ratios and URI were estimated. Humic substances were extracted from soil fractions using NaOH. <sup>1</sup>H and <sup>13</sup>C were analyzed using NMR spectrometer.

The properties of HWP dissolved organic carbon in the most of soil samples were similar. The process followed from the two term order kinetics equation which is:

$$Y = A_1[1 - exp(-k_1x)] + A_2[1 - exp(-k_2x)]$$

Where "Y" is the measured DOC, "t" is the time, "A<sub>1</sub>" is the maximum amount of DOC in the first process, "A<sub>2</sub>" is the maximum amount of DOC in the second process, "K<sub>1</sub>" is the rate constant of the first process and "K<sub>2</sub>" is the rate constant of the second process. There was a good correlation between the content of HWP dissolved organic carbon and the absorbance at 254 nm. URI, SUVA,  $E_2/E_3$  and  $E_4/E_6$  indicated that the most of HWP dissolved organic carbon of samples was fulvic acid components with low molecular weight. A few amount of humic acid contain a mixture of weak aliphatic and aromatic organic acids was extracted with HWP method.

The soil sample of virgin forest contained the most total organic carbon, nitrogen and the minimum value of C:N ratio. Clay fraction showed the maximum amounts of total organic carbon and nitrogen. The most value of C:N ratio was estimated in sand fraction. There was a good correlation between dissolved organic carbon in NaHCO<sub>3</sub> extracts and the absorbance at 254 and 365 nm. The  $E_2:E_3$ ,  $E_4:E_6$  ratios and URI showed the presence of humic acid in most of samples. The result of <sup>1</sup>H and<sup>13</sup>C NMR indicated a wide range of organic components with aliphatic and carbohydrate structure in different samples and the sample with non-native species showed lower degree of organic matter degradation and humification. However there was low and high proportions of alkyl and aromatic carbons in the sample with non-native species.

### 7. RELATED PUBLISHMENTS

1. **Marieh Nadi**, Ebrahim Sedaghati and György Füleky. (2012): Characterization of organic matter content of Hungarian agricultural soils. Acta Agronomica Hungarica No:4/2012,ISSN: 0238-0161, IF: 0.172.

2. **Marieh Nadi**, Ebrahim Sedaghati and György Füleky. (2012): Evaluation of humus quality of forest soils with two extraction methods. Published in International Journal of Forest, Soil and Erosion. (IJFSE), 2 (3): 124-127. ISSN 2251-6387.

4. **M. Nadi**, A. Golchin, S.J. Hosseinifard, S.Shafie, E Sedaghati and G. Füleky. (2012): Characterization of soil particles organic matter in a mixed Alnus and Populus cultivation using<sup>1</sup>H and <sup>13</sup>C NMR., J. Agr. Sci. Tech. (JAST). ISSN: 1680-7073. IF: 0.436. (revised submission processing)

5. **M. Nadi**, A. Golchin, S. J. Hosseinifard, E. Sedaghati, S. Shafiei, G. Füleky. (2012): The effects of reforestation on soil organic carbon. Caspian Journal of Environmental Science (CJES). ISSN: 1735-3033. (under review).

6. **Marieh Nadi**, Gabriella Rethati and György Füleky. (2011): Characterization of dissolved organic carbon extracted with HWP in some typical Hungarian soils. Current research in chemical, physical and biological characterization of peat. Zittau, Germany. Heft 5: 53-56. ISBN: 978-3-941521-01-8.

7. Marieh Nadi, Ebrahim Sedaghati, György Füleky (2012). Reforestation and Soil organic matter
 . 3<sup>nd</sup> International Conference for "Development of Environmental Engineering Education", Budapest, Hungary. 109-112. ISBN: 978-80-8086-185-8.

87

### 8. ACKNOWLEDGMENT

Hereby I would like to thank Prof. Dr. György Füleky for his guidance constant encouragement, advices, constructive criticism and scientific challenges. Doubtless without his unwavering support I could not finish this study.

I appreciate department staff members, Török Zsuzsa, Vejzer Tiborne Erzsika, Prokaine Agnes, Rethati Gabrilla and the other members of the institute of Environmental Science, Faculty of Agricultural and Environmental Science at Szent Istvan University for their support and help during my study.

I woul like to thank Torokne Hajdu Monika and Kamenszki Anita and the other staff members of Doctoral School.

My appreciation is extended to Prof. Dr. Ahmad Golchin for helpful scientific suggestion and advices and Saeid Shafiei for friendly help in some parts of this research. My thanks to Dr. Seyed Javad Hosseinifard and Department of Water and Soil, Iranian Pistachio Research Institute for the helpful cooperation in some parts of this research.

I would like to extend my appreciate Physics Department of Vali-e- Asr University of Rafsanjan and Mr. Mohammad Bagher Marashi because of their assistances in progressing some parts of this research.

I appreciate the evaluation committee members specially Prof. Dr. Heltai György, Prof. Dr. Czinkota Imre and Dr. Tünde Nyilas for useful scientific suggestions and evaluating this study.

I appreciate my family for laughter, continuous encouragement and moral ethic. I would like to thank my spouse for his strongly undying, moral, scientific and financial supports during this period. It was very hard these last few years without his unwavering supports.

I would like to thank all my friends in Hungary and Iran for their moral support.

88

#### 9. USED REFERENCE

- Adani F, Genevini P, Zaccheo P, Zocchi G. (1998): The effect of commercial humic acid on tomato plant growth and mineral nutrition. Journal of plant nutrition. 21: 561-575.
- Amer F, Bouldin D, Black C, Duke F. (1955): Characterization of soil phosphorus by anion exchange resin adsorption and P 32-equilibration. Plant and Soil. 6: 391-408.
- Anderson D, Saggar S, Bettany J, Stewart J. (1981): Particle size fractions and their use in studies of soil organic matter: I. The nature and distribution of forms of carbon, nitrogen, and sulfur. Soil Sci Soc Am J. 45: 767-772.
- Augris N, Balesdent J, Mariotti A, Derenne S, Largeau C. (1998): Structure and origin of insoluble and non-hydrolyzable, aliphatic organic matter in a forest soil. Organic geochemistry. 28: 119-124.
- AWWA A. (1998): WEF. Standard Methods for the Examination of Water and Wastewater 20th Edition, Washington, DC, New York.
- Ayanaba A. (1976): The effects of clearing and cropping on the organic reserves and biomass of tropical forest soils. Soil Biology and Biochemistry. 8: 519-525.
- Baglieri A, Ioppolo A, Negre M, Gennari M. (2007): A method for isolating soil organic matter after the extraction of humic and fulvic acids. Organic Geochemistry. 38: 140-150.
- Baldock J, Skjemstad J. (2000): Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic geochemistry. 31: 697-710.
- Baldock JA, Oades JM, Nelson P, Skene T, Golchin A, Clarke P. (1997): Assessing the extent of decomposition of natural organic materials using solid-state super (13) C NMR spectroscopy. Australian Journal of Soil Research. 35: 1061-1083.
- Berger K, Truog E. (1944): Boron tests and determination for soils and plants. Soil Science. 57: 25-36.
- Berliner R, Torrey JG. (1989): On tripartite Frankia-mycorrhizal associations in the Myricaceae. Canadian Journal of Botany. 67: 1708-1712.
- Beznosikov V, Lodygin E. (2009): Characteristics of the structure of humic substances of podzolic and peaty podzolic gleyey soils. Russian Agricultural Sciences. 35: 103-105.

- Bonan GB. (2008): Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science. 320: 1444-1449.
- Bot A, Benites J. (2005): The Importance of Soil Organic Matter: Key to Drought-resistant Soil andSustained Food Production. Food & Agriculture Organazation. 78 pp.
- Brown S, Lugo AE. (1990): Effects of forest clearing and succession on the carbon and nitrogen content of soils in Puerto Rico and US Virgin Islands. Plant and Soil. 124: 53-64.
- Buzás I. (1988): Talaj-és agrokémiai vizsgálati módszerkönyv. (Manual of soil and agricultural chemical analysis). Mezőgazdasági Kiadó, Budapest. 242 pp.
- Carter MR. (2002): Soil quality for sustainable land management: Organic matter and aggregation interactions that maintain soil functions. Agronomy Journal. 94: 38-47.
- Cervantes E, Rodríguez-Barrueco C. (1992): 22 Relationships between the Mycorrhizal and Actinorhizal Symbioses in Non-legumes. Methods in microbiology. 24: 417-432.
- Chefetz BS, Deshmukh MJ, Hatcher AP, Patrick G. (2002): Structural components of humic acids as determined by chemical modifications and carbon-13 NMR, pyrolysis-, and thermochemolysis-gas chromatography/mass spectrometry. Soil Science Society of America Journal. 66: 1159- 1171.
- Chen C, Wang S. (2004): Ecology of mixed plantation forest. Science, Beijing (in Chinese).
- Chen C, Zhang J, Zhou C, Zheng H. (1990): Researches on improving the quality of forest land and the productivity of artificial Cunninghamia lanceolata stands. Journal of Applied Ecology. 1: 97-106.
- Chen J, Gu B, LeBoeuf EJ, Pan H, Dai S. (2002): Spectroscopic characterization of the structural and functional properties of natural organic matter fractions. Chemosphere. 48: 59-68.
- Chen Y, Senesi N, Schnitzer M. (1977): Information provided on humic substances by E4/E6 ratios. Soil Sci Soc Am J. 41: 352-358.
- Cheshire M. (1979): Nature and origin of carbohydrates in soils. Academic Press London. 216 pp.
- Chodak M, Khanna P, Beese F. (2003): Hot water extractable C and N in relation to microbiological properties of soils under beech forests. Biology and fertility of soils. 39: 123-130.

- Christensen JB, Jensen DL, Gron C, Filip Z, Christensen TH. (1998): Characterization of the dissolved organic carbon in landfill leachate-polluted groundwater. Water research. 32: 125-135.
- Coates JD, Cole KA, Chakraborty R, O'Connor SM, Achenbach LA. (2002): Diversity and ubiquity of bacteria capable of utilizing humic substances as electron donors for anaerobic respiration. Applied and Environmental Microbiology. 68: 2445-2452.
- Coble PG. (1996): Characterization of marine and terrestrial DOM in seawater using excitationemission matrix spectroscopy. Marine Chemistry. 51: 325-346.
- Cooperband L. (2002): Building soil organic matter with organic amendments. Center for Integrated Agricultural Systems.
- Cusack D, Montagnini F. (2004): The role of native species plantations in recovery of understory woody diversity in degraded pasturelands of Costa Rica. Forest Ecology and Management. 188: 1-15.
- De Leeuw JW, Largeau C. (1993): A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal, and petroleum formation. In: M.H. E, S.A. M, editors. Organic geochemistry: Principles and applications. Plenum Press, , New York. pp 23-72.
- De Souza Sierra M, Donard O, Lamotte M, Belin C, Ewald M. (1994): Fluorescence spectroscopy of coastal and marine waters. Marine Chemistry. 47: 127-144.
- Debska B, Drag M, Banach-szott M. (2007): Molecular size distribution and hydrophilic and hydrophobic properties of humic acids isolated from forest soil. Soil and Water Research-UZPI. 2: 45-53.
- Deshmukh AP, Chen Y, Tarchitzky J, Chefetz B, Hatcher PG. (2002): Structural characterization of soil organic matter and humic acids in particle-size fractions of an agricultural soil. Soil Science Society of America Journal. 66: 129-141.
- Devine WD, Harrington CA, Station PNR. (2006): Effects of vegetation control and organic matter removal on soil water content in a young Douglas-fir plantation. US Dept. of Agriculture, Forest Service, Pacific Northwest Research Station. 28 pp.

- Doyle GL, Rice CW, Peterson DE, Steichen J. (2004): Biologically defined soil organic matter pools as affected by rotation and tillage. Environmental Management. 33: 528-538.
- Dria KJ. (2004): Carbon and nitrogen distribution and processes in forest and agricultural ecosystems: A study involving solid-and liquid-state NMR and pyrolysis GC/MS: The Ohio State University. 207 p.
- Ekblad A, Huss-Danell K. (1995): Nitrogen fixation by Alnus incana and nitrogen transfer from A. incana to Pinus sylvestris influenced by macronutrients and ectomycorrhiza. New Phytologist. 131: 453-459.
- Farenhorst A. (2006): Importance of soil organic matter fractions in soil-landscape and regional assessments of pesticide sorption and leaching in soil. Soil Science Society of America Journal. 70: 1005.
- Feller C, Beare M. (1997): Physical control of soil organic matter dynamics in the tropics. Geoderma. 79: 69-116.
- Flaig W. (1988): Generation of model chemical precursors. Humic substances and their role in the environment. 41: 75-92.
- Flaig W, Beutelspacher H, Rietz E. (1975): Chemical composition and physical properties of humic substances. In: Gieseking J E S-V, editor. Soil components, Organic Components. Springer-Verlag, New York. pp 1-211.
- Fong SS, Mohamed M. (2007): Chemical characterization of humic substances occurring in the peats of Sarawak, Malaysia. Organic geochemistry. 38: 967-976.
- Füleky G, Czinkota I. (1993): Hot water percolation (HWP): A new rapid soil extraction method. Plant and Soil. 157: 131-135.
- Grøn C, Wassenaar L, Krog M. (1996): Origin and structures of groundwater humic substances from three Danish aquifers. Environment international. 22: 519-534.
- Guariguata MR, Rheingans R, Montagnini F. (1995): Early woody invasion under tree plantations in Costa Rica: implications for forest restoration. Restoration Ecology. 3: 252-260.

- Harborne JB. (1997): Role of phenolic secondary metabolites in plants and their degradation in nature. In: Cadisch G, Giller K E, editors. Driven by nature: plant litter quality and decomposition. CAB International Wallingford, UK. pp 67-74.
- Harvey G, Boran D. (1985): Geochemistry of humic substances in seawater. Humic Substances in Soil, Sediment, and Water: Geochemistry, Isolation, and Characterization John Wiley and Sons, New York NY 1985 p 233-247, 9 fig, 3 tab.
- Hassan RM, Scholes R, Ash N. (2005): Ecosystems and Human Well-being: Current State and Trends
  : Findings of the Condition and Trends Working Group of the Millennium Ecosystem Assessment. Island Press, Washington. 921 pp.
- Hassink J. (1997): The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil. 191: 77-87.
- Hatcher P, Spiker E. (1988): Selective degradation of plant biomolecules. In: F.H. F, R.F. C, editors. Humic substances and their role in the environment. John Wiley,, New York. pp 59-74.
- Hedges J. (1988): Polymerization of humic substances in natural environments. In: F.H. F ,R.F. C, Hedges J, editors. Humic substances and their role in the environment John Wiley,, New York. pp 45-58.
- Helms JR, Stubbins A, Ritchie JD, Minor EC, Kieber DJ, Mopper K. (2008): Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnology and Oceanography. 53: 955-969.
- Hempfling R, Schulten HR. (1989): Selective preservation of biomolecules during humification of forest litter studied by pyrolysis-field ionization mass spectrometry. Science of the total environment. 81-82: 31-40.
- Her N, Amy G, Park HR, Song M. (2004): Characterizing algogenic organic matter (AOM) and evaluating associated NF membrane fouling. Water research. 38: 1427-1438.
- Her N, Amy G, Sohn J, Gunten U. (2008): UV absorbance ratio index with size exclusion chromatography (URI-SEC) as an NOM property indicator. Journal of Water Supply Research and Technology—AQUA. 57: 35-44.

- Hibbs DE, Cromack Jr K. (1990): Actinorhizal plants in Pacific Northwest forests. The Biology of Frankia and Actinorhizal Plants Eds CR Schwintzer and JD Tjepkema: 343-363.
- Hur J, Kim G. (2009): Comparison of the heterogeneity within bulk sediment humic substances from a stream and reservoir via selected operational descriptors. Chemosphere. 75: 483-490.
- Islam K, Weil R. (2000): Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. Agriculture, Ecosystems & Environment. 79: 9-16.
- Keeney D. (1966): Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron J 58: 498-503.
- Kiem R, Knicker H, Körschens M, Kögel-Knabner I. (2000): Refractory organic carbon in C-depleted arable soils, as studied by 13C NMR spectroscopy and carbohydrate analysis. Organic geochemistry. 31: 655-668.
- Klasnja B, Kopitovic S, Orlovic S. (2003): Variability of some wood properties of eastern cottonwood (Populus deltoides Bartr.) clones. Wood Science and Technology. 37: 331-337.
- Kogel-Knabner I. (1997): 13C and 15N NMR spectroscopy as a tool in soil organic matter studies. Geoderma. 80: 243-270.
- Kogel-Knabner I. (2000): Analytical approaches for characterizing soil organic matter. Organic geochemistry. 31: 609-626.
- Kögel-Knabner I. (1992): Forest soil organic matter: structure and formation. Lehrstuhl für Bodenkunde und der Universität Bayreuth Bodengeographie. 103 pp.
- Kolattukudy P, Espelie K. (1985): Biosynthesis of cutin, suberin, and associated waxes. In: Higuchi T, editor. Biosynthesis and biodegradation of wood components. Academic Press, London.
- Kononova MM, Nowakowski T, Newman A. (1961): Soil organic matter: Its nature, its role in soil formation and in soil fertility. Pergamon Press London, England. 450 pp.
- Kouki J, Löfman S, Martikainen P, Rouvinen S, Uotila A. (2001): Forest fragmentation in Fennoscandia: linking habitat requirements of wood-associated threatened species to landscape and habitat changes. Scandinavian Journal of Forest Research. 16: 27-37.

- Lapola DM, Schaldach R, Alcamo J, Bondeau A, Koch J, Koelking C, Priess JA. (2010): Indirect land-use changes can overcome carbon savings from biofuels in Brazil. Proceedings of the National Academy of Sciences. 107: 3388-3393.
- Lavrik N, Sagdiev A, Dergacheva M. (2004): Fluorescence and Electron Absorption Studies of the Structure of Humic Acids Extracted from the A Horizon of Soils. Chemistry. 12: 437-442.
- Li LH, Peng W, Sheng P, Guoying Fu J. (2003): Chemical and molecular heterogeneity of humic acids repetitively extracted from a peat. Soil Science Society of America Journal. 67: 740-746.
- Mackowiak CL, Grossl PR, Bugbee BG. (2001): Beneficial effects of humic acid on micronutrient availability to wheat. Soil Sci Soc Am J. 65: 1744-1750.
- Mahieu N, Powlson D, Randall E. (1999): Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. Soil Sci Soc Am J. 63: 307-319.
- Maillard L. (1913): Formation de matieres humiques par action de polypeptides sur sucres. CR Acad Sci. 156: 148-149.
- Makarov M, Haumaier L, Zech W. (2002): The nature and origins of diester phosphates in soils: a 31 P-NMR study. Biology and fertility of soils. 35: 136-146.
- Malcolm RL. (1989): Applications of solid-state 13C NMR spectroscopy to geochemical studies of humic substances. Humic substances II In search of structure. 2: 339-372.
- Mao JD, Xing B, Schmidt-Rohr K. (2001): New structural information on a humic acid from twodimensional 1H-13C correlation solid-state nuclear magnetic resonance. Environmental science & technology. 35: 1928-1934.
- McDonald S, Bishop AG, Prenzler PD, Robards K. (2004): Analytical chemistry of freshwater humic substances. Anal Chimi Acta. 527: 105-124.
- Monzón A, Azcón R. (2001): Growth responses and N and P use efficiency of three Alnus species as affected by arbuscular-mycorrhizal colonisation. Plant growth regulation. 35: 97-104.
- Müller MB, Schmitt D, Frimmel FH. (2000): Fractionation of natural organic matter by size exclusion chromatography-properties and stability of fractions. Environmental science & technology. 34: 4867-4872.

- Myneni SCB. (2002): Soft X-ray spectroscopy and spectromicroscopy studies of organic molecules in the environment. Reviews in mineralogy and geochemistry. 49: 485-579.
- Neill C, Melillo JM, Steudler PA, Cerri CC, de Moraes JFL, Piccolo MC, Brito M. (1997): Soil carbon and nitrogen stocks following forest clearing for pasture in the southwestern Brazilian Amazon. Ecological applications. 7: 1216-1225.
- Olk D, Cassman K, Fan T. (1995): Characterization of two humic acid fractions from a calcareous vermiculitic soil: implications for the humification process. Geoderma. 65: 195-208.
- Önemli F. (2004): The effects of soil organic matter on seedling emergence in sunflower (Helianthus annuus L.). Plant, Soil and Environment-UZPI. 50: 494-499.
- Orlov DS. (1995): Humic substances of soils and general theory of humification. Taylor & Francis. 266 pp.
- Parrotta JA, Knowles OH. (1999): Restoration of Tropical Moist Forests on Bauxite-Mined Lands in the Brazilian Amazon. Restoration Ecology. 7: 103-116.
- Parrotta JA, Turnbull JW, Jones N. (1997): Catalyzing native forest regeneration on degraded tropical lands. Forest Ecology and Management. 99: 1-7.
- Paul E, Van Veen J. (1978): The use of tracers to determine the dynamic nature of organic matter. p1-43.
- Pelekani C, Newcombe G, Snoeyink VL, Hepplewhite C, Assemi S, Beckett R. (1999): Characterization of natural organic matter using high performance size exclusion chromatography. Environmental science & technology. 33: 2807-2813.
- Peña-Méndez EM, Havel J, Patočka J. (2005): Humic substances-compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. J Appl Biomed. 3: 13-24.
- Perminova IV, Frimmel FH, Kovalevskii DV, Abbt-Braun G, Kudryavtsev AV, Hesse S. (1998): Development of a predictive model for calculation of molecular weight of humic substances. Water research. 32: 872-881.
- Peuravuori J, Pihlaja K. (1997): Molecular size distribution and spectroscopic properties of aquatic humic substances. Analytica Chimica Acta. 337: 133-149.

- Piccolo A, Mbagwu JSC. (1999): Role of hydrophobic components of soil organic matter in soil aggregate stability. Soil Sci Soc Am J. 63: 1801-1810.
- Preston CM. (1996): Applications of NMR to soil organic matter analysis: history and prospects. Soil Science. 161: 143-200.
- Preston CM. (2001): Carbon-13 solid-state NMR of soil organic matter-using the technique effectively. Canadian Journal of Soil Science. 81: 255-270.
- Provin TL, Wright AL, Hons FM, Zuberer DA, White RH. (2008): Seasonal dynamics of soil micronutrients in compost-amended bermudagrass turf. Bioresource technology. 99: 2672-2679.
- Reeves D. (1997): The role of soil organic matter in maintaining soil quality in continuous cropping systems. Soil and Tillage Research. 43: 131-167.
- Ritchie G, Dolling P. (1985): The role of organic matter in soil acidification. Soil Research. 23: 569-576.
- Roggy J, Moiroud A, Lensi R, Domenach A. (2004): Estimating N transfers between N 2-fixing actinorhizal species and the non-N 2-fixing Prunus avium under partially controlled conditions. Biology and fertility of soils. 39: 312-319.
- Schmitt P, Garrison A, Freitag D, Kettrup A. (1997): Capillary isoelectric focusing (CIEF) for the characterization of humic substances. Water research. 31: 2037-2049.
- Schnitzer M. (1977): Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones. Soil Organic Matter Studies. 2: 117-132.

Schnitzer M. (1991): Soil organic matter-the next 75 years. Soil Science. 151: 41-58.

Schnitzer M, Khan SU. (1972): Humic substances in the environment. M. Dekker New York. 327 pp.

- Schnitzer M, Khan SU. (1978): Soil Organic Matter. Elsevier Science Ltd, New York. 319 pp.
- Schulten HR, Plage B, Schnitzer M. (1991): A chemical structure for humic substances. Naturwissenschaften. 78: 311-312.
- Schwencke J, Carú M. (2001): Advances in actinorhizal symbiosis: host plant-Frankia interactions, biology, and applications in arid land reclamation. A review. Arid Land Research and Management. 15: 285-327.

- Senesi N. (1990): Molecular and quantitative aspects of the chemistry of fulvic acid and its interactions with metal ions and organic chemicals:: Part II. The fluorescence spectroscopy approach. Analytica Chimica Acta. 232: 77-106.
- Senesi N, Miano T, Provenzano M. (1991): Fluorescence spectroscopy as a means of distinguishing fulvic and humic acids from dissolved and sedimentary aquatic sources and terrestrial sources. Humic Substances in the Aquatic and Terrestrial Environment. 31: 63-73.
- Shin H, Rhee S, Lee B, Moon C. (1996): Metal binding sites and partial structures of soil fulvic and humic acids compared: aided by Eu (III) luminescence spectroscopy and DEPT/QUAT 13C NMR pulse techniques. Organic geochemistry. 24: 523-529.
- Shon HK, Kim SH, Erdei L, Vigneswaran S. (2006): Analytical methods of size distribution for organic matter in water and wastewater. Korean Journal of Chemical Engineering. 23: 581-591.
- Siuda W, Chrost R. (2002): Decomposition and utilization of particulate organic matter by bacteria in lakes of different trophic status. Polish Journal of Environmental Studies. 11: 53-66.
- Smith D, Lorimer J. (1964): An examination of the humic acids of Sphagnum peat. Canadian Journal of Soil Science. 44: 76-87.
- Solomons TWG. (1992): Solomons Organic Chemistry. John Wiley and Sons New York. 1272 pp.
- Stevenson FJ. (1994): Humus chemistry: genesis, composition, reactions. John Wiley & Sons Inc. 497 pp.
- Surampalli RY. (2004): Advances in Water and Wastewater Treatment. American Society of Civil Engineers, Reston, Virginia. 585 pp.
- Sylvia D, Williams S. (1992): Vesicular-arbuscular mycorrhizae and environmental stress. ASA special publication: 101-124.
- Takács M, Füleky G. (2010): Characterization of dissolved organic matter (DOM) extracted from soils by hot water percolation (HWP). Agrokémia és Talajtan. 59: 99-108.
- Taleshi S, Dhumal K, Alipour A, Espahbodi K, Ghasemi O. (2009): Impact of Alder(Alnus subcordata) in Fertility of Forest Soil. Research Journal of Environmental Sciences. 3: 640-644.

Tan KH. (2000): Environmental soil science. M. Dekker. 452 pp.

- Tegelaar E, De Leeuw J, Derenne S, Largeau C. (1989a): A reappraisal of kerogen formation. Geochimica et Cosmochimica Acta. 53: 3103-3106.
- Tegelaar E, De Leeuw J, Saiz-Jimenez C. (1989b): Possible origin of aliphatic moieties in humic substances. Science of the total environment. 81: 1-17.
- Tegelaar E, Hollman G, Van der Vegt P, De Leeuw J, Holloway P. (1995): Chemical characterization of the periderm tissue of some angiosperm species: recognition of an insoluble, non-hydrolyzable, aliphatic biomacromolecule (Suberan). Organic geochemistry. 23: 239-251.
- Thurman EM. (1985): Organic geochemistry of natural waters. Science & BusinessSpringer. 497 pp.
- Tiessen H, Cuevas E, Chacon P. (1994): The role of soil organic matter in sustaining soil fertility. Nature. 371: 783 -785.
- Tranvik LJ. (1992): Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. Hydrobiologia. 229: 107-114.
- Wang Q, Wang S, Fan B, Yu X. (2007): Litter production, leaf litter decomposition and nutrient return in Cunninghamia lanceolata plantations in south China: effect of planting conifers with broadleaved species. Plant and Soil. 297: 201-211.
- Wattel-Koekkoek E, Van Genuchten P, Buurman P, Van Lagen B. (2001): Amount and composition of clay-associated soil organic matter in a range of kaolinitic and smectitic soils. Geoderma. 99: 27-49.
- Wauchope RD, Yeh S, Linders JBHJ, Kloskowski R, Tanaka K, Rubin B, Katayama A, Kördel W, Gerstl Z, Lane M. (2002): Pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. Pest management science. 58: 419-445.
- Wheeler C, Miller I. (1990): Current and potential uses of actinorhizal plants in Europe. The Biology of Frankia and Actinorhizal Plants Eds CR Schwintzer and JD Tjepkema. 1 ed. Academic Pr. pp 365-389.
- Willis KG, Garrod G, Scarpa R, Powe N, Lovett A, Bateman IJ, Hanley N, Macmillan DC. (2003):The social and environmental benefits of forests in Great Britain. UK: Centre for Research inEnvironmental Appraisal & Management University of Newcastle. p 36.

- Wilson MA. (1987): NMR techniques and applications in geochemistry and soil chemistry. Pergamon Press Oxford. 367 pp.
- Wilson MA, Hatcher PG. (1988): Detection of tannins in modern and fossil barks and in plant residues by high-resolution solid-state 13C nuclear magnetic resonance. Organic geochemistry. 12: 539-546.
- Wright RF. (1989): Rain project: role of organic acids in moderating pH change following reduction in acid deposition. Water, Air, & Soil Pollution. 46: 251-259.
- Yang K, Xing B. (2009): Adsorption of fulvic acid by carbon nanotubes from water. Environmental Pollution. 157: 1095-1100.
- Yates LM, von Wandruszka R. (1999): Effects of pH and metals on the surface tension of aqueous humic materials. Soil Science Society of America Journal. 63: 1645-1649.
- Zavarzina A, Vanifatova N, Stepanov A. (2008): Fractionation of humic acids according to their hydrophobicity, size, and charge-dependent mobility by the salting-out method. Eurasian Soil Science. 41: 1294-1301.
- Zech W, Senesi N, Guggenberger G, Kaiser K, Lehmann J, Miano TM, Miltner A, Schroth G. (1997): Factors controlling humification and mineralization of soil organic matter in the tropics. Geoderma. 79: 117-161.
- Zech W, Ziegler F, Kögel-Knabner I, Haumaier L. (1992): Humic substances distribution and transformation in forest soils. Science of the total environment. 117-118: 155-174.
- Ziegler F, Zech W. (1989): Distribution pattern of total lipids and lipid fractions in forest humus. Zeitschrift für Pflanzenernährung und Bodenkunde. 152: 287-290.

## APPENDIX

			-	or game		-			-		4.0
*	Fractions	l	2	3	4	5	6	7	8	9	10
а	ΣDOC	31.74	38.30	43.41	48.34	50.52	51.80	54.72	56.0	57.09	57.27
	Time (min)	0.77	1.77	3.03	4.47	6.17	7.83	9.53	11.3	13.1	15.58
1	ΣDOC	27.39	34.19	39.15	41.17	42.82	43.93	45.95	46.13	47.05	47.24
b	Time (min)	0.65	1.57	2.78	4.18	5.75	7.38	9.25	11.07	13.03	15.33
0	ΣDOC	54.77	65.06	73.70	81.42	85.47	88.59	90.25	91.72	92.63	92.82
C	Time (min)	0.72	1.75	3	4.45	5.95	7.62	9.38	11.22	13.17	15.17
d	ΣDOC	37.25	48.42	56.2	62.46	67.45	71.33	75.03	77.62	80.02	82.79
u	Time (min)	0.38	0.95	1.62	2.4	3.33	4.28	5.42	6.38	7.52	8.68
e	ΣDOC	21.57	29.13	34.48	37.43	40.01	42.23	44.62	46.84	49.23	51.08
C	Time (min)	0.83	1.95	3.42	4.9	6.42	8.03	9.8	11.45	13.22	15.15
f	ΣDOC	29.62	36.58	42.94	48.90	54.27	57.85	61.03	64.01	67.19	69.98
1	Time (min)	0.9	2.17	3.68	5.3	7.15	9.02	10.88	12.77	14.62	16.65
σ	ΣDOC	39.83	41.81	43.19	44.57	45.16	45.75	46.14	46.34	46.54	46.74
D	Time (s)	6	16	29	41	57	73	89	104	119	137
h	ΣDOC	12.03	14.4	14.99	14.99	14.99	14.99	14.99	14.99	14.99	14.99
	Time (s)	9	21	35	66	108	-	-	-	-	-
i	ΣDOC)	20.33	24.48	27.83	30.99	33.76	36.32	37.90	38.89	39.87	42.24
1	Time (min)	1.15	2.72	4.5	6.38	8.45	10.67	12.88	15.23	17.57	19.85
i	ΣDOC)	50.24	61.52	69.82	72.79	75.16	76.94	78.72	80.31	81.1	82.28
J	Time (min)	1.32	3	5.15	7.48	9.63	11.98	14.37	16.77	19.47	22
k	ΣDOC	34.31	46.57	52.26	56.4	59.37	61.53	64.68	67.05	69.81	71.58
ĸ	Time (min)	0.65	1.52	2.6	3.85	5.22	6.62	7.98	9.38	10.83	12.32
1	ΣDOC)	35.08	45.59	52.92	59.26	64.22	68.18	70.96	72.74	75.32	78.88
-	Time (min)	1.43	3.12	5.17	7.57	10.28	12.95	15.52	18.05	20.5	23.22
m	ΣDOC	49.2	60.61	67.9	72.82	77.15	80.29	82.85	85.41	87.38	88.76
111	Time (min)	0.65	1.5	2.28	3.42	4.65	6	7.23	8.5	9.72	11.03
n	ΣDOC	40.34	50.76	57.39	61.93	64.77	65.53	66.86	68.37	70.65	72.16
	Time (min)	0.9	2.22	3.42	5	6.7	8.5	10.07	11.68	13.53	15.38
0	ΣDOC	33.89	43.42	49.11	53.41	56.32	59.04	62.12	64.37	66.55	66.55
	Time (min)	0.85	2.306	3.989	6.244	8.6	11.139	13.411	16.211	18.883	-
n	ΣDOC	58.76	76.03	89.05	99.38	107.63	114.56	121.37	127.45	132.62	132.62
Р	Time (min)	0.911	2.372	3.956	6.311	8.883	11.633	14.394	16.811	19.728	-

ΣDOC :sum of dissolved organic carbon (mg/100g), \* Samples including: a) Magyaróvár, b) Nagyszentjános, c) Homokszentgyörgy, d) Mezőhegyes, e) Új-Szeged, f) Szeged-Öthalom, g) Nyíregyháza, h) Kecskemét, i) Magyoregregy, j) Szilvásvárad, k) Etes, l) Szarvas, m) Gagyvendégi, n) Putnok, o) Nagykanizsa, p) Kompolt.

Samples	210 nm	254 nm	365 nm	465 nm	665 nm
Magyaróvár	3.00	1.61	0.374	0.054	0.006
Szarvas	3.00	1.40	0.305	0.032	0.004
Új-Szeged	3.00	0.91	0.191	0.029	0.004
Homokszentgyörgy	3.00	2.65	0.649	0.086	0.010
Gagyvendégi	3.00	2.52	0.539	0.086	0.029
Nagykanizsa	3.00	1.30	0.304	0.039	0.009
Nyíregyháza	3.00	2.22	0.677	0.354	0.165
Magyaregregy	1.63	0.60	0.126	0.016	0.002
Szilvásvárad	3.00	2.57	0.564	0.073	0.006
Etes	3.00	1.40	0.290	0.054	0.004
Putnok	2.73	2.20	0.470	0.033	0.012
Mezőhegyes	3.00	1.92	0.431	0.05	0.008
Kompolt	3.00	2.76	0.675	0.083	0.030
Nagyszentjános	3.00	1.70	0.421	0.058	0.020
Szeged-Öthalom	3.00	1.50	0.355	0.047	0.008
Kecskemét	1.62	0.49	0.123	0.026	0.007

Table 2: UV VIS absorbance of HWP extracts.

Forest type*	Alnus:Populus%	TOC (%)	N (%)	C:N
VF		<sup>1</sup> a 3.28	a 0.127	b 26.18
	100:0	b 2.71	ab 0.1	b 27.43
	70:30	b 2.53	bc 0.073	b 30.64
PF	50:50	c 2.24	c 0.058	ab 39.08
	30:70	c 2.23	c 0.052	a 50.39
	0:100	d 1.92	c 0.054	ab 35.95
LSD		0.3522	0.0222	55.553

Table 3: The differences of TOC, N and C:N ratio in different samples.

1: Values followed by the same letter are not significantly different at P < 0.05.

\*: VF: virgin forest, PF: planted forest.

Fraction	TOC (%)	N (%)	C:N
Sand	<sup>1</sup> b	b	а
Saliu	2.71	0.026	140.81
S:1+	с	b	b
SIII	20.95	0.021	53.98
Clay	a	a	b
Clay	4.46	0.22	20.89
LSD	0.249	0.0157	39.282

Table 4: The differences of TOC, N and C:N ratio in the fractions.

1: Values followed by the same letter are not significantly different at P < 0.05.


Figure 2: Diagrams of DOC kinetics of different samples a) Nagyszentjános, b) Homokszentgyörgy, c) Mezőhegyes, d) Új-Szeged, e) Szeged-Öthalom, f) Nyíregyháza, g) Kecskemét, h) Magyoregregy, i) Szilvásvárad, j) Etes, k) Szarvas, l) Gagyvendégi, m) Putnok, n) Nagykanizsa, o) Kompolt.



Figure 1: The correlation between HWP dissolved organic carbon (HWPDOC) and absorbance value at 254 nm in different fractions (×: first fraction, +: second fraction and•: the last fraction) of different samples a) Magyaróvár, b) Nagyszentjános, c) Mezőhegyes, d) Új-Szeged, e) Szeged-Öthalom, f) Nyíregyháza, g) Kecskemét, h) Magyoregregy, i) Szilvásvárad, j) Etes, k) Szarvas, l) Gagyvendégi, m) Putnok, n) Nagykanizsa, o) Kompolt.