Indian J. Hort. 71(3), September 2014: 360-366

Chemical and structural characterization of soil humic substances under different land use systems in sub-tropical regions of northeast India

T. Ramesh*, K.M. Manjaiah, S.V. Ngachan* and K. Rajasekar*****
Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

Four different tree or crop species (Champak, tree bean, alder and *Khasi* pine in agroforestry land use; maize, potato, rice and turmeric in agriculture land use; pear, peach, *Khasi* mandarin and guava in horticulture land use) were selected and compared with control plots. Soil humic acids (HAs) were extracted from 0-15 cm layer and characterised by Fourier Transform Infrared Spectroscopy (FTIR), elemental composition, total acidity and functional groups analyses. The HAs showed an increase in the carbon and nitrogen contents, followed by a reduction in C/N ratios in agroforestry and horticulture land use systems over agriculture and fallow lands. Generally, C and N contents of HAs were decreasing in the order: agroforestry > horticulture > agriculture > fallow lands. The relatively low E4/E6 values (4.86) of HAs in the agroforestry land use indicated the prominence of aromatic components, suggesting that the HAs were of high molecular weight. The relatively high E4/E6 ratios (5.63) of HAs under agriculture land use indicated the dominance of aliphatic components, suggesting that HAs were of low in molecular weight. The total acidity, carboxylic (-COOH) and phenolic (-OH) groups of HAs were found to be significantly higher under agroforestry land use followed by horticulture and agriculture land uses. Conversely, adoption of various agroforestry tree species, horticulture fruit trees and agriculture crop species increased the C and N contents, total acidity, carboxylic OH and phenolic OH groups of HAs compared to fallow lands.

Key words: Elemental composition, E4/E6 ratio, functional groups, humic acids, land use systems.

INTRODUCTION

North-eastern region of India is characterised by diverse agro-climatic and geographical situations. About 54.1% of the total geographical area is under forests, 16.6% under crops, and the rest either under non-agricultural uses or uncultivated land. The low area under agricultural crops is due to natural corollary of the physiographic features of the region, as major chunk of the land has more than 15% slope, undulating topography, highly eroded and degraded soils, and inaccessible terrains. Intensive forestry or agricultural uses without appropriate soil and water conservation measures in northeast Indian soils have often led to severe changes in soil organic matter (SOM) turnover. This depends on climatic constraints and the impact of local management practices ranging from extensive commercial farming to low-input traditional agriculture. Most soils in northeast India are acidic and weathered with a productivity closely linked to SOM levels. Clearance of natural vegetation by 'shifting cultivation' for arable agriculture modifies the amount and quality of SOM, whereas soils temperature/moisture regimes and biological processes affect litter decomposition and SOM dynamics (Moorhead *et al*., 10).

Studies evaluating the effect of land use and management practices on SOC quality are need to be focussed on the investigation of chemical and structural characterization of humic substances, which represent around 90% of total organic carbon, constituting the major organic reserve in the soil (Stevenson, 17). However, in northeast India there are no such conclusive studies so far showing the occurrence of both qualitative and quantitative changes due to land use changes. The best method for measuring the degree of humification is still being debated because there is no well-defined model of humic substances (HS) structure (Piccola, 13). Several techniques have been used to characterize the progress of humification, including measurement of the E4/E6 ratio; elemental composition; functional groups analysis and C/H, C/O, and C/N ratios (Stevenson, 17).

Due to the constant input of organic materials from leaf litter and root biomass, it was assumed that agroforestry, agriculture and horticulture systems induce the chemical and structural changes in HS in comparison to the fallow lands, which in turn result in to less humified humic substances. Nevertheless, there is no information available on the chemical composition

*Division of Natural Resource Management, ICAR Research Complex for NEH Region, Umiam, Meghalaya 793103
**Corresponding author's E-mail: manjaiah@iari.res.in
***ICAR Research Complex for NEH Region, Mizoram centre, Mizoram 793103

and spectral characteristics of the SOM under different land use systems in north-east India. Therefore, the study was conducted to characterize the humic acids under agroforestry, agriculture and horticulture land use systems in the sub-tropical hilly ecosystems of northeast India.

MATERIALS AND METHODS

The experimental sites with different agroforestry tree species planted under agri-silvi-pastoral system in 1983, horticulture tree species planted in 1994 and agricultural crops continuously cultivated for the past 10 years is located at Indian Council of Agricultural Research (ICAR) Complex for North-East Hill (NEH) Region, Umiam (Table 1). The climate of the study area is seasonal with distinct warm-wet and cold-dry seasons in a year. During the study period, the mean annual rainfall was about 2208.5 mm, of which more than 90% is received during May to October. While, the mean minimum and maximum temperature were 6.8^o and 29.7^{o}C, respectively the mean relative humidity varied from 40-88% during the study period. The soil of the area is a *Typic Hapludalf* and is highly acidic (pH: 4.36 to 6.24) with adequate levels of organic matter (Table 2). Surface soil samples were collected during October-November, 2009 by using a eight cm bulk density corer at five places in different blocks under all the treatments in each land use system including fallow lands. The soil samples were brought to the laboratory, air-dried at room temperature, ground to pass through 2 mm sieve and used for further analysis. The physico-chemical and biological parameters of the soils were determined by following the standard procedures.

The humic substances were extracted from soil (Swift, 18), purified by successive washing with a solution of 10% HF + 0.5% HCl to eliminate the influence of paramagnetic ions, freeze-dried and used for estimation of C and N contents, functional groups and E4/E6 ratio (Schnitzer, 14; Stevenson, 17). The FTIR spectra of HA samples were recorded on a Win-IRrez (Bio-Rad Hercules CA USA) using the potassium bromide (KBr) disc technique. The data on chemical composition of HAs were statistically analysed by one-way analysis of variance (ANOVA) technique using software SPSS for Windows (SPSS Inc. USA). The significance of the difference between means for different land use systems was evaluated at 95% confident interval using Tukey's Honestly Significant Difference (HSD) post hoc test. Correlation and regression tests were carried out to test the functional relationships among the soil variables and HAs characteristics.

RESULTS AND DISCUSSION

All the crops and tree species had significant effects on carboxylic- and phenolic- OH groups, whereas only agriculture crops showed significant influence on the concentration of total acidity (Table 3). In general, total acidity, carboxylic- and phenolic- OH groups followed the order: agroforestry > horticulture > agriculture, whereas E4/E6 ratio showed the opposite trend (Table 3). Overall, agroforestry tree species showed higher total acidity, carboxylic OH and phenolic OH concentration in HAs by 48, 74 and 19%, respectively compared to agriculture crops, the values being within the range reported by previous studies (Ch'ng *et al*., 4; Moraes *et al*., 11; Seddaiu *et al*., 15). The carboxylic OH groups contributed 62, 52 and 56% of total acidity in agroforestry, agriculture and horticulture land use systems, respectively. The mean E4/E6 ratio of HAs from agriculture land use was 14% higher than that of agroforestry land use, so confirming that HAs under agroforestry are associated with a relatively large molecular size or high molecular weight (*i.e.* a large molecule) with high degree of aromatic condensation. This molecule has high C content, but relatively low in oxygen (not

Table 1. Land use systems selected for the present study in Northeast India.

Main land use	Agriculture	Horticulture	Agroforestry
Age (years)	10	15	26
Sub-land use	-Maize (*Zea mays*)	-Pear (*Pyrus communis*)	-*Champak* (*Michelia oblonga*)
	-Potato (*Solanum tuberosum*)	-Peach (*Prunus persica*)	-Tree bean (*Parkia roxburghii*)
	-Rice (*Oryza sativa*)	-*Khasi* mandarin (*Citrus reticulata*)	-Alder (*Alnus nepalensis*)
	-Turmeric (*Curcuma longa*)	-Guava (*Psidium guajava*)	-*Khasi* pine (*Pinus kesiya*)
	-Control (No crop)	-Control (No tree)	-Control (No tree)

Table 2. Initial soil properties of different land uses selected for the study in Northeast India.

Land use system	Soil properties							
	pH	OC (g 100g^{-1})	*MBC (mg kg^{-1})	**MWD (mm)	Moisture content (g 100g^{-1})	N	P	K
						(kg ha^{-1})		
				Agroforestry				
Champak	4.60	2.45	455.3	2.25	31.0	522.3	32.1	313.6
Tree bean	4.59	2.32	408.7	1.98	26.1	496.3	30.1	286.7
Alder	4.38	3.12	548.0	2.36	31.8	584.3	47.2	361.2
Khasi pine	4.36	2.27	378.0	2.44	33.8	464.8	23.7	276.0
Control (No tree)	4.76	1.91	335.3	1.37	28.4	403.4	19.3	248.4
				Agriculture				
Maize	5.12	1.62	262.7	1.50	29.9	394.4	49.1	292.7
Potato	5.24	1.72	301.3	1.57	28.9	414.6	65.6	365.1
Rice	5.09	1.50	274.7	1.48	32.2	421.4	23.5	322.2
Turmeric	5.29	1.71	308.7	1.69	26.7	398.9	36.9	306.1
Control (No crop)	4.81	1.37	217.3	1.02	24.8	387.7	20.7	203.1
				Horticulture				
Pear	5.71	1.71	361.0	1.77	31.6	437.1	43.2	323.6
Peach	6.24	1.81	339.0	1.85	32.7	455.1	39.1	495.2
Khasi mandarin	5.75	1.89	401.3	1.97	28.7	428.1	45.3	419.6
Guava	5.43	1.78	319.3	1.70	29.9	446.1	24.6	343.5
Control (No tree)	4.82	1.61	282.3	1.15	27.1	434.9	26.9	328.5

*MBC = Microbial biomass carbon; **MWD = Mean weight diameter

measured), COOH groups, and total acidity (Hanmin *et al.*, 7; Campitelli *et al.*, 3).

The C and the C/N ratios of HA were within the ranges reported for other soils (Stevenson, 17) and did not substantially change from agroforestry to cultivated soils however, the HA-N content substantially differed within and between the land uses. The mean HA-C content was 62.7% in agroforestry, 57.2% in agriculture and 60.7% in horticulture land uses. HA-N content also followed the similar pattern of HA-C in all the land uses. However, the HA-C and -N contents in agroforestry land use were increased by 10 and 34%, correspondingly over agriculture land use as given in Table 3. With cultivation, both C and N contents of HAs in agriculture lands decreased due to oxidative degradation of C and N compounds upon cultivation. In contrary to HA-C and -N contents, the mean C/N ratio was highest under agriculture (13.86) compared to horticulture (12.39) and agroforestry (11.44) land uses (Table 3) indicating a higher degree of humification (Guggenberger *et al.*, 6) and/or a greater microbial contribution for agroforestry land use in relation to agriculture land use. The general constancy of C/N values for the different humic fractions suggests a resistance to microbial decomposition attributable to their recalcitrant nature deriving from either physical protection in micro-aggregates (Dutarte *et al.*, 5) or chemical protection by their hydrophobic composition (Spaccini *et al.*, 16).

The Fourier Transform Infra-Red (FTIR) spectra of the representative HAs extracted from soils under all land use systems are illustrated in the Figure 1 a, b & c for agroforestry, agriculture and horticulture land uses, respectively. The FTIR spectra of HAs presented a number of common absorption bands with smaller differences in their relative intensity. The relatively less important bands of FTIR at 3,400 cm^{-1} which is associated to OH-stretch of OH groups, and the bands at 2,933 cm^{-1} associated to aliphatic C-H stretching could not be detected in none of the HAs extracted from each land use systems however, they were visible with less intensity in agroforestry and horticulture land uses. All the HAs extracted from each land uses, on the other hand, showed many bands which are characteristics to the original humic acid substances.

The different characteristics observed in the area below 1,800 cm^{-1} of the spectra of FTIR were

Table 3. Properties of soil humic acids extracted from different land uses in Northeast India.

Land use system	Total acidity	Carboxylic OH	Phenolic OH	C	N	C/N ratio	E4/E6 ratio
	(meq g^{-1} Humic acid)			(g $100g^{-1}$ Humic acid)			
			Agroforestry				
Champak	10.0a	6.30a	3.70bc	63.9a	5.94a	10.76ab	5.22a
Tree bean	9.50a	6.20ab	3.30cd	61.6a	5.43b	11.34ab	5.13a
Alder	9.30a	5.20b	4.10ab	62.3a	6.32a	9.86b	4.76b
Khasi pine	11.0a	6.80a	4.20a	64.0a	5.10bc	12.55a	4.80b
Control (No tree)	9.01a	5.80ab	3.20d	61.9a	4.87c	12.71a	4.41c
Mean	9.77	6.06	3.70	62.7	5.53	11.44	4.86
			Agriculture				
Maize	6.80b	4.00b	2.80c	57.3a	4.51a	12.71a	5.02c
Potato	5.20c	2.00d	3.20b	56.0a	4.11bc	13.63a	6.07ab
Rice	6.60bc	3.40bc	3.20b	55.5a	3.93cd	14.12a	6.37a
Turmeric	8.40a	4.80a	3.60a	59.7a	4.27b	13.98a	4.89c
Control (No crop)	6.00bc	3.20c	2.80c	56.8a	3.82d	14.87a	5.81b
Mean	6.60	3.48	3.12	57.2	4.13	13.86	5.63
			Horticulture				
Pear	9.40a	6.20a	3.20b	60.8a	4.94ab	12.31a	5.52a
Peach	8.00ab	4.80b	3.00b	61.4a	5.48a	11.20a	5.31a
Khasi mandarin	7.40b	3.80c	3.60b	58.3a	4.76b	12.25a	5.26a
Guava	7.80b	3.20c	4.60a	63.0a	5.04ab	12.50a	4.88ab
Control (No tree)	8.30ab	5.10b	3.20b	59.9a	4.38c	13.68a	4.43b
Mean	8.18	4.62	3.52	60.7	4.92	12.39	5.08

concentrated in the 1,720 to 1,705 cm^{-1} region, attributed to the stretching of C=O of carboxylic acids, aldehydes and ketones, whose relative intensities are strong for the HAs extracted from soils under agroforestry and horticulture land uses. The bands in this range of the infrared spectrum have several assignments including aromatic C=C stretching, amide group ($RCONH_2$) C=O stretching and amide -NH bending. Absorption band at 1,720 cm^{-1} wave number appeared to be more intense in agroforestry and horticulture land uses, and shifted towards lower number in agriculture land use (Fig. 1c). Similarly, the bands in the region of 1,650 to 1,400 cm^{-1} were more pronounced in both land uses compared to agriculture land use. Absorption bands between 1,661 and 1,628 cm^{-1} were attributed to the stretching C=O of groups amide (band I of the amide), and also C=O of quinones and H tied to conjugated ketones, which are also mostly seen in all the land uses; a fourth peak between 1,614 and 1,612 cm^{-1} was generally attributed to the stretching of aromatic C=C and symmetrical stretching of the anion COO^-, that are relatively intense in agroforestry land use. Carbon-carbon stretching usually appears at about 1,650 cm^{-1} for doubled bonds, but is shifted to about 1,600 cm^{-1} by conjugation. Substituted or unsubstituted amides, on the other hand, showed the C=O band in the 1,640-1,690 cm^{-1} region and the -NH bending at 1,600-1,640 cm^{-1} (Marinari *et al*., 9). All these bands are produced by aromatic C=C, C=O in amide (I), ketone and quinone groups of the HAs (Amir *et al*., 2). Absorption bands in 1,540-1,550 cm^{-1} were attributed to the N-H deformation of the amide (band II of the amide) and stretching of C=N just as observed in the spectra of the HAs of agroforestry land use; a band in the region from 1,354 to 1,327 cm^{-1} was attributed to the symmetric deformation of CH_3. The band at 1,220 cm^{-1} became more intense in agroforestry land use than those of horticulture and agriculture land uses. The strong absorption in 1,015-1,037 cm^{-1} represents C-O of alcohol and polysaccharide (Adekunle *et al*., 1); absorption in the region from 762 to 669 cm^{-1} could be attributed to the angular deformation out of the plan of connections C-H of aromatic rings. In general, the FTIR spectra showed some characteristics and strong absorption bands in the regions from 1,700-1,725,

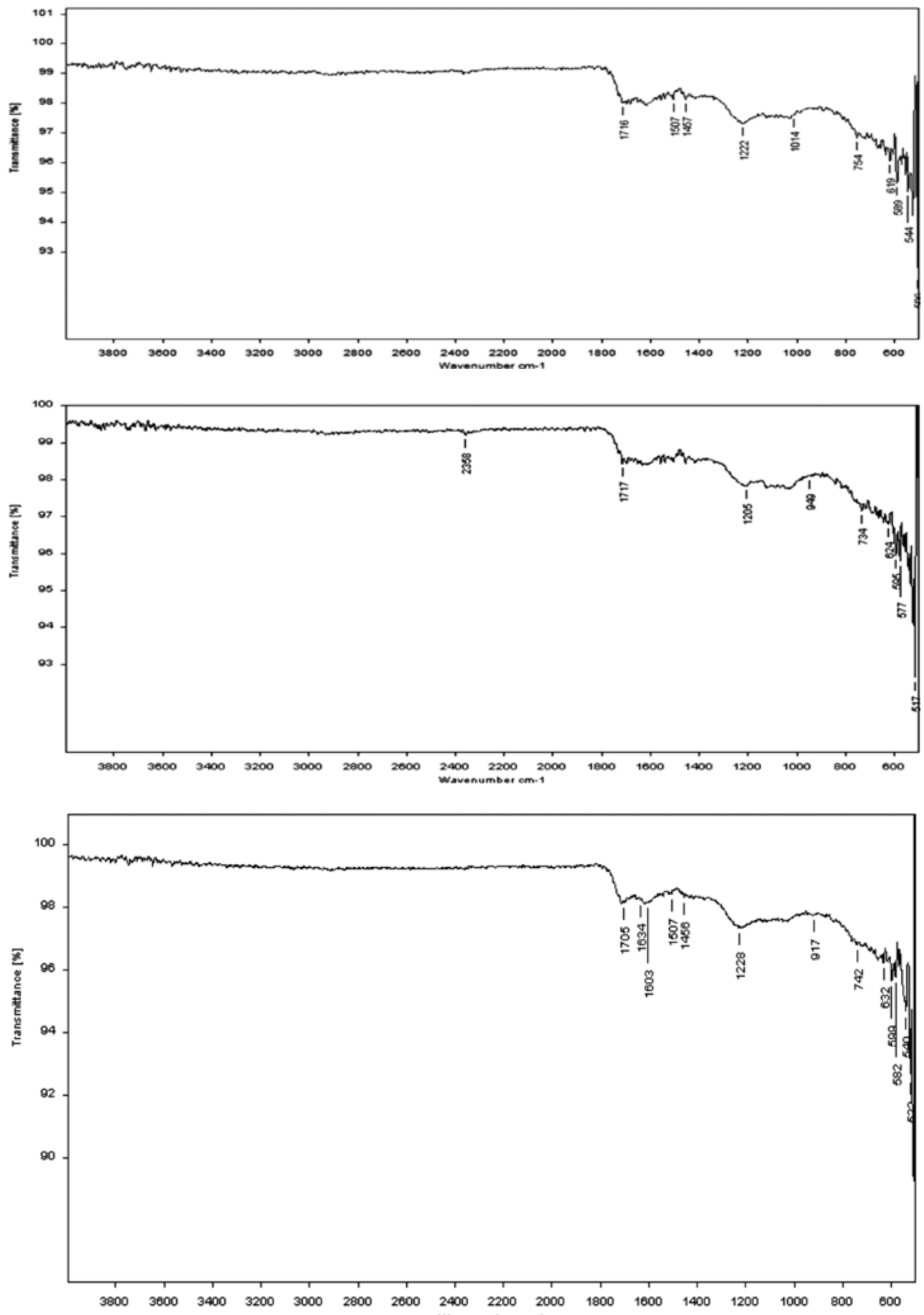

Fig. 1. FTIR spectra of humic acids extracted from the soils under (A) agroforestry, (B) agriculture and (C) horticulture land use systems in northeast India.

1,457-1,640, 1,215-1,225, 1,014-1,030, 719-754 and 517-533 cm^{-1} wave number and suggest a mixture of aromatic/aliphatic characteristics, a great amount of carboxylic groups and more numbers of N-containing groups for HAs of the studied land use systems.

The extracted HA-C and -N contents were strongly correlated with SOC and available N ($P<0.01$). Similarly, total acidity, phenolic OH and carboxylic OH groups had positive and significant relationships with SOC contents, whereas with available N, only carboxylic OH content showed the significant correlation (r = 0.393**). This shows that the quality of SOM or humification degree was primarily driven by the SOC content (Szajdak *et al.*, 19). It also indicates that quantities of all SOM pools are heavily influenced by the net balance between C inputs to the systems and the rate of the C decomposition of the added C inputs. Soil OC and MBC explained about 20% of the variation of HA-C content, whereas these variables along with available N showed 66.7% variation on HA-N content (data not shown). All the HAs properties, except E4/E6 ratio, were significantly related with soil MBC. There was a strong and negative correlation between soil pH, and total acidity (P<0.01) and phenolic OH (P<0.05). All the soil properties except available N explained 38.8% variation on total acidity. Amongst the SOM quality variables, E4/E6 ratio was strongly correlated with all the quality parameters except carboxylic OH groups. Increase in total acidity, carboxylic- and phenolic-OH groups of HAs, as evident from the Table 3, resulted in reduced E4/E6 ratios, and had strong and inverse relationships with these acidic groups. The HA-C and -N contents had strong relationships with total acidity, carboxylic and phenolic-OH groups (P<0.01), whereas C/N ratio showed strong correlation with HA-N content, and to a lesser degree with total acidity. The stepwise regression model showed greatest variations in N content of HAs included SOC, total acidity and C/N ratio of HAs. Soil OC accounted 63.7% variation, whereas the addition of total acidity and C/N ratio of HAs in the model increased the variation to 75.6%. The contribution of HAs-N, phenolic-OH and carboxylic-OH was very small since the variation explained by HA-N, and phenolic and carboxylic-OH groups has been already accounted for by HA-C and total acidity, respectively. Soil MBC, one of the important factors controlling the decomposition of SOM, showed strong influence on HAs characteristics (Nierop *et al*., 12; Jolivet *et al*., 8).

All the studied land use systems significantly influenced the chemical and structural characteristics of the humic acids. Agricultural crops cultivation reduced the C and N contents of HAs. The results also signifies that adoption of various agroforestry, horticulture fruit trees and agriculture crops species in the fallow lands increased the degree of humification thus resulted in the higher aromatic condensation of HAs and increased stabilization or quality of SOM. Amongst the three land use systems, agroforestry land use showed higher aromatic condensation and increased stabilization or quality of SOM.

REFERENCES

1. Adekunle, I.M., Arowolo, T.A., Ndahi, N.P., Bello, B. and Owolabi, D.A. 2007. Chemical characteristics of humic acids in relation to lead, copper and cadmium levels in contaminated soils from Southwest Nigeria. *Ann. Env. Sci.* **1**: 23-34.
2. Amir, S., Hafidi, M., Merlina, G., Hamdi, H. and Revel, J.C. 2004. Elemental analysis, FTIR, ^{13}C-NMR of humic acids from sewage sludge composting. *Agronomies*, **24**: 13-18.
3. Campitelli, P.A., Velasco, M.I. and Ceppi, S.B. 2006. Chemical and physicochemical characteristics of humic acids extracted from compost, soil and amended soil. *Talanta*, **69**: 1234-39.
4. Ch'ng, H.Y., Ahmed, O.H. and Majid, N.M.A. 2011. Qualitative assessment of soil carbon in a rehabilitated forest using fourier transform infrared spectroscopy. *Scientific World J.* **11**: 532-45.
5. Dutarte, P., Bartoli, F., Andreux, F., Portal, J.M. and Ange, A. 1993. Influence of content and nature of organic matter on the structure of some sandy soils from West Africa. *Geoderma*, **56**: 459-78.
6. Guggenberger, G., Zech, W. and Thomas, R.J. 1995. Lignin and carbohydrate alteration in particle-size separates of an oxisol under tropical pastures following native savannah. *Soil Biol. Biochem.* **27**: 1629-38.
7. Hanmin, A.N., Muhamad, A.M.N., Ahmed, O.H., Susilawati, K. and Khairulmazmi, A. 2012. Physico-chemical properties of indigenous micro organism-composts and humic acid prepared from selected agro-industrial residues. *African J. Biotech.* **11**: 8456-63.
8. Jolivet, C., Guillet, B., Karroum, M., Andreux, F., Bernoux, M. and Arrouays, D. 2001. Les phenols de la lignine et le ^{13}C, traceurs de l'origine des matie`res organiques du sol. *C R Acad. Sci. Paris*, **333**: 651-57.
9. Marinari, S., Dell Abate, M.T., Brunetti, G. and Dazzi, C. 2010. Differences of stabilized organic carbon fractions and microbiological activity along Mediterranean vertisols and alfisols profiles. *Geoderma*, **156**: 379-88.
10. Moorhead, D.L., Currie, W.S., Rastetter, E.B., Parton, W.J. and Harmon, M.E. 1999. Climate and litter quality controls on decomposition: An analysis of modelling approaches. *Global Biogeochem. Cycling*, **13**: 575-89.

11. Moraes, G.M., Xavier, F.A., Mendonca, E., Filho, J.A. and Oliveira, T.S. 2011. Chemical and structural characterization of soil humic substances under agroforestry and conventional systems. *Revista Brasileria de Ciencia do Solo*, **35**: 1597-1608.

12. Nierop, K.G.J., Van Lagen, B. and Buurman, P. 2001. Composition of plant tissues and soil organic matter in the first stages of a vegetation succession. *Geoderma,* **100**: 1-24.

13. Piccolo, A. 2001. The supramolecular structure of humic substances. *Soil Sci.* **166**: 810-32.

14. Schnitzer, M. 1982. Organic matter characterization. **In**: *Methods of Soil Analysis Part 2: Chemical and Microbiological Properties*, Page, A.L., Miller, R.H. and Keeny, D.R. (Eds.), American Society of Agronomy, No. 9, Part 2, USA, pp. 581-94.

15. Seddaiu, G., Porcu, G., Ledda, L. and Roggero, P.P. 2013. Soil organic matter content and composition as influenced by soil management in semi-arid Mediterranean agro-silvo-pastoral system. *Agric. Ecosys. Env.* **167**: 1-11.

16. Spaccini, R., Mbagwub, J.S.C., Contea, P. and Piccolo, A. 2006. Changes of humic substances characteristics from forested to cultivated soils in Ethiopia. *Geoderma*, **132**: 9-19.

17. Stevenson, F.J. 1994. *Humus Chemistry-Genesis, Composition, Reactions* (2^{nd} edn.), John Wiley and Sons, New York, USA.

18. Swift, R.S. 1996. Organic matter characterization. **In**: *Methods of Soil Analysis,* Sparkers *et al*. (Eds), Soil Science Society of America, Part 3, USA, pp. 1011-20.

19. Szajdak, L., Brandyk, T. and Szatylovicz, J. 2007. Chemical properties of different peat-moorsh soils from Biebrza river valley. *Agron. Res.* **5**: 165-74.

Received: September, 2013; Revised: February, 2014;
Accepted: May, 2014