HUMUS-SOIL MINERAL INTERFACE IN ORCHARD AND FARM SOILS: NANOSCALE APPROACH

Thesis

Submitted to Punjab Agricultural University
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(Minor Subject: Chemistry)

By
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(L-2008-A-78-M)

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CERTIFICATE-I

This is to certify that the thesis entitled "**Humus-Soil Mineral Interface in Orchard and Farm Soils: Nanoscale Approach**", submitted for the degree of **M.Sc.** in the subject of **Soils** (Minor subject: **Chemistry)** of the Punjab Agricultural University, is a bonafide research work carried out by **Gurjit Singh (L-2008-A-78-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been duly acknowledged.

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CERTIFICATE-II

This to certify that the thesis entitled, **“Humus-Soil Mineral Interface in Orchard and Farm Soils: Nanoscale Approach”,** submitted by **Gurjit Singh (L-2008-A-78-M)** to the Punjab Agricultural University, Ludhiana in partial fulfillment of the requirement for the degree of **M.Sc.** in the subject of **Soils** (Minor subject: **Chemistry)** has been approved by the Student’s Advisory Committee after an oral examination of the same.

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ABSTRACT

Clay-Humus complexes are pivotal for understanding long-term carbon retention in soils and founding protocols for nutrients, water and tillage management. Very little attempts were made to decipher the complexes formed under cultivation. Therefore, the study was undertaken for looking at bonding that different clay-minerals made with humus, imaging and deciphering interfaces of clay–humus complexes, and their association with land-use. For this, clays were extracted from surface soils of mango orchard (S1), guava orchard (S2) and rice-wheat cultivated field (S3) of Bahadurgarh (30°21′45″N; 76°28′03″E; 255 m) in Punjab, India. Clays were untreated, organic matter (OM) was removed, $CaCO_3$ was removed, and both OM and $CaCO_3$ were removed. They were analyzed by chemical means, by Infra-Red Spectroscopy (IR), and by Scanning Electron Microscopy and Energy Dispersive Spectroscopy. IR spectroscopy identified dioctahedral and trioctahedral illites, and vermiculite, kaolinite, Al-chlorite, and montmorillonite in clays. Humic acids, amides, quinine, ketones and polysaccharides, and fulvic acids were identified in OM. S1 had phenol, and CH_2 and CH_3 groups, while S2 had aliphatic humic and fulvic acids, and aldehyde of humic acids, and carboxyl and ketonic groups in fulvic acid, and alkenes. The ratio of Oxydizable OC to clay-humus OC was: S1 (0.049) < S3 (0.054) < S2 (0.060). Micrographs portrayed uniform spread, irregular spread, and scattered lump of humus on various clay-minerals. The humus from guava field had higher C content (15.60 by atom %) than humus from mango (12.1 by atom %), and cultivated fields (9.78 by atom %). Strong bond between clay minerals and humus deforms OH of clay minerals, and causes COO^- anti-symmetric stretching in humus. Presence of phenol compounds in humus on clay surfaces tender resistance of these complexes to microbial degradation, and could be a crucial factor for N management in soils.

Key Words: Clay humus complexes, clays, electron microscopy, inorganic carbon, IR spectroscopy, organic carbon

Signature of the major advisor **Signature of the student**

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ਚੀਕਨੀ ਮਿੱਟੀ-ਹਊਮਸ ਦੇ ਮਿਸ਼ਰਣ ਮਿੱਟੀ ਵਿੱਚ ਕਾਰਬਨ ਦੀ ਲੰਮੇ ਸਮੇਂ ਲਈ ਹੋਂਦ ਅਤੇ ਪੌਸ਼ਟਿਕ ਤੱਤਾਂ, ਪਾਣੀ ਅਤੇ ਖੇਤੀ ਪ੍ਰਬੰਧਾਂ ਦੇ ਖਰੜੇ ਨੂੰ ਸਮਝਣ ਲਈ ਮਹੱਤਵਪੂਰਣ ਹਨ । ਖੇਤੀ ਦੌਰਾਨ ਬਣੇ ਇਹਨਾਂ ਮਿਸ਼ਰਣਾਂ ਨੂੰ ਸਮਝਣ ਲਈ ਬਹੁਤ ਘੱਟ ਕੋਸ਼ਿਸ਼ਾਂ ਹੋਈਆਂ । ਇਸ ਲਈ ਫੋਟੋਆਂ ਦੁਆਰਾ ਚੀਕਨੀ ਮਿੱਟੀ-ਹਊਮਸ ਦੇ ਮਿਸ਼ਰਣ ਅਤੇ ਇਸਦੇ ਵਰਤੋਂ ਵਾਲੀ ਜ਼ਮੀਨ ਨਾਲ ਸਬੰਧ ਨੂੰ ਸਮਝਣ, ਚੀਕਨੀ ਮਿੱਟੀ ਦੇ ਪੌਸ਼ਟਿਕ ਤੱਤਾਂ ਦੁਆਰਾ ਹਊਮਸ ਨਾਲ ਬਣੇ ਵੱਖ-ਵੱਖ ਜੋੜਾਂ ਨੂੰ ਸਮਝਣ ਲਈ ਇਹ ਖੋਜ ਕੀਤੀ ਗਈ । ਪੰਜਾਬ ਦੇ ਬਹਾਦਰਗੜ੍ਹ ਤੋਂ ਅੰਬ (S1), ਅਮਰੂਦ (S2) ਦੇ ਬਾਗਾਂ ਅਤੇ ਕਣਕ ਝੋਨੇ ਫ਼ਸਲੀ ਚੱਕਰ (S3) ਵਾਲੀਆਂ ਜ਼ਮੀਨਾਂ ਵਿੱਚੋਂ ਮਿੱਟੀ ਦੇ ਨਮੂਨੇ ਲਏ ਗਏ ਅਤੇ ਇਹਨਾਂ ਵਿੱਚੋਂ ਚਿਕਨੀ ਮਿੱਟੀ ਨੂੰ ਕੱਢਿਆ ਗਿਆ । ਚੀਕਨੀ ਮਿੱਟੀ ਨੂੰ ਚਾਰ ਪ੍ਰਬੰਧਾਂ ਵਿੱਚ ਵੰਡਿਆ ਗਿਆ। ਪਹਿਲਾਂ ਜਿਸ ਵਿੱਚੋਂ ਕੈਲਸ਼ੀਅਮ ਕਾਰਬੋਨੇਟ ($CaCO_3$), ਦੂਜਾ ਜਿਸ ਵਿੱਚੋਂ ਜੈਵਿਕ ਪਦਾਰਥ, ਤੀਜਾ ਜਿਸ ਵਿੱਚੋਂ ਦੋਵੇਂ ਕੈਲਸ਼ੀਅਮ ਕਾਰਬੋਨੇਟ ਅਤੇ ਜੈਵਿਕ ਪਦਾਰਥ ਕੱਢੇ ਗਏ ਅਤੇ ਚੌਥੇ ਵਿੱਚ ਕੋਈ ਬਦਲਾਅ ਨਹੀਂ ਕੀਤਾ ਗਿਆ। ਇਹਨਾਂ ਨੂੰ ਰਸਾਇਣਿਕ, ਇਨਫਰਾਰੈਡ ਸਪੈਕਟਰੋਸਕੋਪੀ, ਸਕੈਨਿੰਗ ਇਲੈਕਟਰੋਨ ਮਾਈਕਰੋਸਕੋਪੀ ਅਤੇ ਇਨਰਜੀ ਦਿਸਪਰਸਿਵ ਸਪੈਕਟਰੋਸਕੋਪੀ ਵਿਧੀਆਂ ਦੁਆਰਾ ਪਰਖਿਆ ਗਿਆ । ਇਨਫਰਾਰੈਡ ਸਕੈਕਟਰੋਸਕੋਪੀ ਦੁਆਰਾ ਚਿਕਨੀ ਮਿੱਟੀ ਵਿੱਚੋਂ ਡਾਈਔਕਟਾਹੈਦਰਲ ਅਤੇ ਟਰਾਈਔਕਟਾਹੈਡਰਲ ਇਲਾਈਟ, ਵਰਮੀਕੁਲਾਈਟ, ਕਔਲੀਨਾਈਟ, Al-ਕਲੋਰਾਈਟ ਅਤੇ ਮੌਂਟਮੋਰੀਲੋਨਾਈਟ ਪਦਾਰਥਾਂ ਦੀ ਸ਼ਨਾਖਤ ਹੋਈ । ਜੈਵਿਕ ਪਦਾਰਥਾਂ ਵਿੱਚ ਹਊਮਿਕ ਅਤੇ ਫੁਲਵਿਕ ਤੇਜ਼ਾਬ, ਅਮਾਈਡ, ਕਿਊਨਾਂਈਨ, ਪੋਲੀਸੈਕਾਰਾਈਡ ਅਤੇ ਕਿਟੋਨਸ ਆਦਿ ਗਰੁੱਪਾਂ ਦੀ ਸ਼ਨਾਖਤ ਹੋਈ । ਅੰਬਾਂ ਦੀ ਮਿੱਟੀ (S1) ਵਿੱਚ ਫਨੌਲ ਅਤੇ ਮਿਥਾਈਲ ਗਰੁਪ, ਜਦਕਿ ਅਮਰੂਦਾਂ ਦੇ ਬਾਗਾਂ ਵਾਲੀ ਮਿੱਟੀ (S2) ਵਿੱਚ ਹਊਮਿਕ ਅਤੇ ਫੁਲਵਿਕ ਤੇਜ਼ਾਬ ਨਾਲ ਕਾਰਬੋਖਸੀਅਲ, ਕਿਟੋਨਿਕ ਅਤੇ ਅਲਕੀਨਸ ਗਰੁੱਪ ਪਾਏ ਗਏ । ਕੁਸ਼ਤਾ ਬਣਨ ਯੋਗ ਜੈਵਿਕ ਕਾਰਬਨ ਅਤੇ ਚੀਕਨੀ ਮਿੱਟੀ-ਹਊਮਸ ਵਿੱਚ ਜੈਵਿਕ ਕਾਰਨ ਦਾ ਅਨੁਪਾਤ, S1(0.049 < S3(0.054) < S2(0.060) ਹੈ । ਤਸਵੀਰਾਂ ਦੁਆਰਾ ਚੀਕਨੀ ਮਿੱਟੀ ਤੇ ਹਊਮਸ ਦੇ ਇਕਸਾਰ, ਅਸੰਬਤ ਅਤੇ ਢੇਲਿਆਂ ਦੇ ਰੂਪ ਵਿੱਚ ਖਿਲਰੇ ਹੋਣ ਦਾ ਸਪੱਸ਼ਟੀਕਰਨ ਕੀਤਾ ਗਿਆ । ਅਮਰੂਦ ਦੇ ਬਾਗਾਂ ਦੀ ਮਿੱਟੀ ਦੇ ਹਊਮਸ ਵਿੱਚ ਸਭ ਤੋਂ ਵੱਧ ਕਾਰਬਨ ਦੀ ਮਾਤਰਾ (15.60% ਪ੍ਰਮਾਣੂ), ਅੰਬਾਂ ਦੇ ਬਾਗਾਂ (12.1% ਪ੍ਰਮਾਣੂ) ਅਤੇ ਝੋਨਾ-ਕਣਕ ਫ਼ਸਲ ਵਾਲੀ ਜ਼ਮੀਨ ਵਿੱਚ (9.78% ਪ੍ਰਮਾਣੂ) ਹੈ । ਚੀਕਨੀ ਮਿੱਟੀ ਅਤੇ ਹਊਮਸ ਵਿੱਚ ਮਜਬੂਤ ਬੰਧਨ ਨੇ ਚੀਕਨੀ ਮਿੱਟੀ ਪਦਾਰਥਾਂ ਦੇ ਹਾਈਡ੍ਰੋਕਸੀਅਲ (OH) ਨੂੰ ਵਿਗਾੜ ਦੇ ਹੋਏ ਉਹਨਾਂ ਦੀ ਹਊਮਸ ਵਿੱਚ ਕਾਰਬੋਕਿਸੀਅਲ (COO^-) ਐਂਟੀਸਮਿਟਰਿਕ ਸਟਰੈਚਿੰਗ ਕੀਤੀ । ਚੀਕਨੀ ਮਿੱਟੀ ਦੀ ਸਤ੍ਹਾ ਤੇ ਹਊਮਸ ਵਿੱਚ ਫੀਨੋਲ ਇਹਨਾਂ ਮਿਸ਼ਰਣਾਂ ਨੂੰ ਜੀਵਾਣੂ ਅਧਗੋਤੀ ਤੋਂ ਬਚਾਉਂਦੀ ਹੈ ਅਤੇ ਮਿੱਟੀ ਵਿੱਚ N ਦੇ ਪ੍ਰਬੰਧ ਲਈ ਬਹੁਤ ਨਿਰਣਾਇਕ ਸਿੱਧ ਹੁੰਦੀ ਹੈ ।

ਮੁੱਖ ਸ਼ਬਦ: ਚੀਕਨੀ ਮਿੱਟੀ-ਹਊਮਸ ਦੇ ਮਿਸ਼ਰਣ, ਚੀਕਨੀ ਮਿੱਟੀ, ਇਲੈਕਟਰੋਨ ਮਾਈਕਰੋਸਕੋਪੀ, ਅਜੈਵਿਕ ਕਾਰਬਨ, ਇਨਫਰਾਰੈਡ ਸਪੈਕਟਰੋਸਕੋਪੀ, ਜੈਵਿਕ ਕਾਰਬਨ ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਦਸਤਖਤ

ਵਿਦਿਆਰਥੀ ਦੇ ਦਸਤਖਤ

CONTENTS

CHAPTER I

INTRODUCTION

Humus is the dark coloured more or less stable fraction of soil-organic-matter (SOM), derived from microbial decomposition of plant and animal substances. The humus formed from the decomposition of fresh organic material contains approximately 50 per cent Carbon and 5 per cent Nitrogen. In other words, the C: N ratio in the freshly-formed humus is 10:1**.** It includes sugars, amines, nucleic acids, phospholipids, vitamins, sulpholipids, polysaccharides and some other unclassified compounds. The humus or humic substances comprise of about 60 to 80 per cent of the organic matter present in mineral soils. They consist of huge molecules with variable structures and composition, and characterized by aromatic, ring-type structures that include polyphenols and comparable polyquinones. The cation exchange capacity of the humus colloids (150-300 cmol kg^{-1}) is much higher than that of clays (4-150 cmol kg^{-1}). But, surface area of the humus colloids is variable (grossly estimated to be 20-800 $m^2 g^{-1}$), and comparable with the colloidal clays (10-800 $m^2 g^{-1}$). Therefore, inspite of being in small amounts in mineral soils, the colloidal surfaces of humus provides great amounts of charges mostly negative, but also some amounts of positive charges. The humic substances are dark coloured, non-crystalline (amorphous) materials, and based on their solubility in acid and alkali, they are classified into three chemical groups, namely *viz.*, fulvic acid (FA), humic acid (HA), and humin (Hu). Fulvic acid is lightest in both molecular weight and colour amongst the three fractions.

Interaction of humus with clay minerals provides means of stabilizing soil organic matter by adsorption on the surfaces of soil minerals (Mayer 1994, Mayer and Xing 2001, Kahle *et al* 2002 and Kennedy *et al* 2002), and regulates nitrogen cycle; a crucial global issue of sustainability. Thermodynamically, both colloidal clays and colloidal organic matter (i.e., humus) would form complexes so as to attain stability ($dG < 0$), and therefore, it is one such natural phenomenon that protects earth system. This explains why clay-humus complexes amongst all pools of C in soils are stable over thousands of years (in some soils they are stable over billion of years) (Kennedy *et al* 2002, 2006). In turn, the sustainability of agriculture would be dependent on retaining stability of clay-humus complexes. Not all mineral surfaces are equally important in the sorptive preservation of organic carbon. Among the phyllosillicates, smectites have been shown to be more intimately associated with SOM than other clay minerals (Wattel- Koekkoek *et al* 2003) due to their large specific surface area. They especially attract and hold amino acids, peptides and proteins. Organic matter that is entrapped in the very small (<1μm) pores formed by clay particles is physically inaccessible to decomposing organisms, and such pores are large in smectites and in other fine clays, and moderate in coarse clays. Layer silicates (e.g., vermiculite) bind humus in their inter layers that strongly resist its decomposition. Similarly, oxides of iron and aluminum in highly weathered soils (e.g., Oxisols and Ultisols) and allophones in volcanic soils (e.g., Andisols) bind nitrogenous organic molecules and protect them from decay.

The world's soils contain more than 1.5 trillion tonnes of C or roughly three times the carbon contained in all the world's vegetation and twice the amount of carbon (as CO_2) in the earth's atmosphere (Knorr and Prentice 2005). Hence, relatively small changes in SOM can have large effects on greenhouse gas (GHG) concentrations - either positively or negatively. At one hand, decay in clay-humus complexes may increase GHS emission to the atmosphere, and on the other hand effort on C sequestration in soils will be tangible only if humification process on SOM leads to the formation of clay-humus complexes. On other words, the transformation of plant residues into stabilized clay–humus complexes is driving determinant of C sequestration efforts, and therefore all soil management practices must aim for it. It is nevertheless important that there is a limit upto which C can be sequenced in soils (Schlesinger 1990), which is governed by climate, soil properties (e.g., texture, structure) and mineralogical make-up (Davidson and Janssens 2006). It is also important to recognize that many attempts to raise C content in soils remained largely elusive (Wiseman and Puttmann 2005).

There are, however, little or no information available on the nature and behavior of clay-humus complexes in naturally occurring soils. Almost all studies involved synthetic clays or, soil-clays and synthetic organic materials, which have great academic value, but may or may not reflect real soil situations. The C sequestration in soils under various land use systems (e.g., agricultural, managed forest, urban and under shallow water ecosystems) can be understood and informed management decisions can be implemented only if clay-humus complexes are understood from real soil data. Apart from it, there is very little understanding on clay-humus complexes in mixed mineralogy systems, inspite of the fact that most of the global soils, including soils of Punjab belong to it.

Sen (1964) suggested two types of linkages between clay and humus; one with weak bonding and the other with strong bonding. He also noted that clay bound humus was more resistant to microbial attack than free humus. Varadachari *et al* (1984) observed that smaller and more flexible humic molecules were less susceptible to microbial decomposition than larger and structurally more rigid molecules. Sen (1960) showed that clays with higher CEC formed stronger bonds. He had also demonstrated that Mg^{2+}, Al^{3+} and Fe^{3+} caused considerable increase in HA adsorption than to Ca^{2+} and H^+. Ahmed *et al* (2002a, b) concluded that there were wide differences in the nature of clay-humus bonding amongst soils of Entisols, Alfisols, Vertisols and Mollisols. Electron exchange behaviours of humic substances with iron, copper and manganese showed that apart from the simultaneous formation of chelates, humic substances were independently capable of changing the valence state of metal ions (Ghosh *et al* 1983). They also observed that there was decrease in fixation with increase in the acidity of the humic samples. They proposed that the decreasing content of acidic groups in humic substances promoted their fixation by montmorillonite because of lowering of interparticle repulsive forces as well as a reduction in hydration energies and consequent decrease in net energy available for breaking down the clay–humus bonds.

Challa and Raman (1985) reported high amounts of adsorption of humic acid on Cu-montmorillonite. They ascribed it to the vacant d-orbital of Cu^{2+}, which forms coordination compounds with humic acids. Basak and Ghosh (1999) studied the adsorption of HA on amorphous aluminosilicates of varying Si/Al ratios. All the above inferences were drawn on clay-humus complexes artificially prepared in the laboratory and they were subjected to study generally in their suspension state (Kumar *et al* 2002). Sengupta and Banerjee (1970) grouped clay-humus complexes into 'fertile' and 'infertile' fractions, and demonstrated that organic matter incorporation in soils favoured the formation of the fertile fraction, especially in the presence of montmorillonite. Electron exchange behaviours of humic substances with iron, copper and manganese revealed that apart from the simultaneous formation of chelates, humic substances were independently capable of changing the valence state of metal ions (Ghosh *et al* 1983).

Varadachari *et al* (1991) experimented with the effect of adsorption of HA on variable-lattice-charge montmorillonite, and observed an initial increase in fixation of HA, and subsequent decrease of fixation of HA on reduction of layer charge. Varadachari *et al* (2000) opined that analysis of crystal structures at broken surfaces and edges can provide information on the primary factors controlling oxide-HA complexation. Ahmed *et al* (2002) indicated that the nature of clay-humus bonding in soils varies significantly from one soil to another and such variation is most likely due to the nature of the clay minerals and the bridging cations. They also observed that in the Vertisols, montmorillonite-humus complexes were very much reduced than the clay itself due to the large stack size of clays.

Clay-humus complexes played crucial role in the evolution of multicellular animals from one-celled eukaryotes in the last one billon years (Derry 2006), and for the expansion of metazoans and pedogenesis of clay-minerals (Kennedy *et al* 2006). Derry (2006) warned that if this process of formation of clay-humus complex is reversed, or in other words if we remain passive spectator of humus oxidation from its protective soil mineral surfaces, then we would lose what we could gain in the last billion years.

The land use is of prime concern for global carbon and nitrogen cycles (Cotrufo *et al* 2011). It is generally perceived that the change in land use alters clay-humus complexes. On the basis of information available in the literature on the soil organic carbon contents in conjuncture with the FAO-UNESCO Soil Map of the Word, the organic carbon stocks in the upper 30 cm of native soils of the Central Asia were pegged at 20,172 peta gram (Pg). The extent of conversion of native land into agricultural land and the degradation of rangelands was assessed by a land use and land cover change map of the region. The land use change in the region was responsible for a reduction of the soil organic carbon by about 8,28 Tg C, or on average 4.1 per cent of the total stocks (Sommers and Pauw 2011). Apart from change in land use, agricultural practices also affect the soil organic carbon pools and fluxes. Conversion of natural to agriculture ecosystems decreases SOC pools and magnitude of decrease depends on land use management and ecological factors. However, the conversion of natural ecosystem to cropland in USA has led to release of 5000 Tg (million metric tons) of carbon to atmosphere.

Scanning Electron Microscope (SEM) is a powerful tool to obtain images of the morphology of the clay–humus complexes, while Energy Dispersive Spectroscope (EDS) maps spatial distribution of the elements at desired region. Using these techniques, Laird *et al* (2001) identified two distinct types of clay-associated humic substances. The first type was diffuse filamentous films that covered basal surfaces of 2:1 phyllosilicates in the medium and fine clay fractions. The second type was discrete particles of high density metal-humic substance complexes in the coarse clay fractions.

Infra-Red spectroscopy (IR) could depict clay-humus complexes with respect to functional groups and masking of charge sites on clays. The overall infrared region may be subdivided into Near-Infra-Red (NIR; 13300 to 4000 cm^{-1}; 0.75-2.5μm); Mid-Infra-Red (MIR; 4000 to 400 cm^{-1}; 2.5- 25 μm) or fundamental (rotation vibration); and Far-Infra-Red (FT-IR; 400 to 20 cm^{-1}; 25– 500 μm) or, skeletal vibration. The region of primary interest in the study of soil components is between wave numbers of 4000 and 200 cm^{-1} (2.5- 25 μm).

Both organic (humus) and inorganic (clay) components of soils respond to Infrared waves. The infrared spectra of humic substances are classified into three general types such as humic acid with presence of strong bands at 3400, 2900, 1720, 1600 cm^{-1}, fulvic acid with presence of strong bands at 1720 cm^{-1}, and protein and carbohydrates with presence of strong bands around 1540 cm^{-1}. These observations highlighted an urgency to study humus-clays complexes, especially to determine the nature of humus that is association with clays under the land use of managed orchard and intensively-cultivated-irrigated farm. If the mechanisms, bonding, and humus-soil mineral interface are understood properly, possibly new farming protocols can be worked out. The nature of bonding between humus and soil minerals, and the way humus laminates over soil minerals can be elucidated through nanoscale approach. Therefore, the present study was undertaken with the following objectives:

(i) To study the nature of bonding at the interfaces of humus and soil minerals,

(ii) To interpret interfaces of soil – minerals and humus through high resolution images.

(iii) To delve the impact of land use on interfaces of humus and soil minerals.

(iv) To discern the relationship of soil minerals with humus.

CHAPTER II

REVIEW OF LITERATURE

2.1 Nature and properties of humus

Humus is the organic portion of the soil remaining after prolonged microbial decomposition. It is the major component of organic matter in soil, making up between 65 percent and 80 percent of the total. It is formed by the decomposing action of soil microorganisms (various bacteria and fungi), which break down animal and vegetable material into elements that can be used by growing plants. The decomposition may take place under aerobic and anaerobic conditions - usually in soils, composts, peat bogs, and water basins. Because of its low specific weight and high surface area, humus has a profound effect upon the physical properties of mineral soils with regard to improved soil structure, water intake and reservoir capacity, ability to resist erosion, and the ability to hold chemical elements in a form readily accessible to plants. It also plays an important role as a fertility component in soils, far in excess of its percent occurrence to the total soil mass.

Humus or humic substances are used as synonymous in soil science literature. The most important function of humic substances in soil is their ability to hold and make water available to plants. Soils that contain high concentrations of humic substances hold water for crop-use during periods of drought. Growers who routinely apply humate-based fertilizers, and conscientiously integrate production practices that preserve humic substances, can frequently harvest a crop even during periods of dry weather. Complex carbohydrates synthesized by bacteria function together with humic substances, silt, and clay to form soil aggregates. As the humic substances become intimately associated with the mineral fraction of the soil, formation of colloidal complexes of humus-clay and humus-silt, occurs. These aggregates, formed by electrical processes, increase the cohesive forces that cause clay components and the very fine soil particles to become attracted to one another. Once formed, these aggregates help create more friable topsoil, or crumb structure. 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. Humus adds to the soil structure by coating these mineral particles and holding them together; and thus serves as a major reservoir of plant nutrients. Based on chemical nature, humus is divided into three major components: (i) humic acids, (ii) fulvic acids and (iii) humin. Humic acid is completely decomposed organic matter and it is readily soluble in acids or bases. Humification is the biological process of converting organic matter into humic substances. Humic substances are high molecular weight compounds that together form hydrophilic, molecularly flexible, polyelectrolyte components of humus. The elemental analysis of humic substances showed that they are primarily composed of carbon, oxygen, hydrogen, nitrogen, and sulfur in complex carbon chains. Specifically, humic matter has been shown to increase the uptake of nitrogen by plants, and to increase soil nitrogen utilization efficiency. Nutritionally, humic substances are also sources phosphorus, as well as, enhancers to the up-taking of potassium, calcium, and magnesium, benefiting micro-organisms that benefit the plants in turn. All of these effects increase the productivity of the soil. In addition, chlorosis in plants has been prevented, or corrected by humate application, probably the result of the ability of humate to hold soil iron in an assimilatable form. Humic substances have to contain a wide variety of molecular components. Some typical components are: aliphatic compounds (carbon chains) carbonyls, esters, ethers, fatty acids, furan-ringed compounds (aromatic) include various combinations of benzene, acetal, ketal, and lactol, lignin, lipids, peroxides, phenols polypeptides polysaccharides, and quinines.

Interaction with clay minerals provides means of stabilizing soil nitrogen and organic matter. High activity clays especially attract and hold such substances as amino acids, peptides and proteins forming complexes that protect the carbon containing compounds. Organic matter that is entrapped in the very small (< 1µm) pores formed by clay particles is physically inaccessible to decomposing organisms. It is also possible that layer silicates such as vermiculite, may bind in their interlayer's in forms that strongly resist decomposition of organic matter. Iron and Aluminum oxide coating in highly weathered soils and allophone in volcanic soils can bind with nitrogenous organic molecules protect them from decay.

2.2 Carbon fractions in the soils of north-west India

The SOC is a complex heterogeneous mixture of organic substances which imparts the physical, chemical and biological fertility of soil. Currently the scientists are gaining interest in the role of SOC as a potential sink for atmospheric CO_2. The land use affects the distribution pattern of litter carbon to functionally different SOM pools. Actually the dynamics of SOC can be better understood by dividing it into various pools or fractions that have a wide range of chemical properties and turnover times and consequently respond differently to land use changes. Light fractions (LF) generally represent only a small proportion of total SOC in biologically active soils, however changes in carbon stocks following land use changes can be more pronounced in LF compared with bulk soil. Spatial distribution of different (SOC) fractions is influenced by land use and management.

Bhattacharyya et al (2000) studied the organic carbon stock in Indian soils and their geographical distribution. In the 48 soil series of a few major soils of India, the organic carbon (OC) stock in the top 150 cm depth of soils was 24.3 Pg. SOC stock appears to be a single parameter that can help effectively in prioritizing the area for restoring soil health. The Indian sub-continent comprises three major physiographic regions, namely Mountain and Hill Region of the Himalayas, Indo-Gangetic Plain and Peninsular Plateau. The SOC reserves in this physiographic region based on 40 soil series was 7.89 Pg in the top 30 cm depth, and

18.31 Pg in the top 150 cm soil depth. On the basis of about 36 soil series, the SOC stock of the Great Plains has been found to vary from 3.28 to 10.53 Pg from first 30 cm to the first 150 cm soil depths. Peninsular India covers the central uplands, Aravalli range, Eastern Rajasthan uplands, Madhya Bharat Plateau, Malwa Plateau, Vindhyan Scarpland, Vindhyan range, Narmada valley and the Bundelkhand upland. This physiographic region covers an area of 54.7 mha. SOC reserves are found to vary from 3.64 to 13.34 Pg in the first 30 cm and 150 cm soil depths respectively. To sustain the quality and productivity of soils the knowledge of OC in terms of its amount and quality in soils is essential. Further Sheik *et al* (2009) investigated altitudinal variation in soil organic carbon stock in coniferous subtropical and broadleaf temperate forests in the Garhwal Himalaya. The current global stock of soil organic carbon is estimated to be 1,500–1,550 Pg. This constituent of the terrestrial carbon stock is twice that in the earth's atmosphere (720 Pg), and more than triple the stock of organic carbon in terrestrial vegetation (560 Pg). The Himalayan zones, with dense forest vegetation, cover a fifth part of India and store a third part of the country reserves of soil organic carbon (SOC). The stocks of SOC were found to be decreasing with altitude: from 185.6 to 160.8 t C ha^{-1} and from 141.6 to 124.8 t C ha^{-1} in temperature (*Quercus leucotrichophora*) and subtropical (*Pinus roxburghii*) forests, respectively. A comparison of the soil organic carbon stock values of different sites in both forests show that the carbon stock tonnes per hectare decrease with increasing altitudes. The tendency of carbon density to increase as altitude decreases may be due to better stabilization of SOC at lower altitudes.

In Punjab Benbi and Brar (2009) studied 25-year record of carbon sequestration and soil properties under intensive agriculture. Several studies have shown that continuous cropping decreases soil organic carbon stocks, rapidly in the initial years then at a slower rate, approaching a new equilibrium after 30 to 50 years. For instance, a study of intensive corn cropping for 35 years on temperate soils showed a 50 percent decrease in soil organic carbon. The study site is located in the north Indian state of Punjab. It is the most intensively cultivated region in the country with a cropping intensity of 190 percent, predominantly of a rice-wheat system. Due to high nutrient demand and its continuous cultivation, the cropping system is presumed to adversely affect soil organic carbon and other soil properties. Therefore, they evaluated soil data for 25 years from 1981/82 to 2005/06 to investigate the impact of intensive agriculture on C sequestration and soil properties on a regional scale. The analysis showed that intensive cultivation of a rice-wheat system unexpectedly resulted in improved C sequestration, a favorable pH environment and amelioration of the soil salinity.

2.3 Mineralization and humification processes of plant sourced substances

The most important function of soil microorganism is the decomposition of various kinds of organic matter present in the soil. Virtually all type of organic matter eventually finds their way to the soil or to the sea. The soil organic matter chiefly consists of residues of dead plant and animals, and the excretory products of the living beings. These organic constituents need to be converted into simple inorganic forms (minerals) to make them available to the autotrophic organisms. This conversion of organic matter into simple inorganic forms is called mineralization. The mineralization is rendered mainly through decomposition of organic matter by soil microorganism, mainly fungi and bacteria. It is estimated that 90 percent of the mineralization of organic matter is the result of the metabolism of all other organisms, as well as the combustion of fuel and other materials. The overwhelming contribution of microorganism to the process of mineralization reflects their ubiquity, their significant contribution to the bulk of living material (their biomass), their high rates of growth and metabolism, and their collective ability to degrade a vast variety of naturally occurring organic materials.

In general, the organic compounds of organism residues are attacked by organotrophic microorganisms capable of digesting and oxidizing them. As the oxygen is consumed, anaerobic microorganisms capable of reducing nitrates and sulphates or carbonates commence their activities ultimately creating an aerobic condition. The various simple inorganic substances such as ammonia, sulphide, hydrogen, etc. are avidly oxidized by lithotrophs. Thus, the organic compounds of organism-residues re completely decomposed to simple organic forms and subsequently oxidized. This cycle of events occur where environment is suitable. Extreme cold and heat are unsuitable for normal turnover of the matter. In cooler parts, decomposition rate is accelerated in spring, where in warmer parts rainy conditions favor the activity.

While investigating the organic carbon (C) content and its chemical fractions like humic acid (HA), fulvic acid (FA), and humin fractions in soils under forest, grass, and agricultural lands. It is observed that the higher amounts of total SOC as well as different SOC fractions, (FA, HA, humin) existed in the forest soils compared to those from grassland and agricultural soils. The carbon proportion of FA, HA, and humin fractions accounted for 25, 29, and 37 percent of the total SOC respectively in forest soils. The corresponding values were 34, 27, and 36 percent in grassland, and 28, 18, and 54 percent in agricultural land. The grassland soils showed higher FA fraction, while the agricultural soils contained lower HA fraction and higher humin fraction as compared to the forest soil. Higher HA/FA ratio and lower (HA + FA)/SOC ratio in forest soils indicates higher degree of humification and larger humification rate in this soil.

2.4. Interaction of humus with clay- minerals and soil- clays

The extant of adsorption of humic substances on the external mineral surfaces depends on the physical and chemical characteristics of the surface, pH of the system, and its water content. The formation of a wide range of clay mineral–humic association involves chemical bonding with widely differing strengths. The complexation reactions of humic acid

(HA) and fulvic acid (FA) with structural cations of edges and hydroxyl Al (or Fe) coatings on mineral colloids are important binding mechanisms. Hydrogen bonding is also of considerable importance as clearly indicated in IR spectra of HA and FA mineral complexes (Schnitzer and Khan 1972). These reactions apparently involves -H and -O- of COOH and -OH groups in HA and FA and O and H of external plannar surfaces and edges. Cations with high hydration energies on mineral surfaces react via water bridges with HA and FA functional groups.

The influence of crystal edges on clay- humus complexes was studied by Varadachari *et al* (1995).The extent and nature of humic acid (HA) bonding at the crystal edges relative to basal surfaces, in dried complexes of montmorillonite, illite and kaolinite were studied by blocking the edges with sodium hexametaphosphate (HMP) or triethanolamine (TEA). Decreases in the fixation of HA caused by the edge blocking was observed in every case except the HMP- Ca- montmorillonite complex, which showed an exceptional increase in fixation. It was concluded that direct bonding of HA to exposed structural ions at the crystal edges of kaolinite does not occur, the linkage is always through a cation bridges. The efficiency of HA fixation by the cations on illite is greater than that on montmorrilonite as a result of the stronger attractive forces of the cation – illite bond. It has been further been inferred that the unique behaviour of HMP-Ca- montmorillonite is caused by its extensive swelling character, which makes available numerous basal surfaces for bonding with HA. Destruction of silicate layer stacking on HA complexation has been observed by X ray diffraction (XRD).

Greenland (1971) studied the interaction between Humic and Fulvic acids and Clays and concluded that the HA and FA can be adsorbed by clay minerals through association with polyvalent metals at the clay surface. When iron and aluminium hydroxides are polymerized at clay surfaces, opportunity arises for strong bonding between clay and organic material to develop. The adsorption of well defined organic materials by montmorillonite has been extensively used as a model of the interaction between clays and organic compounds. It is probable that in many soils surfaces of hydrous oxides are at least as important as mica – type surfaces. Adsorption behaviour at a montmorillonite surface may differ considerably from behaviour at the hydroxylic surfaces of such materials.

The decomposition of humus in clay – humus complexes was investigated by Varadachari *et al* (1984). The result reveals that as the proportion of clay in the humus – clay complexes increase the decomposition of HA is markedly decreased. The extent of such decrease is more pronounced at lower clay concentrations than at higher ones, where the leveling effect appears. On increasing the clay concentration more surface will be available to the HA whereby the extent of multilayer formation decreases. It is also observed that in the presence of relatively small amount of clay that is 1:2.5 and 1:5.0 HA: clay complexes, more

CO_2 is evolved with brown forest HA than with the lateritic HA, where as in the presence of larger amounts of amount of clay decomposition is more marked with the lateritic HA. Similar to this study Varadachari *et al* (1997) also observad the complexation of humic substances with oxides of iron and aluminium. Complexation of HA and FA by goethite, hematite, gibbsite, and boehemite was studied in predried systems, Where hematite showed the highest fixation of HA at various oxides. The influence of pH on the fixation of HA and FA were investigated. Although at pH 7.0 and above, hematite showed strongest fixation and at lower pH fixation was highest by gibbsite. This was attributed to the competitive effect of OH ions in the blocking the coordination sites on the gibbsite surface. In the other three minerals some cation bridging through H ions is involved in fixation such a mode of interaction is significant in gibbsite. It was inferred that two major modes of HA bonding are operative in hematite and gibbsite that are the cation bridges forming oxide -M-HA links and direct bonding of HA to coordination centers ate the oxide surface, forces of such bonding are strongest in hematite. In the boehmite cation bridge is the major interactive mode, where as in gibbsite, HA fixation occurs primarily to coordination centers at the surface.

Further the effect of exchangeable cations and lattice charge on Clay- humus complexation was studied by Vardachari *et al* (1991). The influence of exchangeable cations on montmorillonite, illite and kaolinite and of lattice charge of montmorillonite on clay humic acid complexation was studied and various conclusion were drawn. The exchangeable cations form the predominant bonding link between HA and all three clay minerals, with montmorillonite the bonding strength of cations to clay as well as cations to HA determines the order of effectiveness of cations, direct bonding of HA to crystal edges of kaolinite and illite is not significant, electrostatic repulsive forces are a major obstacle to clay humus bonding, loss of swelling property of montmorillonite causes drastic reduction of HA fixation due to reduced availability of basal surfaces and interaction of HA with montmorillonite causes disruption of its stacking arrangement due to the prying open of the interlayer's by HA molecules.

In Kenya, Wada *et al* (1987) studied the clay minerals and humus complexes in five soils derived from volcanic ash. Clay minerals and humus complexes and their effects on soil chemical properties were studied for five profiles around the Longmont and Susua volcanoes in Kenya. Morphologically, all the profiles have some resemblance to Adepts, but there were clear cut differences in clay contents and mineral composition as well as pH between profiles 1 through 4 and profile 5, which occur under semi arid to sub humid climatic conditions, respectively. Profile 1 through 4 contained embryonic halloysites with X rays amorphous iron oxide and silica, where as profile 5 contained a kaolin mineral, chloritized 2:1 layer silicates, gibbsite and hematite. The carbon content of A horizons ranged from 100 to 112 g kg^{-1} in profile 2, 3 and 5. Humus complexes increased in order, Fe-humus <Al humus<Ca-humus in

profiles 2 ad 3, whereas Al- and Fe-humus were found exclusively in profile 5.

Soil-clay humus complexes isolated from 5 different soils (one each from Entisols, Alfisols, and Vertisols, and two from Mollisols) was investigated by Ahmed *et al* (2002) studied. In Alfisols sample, Ca^{2+}, and Mg^{2+} had small role in clay–HA bonding, and monovalent cation bonding was mainly through Fe^{3+} and Al^{3+}. Extract from Vertisols sample contains little Fe^{3+} or Fe^{2+} or Al^{3+} and major bonding was through Ca^{2+}. In Mollisols, Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} were involved in bonding and were highly correlated to extracted HA. The findings of this investigation showed that clay humus bonding in soils is dominantly influenced by the nature of the clay minerals and the nature of exchangeable cations. Schulthess and Haung (2009) studies the humic and fulvic acid adsorption by oxides of silicon and aluminium surfaces on clay minerals. The surface of the clay was modelled as mixture of amorphous Al and Si oxides. The result showed a strong adsorption of the organics by the Al sites on the Al oxide and a weak adsorption of organics by the Si sites on the Si oxide, mordenite and montmorillonite. At low pH values, the Si sites on the Si-oxide, mordenite and montmorillonite adsorbed fulvic acid (FA). The adsorption behaviour of HA and FA by montmorillonite, kaolonite and mordenite is closely related to the adsorption behaviour by Si- and Al- oxides. Multivalent cations will form organo-metallic complexes that significantly increases adsorption, particularly on Si sites, exception were found with some FA-metal complexes, which were attributed to the degree of complexation. The implication of these observations is that, in natural systems the adsorption of aqueous compounds is highly dependent on the type of amorphous surface present at the outermost layer of the solid phase in contact with the liquid phase. Laird *et al* (2001) studied the nature of clay-humic complexes in an agricultural soil. Soil management systems that encourage the formation and stabilization of recalcitrant clay–humic complexes will have the greatest long-term impact on C sequestration and soil quality. The objective of this study was to determine the relationship between the chemical, biochemical, and spectroscopic characteristics of humic substances and clay mineralogy. The samples were analyzed for mineralogy by XRD (x-ray diffraction), chemical composition by inductively coupled plasma-atomic emission spectorscopy (ICP-AES). The coarse, medium, and fine clay fractions are dominated by quartz, a low-charged interstratified phase, and smectite, respectively. Extractable organic compounds, 30 to 52% of the total C, are dominated by basic amino acids and polyunsaturated fatty acids. Less than 3% of the extractable C is monosaccharides and amino sugars and only trace levels of phenolic acids were found. The result indicates that the biochemistry of the clay–humic complexes differs substantially from that of whole soils and that soil clay mineralogy strongly influences humification.

Laird (2001) studied the nature of clay–humic complexes in an agricultural soil by using Scanning Electron Microscopy (SEM). The most stable forms of organic matter in soils are the clay–humic complexes. An understanding of mechanisms and processes influencing the formation of clay–humic complexes may facilitate development of agricultural management systems that increase the long-term sequestration of C in soils and improve soil quality. The specific objective of this study was to visualize associations between humic substances and clay minerals separated from a typical agricultural soil. All samples were Ca-saturated, dialyzed, and freeze-dried. The samples were analyzed by scanning electron microscopy to obtain images of clay–humic complexes and energy dispersive x-ray analysis to obtain maps of elemental distributions. Two distinct types of clay-associated humic substances were identified. The first type exists as diffuse filamentous films that cover basal surfaces of 2:1 phyllosilicates in the medium and fine clay fractions. The second type exists as discrete particles of high-density metal–humic substance complexes in the coarse clay fraction.

The spectroscopic, thermal and electron microscopy investigation on clay humus complexes was done by Mondal *et al* (1997). Montmorillonite-HA, illite-HA and kaolinite-HA complexes were studied by infrared spectroscopy (IR), Transmission Electron Microscopy (TEM) methods. From IR spectroscopy and TG analysis, no significant involvement of the OH groups of the clay minerals in complexation with HA (humic acids) could be detected. Furthermore, there is no detectable interaction of the free radicals of HA with Fe^{3+}/Mn^{2+} in the clay minerals. TEM showed sheet-like forms of free HA in kaolinite-HA complexes and surface deposits in illite-HA complexes. Montmorillonite-HA complexes showed extensive coating by HA as well as a reduction in the size of the clay particles as a consequence of the breaking up of clay platelets by HA.

2.5 Carbon sequestration in soil

Soil carbon sequestration is the process of transferring carbon dioxide from the atmosphere into the soil through crop residues and other organic solids, and in a form that is not immediately reemitted. This transfer or “sequestering” of carbon helps off-set emissions from fossil fuel combustion and other carbon-emitting activities while enhancing soil quality and long-term agronomic productivity. Soil carbon sequestration can be accomplished by management systems that add high amounts of biomass to the soil, cause minimal soil disturbance, conserve soil and water, improve soil structure, and enhance soil fauna activity. Continuous no-till crop production is a prime example. Carbon is a key ingredient in soil organic matter (57% by weight). Plants produce organic compounds by using sunlight energy and combining carbon dioxide from the atmosphere with water from the soil. Soil organic matter is created by the cycling of these organic compounds in plants, animals, and microorganisms into the soil. Well-decomposed organic matter forms humus, a dark brown, porous, spongy material that provides a carbon and energy source for soil microbes and plants. When soils are tilled, organic matter previously protected from microbial action is decomposed rapidly because of changes in water, air, and temperature conditions, and the breakdown of soil aggregates accelerates erosion. A soil with high organic matter is more productive than the same soil where much of the organic matter has been “burned” through tillage and poor management practices and transported by surface runoff and erosion. However, organic matter can be restored to about 60 to 70 percent of natural levels with best farming practices. Adding organic matter to farmland is good for soil quality and crop yields, both short-term and long-term. Continuous no-till is an efficient way of doing this. Cover crops and manure also help raise carbon levels. If you want to sequester carbon to reduce global warming (and possibly receive a small annual payment) think of it as a bonus for being a good farmer. Soil carbon sequestration is a natural, cost-effective, and environ-mentally-friendly process. Once sequestered, carbon remains in the soil as long as restorative land use, continuous no-till and other best management practices are followed. It also improves quality of soil and water resources, enhances agronomic productivity, and buys us time to identify and implement viable alternatives to fossil fuel.

Studies for a low carbon-storage potential of soils was done by Schlesinger (1990). Over most of the Earth's land surface, the amount of carbon stored in soil organic matter exceeds by a factor of two or three the amount stored in living vegetation. This pool of soil carbon is large (1.5×10^{18} g) and plays a dynamic part in the geochemical carbon cycle. Under terrestrial vegetation and soils would act as a large sink for atmospheric carbon dioxide if its concentration were twice the present level. He uses data from chronosequence studies to show that the production of refractory humus substances in soils sequesters only $\sim 0.4 \times 10^{15}$ g C yr^{-1} from the atmosphere, accounting for just 0.7 percent of terrestrial net primary production. Moreover, agricultural practices tend, on balance, to cause a release of soil carbon to the atmosphere. Thus if the terrestrial biosphere is indeed to act as a carbon sink under future elevated levels of carbon dioxide, this would be more likely to be the result of changes in the distribution and biomass of terrestrial vegetation than of changes in the accumulation of soil organic matter.

Lal (2004a) observed that with a large land area and diverse ecoregions, there is a considerable potential of terrestrial/soil carbon sequestration in India. Of the total land area of 329 million hectares (Mha), 297 Mha is the land area comprising 162 Mha of arable land, 69 Mha of forest and woodland, 11 Mha of permanent pasture, 8 Mha of permanent crops and 58Mha is other land uses. The soil organic carbon (SOC) pool was estimated as 21 Pg at 30-cm depth and 63 Pg to 150-cm depth. The soil inorganic carbon (SIC) pool was estimated as 196 Pg to 1-m depth. The SOC concentration in most cultivated soils was less than 5 g kg^{-1} compared with 15 to 20 g kg^{-1} in uncultivated soils. Low SOC concentration is attributed to plowing, removal of crop residue and other biosolids, and mining of soil fertility. Accelerated soil erosion by water leads to emission of 6 Tg Cyr^{-1}. Important strategies of soil C sequestration include restoration of degraded soils, and adoption of recommended management practices (RMPs) of agricultural and forestry soils. Further Marland *et al* (2004) Studied on enhancing carbon sequestration in soils. Both the quantity and the quality of soil Carbon inputs influencing Carbon storage and the potential for Carbon sequestration. Changes in tillage intensity and crop rotations can also affects C sequestration by changing the soil physical and biological conditions and by changing the amounts and types of organic inputs to the soil. Analyses of changes in soil C and N balances are being supplemented with studies of the management practices needed to manage soil carbon and the implications for fossil-fuel use, emission of other greenhouse gases (such as NO_2 and CH_4), and impacts on agricultural productivity. Ecosystem and landscape-scale studies of soil C and N are important to develop sound strategies for enhancing C sequestration in terrestrial ecosystems. Rates of C sequestration following a change in tillage are predicted to reach a maximum in 5–10 years and decline toward 0 kg C ha yr^{-1} in 15–20 years as a new steady state for soil C is approached. A decrease in tillage results in increased rates of C sequestration by changing soil physical and biological conditions, thereby promoting the formation of soil aggregates and influencing decomposition rates of soil organic C. Changes in crop rotations affect the quantity and quality of litter and thus the input of organic matter to the soil. Increasing the quantity of litter in wheat-fallow systems by decreasing the number of fallow periods resulted in increased C sequestration rates. Changing the quality of litter by moving from continuous cropping to rotation cropping increased C sequestration rates in most cases, regardless of whether there was a simultaneous increase in litter quantity (with the exception of a move from continuous corn to corn-soybean rotation).

The scope of horticultural land use on carbon sequestration in semi-arid tropics of Andhra Pradesh, India was studied by Chandran *et al* (2009). The carbon stock changes expressed per unit area indicate that the forest system may have twice (169.8 Mg ha^{-1}) the amount of SOC at all depths than the horticultural system (73.1 Mg ha^{-1}). The agricultural system had the lowest SOC stock (63.1 Mg ha^{-1}). Organic carbon in tropical soils appears to be more easily degradable than in temperate soils and hence increasing SOC content of soils of the tropics and subtropics is a huge task .They studied the spatial distribution of C stock in different agricultural land-uses. The total soil C in the agricultural lands amounted to 12 Tg, of which land-uses, such as para rubber, mixed orchard, pineapple, cassava and sugarcane–cassava contributed 27.8, 18.2, 17.7, 13.3 and 10.6 percent, respectively. The results of soil C distribution in combination with biomass of the respective land-uses of an agro ecological zone could be used as valuable information in selecting land-use options that contribute in C sequestration. The study reveals that tree crop species improve biomass C while shrub crop species enhance soil C. This indicates the potential for adopting a mixed land-use of tree and shrubs for better C sequestration due to complementary effect of combining them.

The catchments may behave as sources or sinks of soil carbon, depending on the magnitude and type of land use changes within their drainage area, on the intensity of erosion processes and on the fate of eroded sediments. The impact of changing land use on the organic soil carbon (C) stock and the soil C transported by water erosion and buried in depositional wedges behind check-dams was estimated in a Mediterranean catchment in Spain. Changes in land use patterns in the catchment between 1956 and 1997 (57% decrease in areas dedicated to agriculture and 1.5-fold increase of the total forest cover) induced an accumulation rate of 10.7 g m^{-2} $year^{-1}$ of total organic carbon (TOC) in the soil. Mineral-associated organic carbon (MOC) was the main soil carbon pool (70%). Particulate organic carbon (POC) was highest in the scrubland soils (33%). The average sediments/soil enrichment ratio at the sub-catchment scale (8–125 ha) was 0.59 ± 0.43 g kg^{-1}. The soil C replacement due to increased vegetation cover between 1974 and 1997 represented a 36 percent of the original soil organic C stock. All together represent an erosion-induced sink of soil organic C of 40% compared to the original levels of 23 years ago.

Lichter *et al* (2005) studied the soil carbon sequestration and turnover in a pine forests after six years of atmospheric CO_2 enrichment. During the first six years of atmospheric CO_2 enrichment at the Duke Forest free-air CO_2 enrichment experiment, an additional sink of 52 +_ 16 g C m^{-2} yr^{-1} accumulated in the forest floor (O-horizon) of the elevated CO2 treatment relative to the ambient CO_2 control in an aggrading loblolly pine (*Pinus taeda* L.) forest near Chapel Hill, North Carolina, USA. Results indicate that forest soils such as these will not significantly mitigate anthropogenic C inputs to the atmosphere. The organic matter pools receiving large annual C inputs have short mean residence times, while those with slow turnover rates receive small annual inputs.

Lal (2004b) studied the potential of carbon sequestration in the soils of south Asia. Assessment of the potential of carbon (C) sequestration in soils of 8 countries in South Asia (Afghanistan, Bangladesh, Bhutan, India, Iran, Nepal, Pakistan and Sri Lanka) is made on the basis of the available information on the area and soil C dynamics for different land use and soil management practices. Out of a total land area of 642 million hectares (Mha), 218 Mha is cropland including 89 Mha of irrigated cropland, 85 Mha is forest and woodland, 13 Mha is permanent crops and 94 Mha is permanent pasture. Estimates of area affected by soil degradation processes include 82 Mha by water erosion, 11 Mha by wind erosion, 11 Mha by fertility decline, 13 Mha by water logging, 33 Mha by salinization, and 83 Mha by desertification. Land use, management practices, and degradation processes in different countries are studied such as in Bangladesh soil degradation and nutrient depletion, Intensive tillage, puddling, accelerated erosion etc. In India residue removal for fodder and fuel, excessive grazing, and using dung for fuel, Unbalanced fertilizer use, accelerated erosion, desertification, and soil degradation. In Pakistan accelerated erosion, monoculture and in Sri

Lanka accelerated erosion, deforestation and shifting cultivation leads to decline in soil organic matter content in soil. Important processes are recommended in the countries for enhancing C percentage in soil these are restoration of degraded soils and ecosystems, agroforestry and a forestation, grazing management and adoption of RMPs on cropland. The latter includes integrated nutrient management such as use of manure, compost, green manuring and other biosolids including city sludge, mulch farming, conservation tillage, and diverse crop rotations based on legumes and cover crops in the rotation cycle. Soil conservation and water management, water harvesting and recycling, are important strategies of minimizing losses and restoring soil quality.

The effect of land use processes on soil carbon sequestration was studied by Post and Kwon (1999). Soil organic carbon can accumulate by processes that essentially reverse some of the effects responsible for soil organic carbon losses from soil when the land was converted from perennial vegetation. There are many factors and processes that determine the direction and rate of change in SOC content when vegetation and soil management practices are changed. Ones that may be important for increasing SOC storage include (1) increasing the input rates of organic matter, (2) changing the decomposability of organic matter inputs that increase LF-OC in particular, (3) placing organic matter deeper in the soil either directly by increasing belowground inputs or indirectly by enhancing surface mixing by soil organisms, and (4) enhancing physical protection through either intra-aggregate or organomineral complexes.

Davidson and Janssens (2006) studied the temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Significantly more carbon is stored in the world’s soils-including peat lands, wetlands and permafrost-than is present in the atmosphere. Disagreement exists, however, regarding the effects of climate change on global soil carbon stocks. If carbon stored belowground is transferred to the atmosphere by a warming-induced acceleration of its decomposition, a positive feedback to climate change would occur. Conversely, if increases of plant-derived carbon inputs to soils exceed increases in decomposition, the feedback would be negative. Despite much research, a consensus has not yet emerged on the temperature sensitivity of soil carbon decomposition. Unraveling the feedback effect is particularly difficult, because the diverse soil organic compounds exhibit a wide range of kinetic properties, which determine the intrinsic temperature sensitivity of their decomposition. Moreover, several environmental constraints obscure the intrinsic temperature sensitivity of substrate decomposition, causing lower observed ‘apparent’ temperature sensitivity, and these constraints may, themselves, be sensitive to climate.

Further the long-term sensitivity of soil carbon turnover to warming was studied by Knorr *et al* (2005). The sensitivity of soil carbon to warming is a major uncertainty in projections of carbon dioxide concentration and climate. Their studies overwhelmingly indicate increased soil organic carbon (SOC) decomposition at higher temperatures, resulting in increased carbon dioxide emissions from soils. They present a simple three-pool model that partitions SOC into components with different intrinsic turnover rates. Using this model, we show that the results of all the soil-warming experiments are compatible with long-term temperature sensitivity of SOC turnover: they can be explained by rapid depletion of labile SOC combined with the negligible response of non-labile SOC on experimental timescales.

Senapati *et al* (2010) investigated the impact of soil amendments on organic carbon pools under a rice-wheat cropping system. Rice-wheat cropping is the dominant cropping sequence in the Indo-Gangetic plains (IGP) of India. An experiment was conducted to study the impact of continuous application of farmyard manure (FYM) and rice straw (RS), either alone or in conjunction with fertilizer nitrogen (N), under a rice-wheat cropping system on i) total soil organic carbon (SOC) and slow pool C, and ii) stabilization of cumulative input C. Application of FYM, after seven years of rice-wheat cropping cycles, increased total SOC and slow pool C at 0-0.15 m soil depth by 6.7 t ha^{-1} and 1.5 t ha^{-1}, respectively, with the highest effect when FYM, RS and fertilizer N were applied together. About 18.5percent and 4.2 percent of the cumulative input C were stabilized as total SOC and slow pool C, respectively, due to application of FYM; values for RS were 17.9 percent and 3.3 percent, respectively.

Liu *et al* (2006) studied the Effects of agricultural management on soil organic matter and carbon transformation. Soil organic carbon (SOC) is the most often reported attribute and is chosen as the most important indicator of soil quality and agricultural sustainability. In this review they summarized how cultivation, crop rotation, residue and tillage management, fertilization and monoculture effect soil quality, soil organic matter (SOM) and carbon transformation. The results confirms that SOM is not only a source of carbon but also a sink for carbon sequestration. Cultivation and tillage can reduce soil SOC content and lead to soil deterioration. Tillage practices have a major effect on distribution of C and N, and the rates of organic matter decomposition and N mineralization. Proper adoption of crop rotation can increase or maintain the quantity and quality of soil organic matter, and improve soil chemical and physical properties. Adequate application of fertilizers combined with farmyard manure could increase soil nutrients, and SOC content. Manure or crop residue alone may not be adequate to maintain SOC levels. Crop type influences SOC and soil function in continuous monoculture systems. SOC can be best preserved by rotation with reduced tillage frequency and with additions of chemical fertilizers and manure. The Soil organic carbon sequestration with conservation agriculture in the south eastern USA was revived by Franzluebbers (2011). The carbon (C) cycle is the basis for greenhouse gas emissions and global warming. Globally, agriculture is responsible for about 20 percent of the greenhouse gas emissions. Mean soil organic C sequestration rate in cotton production systems was estimated at 0.48 +

0.56 Mg C ha^{-1} yr^{-1} from 41 observations throughout the southeastern USA. Increased cropping system complexity would be expected to create a diversity of crop residue qualities, crop rooting depths and patterns, and possibly greater C inputs. Conservation tillage, increased cropping system complexity, cover cropping, animal manure application, optimum fertilization, and rotation of crops with pastures are effective strategies to enhance soil organic C sequestration.

Sun *et al* (2010) investigated the Carbon sequestration and its potential in agricultural soils of China. Agricultural soils hold potential for the expansion of carbon sequestration. Results suggest that a significant increase in the SOC occurred in east and north China, while a decrease appeared in northeast China. As a whole, the organic carbon density in the topsoil to 30 cm depth increased by 3.36 (2.54 to 4.26) Mg ha^{-1} between 1980 and 2000. Accordingly, the croplands in China that cover an area of over 130 Mha sequestered 437 (331 to 555) Tg C, with an average rate of 21.9 (16.6 to 27.8) Tg yr^{-1}, during this period. The potential of SOC sequestration in China was estimated to be 2–2.5 Pg C, which could be achieved by the 2050s if crop production and field management are improved.

2.6 Sample preparation protocols to obtain high resolution images of clay humus complexes under electron microscopy

Keat (2010) studied the microstructure and mineralogical study of organic soils. Organic soils normally are one of the soft soils. Constructions on these soils are often affected by stability and settlement problems. In order to counter these problems, one has to know the mineral content of the organic soils. Therefore, the objective of their study was to determine the microstructure, mineral content, and functional group of organic soils. This study is carried out to identify the mineral content of organic soils such as kaolinite, smectite (momtmorillonite) and biotitie, attapulgite, gibbsite, and also the functional group. X-rays diffraction (XRD), X-rays fluorescence (XRF), Fourier transform infra-red (FT-IR), thermogravimetric analysis (TGA), and energy dispersive analysis by X-rays (EDAX) are used to determine the mineralogy of organic soils. Scanning electron microscopy (SEM) is used to study the microstructure of organic soils. The results show that quartz and kaolinite are two major minerals. The percentages of SiO_2 found in the most soil samples are higher than the total percentages of Al_2O_3 and Fe_2O_3, which indicates the major presence of quartz and kaolinite.

Sample preparation: To avoid the loss of moisture content, soil samples were being seal in the transparent plastic bag once they were taken out from the land. Once the soil samples were reached to laboratory, the soil samples should move to store room immediately. The store room should be under air-condition with temperature around 20° C. A clean spatula should be used to move out the soil samples from tube. About first 100mm in the end of soil samples should be throwing away due to the disturbance possibility.

Fourier Transform Infra-red (FT-IR): Potassium Bromide (KBr) pressed disk technique was useful for a routine characterization of both di and tri octahedral clay minerals and for examination of structural modification of smectite during acid treatment. Samples for infrared analysis were prepared in a suspension form, called mull. The soil sample was made into a thick slurry by mixing it with a viscous liquid, e.g. nujol (paraffin oil), or chlorofluorocarbon grease. By viewing the spectrum, mineral content in soil can be determined such as kaolinite, smectite (momtmorillonite) and biotitie, attapulgite, gibbsite.

Scanning electron microscopy (SEM) and energy dispersive analysis by X-rays (EDAX): The soil-clay could be extracted and mouted on carbon-shielded SEM stubs. After coating twice with carbon or gold, the samples could be scanned with the SEM equipped with EDAX. The SEM and EDAX micrographs of the soil samples would show the strong peak of certain minerals. Identification of soil minerals could be made through these experiments. In EDAX, the specimen was first viewed by SEM, and a tiny spot or a line or an area selected for determination of the element concentrations. For this purpose, the electron beam was focused at the specimen surface exactly on the selected spot to be analyzed. Laird (2001) studied the nature of clay- humus complexes in an agricultural soil: Scanning Electron Microscopy Analysis. The specific objective of this study was to visualize associations between humic substances and clay minerals separated from typical agricultural soil. The samples were analyzed by scanning electron microscopy to obtain the images of clay- humus complexes and energy dispersive X ray analysis to obtain maps of element distribution. The whole soil clay fractions was separated by a relatively mild sedimentation technique and coarse, medium, and fine clay fractions (0.2- 2.0, 0.02- 0.2 and <0.02µm size fractions, respectively) were separated from a portion of the whole clay sample by an aggressive sonication- centrifugation technique. All the samples were Ca saturated, dialyzed and freeze dried.

Mcatee *et al* (1982) studied the electron microscopy of smectites prepared by low temperature freeze-drying. A low temperature freeze-drying method was used for the preparation of clay samples for transmission electron microscopy. After depositing a carbon film on freshly cleaved mica, the sample is freeze-dried, shadowed and a second carbon film deposited. Electron micrographs obtained of dispersions of smectites and organosmectites prepared by this method show pronounced "stringing" or end-to-end association of the clay particles.

The SEM-EDX characterization of Iron- rich kaolinite clay was investigated by Sengupta *et al* (2008). Kaolin clay from Deopani deposit of Assam contain high amount of Iron, Kaolinite particles, characterize by scanning electron microscopy (SEM) – EDX are pseudo hexagonal and arranged in face to face pattern. Clay particles are coated with Iron (Fe) and Titanium (Ti) bearing minerals which can be separated by wet high intensity magnetic separator. Titaniferrous impurities present as coating on kaolinite particles are difficult to remove by oxalic acid treatment. Fe and Ti contents of samples were estimated by wet chemical analysis and X ray fluorescence (XRF) method. Samples were prepared by dispersing dry powder by on double sided conductive adhesive tape. Samples were coated with carbon by arc discharge method of SEM. Samples were scanned in scattered electron (SE) for morphology analysis and back scattered electron (BSE) modes for compositional images. Particles with white patches were analyzed by EDX to ascertain presence of Fe and Ti.

Sokolov *et al* (2007) studied the Quantitative Analysis of Pore Space of Moraine Clay Soils by SEM Images. Problems of studying the pore space of moraine clay soils are considered by analyzing SEM images of the surface of samples prepared using a special technique. A new algorithm for estimating the pore shape is described. The study of the microstructure of moraine clay soils showed that they are characterized by five pore types. These micropores are formed at the interface between the surface of large sand–dust quartz or feldspar grains and the finely divided unoriented clay matrix. Such pores can be ways of localized groundwater filtration through soils, significantly affecting the soil properties. To obtain correct information on the size and shape of large pores at low SEM magnifications (50–1000) shooting was performed in the cathodoluminescence (CL) mode on samples prepared as polished sections impregnated with luminescent polymer. In this case, pores filled with such polymer are reproduced in SEM images as white areas with distinct boundaries. Images of small pores were obtained when studying the cleavage surfaces at high magnifications (2000–32 000) in the secondary electron (SE) mode.

Nanomorphology of kaolinite was observed by Zbik and Smart (1998) by SEM and AFM. Nanomorphological structure of well-crystallized Georgia and poorly crystallized North Queensland kaolinite particles have been compared using field emission scanning electron microscopy (SEM) and atomic force microscopy (AFM). The SEM studies were carried out using a microscope with a field emission gun operating normally at 20 kV acceleration voltage. The kaolinite particles were coated by gold/palladium films to a thickness of 2-3 nm using a Denton Magnetron Sputter Coater system. A Nanoscope II AFM (Digital Instruments Inc.) was used with scan heads A (1X1 μm) and E (14 X 14 μm) and scan rate between 5 and 15 Hz, in height and deflection modes. The AFM was calibrated on a gold grid with 5 μm pits separated by 5 μm. The standard pyramidal silicon nitride tip with a solid angle of 70^0 C and a radius of curvature at the end of the tip of 50 nm was mounted on a cantilever of nominal spring constant 0.06-0.58 Nm^{-1}. For the AFM studies, kaolinite particles were immobilized on an atomically flat mica surface (freshly cleaved) from a dilute suspension in water. Three drops of ammonia solution were added to disperse the kaolinite, the sample was sonicated and allowed to sediment in a glass cylinder for 24 h. A small amount of suspension from the glass cylinder, (1 or 2 droplets) was placed on the freshly cleaved mica square. The particles were dried by heating to 400^0c for a few minutes. Laird *et al* (2008) studied for distinguishing black carbon from biogenic humic substances in soil clay fractions. The Ca-saturated clay sub fractions and the <1.5 g cm^{-3} density fraction of the coarse clay sub-fraction were prepared for SEM analysis by dispersing the samples in dionized water and transferring one drop of suspension to silicon wafers mounted on SEM studs. Some samples were sputter-coated with Au/Pd to reduce surface charging and enhance image quality. Uncoated samples were analyzed by both secondary electron imaging and energy dispersive X-ray (EDX) analysis using a Kevex Instruments Quantum EDX detector (Thermo Fisher Scientific, Inc., Waltham, MA). The necessity of using uncoated samples and removing the final SEM aperture to obtain EDX spectra degraded image quality. Most of the C in the coarse clay sub fraction was present as discrete particles (0.2–5 μm as seen in SEM images) of black carbon (BC) and consisted of approximately 60% aromatic C, with the remainder being a mixture of aliphatic, anomeric and carboxylic C. The results indicate that the biogenic humic materials in our soils have little aromatic C, which is inconsistent with the traditional heteropolymer model of humic substances.

The Preparation of soil specimens for SEM analysis using freeze-cut-drying was done by Shi *et al* (1999). A new method, freeze-cut-drying, is introduced for preparing a soil specimen for micro structural analysis using scanning electron microscopy (SEM). With this method, moisture in the specimen is removed by freeze-drying and an SEM observation surface is made by cutting the specimen at a temperature below freezing point. Tests on bentonite clay were used to compare the results from this method with those using other existing techniques such as peeling. Image processing was employed to analyze the SEM micrographs quantitatively in terms of distributions of size and orientation of particles and pores in the soil. The results indicate that this method can preserve the original soil microstructure and minimize flaws such as false voids and erroneous orientations which frequently occur in SEM images obtained from specimens prepared by other techniques. This method is particularly suitable for clay soils with high moisture contents and should find wide application in the analysis of soft clays, slurries and marine sediments.

2.7 Impact of land use on complexes of humus with clay-minerals and soil-clays

The effects of land use change on carbon storage are of increasing concern in the context of greenhouse gas emissions mitigation, and the first of all are those associating with the conversion of native forest into agricultural systems, especially in the tropical region. Total SOC stock under different land use systems varies significantly. Many of the studies regarding SOC are restricted to upper 10-15 cm of soil because of higher concentration of SOC in those soil layers. However, it has been shown that important amounts of stable soil organic carbon (SOC) are also stored at greater depths. For understanding the SOC sequestration potential in different land use we must penetrate into deeper soil layers as it is not essential that soils having higher concentration of SOC in surface soils must also have higher SOC content in the deeper soil layers or vice-versa. Some study reveals that SOC stock in the deeper soils showed significant variability among different land uses.

Proper management of croplands, forests, grasslands, and restored lands is the key to any potential C sequestration in the soil. The magnitude of soil disturbance and the amount of residue incorporated into the soil impact the aggregate and SOC dynamics. Lal (2004) estimated that the global potential of SOC sequestration ranges from 0.4 to 0.8 Pg C yr^{-1} in croplands, 0.2 to 0.4 Pg C yr^{-1} in forest and degraded/desertified lands, 0.01 to 0.3 Pg C yr^{-1} in rangelands and grasslands, and 0.01to 0.03 Pg C yr^{-1} in irrigated soils. The world soils can sequester between 0.4 and 1.2 Pg C yr^{-1}. Forest soils favour transformation of carbon in the below-ground portion. Thus, the confinement of SOC in the forest subsoils is essential for long-term storage of C, because it is stabilized in the soil matrix against biological decomposition. The extent of this stabilization is determined by organo-mineral interactions, micro-pores, type and nature of clay surfaces, and C location within the micro-aggregates.

Yang and Singh (2004) investigated the effects of land use (forest, grassland and agricultural lands) and management practices (crop rotation, fertilization and cover crops) in a watershed on SOC fractions. Higher content of SOC existed in forest soils compared to those from grassland and agricultural areas. The rotation of grain with grass showed a significantly higher SOC compared to grain alone. The grassland soil has a significantly higher FA fraction as compared to forest soils and agricultural soils, while agricultural soil contained lower HA fraction and higher humin fraction than the other two land uses. The rotation of grain+ grass led to a higher C content in HA, and humin fractions and a lower C in FA fraction as compared with grain alone. The increase in HA and humin fractions caused by farmyard manure application was higher than that by chemical fertilizers. Forest soil showed higher HA/FA ratio and lower (HA+FA)/SOC ratio, which indicated that higher degree of humification and larger humification rates in forest soil were obvious as compared to other land uses.

Lemenih and Itanna (2004) investigated the soil C stock and turn over in five vegetation types and following deforestration and conversion of each vegetation types into arable lands in southern highlands of Ethiopia. The soils from farmlands had significantly lower soil C stock than that under the natural vegetations. Losses of C from upper 0-10 cm soil depth following conversion of the natural vegetation to farmlands were highest at humid climatic forest and lowest at semiarid climatic forest. The average rate of soil C losses ranged between 2.0% and 3.0% per annum in the sub-humid to humid eco-climatic zone and 0.5-1.0% per annum in the semiarid lowland or the cool sub-afroalpine eco-climatic zones.

Studies in the tropics showed similar decline in soil organic matter following deforestration and conversion into intensive land uses such as agriculture. The effects of land use changes and soil types on the storage and stability of different SOC fractions in the tropics was studied by Paul *et al* (2008). The origin of organic carbon stored in free light (< 1.6 g cm^{-3}) fractions, and in two light fractions (LF) occluded within aggregates of different stability, was determined. Irrespective of parent material, the contribution of the LFs to SOC storage was greater in natural forest (19.2-1.2%) and secondary forest (16.6-1.0%) than in pasture soils (12.8- 1.0%). Changes in SOC stocks were more pronounced in the LFs compared with bulk soil. Thus, LFs can be used as an early indicator for SOC changes induced by land use changes.

Further Yang *et al* (2009) evaluated the effects of forest transition (conversion of natural broadleaf forests into monoculture tree plantations) on soil labile fractions. Soil samples were collected from a natural forest of *Castanopsis kawakamii* Hayata (NF) and two adjacent 36-year old monoculture plantations of *C. kawakamii* (CK) and *Cunninghamia lanceolata* Lamb. They observed that in the 0–10 cm layer, the concentration of LFOC in NF was 41 and 53 percent higher than those in CK and CF, while the concentrations of POC in NF were 2.2– 4.8 times than those of CK and CF. This study established the implication that plantation establishment by converting the natural forest may lead to a reduction in labile organic C in soils and could threaten the negative impact on C cycling as well as the prospects for sustainable management.

Tan *et al* (2007) studied the distribution of light and heavy SOC fractions influenced by land use and management systems and observed that C concentration of light fractions in all aggregate classes were significantly higher in soils under forest than under conventional tillage. This study suggested that conversion from forest to agriculture is attributed to reduction in C concentrations in both heavy and light fractions. Lower concentration of light fractions in macro-aggregates particularly in the conventional tillage than no-tillage is attributed to the destruction of larger aggregates by mechanical disruption and subsequent oxidation of protected light fractions.

Marcos and Juan (2006) observed that cultivation leads to the decrease of different SOC fractions like particulate organic matter, carbohydrate, humic acid contents. The increase in micro- aggregates as a result of long term cultivation also causes complete loss of its particulate organic carbon content due to collapse of the macro-aggregates resulting in rapid oxidation and microbial attack. There were indications suggesting that particulate organic carbon and hot water extractable carbohydrate contents to be valuable indicators of soil structure degradation due to exhaustive cultivation practices. Although it is well known that humic substances are chemically and structurally much more stable than non-humic substances but the results showed a surprising decrease in humic substances under continuous cultivation.

Pikul *et al* (2007) determined the effect of cropping rotation and soil management (including tillage, crop rotation, native grass pasture, and corn) on SOM and its, components in the surface soil. The soil carbon (SC), SOM, fine particulate organic matter (fPOM), and coarse POM were measured in different sized aggregates. No tillage (NT) increased fPOM/SOM by 19 and 37 percent compared with tillage following 4 and 10 years of NT, respectively. There was a 36 percent increase of fPOM/SOM compared with monoculture by a 5-yr diverse rotation. Diversity of rotation or reduction of tillage increased fPOM and this may help to curb soil loss by maintaining surface conditions resistant to erosion.

Wander *et al* (1994) studied whether 10 yr of organic or conventional management generated differences in biologically active SOM pool. The soils receiving organic treatments accumulated biologically active C. In the cover-cropped soil, higher total C and N, particulate SOM, and reduced water dispersible organic C contents indicated that its SOM was more stable than SOM in the other organic treatments. The conventionally managed soil had the lowest biological activity. Their experiment characterised that particulate organic matter is the best index of biologically active SOM because it documented important quality (i.e., biological liability) and quantity aspects of SOM character in the soils.

Ashagrie *et al* (2007) observed that conversion of natural forest to cultivated land always accompanied with a decline in SOC and nutrients, and deterioration of soil structure. The free light fraction C (free LF) and N are more likely to be affected by cultivation than the amounts of intraparticulate organic matter (iPOM) C and N. Similar results have been reported for situations dealing with the conversion of native forest to corn cultivation and native grassland soil to cultivation). With 35 years of continuous cultivation of a soil under virgin brigalow (*Acacia harpophylla*), as much as 95 percent OC loss from the light fraction. also reported losses of substantial amounts of both free and physically protected organic matter following 32 years of cultivation compared with sod. Higher iPOM levels in water stable macro-aggregates sampled from native sod soil than from cultivated soil. In the natural forest, the proportion of whole soil OC and N found in free LF accounted for 55 percent and 47 percent, respectively, and that of iPOM C and N comprised 8 percent and 6 percent, respectively. SOC pool lost during cropping of native pasture soils. The study indicated that POC is the form of organic C that is preferentially lost during cropping; on average it made up 70 percent of the difference in organic C between native pasture and cropped land in 0-5 cm layer. It is also the form of organic C regained when cropped soils were returned to pasture. Significant relationship was found between POC and water stability of the > 250 µm size fraction than that of the < 50 µm fraction.

Yong (2007) reported that conversion of annually cultivated land to forage grasses has potential to increase C and N sequestration. Organic C, total N, POM-C, and POM-N contents in the 0–5 cm layer were significantly greater in alfalfa field than in adjacent cropland but in the entire 0-20 cm profile, there were significant differences in SOC, POM-C and POM-N but not in total N between alfalfa and crop soils. POM-C and POM-N showed greater difference between the two land-use treatments than in SOC and total N.

Ratnayake *et al* (2011) evaluated the effect of cultivation on organic carbon content in the clay mineral fraction of soils. Conversion of native land, into a cultivated system causes precipitous degradation of the soil organic matter. It is reported that C stored in the clay mineral fraction contributes more to the long-term stability of C than sand and silt fraction. This study reports on the soil organic carbon in the clay mineral fraction of some cultivated lands in Sri Lanka and how it deviates from the adjacent natural forests. The field sites included 7 cultivated lands (tea, rubber, coconut, mixed crops, potato, home garden, chena cultivation) and 7 tropical forest types (wet evergreen, semi evergreen, moist monsoon, dry monsoon, montane, dry mixed evergreen). The significant differences in carbon content of the clay fraction between the cultivated land and the adjacent forest in the same location revealed that cultivation has decreased the carbon content in all sites. The highest C content shown by the mixed crop and rubber plantations showed that minimum land management and soil tillage involved with this reduced the rate of decomposition. High temperature of dry mixed evergreen forest and dry monsoon forest may have increased the decomposition rates and lowered the SOC in the clay fraction compared to the other forests. Significant correlations were observed in forests with in clay fraction and climatic parameters but to a lesser extent in cultivated lands. Considerable variations in organic matter input in cultivated lands will be the reason for these weak relationships. The study confirmed that clay mineral fraction was also equally affected during long term cultivation although it was reported to be more stable against rapid decomposition.

Schulten *et al* (1990) studied the characterization of cultivation effects on soil organic matter humus properties in various Ap horizons from field plots, that have been cultivated in long-term experiments under different management conditions, were investigated by pyrolysis-field ionization mass spectrometry (Py-FIMS). The results of Py-FIMS were evaluated by correlation and principal component analysis from reproducible data sets of bulk soil samples and extracted humic substances, and allowed a distinct discrimination on the basis of humus quality and composition. The chemical subunits suitable for discrimination are the major plant constituents carbohydrates, lignin, and proteinaceous materials as well as their humification products. The contribution of these compound classes to soil organic matter decreased with the intensity of management. CPMAS and solution spectra of soils and humic substances demonstrated that with more intense management, both the intensities of the phenolic region (140–160 ppm) and the aromatic region (110–140 ppm) decreased. The combination of both independent methods MS and

NMR, together with microbiological and biochemical data, yields the general result that intensive soil management leads to a less active humus.

Land use systems affect the soil profile carbon stock. Conversion of native ecosystem to cultivated land cause rapid loss of soil C from the upper surface. The C sequestration potential of grassland and forest in a soil profile are quite apart. Forest trees favour the transfer of C into the deeper soil layers due to their deeper root systems. The overall C stock is more in forest soils due to more above-and below-ground biomass. However, most of the studies are concerned with mainly upper few cm of soils although sufficient amount of C might be present in the deeper layers particularly in forest soils which are more protected. Thus, a thorough understanding of the dynamics of the C stock in relation to land use and land management strategies is of paramount important to identify the pathways of C sequestration in soils and for maintaining SOC at a level that is essential for maintaining soil fertility and to cope up with the global worming effects.

CHAPTER III

MATERIALS AND METHODS

3.1 Soil sampling site

The soil samples were collected from Bahadurgarh ($30^{o}21^{/}45^{//}$N latitude, and $76^{o}28^{/}03^{//}$E longitude with an elevation of 255 metres above the mean sea level) of the Patiala district in the State of Punjab in the north-west of India. It belongs to Northern Plain, Dry Sub Humid with Length of Growing Period of 120-150 days Agro-Ecological Sub Region.

3.2 Geology and clay mineralogy

Geology: The study area comprises of the unconsolidated alluvial deposits of the Indo-Gangetic Plain, whose expanse has stretched along the whole length of the Himalayas from Punjab to Assam, and separates the Peninsular block from the Extra-peninsular Mountains. The alluvial tract covers an east-west rift or fore-deep that has orogeny in front of the Himalaya. This fore-deep was filled with alluvial sediments brought by rivers of the Indus and the Ganges systems, and basement topography and geological structures are completely obscured. The exact depth of alluvium has not been ascertained but some gravity, magnetic and seismic explorations showed that the deposits of the Indo-Gangetic Plain vary in thickness from about 1000 m to up to 2000 m, and chronologically of the Late Pleistocene to the recent epoch of the Quaternary period. The Indo-Gangetic Plain in north-western India, particularly of Punjab is formed by the deposition of alluvial sediments brought by rivers of the Indus system. The rivers in the process of formation of land, kept on shifting their channels as evident from the presence of paleochannels in the area. The alluvial deposits, thus, show varied textural compositions; from clayey to sandy. The soils of the study area have developed in the alluvial deposits of generally finer texture (silt loam / silty clay loam / clay loam). The deposits are generally calcareous, and occasionally contain calcium carbonate nodules / concretions.

Clay Mineralogy: X-ray diffractograms of clays showed that illite (77 to 82%) was most dominating mineral. It was predominantly dioctahedral as evident from the ratio of peak intensities between 0.49 and 0.99 nm. Smectite was present in small amount (5-8%), followed by Fe-rich chlorite (4-8%), vermiculite (3-5%), and kaolinite (4%).

3.3 Climate

The climate is Semi-Arid (Semi-Dry) less-hot. The region receives an annual rainfall of 674.2 mm, out of which 77% is received during summer monsoon period (521.2 mm; June through September). The potential evapo-transpiration (PET) is 1398.3 mm, which is 207% of the mean annual rainfall. Rainfall exceeds PET only in January, February and August. The mean annual temperature is 24.5° C, mean summer temperature is 30.4°C, and mean winter temperature is 18.6° C, and mean monthly temperature is more than 20°C in seven months in a year.

3.4 Collection and processing of soil samples

Three samples from genetic surface horizon were collected; two from the fields of the University orchard (one under mango; *Mangifera indica* and other under guava; *Psidium guajava* plantations and one from the nearby cultivated field under rice; *Oryza sativa* –wheat; *Triticum aestivum*) cropping system in the Bahadurgarh. In case of orchard sampling, samples were collected in between the tree rows and in cropped land samples were collected when there was no crop in the field. Samples were dried under shade, ground with wooden pestle and mortar and passed through 2-mm sieve.

3.5 Extraction and purification of clay

3.5.1 Extraction of clay

No pre-treatments were given to the soil samples to avoid any kind of alteration. In each batch, 100 ml of distilled water was added to 30 g soil and stirred for 30 min. To achieve thorough dispersion, soil suspension was sonicated for 5 s at 1s pulse rate with Ultrasonicator (Model: Oscar PR1000 MP). Soil suspension was then sieved through 50 µm (300 mesh inch^{-1}) sieve into the 1 litre sedimentation jar following the procedure described by Jackson (1969). Sand (2-0.02 mm) remained on the sieve, and silt (0.02- 0.002 mm) + clay (< 0.002 mm) passed through it. The sedimentation jar then filled with water, and suspension was dispersed by plunging. Temperature of the suspension was noted after the plunging of silt+clay sample and the clay was decanted up to 10 cm depth after the recommended time adhering to the requirement of Stock's Law. The decantation of clay continued till suspension became free from clay. Saturated NaCl solution was added to the clay to flocculate and to have a concentrated suspension. Once clay flocculated completely, supernatant was discarded. Thus, concentrated suspension of Na saturated clay was obtained.

3.5.2 Purification of clay

Excess of salt was removed by washing once with distilled water and then with 99 percent methanol. After 5-6 washings with methanol samples got dispersed in the medium. Then samples were taken in the dialysis bag and washing with methanol continued till the outer solution became free from sodium (tested for chloride using $AgNO_3$).

3.5.3 Preparation of clay specimens

Four sets of each of three soil-clays were used for the study *viz.* (i) clay free from both calcium carbonate and organic matter (referred as mineral clay; MC) (ii) clay free from organic matter alone (referred as carbonate-clay; CC), (iii) clay free from calcium carbonate alone (referred as humus-clay; HC) and (iv) clay without any treatments (referred as soil-clay; SC). The procedure for the removal of various pre-treatments is described below:

3.5.3.1 Preparation of Mineral Clay (MC)

Mineral clay was prepared by providing two treatments: (i) removal of organic matter, (ii) removal of carbonates. Organic matter was removed from the clays by wet oxidation. For this, 30% hydrogen peroxide was used. Clay sample was taken in 1 litre beaker. About 40 ml of 30% H_2O_2 was added to it, initially at room temperature, and subsequently raising the temperature up to 65°C, to avoid spontaneous decomposition of H_2O_2. The complete removal of OM was confirmed from ceasing of reaction of H_2O_2, and disappearance of dark colour.

Then carbonate was removed from the clay sample. For this, sodium acetate and acetic acid buffer solution at pH 5.0 was used. The solution was prepared by mixing 60 ml of the glacial acetic acid and approx 82 g of sodium acetate in 1 litre of distilled water. The pH of the solution was maintained at 5.0. About 40 ml of buffer solution was added to each of about 10 g clay sample, stirred with policeman, heated to $\leq$ 80°C, and allowed to stand overnight. The suspension was centrifuged at 2500 rpm for 20 minutes, and supernatant was discarded. The process was repeated till clay was free from carbonates. The presence of carbonate was tested by adding a few drops of aliquot to oxalic acid solution.

3.5.3.2 Preparation of Carbonate-Clay, CC

For the preparation of Carbonate-Clay (CC) only soil organic matter was removed from the specimen. Organic matter was removed by the following procedure as described in Section 3.5.3.1.

3.5.3.3 Preparation of Humus-Clay, HC

For the preparation of Humus-Clay (HC), only calcium carbonate was removed from the clays. Carbonate was removed by the following procedure as described in Section 3.5.3.1.

3.5.3.4 Preparation of Soil-Clay, SC

Soil clay is a specimen which was not treated with any pre-treatments. Clay was extracted from the soil samples following the procedure as described in Section 3.5.1.

3.6 Laboratory Analysis

The soil samples were analysed for the following physical and chemical parameters:

3.6.1 Soil reaction

The soil pH was determined in 1:2 soil water suspensions by using an Elico glass electrode pH meter following the procedure described by Jackson (1973).

3.6.2 Electrical conductivity

The electrical conductivity of soil samples was measured at 1:2 soil water ratio at soil-suspension after equilibrated for 24 hours by immersing conductivity bridge in the suspension using Elico Solubridge Type CM-84 Conductivity Meter.

3.6.3 Calcium carbonate

Calcium carbonate of soil samples was determined by rapid titration method as described by Puri (1930). Soil water suspension was titrated against 0.5 N H_2SO_4 using a mixture of bromothymol blue and bromocresol green indicators.

3.6.4 Particle size distribution

Particle size distribution was determined by the pipette method (Day 1965). Twenty gm of soil sample was dispersed by stirring for fifteen minutes using sodium hydroxide solution. The suspension then passed through a 50 µm (300 mesh $inch^{-1}$) sieve. Sand remained on the sieve, while suspension of silt and clay was collected in 1 litre sedimentation jar. Sand was dried in the oven, and quantity was measured. The volume of the suspension was made up-to one litre, dispersed, and silt was allowed to settle. After due settling time for $\geq$ 2µm particles, 25 ml of the suspension was pipetted out from the depth of 10 cm, dried and clay was estimated. Silt was estimated by difference, and particle size fraction was expressed in percent.

3.6.5 Cation exchange capacity

The cation exchange capacity of the soil was determined by saturating soils with Na (using NaOAc) and subsequently replacing Na^+ by NH_4^+ (using NH_4OAc) method as described by Jackson (1973). The Na^+ was determined by flame photometer method. The leaching of samples was done at pH 8.2.

3.6.6 Soil organic carbon

3.6.6.1 Oxidizable organic carbon

Oxidizable organic carbon was determined by following the procedure of wet digestion as proposed by Walkley and Black (1934) and described by Jackson (1973). Soil samples were treated with excess of standard $K_2Cr_2O_7$ solution in the presence of the concentrated H_2SO_4 for 30 min, and then unused $K_2Cr_2O_7$ was titrated back by the standard solution of ferrous ammonium sulphate in the presence of NaF using diphenylamine as an indicator.

3.6.6.2 Extraction of total soil organic matter fractions

The organic matter of soil sample was extracted by following the procedure as described by Jackson (1969). It is described as follows:

10% NaCl at pH 3: For this approximately 100 g of NaCl and 0.4 ml of concentrated HCl were dissolved in 1 litre of distilled water. The pH of the solution was maintained at 3.

10% NaCl at pH 9: Approximately 100 g of NaCl, 2g of Na_2CO_3 and 2g of $NaHCO_3$ dissolved in 1 litre of distilled water. The pH of solution was maintained at 9.

Na_2CO_3-$NaHCO_3$ extracted solution at pH 9: About 0.25 g of Na_2CO_3 and 2 g of $NaHCO_3$ were dissolved, and volume was made up to 1 litre with distilled water. The pH of the solution was maintained at 9.

About 50 gm of soil sample was placed in the 500 ml conical flask and to this 200 ml of NaCl solution (pH 3) was added to each soil samples. The suspension was shaken for 30 minutes on mechanical shaker. Then the suspension of each soil sample was filtered through Whatman No. 2 filter paper. The drop wise leaching was done. The leaching was continued with an addition of 200 ml of same solution of NaCl (pH 3) followed by the 500 ml of NaCl (pH 9). This extraction removes the exchangeable cations, particularly the exchangeable calcium and magnesium and soluble silica from all the soil samples. The filtrates were discarded.

3.6.6.3 Extraction of organic colloids

The soil samples leached previously were then leached drop by drop with the 500 ml of the Na_2CO_3-$NaHCO_3$ solution. The first 100 ml of leachate was discarded to eliminate contamination. Slowly, organic colloids became sufficiently dispersed, and began to pass through the filter paper. This leachate was brown to black in colour, and collected in beaker. It was kept in hot-air oven at 40°C for drying.

3.6.6.4 Analysis of the extracted soil organic matter by Fourier Transformed Infra-Red Spectroscopy

The soil organic matter was dried in hot-air oven at 45°C for about two days, and then further desiccated in desiccators under vacuum. Dried organic matter was analyzed by Fourier Transformed Infrared Spectroscopy (FT-IR Model: Perkin Elmer Spectrum 100) following the procedure similar to clay samples as detailed in Section 3.7.

3.7 FT-IR analysis of the clay samples

Clays extracted from soils were analyzed by Infra-Red Spectroscopy between 400 to 4000 cm^{-1} wavelength using FT-IR Model Perkin Elmer Spectrum 100.

3.7.1 Material used for preparation and analysis of samples by Infra-Red Spectroscopy

Following materials were used for preparation and analysis of samples by Infra-Red Spectroscopy:

(a) Potassium bromide (KBr), spectroscopic grade.

(b) Die for making KBr pellets: The die consists of a ram and an anvil enclosed in such a way that when they are pressed together by means of hydraulic press, the sample is compressed into a pellet or disk in the confining space.

(c) Laboratory hydraulic press for a stroke of at least 10 cm and capable of creating pressure on the confined sample equivalent to 9000 to 11000 kg of force on the ram of a 13 mm diameter pellet pressing die.

(d) A small hand mortar and pestle.

3.7.2 Sample preparation

The clay specimens were sonicated for about 5 second at 1 second pulse rate using Oscar Ultrasonicator (Model: PR1000 MP). The dispersed samples were then smeared on cover slips and dried in a hot-air oven at 45° C for about 4 hours. They were then dried overnight in the desiccators under vacuum.

3.7.3 Procedure

About 1 mm thick and 13 mm diameter of pellets were prepared for clays by mixing approx 0.5 mg of clay with thoroughly dried 300 mg of spectroscopic grade KBr. Satisfactory mixing was carried out by using mortar and pestle (White and Roth 1986). The mixture was further dried in a desiccators under vacuum to ensure proper removal of moisture from the samples. Further the mixture was placed into the die set and pressed with the hydraulic pressure for the stroke of at least 10 cm that created pressure on the confined sample equivalent to 9000 to 11000 kg total force on the die set for about 5 min which makes the 13 mm pellet. Then the pellets were placed in the sample holder and were analyzed by FT-IR.

3.8 Scanning Electron Microscopy (SEM) of clay samples

Clays were viewed under Scanning Electron Microscope (Model: Hitachi S-3400 N). The details are as follows:

3.8.1 Sample preparation

The clays were sonicated for about 5 s at 1 second pulse rate using Ultrasonicator (Oscar Model: PR1000 MP). Then, samples were dried in hot-air oven at 45° C for 4 to 5 hours, and further desiccated overnight under vacuum. Aluminium (Al) stub were washed with the isopropyl alcohol prior to use for the proper removal of dust particles and other contaminants. Then the dried clay particles were scratched from the cover slip and placed on Al stub coated with double sided adhesive tape. Each time 4-5 samples were placed on the single Al stub.

3.8.2 Ion sputter coating with gold

Al stub was placed in the ion sputter coater (Hitachi E-1010) in which the clay samples were subjected to coating with gold ions by vaporizing and depositing gold at 18-20 mA current flow for 20 s. This was done for blocking of negative charges of the clay/clay-humus complex and also for enhancing the conduction of the sample.

3.8.3 Viewing under Scanning Electron Microscopy

The Al stub was placed in the specimen chamber of SEM (Hitachi S- 3400 N) and viewed under the microscope at 15 to 30 kV accelerated voltage at the working distance of 10 mm so as to get best possible image. Secondary electron images were captured and printout was taken.

3.8.4 Electron Dispersive Spectroscopy (EDS) of clay samples

Elemental occurrence of the samples was determined by Electron Dispersive Spectroscopy (EDS) attached with SEM at 30 kV accelerated voltage. Data were collected from the entire surface area of the specimen.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Physical and chemical properties of soils

Soil reaction: The pH of the soils were slightly alkaline (Table 4.1), a feature common to the soils of the north-west part of the Indo-Gangetic Plains. Relatively lower pH was observed in soils of the cultivated field (Soil 3) than soils (Soils 1 and 2) under orchard (mango and guava). The former soils had been under rice-wheat system of cultivation. It is well known that the pH in the alkaline soils under rice tend to go down with time.

Electrical conductivity: EC was in the range of 0.15 to 0.32 dS m^{-1} (Table 4.1). It shows that soils are free from salt problems. The EC was relatively lower in cultivated soils (Soil 3) than soils under orchard (Soils 1 and 2). This could be because of base leaching due to submergence during rice season in the cultivated fields

Lime: Small amount of calcium carbonate (0.25% in Soil 1 and 0.50% in Soil 2) was present in the soils under orchard, but soils under cultivation were free from it (Table 4.1). Absence of calcium carbonate in the cultivated field, despite parent materials being calcareous, further confirms that leaching of calcium carbonate has been going on due to rice cultivation in the soils that are under rice-wheat cultivation for over four decades.

Particle size distribution and texture: Sand content in these soils varied from 24.7 to 48.3 percent, silt content was between 33.0 and 56.3 percent, while clay content was in a narrow range of 16.4 and 19.0 percent. The texture of Soil 1 was loam, while Soil 2 and Soil 3 were silt loam.

Cation exchange capacity (CEC) and extractable bases: The CEC was in the range of 5.67 and 8.76 cmol kg^{-1}. The ratio of CEC/clay was 0.3032 for Soil 1, 0.5341 for Soil 2, and 0.4505 for Soil 3. The high CEC/clay ratio in Soil 2 was accompanied by high OC content (0.94%). The data showed that CEC was contributed by both clay and OC in these soils. Calcium and magnesium constituted more than 90 percent CEC sites. ESP was 4.94 and 5.14 in soils under orchard, while it increased to 5.49 in the cultivated field. Presley et al (2004) reported increase in ESP due to long term irrigation. In the present case both orchard and cereal-farming are managed and irrigated for more than four decades. However, cultivated field has been kept under submergence during paddy growing season for more than four decades. Therefore, the differences in soil properties, especially of pH, EC and ESP are due more to submergence of soil during rice growing season than any other factors. Percent Exchangeable K was 3.35 to 3.54 in soils under orchard, but only 1.05 in cultivated fields. This poor PEK was perhaps because K is regularly applied to soils under orchard, while no K dressing is done in the farmers' field (rice-wheat).

Table 4.1 some physical and chemical properties of soils

Soil sample	1 (mango orchard)	2 (Guava orchard)	3 (Cultivated field)
pH (1:2)	7.7	8.2	7.5
EC (1:2; dS m^{-1})	0.22	0.32	0.15
$CaCO_3$ (%)	0.25	0.50	Nil
Sand (%; 50-2000 µm)	48.3	31.0	24.7
Silt (%; 2-50µm)	33.0	52.6	56.3
Clay (%; < 2 µm)	18.7	16.4	19.0
Texture	loam	Silt loam	Silt loam
CEC (cmol kg^{1})	5.67	8.76	8.56
Extractable bases (cmol kg^{-1}):			
Ca+Mg	5.20	8.00	8.00
Na	0.28	0.45	0.47
K	0.19	0.31	0.09
Exchangeable Sodium (% of CEC)	4.94	5.14	5.49
Exchangeable K (% of CEC)	3.35	3.54	1.05

4.2 Fractionation of Soil Organic Matter

Oxydizable and Clay-Humus Organic Carbon: Organic Carbon in oxydizable form and in the clay-humus form followed a trend: Soil 2(0.94%; 15.60% by atom) > Soil 1 (0.59%; 12.10% by atom) > Soil 3 (0.53%; 9.78% by atom) (Table 4.2). The ratio of oxydizable OC to clay-humus OC was: Soil 2 (0.060) > Soil 3 (0.054) > Soil 1 (0.049). There is little difference in the clay mineralogical composition in the soils. Therefore, it could be concluded that proportionally more amount of raw organic matter is converted to clay-humus complexes in soils under mango than soils under guava and rice-wheat, while in terms of quantity, soils under guava is superior to soils under mango and rice-wheat.

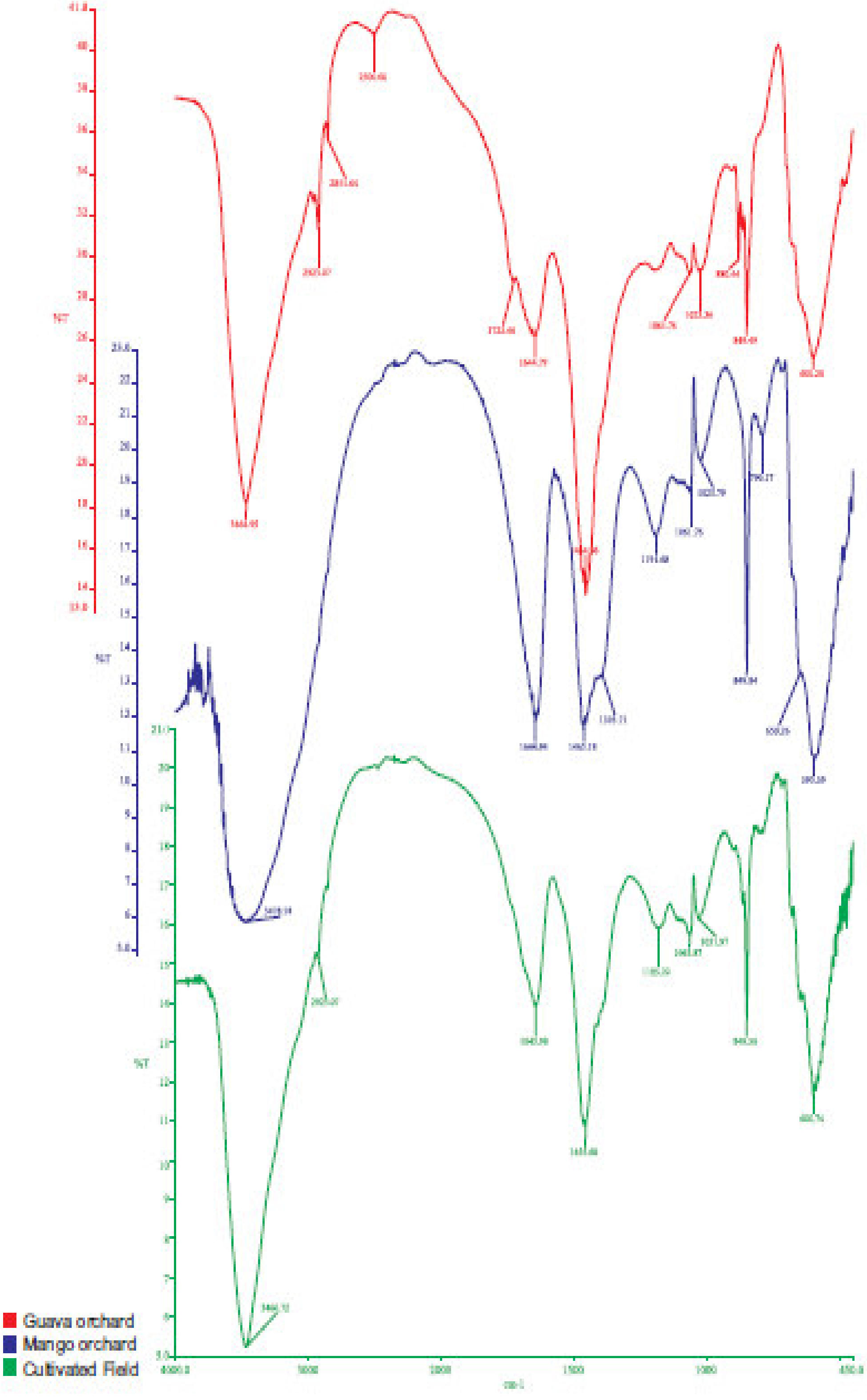

Fig.4.1. IR spectra of organic matter extracted from soils under mango orchard, under guava orchard and from cultivated field.

Table 4.2 Organic carbons in oxydizable form, in clay bound humus, and identification of nature of total organic matter by Infra Red Spectroscopy (absorption peaks in cm^{-1})

Sl. No.	1 (Mango orchard)	2 (Guava orchard)	3 (Cultivated field)	Interpretation
Organic carbon (%) in oxydizable organic matter fraction				
OC (%)	0.59	0.94	0.53	
Organic carbon (atom % by EDS analysis) in clay bound humus fraction				
atom (%)	12.10	15.60	9.78	
Characterization of extractable total organic matter by Infra Red Spectroscopy (absorption peaks in cm^{-1})				
1	3478.74	3464.95	3466.72	OH stretching of humic and fulvic acid
2	2923.07	2923.07	2923.07	Aliphatic C-H stretching of humic acid and fulvic acid
3	-	2851.04	-	C-H aldehyde stretching of humic acid
4	-	2500.00	-	Carboxyl OH stretching
5	-	1725.66	-	C=O stretching of COOH and ketonic groups in fulvic acid
6	1644.94	1644.79	1643.98	C=O stretching of amide groups, quinine C=O and C=C of H bonded conjugated ketonic groups of humic acid
7	1463.18	1454.96	1455.68	Aliphatic C-H stretching
8	1393.18	-	-	OH deformation and C-O stretching of phenolic OH, CH deformation of CH_2 and CH_3 groups.
9	1061.76	1061.76	1063.87	C-O stretching of polysaccharides
10	-	880.44	-	Alkenes C-H bending

Four IR absorption peaks at 3464-3479, 2923.07, 1644, 1455-1463, and 1062 cm^{-1} were common to organic matter extracts of the soils. These peaks are due to OH stretching of humic acid (3464-3479 cm^{-1}), C=O stretching of amide groups, quinine C=O and C=C of H bonded conjugated ketones (1644 cm^{-1}), aliphatic C-H stretching (1455-1463 cm^{-1}), and C-O stretching of polysaccharides (1062 cm^{-1}) (Table 4.2; Fig 4.1). Therefore, it could be concluded that organic matter in these soils irrespective of land use contains humic acid, amides, quinine, ketones and polysaccharides. Soils under rice-wheat had only those constituents. But, more diversified constituents were found in the soils under orchard, where it was also uniquely associated with tree species. For example, organic matter in soils under mango showed additional peak at 1393 cm^{-1}, which is due to presence of phenol, and CH_2 and CH_3 groups. Organic matter in soils under guava was most diversified as it had shown four additional peaks at 2851, 2500, 1726, and 880 cm^{-1}, which are indicative of presence of ketones, aldehydes, fulvic acid and alkenes, and compounds containing aliphatic and carboxyl groups.

4.3 Characterization of mineral-clays by Infrared Spectroscopy and Scanning Electron Microscopy (SEM).

4.3.1 Characterization of mineral-clays by Infrared spectroscopy

Table 4.3 Infra Red absorption peaks (cm^{-1}) of mineral clays

Sl No.	1 (Mango orchard)	2 (Guava orchard)	3 (Cultivated field)	Interpretation
1.	-	3695.95	3690.77	OH stretching of kaolonite
2.	3645.97	-	3646.39	OH stretching of illite
3.	3625.94	3622.02	3626.04	OH stretching of illite (dioctahedral), kaolinite, chlorite, and montmorillonite
4.	3444.23	3435.61	3444.42	OH stretching of trioctahedral illite
5.	-	1651	1650.85	OH bending of vermiculite
6.	1637.88	1632.95	-	OH bending frequency for water molecules in vermiculite and kaolinite
7.	-	1027.54	1027.42	Absorption peak due to illite
8.	1007.23	-	-	Absorption peak of kaolinite
9.	910.88	910.88	913.62	OH liberation from exposed Al_2OH (e.g., gibbsite, kaolinite, and other dioctahedral minerals)
10.	828.58	828.58	828.58	Al-rich chlorite, dioctahedral illite, OH liberation of dioctahedral Mg- Fe^{3+} clay minerals
11.	799.64	799.59	799.32	Bending vibrations of exposed-OH on edges in clay minerals.
12.	752.98	749.02	751.76	OH bending of illite
13.	695.57	695.58	695.34	Bending vibrations of –OH or structural water in clay minerals
14.	467.10	470.89	471.24	Si-O-Si bending of montmorillonite and illite.

The absorption peaks of Infra Red Spectroscopy common to all clays occurred at ~3622, ~3444, ~911, ~828, ~799, ~752, ~695, and ~471 cm^{-1} (Table 4.3; Fig 4.2). These peaks showed the presence of illite (dioctahedral and trioctahedral), vermiculite, kaolinite, Al-chlorite, and montmorillonite in all clays. The occurrence of absorption peaks at 3695, 1007 cm^{-1} is indicative of presence of kaolinite in all clay fractions. Chlorite was Al rich as evident from the occurrence of peak at ~828 cm^{-1} in all clays. Peak at 1650 cm^{-1} in clay 3, and 1633-1638 cm^{-1} in clay 1 and clay 2 confirmed presence of vermiculite. The identification of montmorillonite (instead of by its group name smectite) was also confirmed by Si-O-Si bending at 470 and 3622 cm^{-1}. The IR spectra of mineral clay under three different cultivation systems showed two peaks at 910 cm^{-1} and 799 cm^{-1} indicating that the soils were weathered enough to have exposed surfaces of Al_2OH. These peaks represent halloysite, but it is being refuted, because soils developed under semi-arid climate marked with alkaline pH and high base status, and deep water table cannot support occurrence of halloysite. Similarly, the peak at 695 cm^{-1} could possibly showed water of hydration or structural water in these

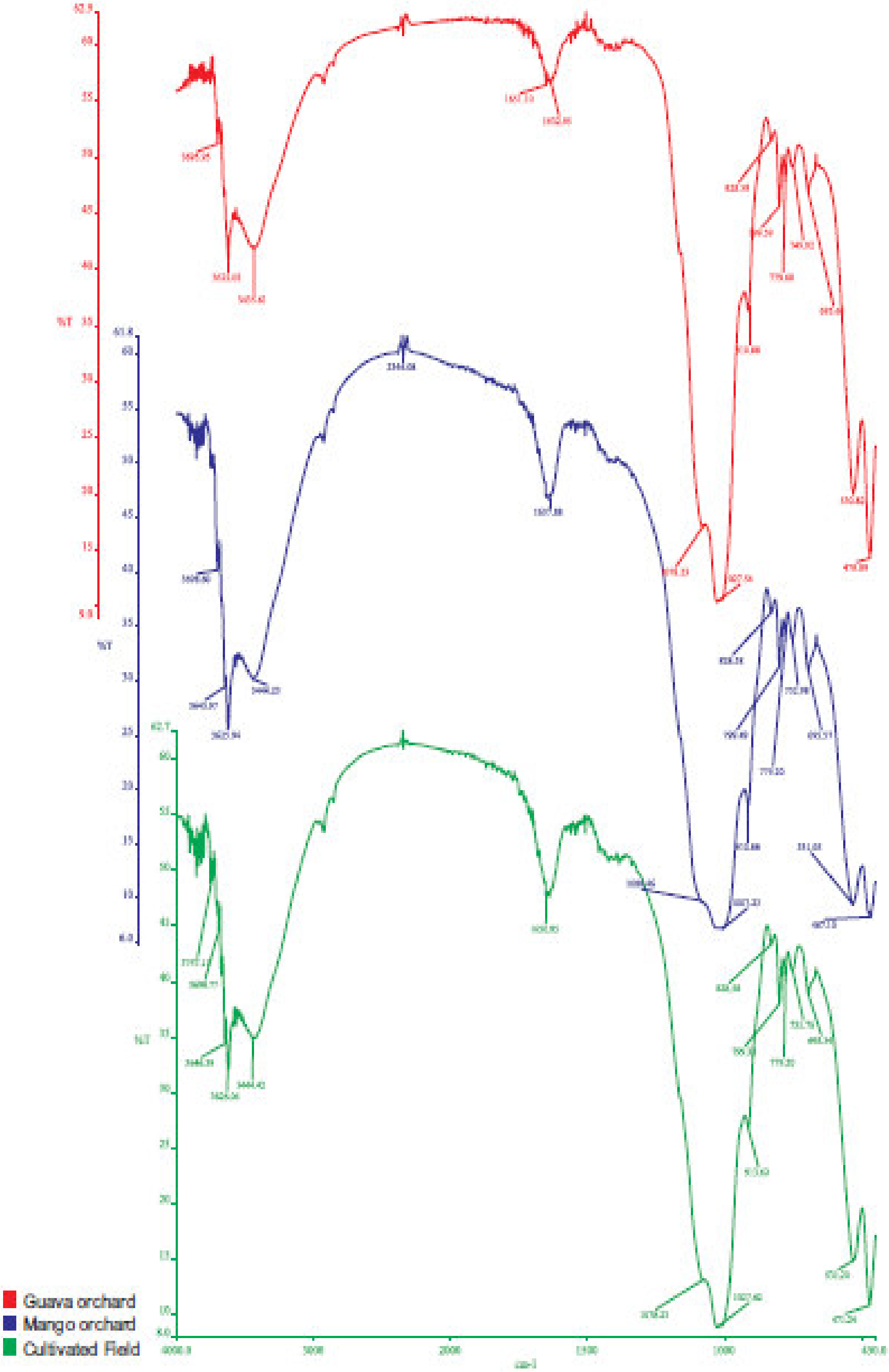

Fig.4.2. IR spectra of mineral clay extracted from soils under mango orchard, under guava orchard and from cultivated field.

phyllosilicates rather than halloysite. The advantage of IR over XRD is that IR deciphered dioctahedral and trioctahedral nature of illite as indicated by absorption frequencies at 3622, 3435 and 828 cm^{-1} in all the cases, and identified specific species of smectite group of mineral like montmorrilonite. The occurrence of gibbsite is also ruled out, because it cannot occur in these slightly alkaline soils with high base saturation, and especially in the presence of carbonates.

4.3.2 Characterization of mineral-clays by Scanning Electron Microscope

SEM micrographs of mineral clay sample of Soil 1(Plate 4.1), illustrate platy and flaky nature of clays. Boundaries of the particles are clear and at weathered faces opened. The shinny appearance at edges is due to –ve charge of the clays. The clay mineral particles are uniformly distributed to form planar structure and there is no formation of aggregates.

SEM micrograph of mineral clay of soil 2 (Plate 4.2) illustrates that clays are platy and flaky; very much similar to clay of soil 1 (Plate 4.1). Boundaries of the particles are clear and at weathered faces opened-up (cleavage opening) indicating low degree of weathering. The shinny appearance at edges is due to –ve charge of the clays. The clay mineral particles are uniformly distributed to form planar structure and there is no formation of aggregates.

SEM micrograph of mineral clay of soil 3 (Plate 4.3) illustrates that the boundary of the clays is bended or irregular lines illustrating greater degree of weathering of these clays. Apart from it, other characters like platy and flaky nature of clays, shinny appearance at edges, planar structure and absence of aggregation are common clays of soils 1 and 2 (Plates 4.1 and 4.2).

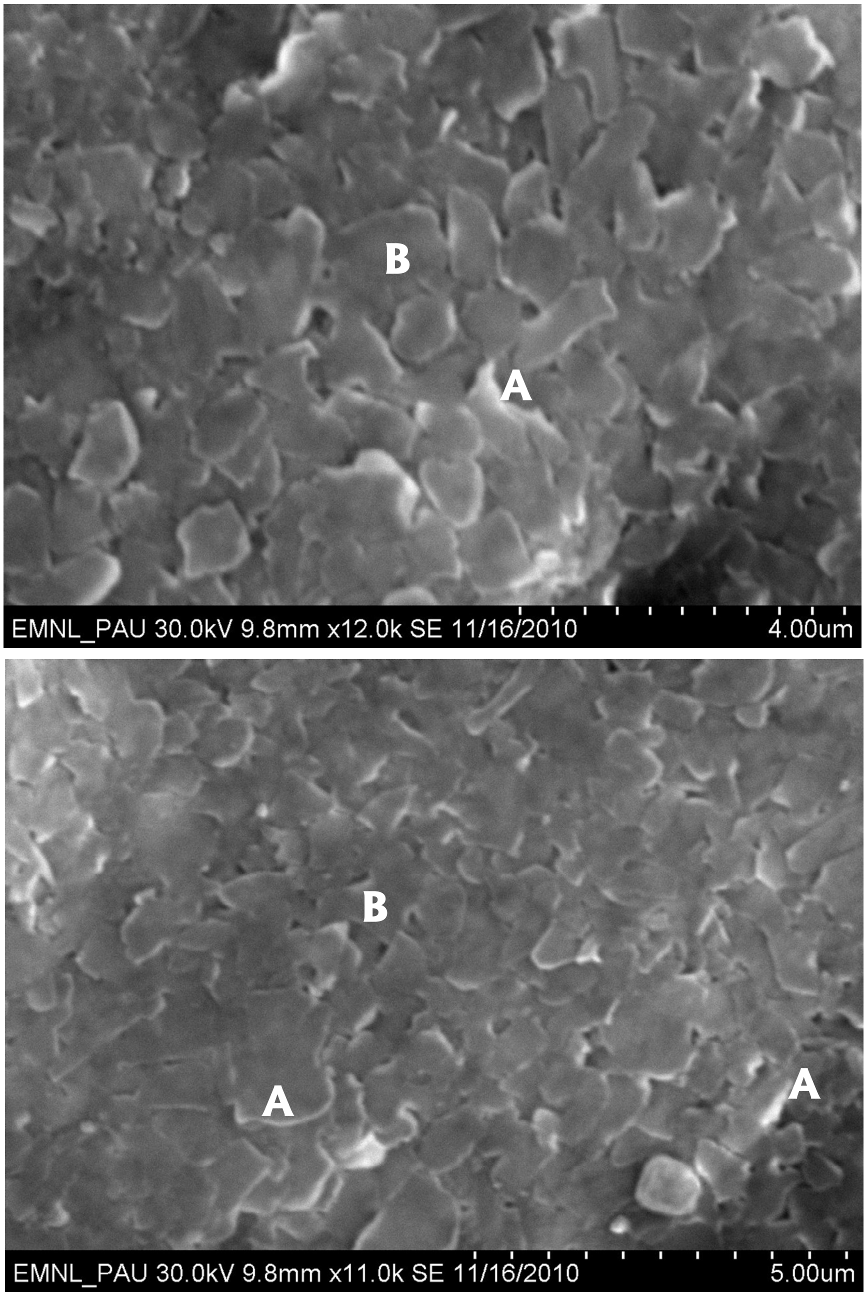

Plate 4.1 SEM micrographs of minerals clay sample of soil 1 illustrating platy and flaky nature of clays. Boundary of the particles is clear and at weathered faces opened. The shinny appearance at edges is due to -ve charge of the clays (A). The clay mineral particles are uniformly distributed to form planar structure and there is no formation of aggregates (B).

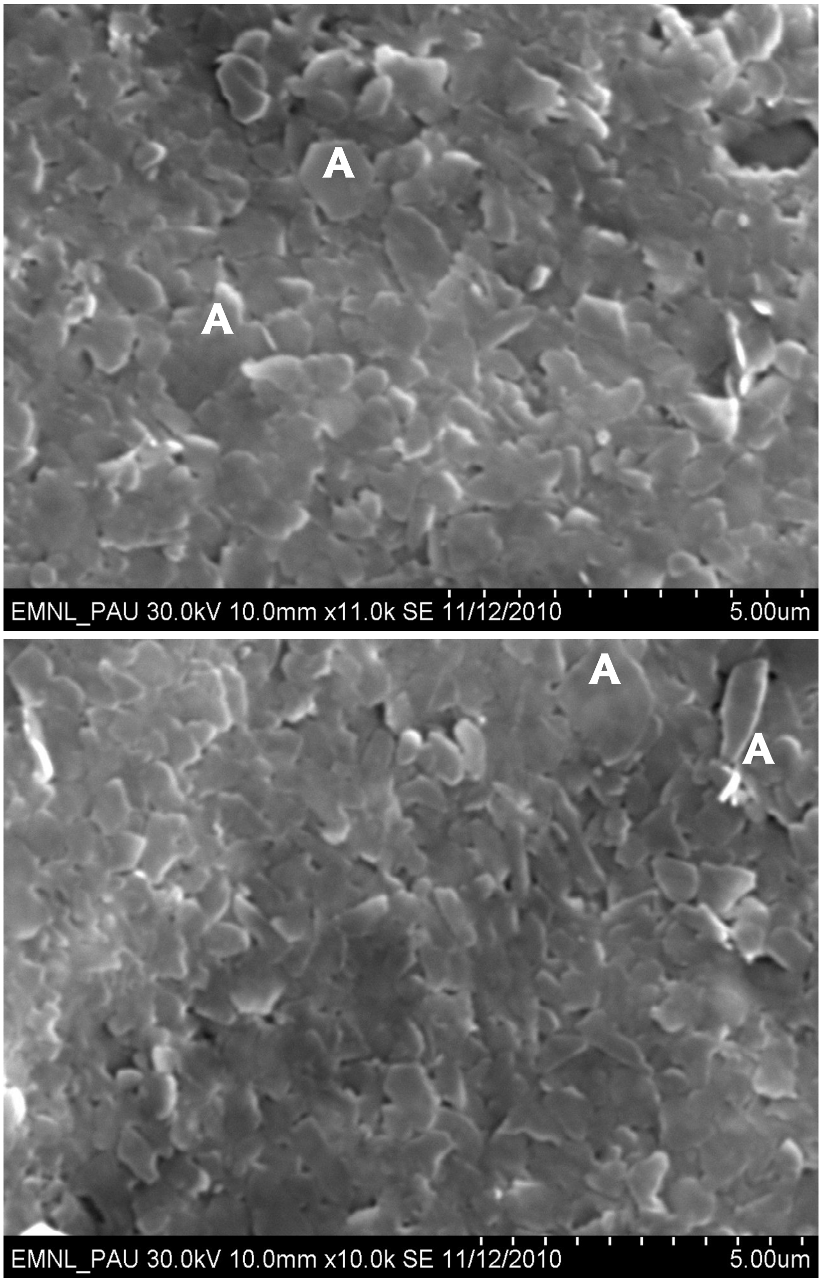

Plate 4.2 SEM micrographs of minerals clay sample of soil 2. Clays are similar to plate 4.1, and illustrating Platy and flaky nature of clays. Boundaries of the particles are clear and at weathered faces opened-up indicating low degree of weathering. The shinny appearance at edges is due to -ve charge of the clays (A). The clay mineral particles are uniformly distributed to form planar structure and there is no formation of aggregates.

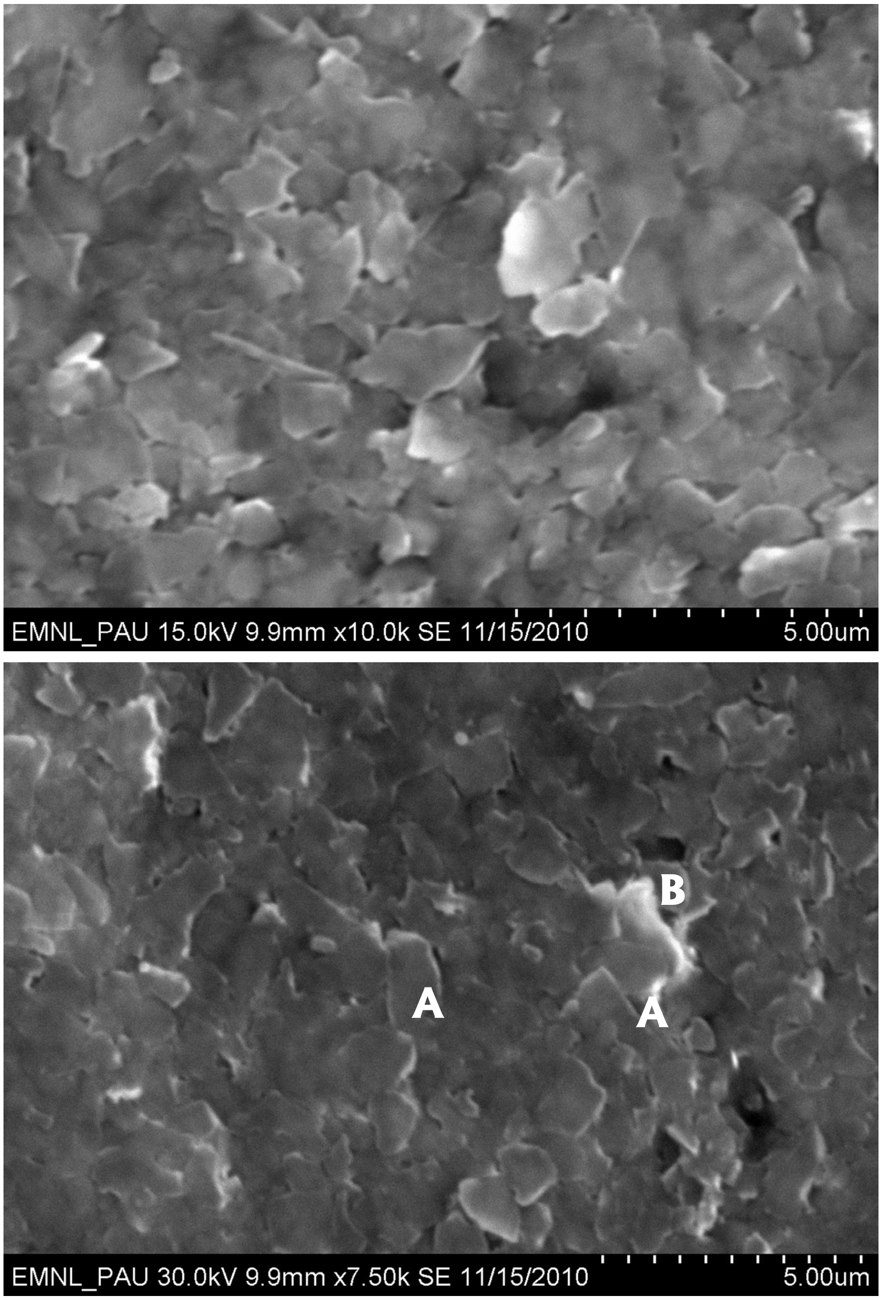

Plate 4.3 SEM micrographs of minerals clay sample of soil 3. The boundary of the clay is bended or irregular lines illustrating greater degree of weathering of these clays (A). The characters common to plates 4.1 and 4.2 are platy and flaky nature of clasy, shinny appearance at edges, plannar structure and absence of aggregation (B).

4.4 Clay humus complexes:

4.4.1 IR characterization of clay- humus complexes

Table 4.4 IR absorption peaks

Samples	Mineral clay	Organic matter	Carbonate clay	Humus clay	Soil clay
1	3444	-	3431	3444	3429
	-	3479	-	-	-
	-	2923		2923	2923
	-			2851	2851
	-	1644	-	1650	1650
	-	1463	-	-	1440
	-	1393	-	1385	1383
	-	1061	-	-	-
	1007	-	1027.35	1027.26	1019.19
2	3435	-	3444	3435	3444
	-	3465	-	-	-
	-	2923	2923	2923	2923
	-	2851	2851	2851	2851
	-	2500	-	-	-
	-	1725	-	-	-
		1645	-	-	-
	1632		1638	1633	1638
	-	1455	-	-	1450
	-		-	1384	1384
	-	-	-	-	1163
	-	1062	-	-	-
	-	880	-	-	-
3	3444	-	3444	3444	3435
	-	3467	-	-	-
	-	2923	2923	2923	2923
	-	2851	2851	2851	2851
	1650	-	1638	1651	1633
	-	1644	-	-	-
	-	1456	-	-	1450
	-	1380	-	1384	1385
	-	1063	-	-	-
	1028	-	1027	1030	1027

A perusal of data in Table 4.4 showed that: (i) peaks between 3431 and 3444 cm^{-1} in Clays 1, 2, and peaks between 1007 and 1027 cm^{-1} in Clays 1, and in 3 were found in MC, CC, HC and in SC, and therefore, it could be discerned that these peaks are characteristics of mineral fraction of clays or, devoid of organic substances. Occurrence of these peaks is irrespective of soil cover, (ii) distinct peaks at 2923 and at 2851 cm^{-1} are common to organic matter and CC, HC and in SC illustrating the properties of carbon that is common to both organic and inorganic sources, and this happens irrespective of land-cover, (iii) peaks that occur between 1644 and 1645 and 1650 cm^{-1} are common to OM, HC and SC in Clay 1, but found only in OM in Clay 2 and 3. This indicates that OM only under mango cover gets converted to clay bound humus. (iv) peaks found between 1455 and 1463 cm^{-1} in OM shifts to

1440 and 1450 cm^{-1} (attributed to the aliphatic C-H stretching of organic matter) in SC in all land-cover systems indicating that OM peaks moved leftward due to interference of clays, and (v) peaks found between 1380 and 1393 cm^{-1} are common to OM, HC and SC irrespective of land of clay 1 and 3. The peak at 1384 cm^{-1} represent fraction of OM that is converted to clay-bound humus.

4.4.2 Characterization of clay-humus complexes by SEM

SEM micrographs of clay-humus complexes in Clay 1 and Clay 2 showed small to large sized clay aggregates (Plates 4.4 and 4.5). The size of the clay aggregates ranged in 5-10 μm. There are three types of humus deposition on clays. Some clay-aggregations are marked with deep dark lumps, where humus is completely spread over the surface. On some other clay surfaces humus is smeared partially and in uneven manner. In some other clay-aggregations, little humus is visible.

SEM micrographs of clay-humus complexes in clays obtained from soil 3 shows that humus was thinner and lighter that those in Clays 1 and 2. Micrographs of Clay 3 (Plate 4.6) also illustrated that humus on clays was more dispersed that the humus observed on Clays 1 and 2. This illustrates lower binding power of humus under cultivation than on clays under orchard (Clays 1 and 2), which were relatively less vulnerable to weathering than the former.

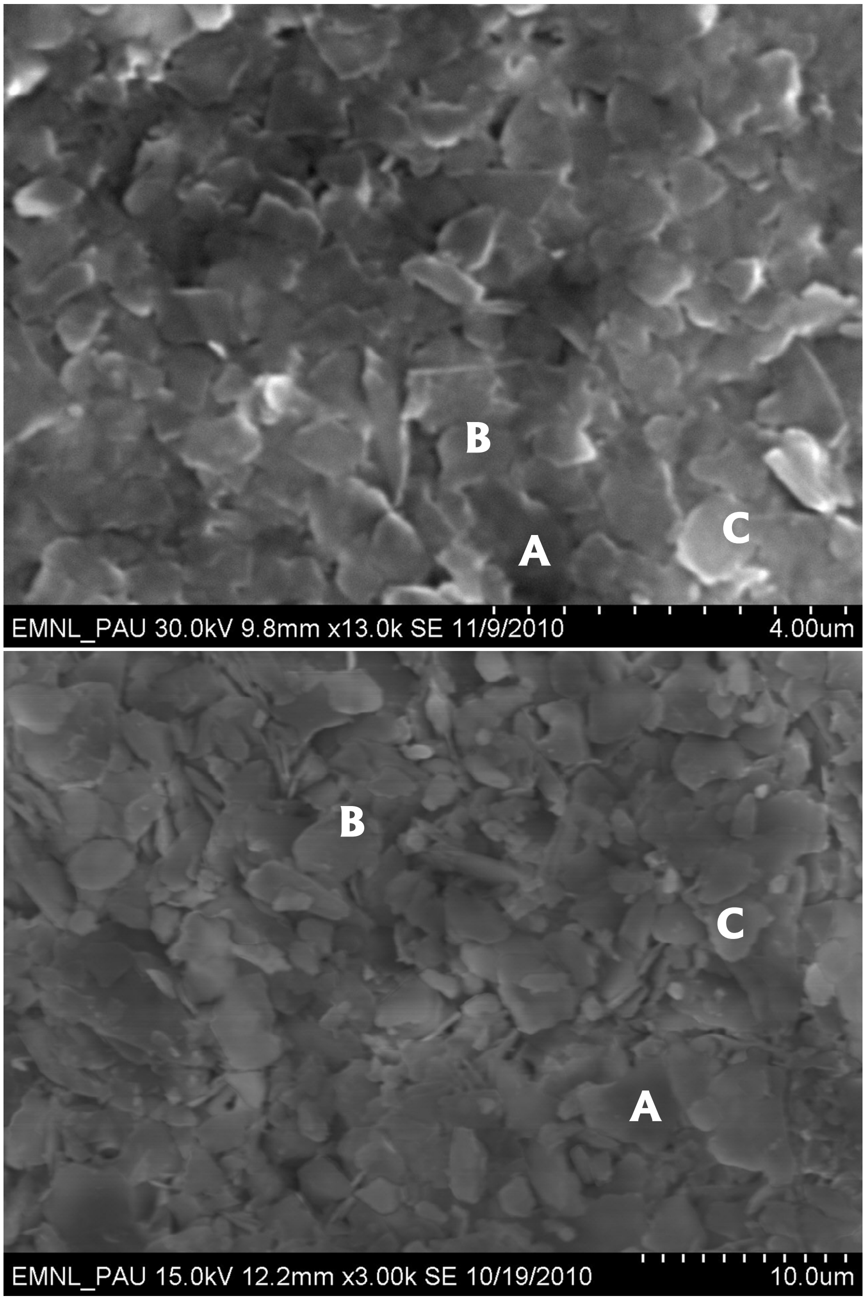

Plate 4.4 **SEM micrographs of humus clay sample of Soil 1 shows small to large sized clay aggregates. There were three types of humus deposition on clays. Some clay-aggregations are marked with deep dark lumps, where humus was completely spread over the surface (A). On some other clay surfaces humus is smeared partially and in uneven manner (B). In some other clay-aggregations, little humus is visible (C).**

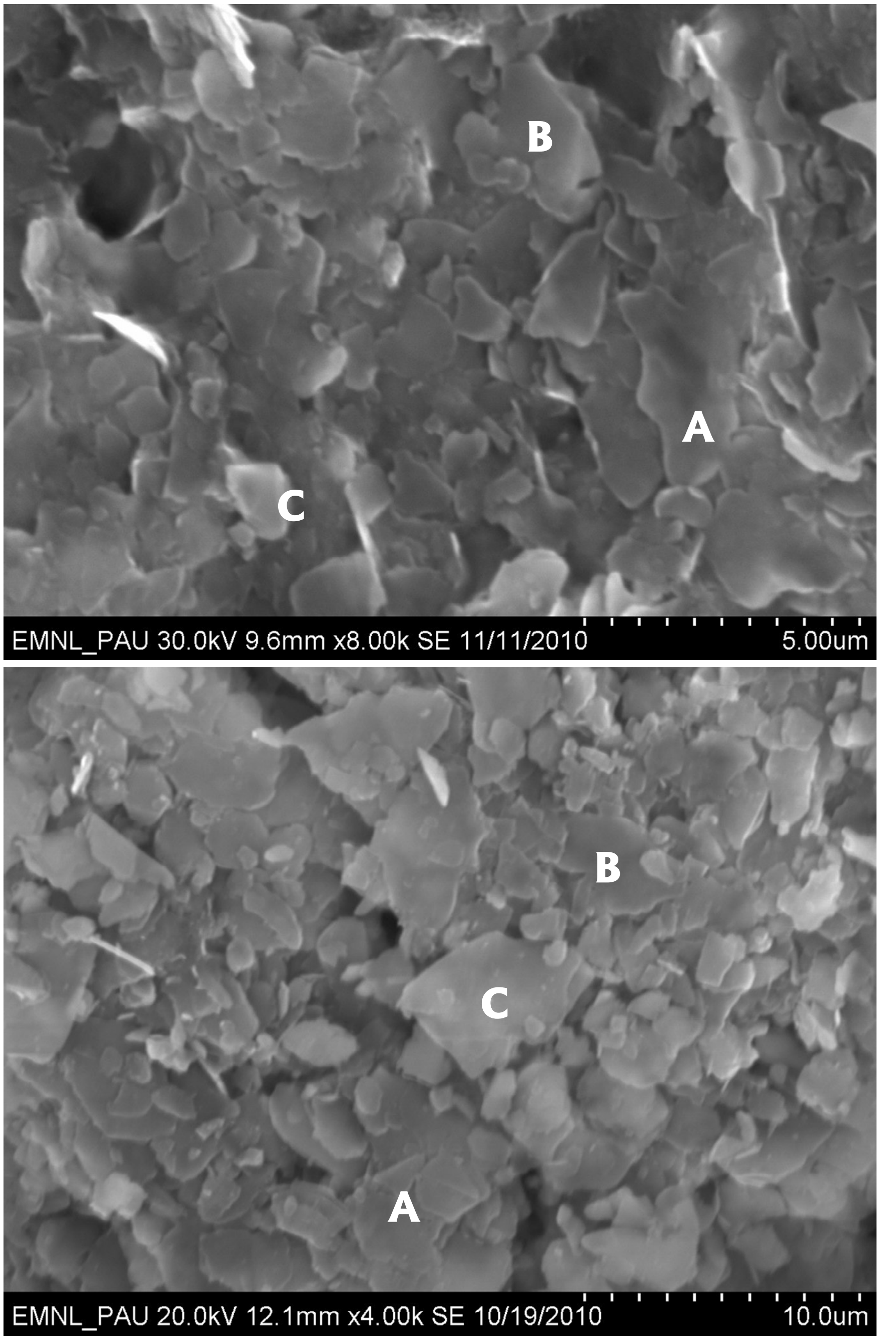

Plate 4.5 **SEM micrographs of humus clay sample of Soil 2 shows that there were three types of humus depositin on clays. Some clay-aggregations are marked with deep dark lumps, where humus was completely spread over the surface (A). On some other clay surfaces humus is smeared partially and in uneven manner (B). In some other clay-aggregations, little humusis visible (C).**

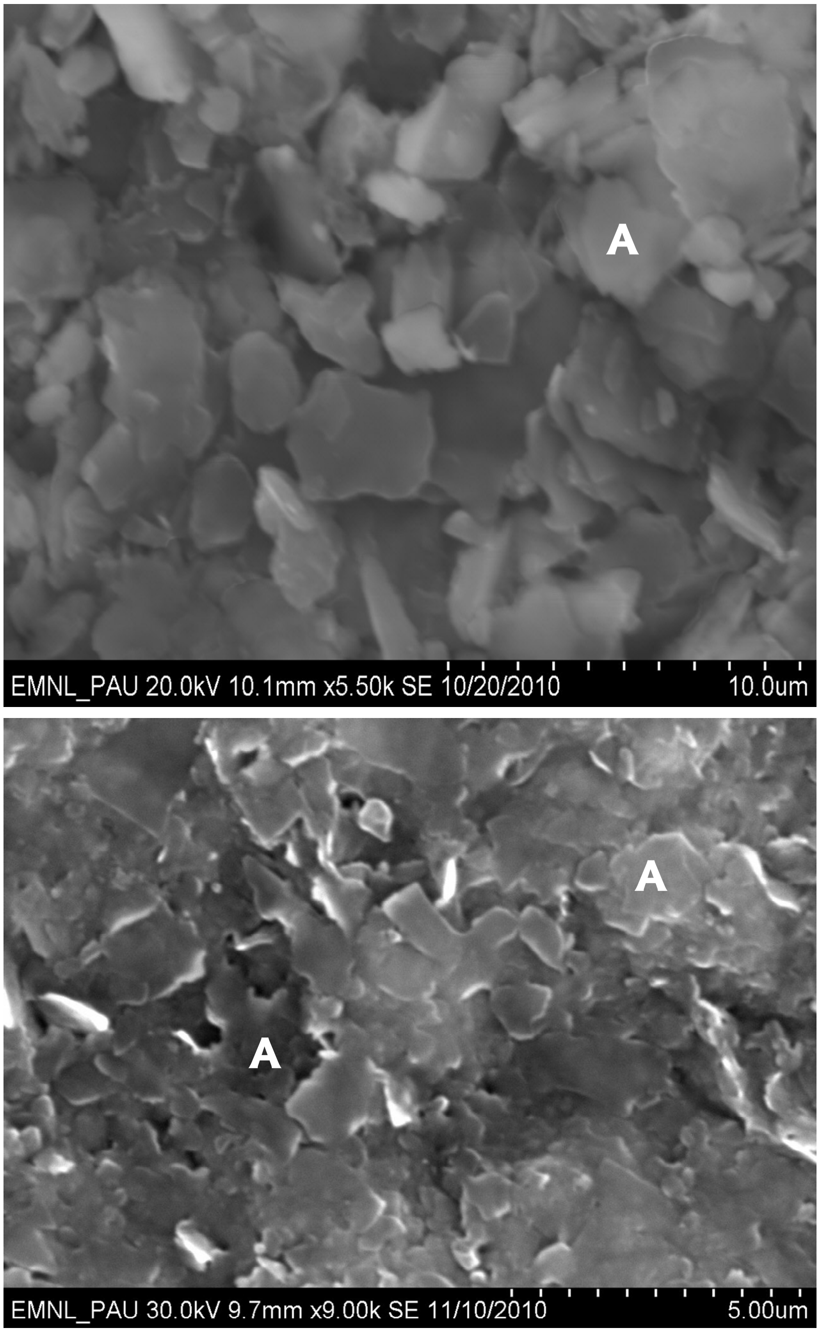

Plate 4.6 **SEM micrographs of humus clay sample of Soil 3 shows that humus appeared thinner and lighter that those in Clay 1. Micrographs of Clay 3 were also more dispersed than Clays 1 and 2, illustrating lower binding power of humus under cultivation than relatively less vulnerable clays under orchard (A).**

4.4.3 Characterization of carbonate-clays by Scanning Electron Microscope

SEM micrograph of carbonate clay of soil 1 (Plate 4.7) and soil 2 (Plate 4.8) illustrates mineral clay properties due to removal of organic matter and charged surfaces on the edges. Aggregations are due to binding by calcium carbonate.

SEM micrographs of carbonate clay sample 3 of cultivated field (Plate 4.9) also shows aggregation of clay particle. Similar shiny edges were also seen as in mango and guava clay samples.

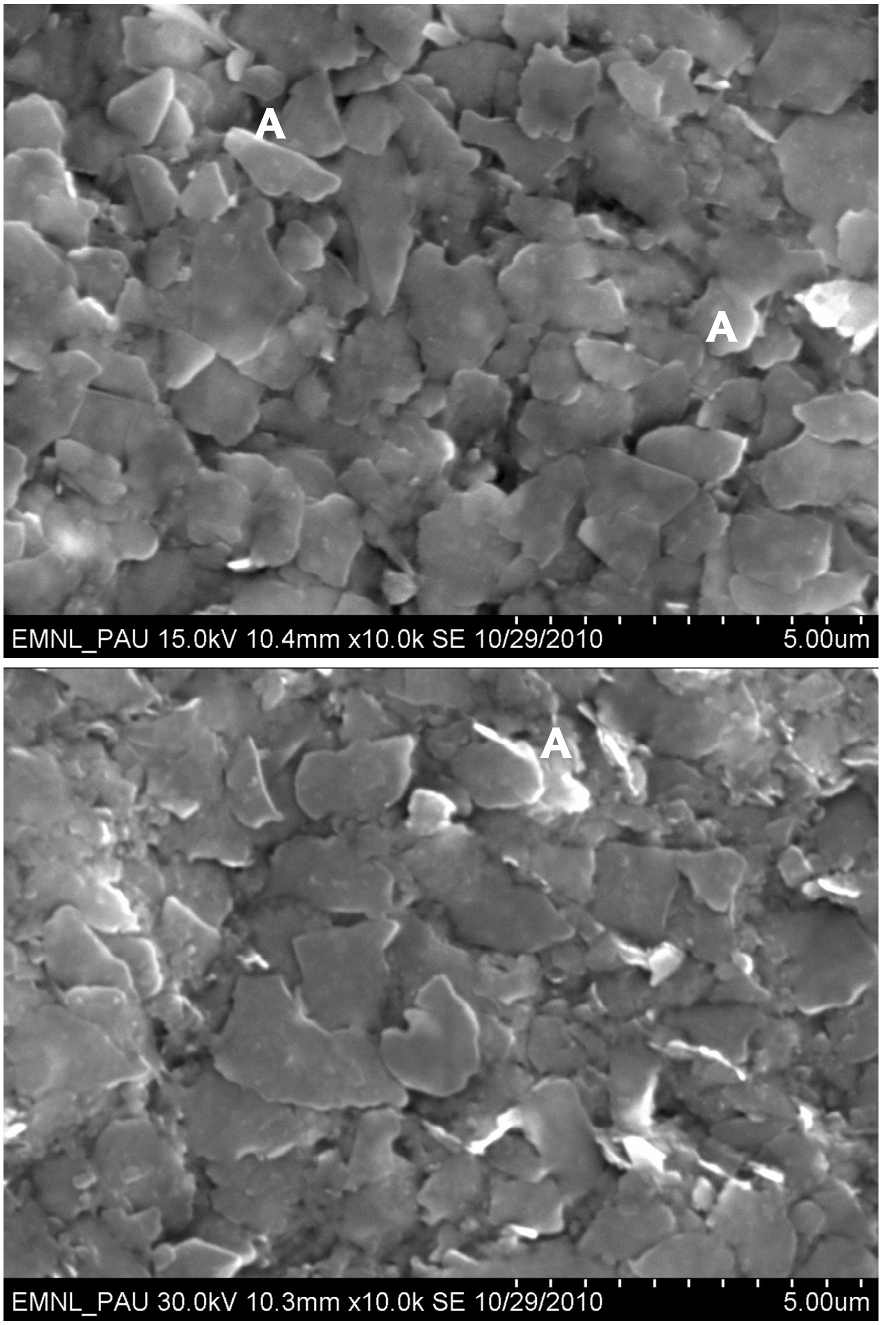

Plate 4.7 **SEM micrographs of carbonate clay sample of Soil 1 illustrating mineral clay properties due to removal of organic matter and charged surfaces on the edges (A). Aggregations are due to binding by calcium carbonate.**

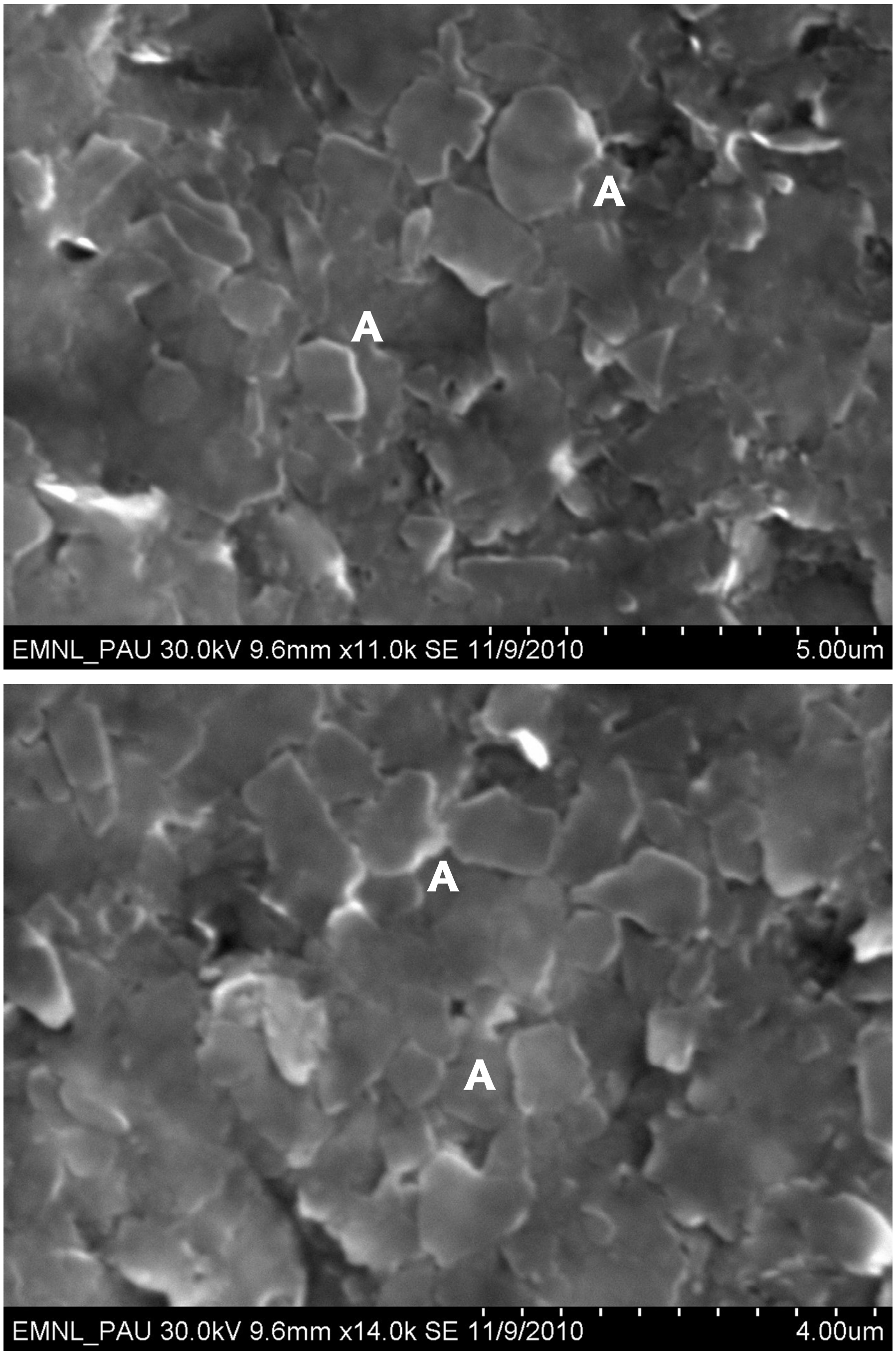

Plate 4.8 SEM micrographs of carbonate clay sample of Soil 2 illustrating mineral clay properties due to removal of organic matter and charged surfaces on the edges (A). Aggregations are due to binding by calcium carbonate.

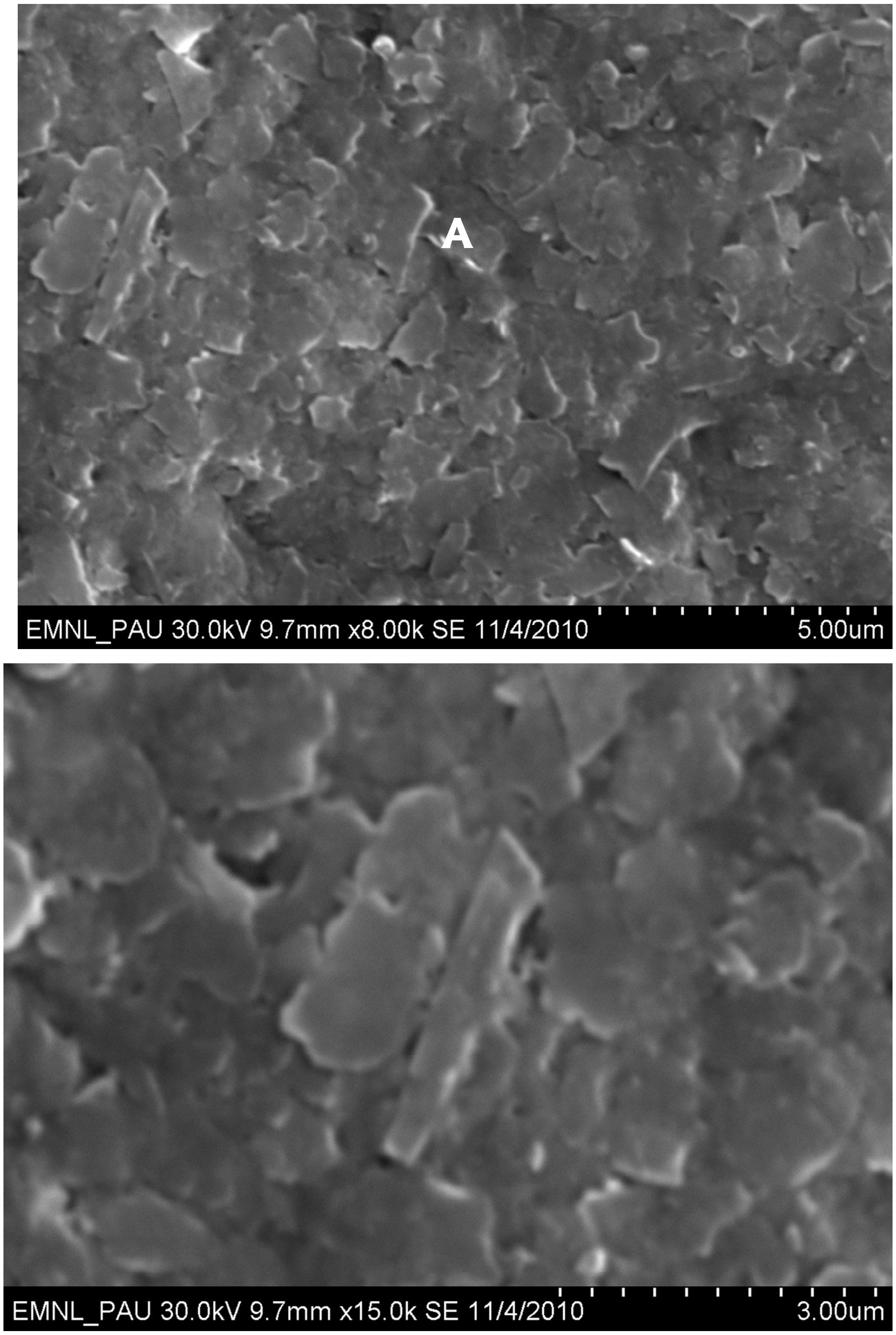

Plate 4.9 **SEM micrographs of carbonate clay sample of Soil 3 illustrate aggregation of clay particle. Similar shiny edges were also seen as in mango and guava clay samples (A).**

4.4.4 Characterization of soil-clays by Scanning Electron Microscopy.

SEM micrographs of soil clay of soil 1 (Plate 4.10) and soil 2 (Plate 4.11) illustrates lumps of organic matter, irregular distribution of organic matter and the partly distribution of organic matter on various clay particles.

SEM micrographs of soil clay of soil 3 (Plate 4.12) illustrates large particles with lumps of organic matter in pores.Spread of organic matter on clay surfaces is visible. Uneven charge distribution is also visible.

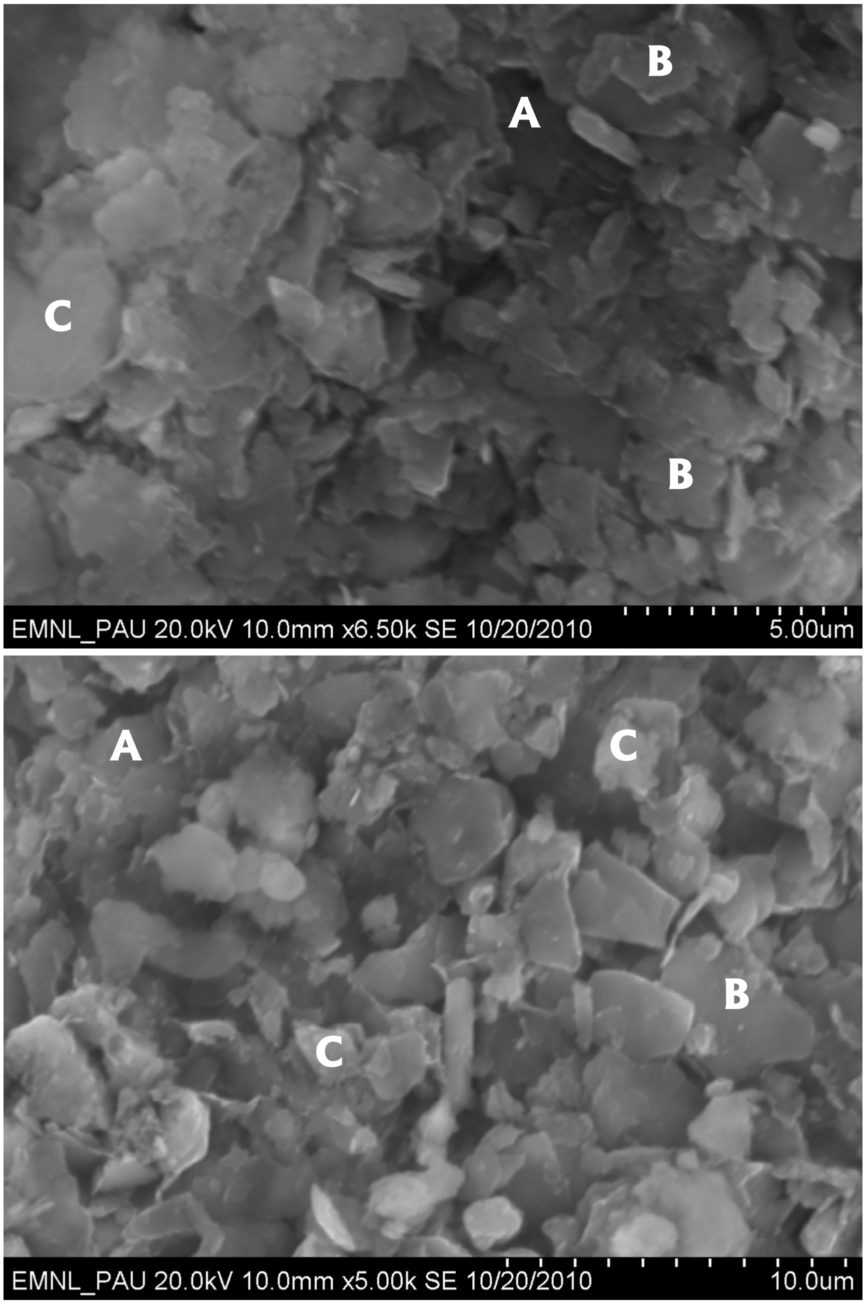

Plate 4.10 SEM micrographs of soil clay sample of Soil 1 illustrates lumps of organic matter (A), irregular distribution of organic matter (B) and the partly distribution of organic matter (C) on various clay particles.

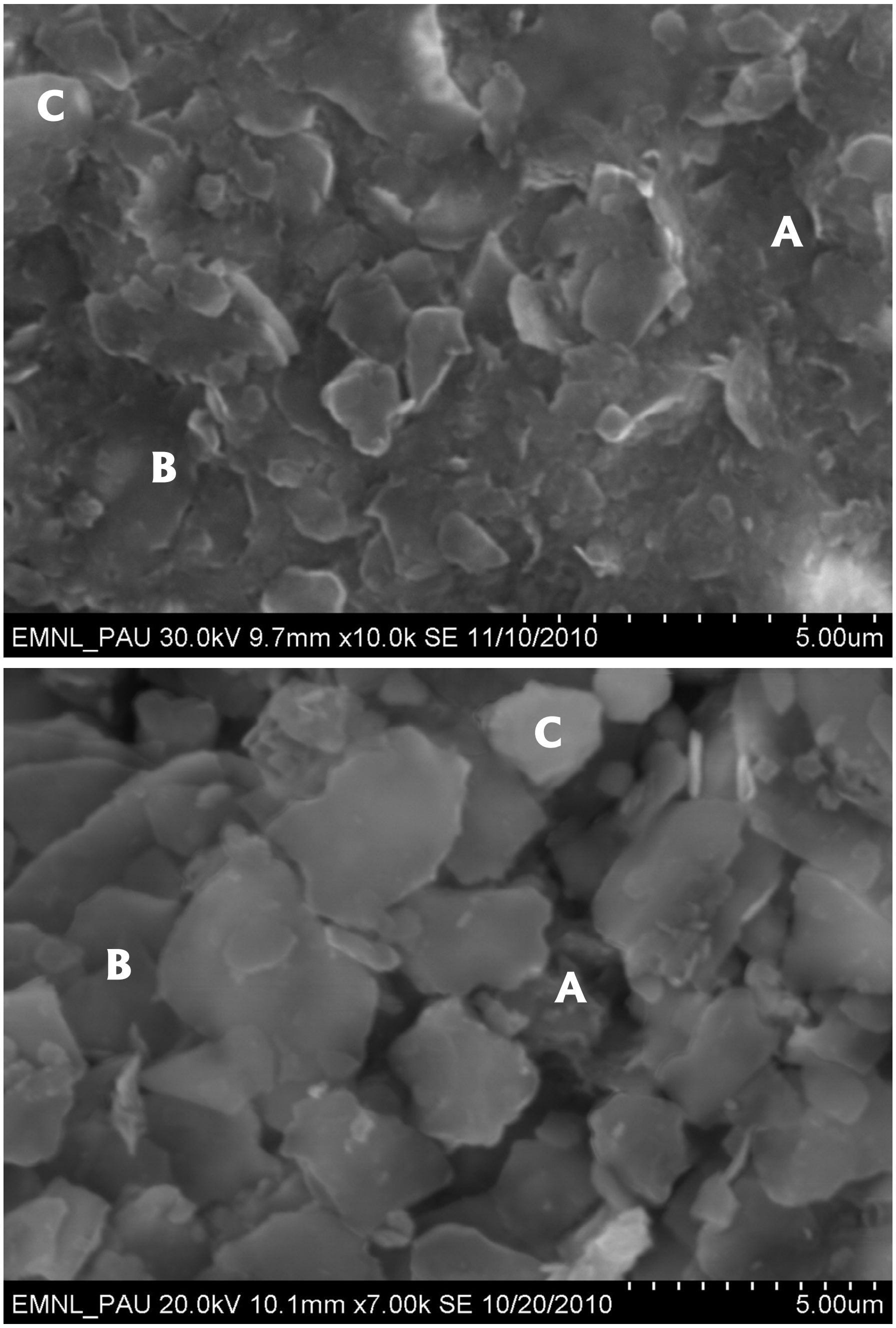

Plate 4.11 **SEM micrographs of soil clay sample of Soil 2 illustrates lumps of organic matter (A), irregular distribution of organic matter (B) and the partly distribution of organic matter (C) on various clay particles.**

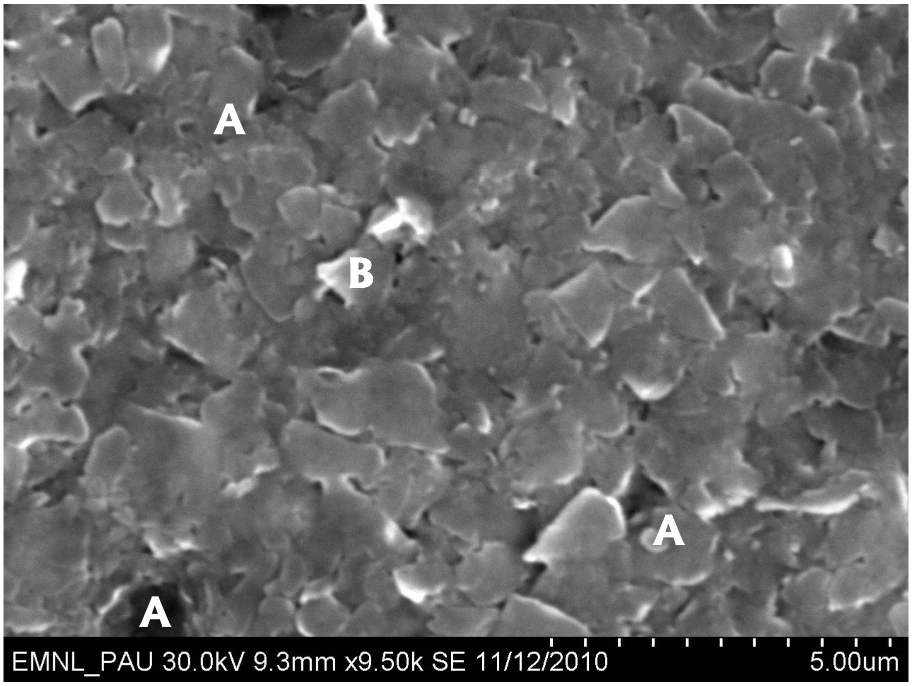

Plate 4.12 SEM micrographs of soil clay sample of Soil 3 illustrating large particles with lumps of organic matter in pores (A). Spread of organic matter on clay surfaces is visible. Uneven charge distribution is also vissible (B).

4.5 Characterization of elemental carbon on clay surfaces by Energy Dispersive Spectroscopy (EDS).

Table 4.5 Distribution of elemental carbon on clay surfaces

Sample No.	**1 Mineral Clay (MC)**	**2 Humus Clay (HC)**	**3 Carbonate Clay (CC)**	**4 Col 2 + Col 3**	**5 Soil Clay (SC)**
	Atom %				
1	Nil	12.10	2.10	14.20	15.25
2	Nil	15.60	2.73	18.33	18.90
3	Nil	9.78	0.00	9.78	11.69

The Energy Dispersive Spectroscopy in association with Scanning Electron Microscopy data on distribution of carbon (atom %) in inorganic form ($CaCO_3$) and in organic clay-humus form over about 5 μm^2 area showed that only 17.5 percent C was present in inorganic form ($CaCO_3$) out of the total amount present in the clays obtained from the soils under orchard (Table 4.5). The soil under cultivation was free from $CaCO_3$. The carbon in clay bound humus content was higher in the soils under guava, followed by the clay bound humus obtained from the soils under mango, and then by the clay bound humus obtained from the soils under cultivation. The small difference in the carbon (atom %) in the clay bound humus form obtained from the soils that were given no pre-treatments (col.5) and total of carbon (atom %) in inorganic and organic forms (col.4) is because of removal of C that got dissolved in the chemicals used at pre-treatment stages. Overall, the data indicated greater accumulation of humus in clay bound form when soils were under guava, followed by soils under mango, and cultivated soils were poorest amongst them. It could, therefore, be discerned that soil cover including tree species played important role in C retention and that present practice of cultivation especially leaving soils barren in between the crop seasons of rice and wheat remains questionable.

CHAPTER V

SUMMARY

Formation of clay-humus complex provides means of stabilizing soil organic matter and often for thousands of years. Global concern for sequestrating carbon in soils and developing farming protocols for achieving it would largely be dictated by nature of clay-humus complexes. Very little is known about these complexes in real farm situations that are under various land-use systems, especially, in vulnerable hot semi-arid regions. Therefore, the present study aimed for looking at bonding that different clay-minerals make with humus, imaging and deciphering interfaces of clay–humus complexes, and their association with land-use. For this, three surface samples, two from orchard (one under mango and other under guava) and one from cultivated field (under rice-wheat system) were collected. Clays were extracted from these soils. Clays were left untreated (Soil Clay, SC), or, given specific treatments like H_2O_2 (Carbonate Clay, CC), NaOAc-Ac (Humus Clay, HC), and both H_2O_2 and NaOAc-Ac (Mineral Clay, MC). They were analyzed by chemical means, by Infra-Red Spectroscopy, and by Scanning Electron Microscopy and Energy Dispersive Spectroscopy.

Fractionation of soil organic matter showed that OC in oxydizable form and in the clay-humus form were in order of Soil 2(0.94%; 15.60% by atom), Soil 1 (0.59%; 12.10% by atom) followed by Soil 3 (0.53%; 9.78% by atom). The conversion of organic matter to clay-humus complexes in soils under mango is more than soils under guava and rice-wheat, while interms of quantity, soils under guava is superior to soils under mango and rice-wheat. Infra-red spectra of organic matter extracts showed common absorption peaks at 3464-3479, 2923, 1644, 1455-1463, and 1062 cm^{-1}. These peaks represented humic acid, amides, quinine, ketones and polysaccharides irrespective of the land use. One additional peak was observed for soils under mango at 1393 cm^{-1}, which was due to presence of phenol, and CH_2 and CH_3 groups, however four additional peaks at 2851, 2500, 1726, and 880 cm^{-1} occurred for soils under guava indicating the presence of ketones, aldehydes, fulvic acid and alkenes, and compounds containing aliphatic and carboxyl groups showing more diversification in organic matter of guava orchard.

Characterization of mineral clays was done by Infra Red Spectroscopy (IRS), Scanning Electron Microscopy (SEM) and Electron dispersive spectroscopy (EDS). Common IR absorption peaks occurred at ~3622, ~3444, ~911, ~828, ~799, ~752, ~695, and ~471 cm^{-1} in all the three clay samples that identified clay minerals like illite (both dioctahedral and trioctahedral), vermiculite, kaolinite, Al-chlorite, and montmorillonite. Chlorite was marked as Al rich due to occurrence of peaks at ~828 cm^{-1} in all clays. Vermiculite and montmorillonite were confirmed by peaks at 1650 cm^{-1} and Si-O-Si bending at 470 and 3622 cm^{-1}, respectively. The IR spectra of mineral clay under three cultivation systems showed two peaks at 910 cm^{-1} and 799 cm^{-1} indicating that soils were weathered enough to have exposed surfaces of Al_2OH in their clay fractions of these soils. Halloysite and gibbsite formation was ruled out because of semi-arid climate marked with alkaline pH and high base saturation. IR also deciphered dioctahedral and trioctahedral nature of illite as indicated by absorption frequencies at 3622, 3435 and 828 cm^{-1} in all the cases, and identified montmorillonite as a specific species in smectite group. SEM micrographs of mineral clay samples in all clay samples showed platy and flaky nature of clays. Greater degree of weathering was found in Clay of soil 3 followed by Clay of soil 2 whereas boundary of the particles was clear and weathered faces opened in Clay of soil 1.

The presence of absorption peaks between 3431 and 3444 cm^{-1} in clays 1 and 2, and peaks between 1007 and 1027 cm^{-1} in clays of soil 1 and 3 in MC, CC, HC and in SC confirmed that these peaks correspond to mineral fraction of clays only. Set of peaks at 2923 and at 2851 cm^{-1} showed properties of carbon common to both organic and inorganic forms as these were absent only in mineral clays irrespective of land-cover. Peaks between 1644 and 1645 and 1650 cm^{-1} common to OM, HC and SC in Clay of soil 1 indicates that OM under mango cover readily gets converted to clay bound humus. Additionally, the peak at 1384 cm^{-1} represent fraction of OM that is converted to clay-bound humus. SEM micrographs of clay-humus complexes of Clays of soil 1 and 2 showed three types of humus deposition on clays, one with deep dark lumps where humus spread over the surface, other where humus was smeared partially in uneven manner and in third type, little humus was visible. Humus appeared thinner and lighter in Clay 3 than that those in Clays of soil 1 and 2. SE micrographs of Clay of soil 3 were also more dispersed than Clays of soil 1 and 2, illustrating lower binding power of humus under cultivation that are vulnerable to weathering due to rigor of cultivation than relatively less vulnerable clays under orchard. The Energy Dispersive Spectroscopy in association with Scanning Electron Microscopy data on distribution of carbon (atom %) in inorganic form ($CaCO_3$) and in organic clay-humus form over about 5 μm^2 area showed that only 2.5 percent C was present in inorganic form ($CaCO_3$) out of the total amount of carbon (17.5) percent present in the clays obtained from the soils under orchard. The soil under cultivation was free from $CaCO_3$. Soils under guava had more accumulation of humus in clay then soils under mango and soils under rice wheat cultivation.

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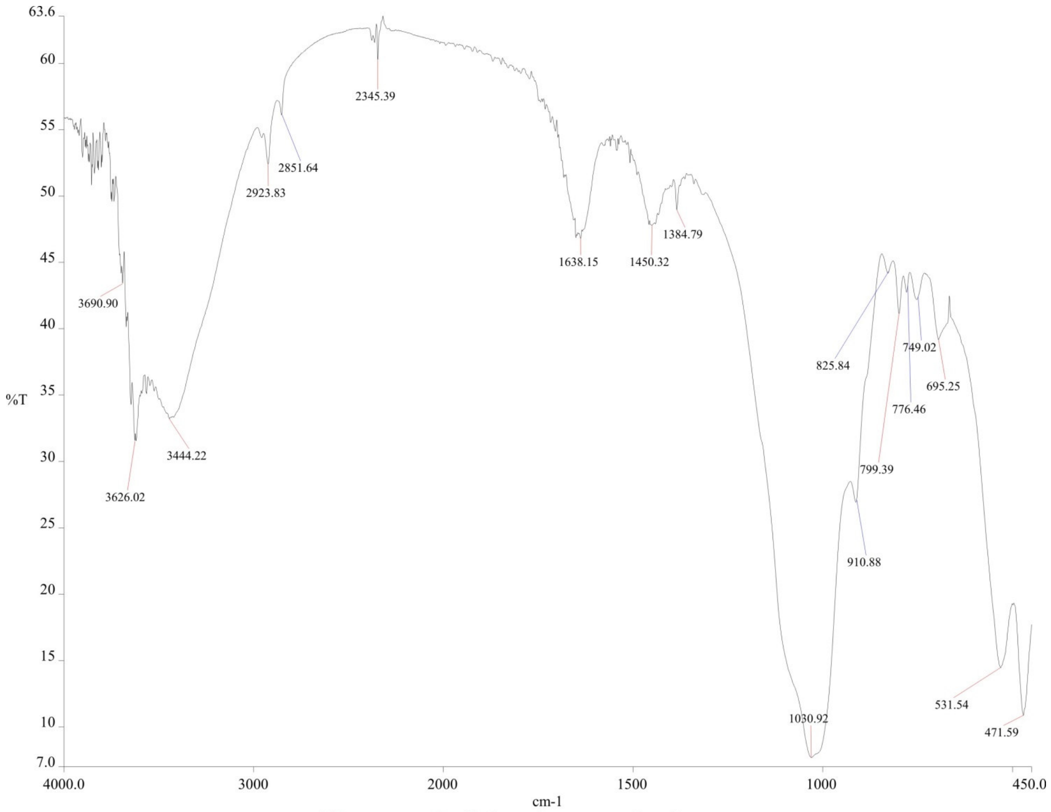
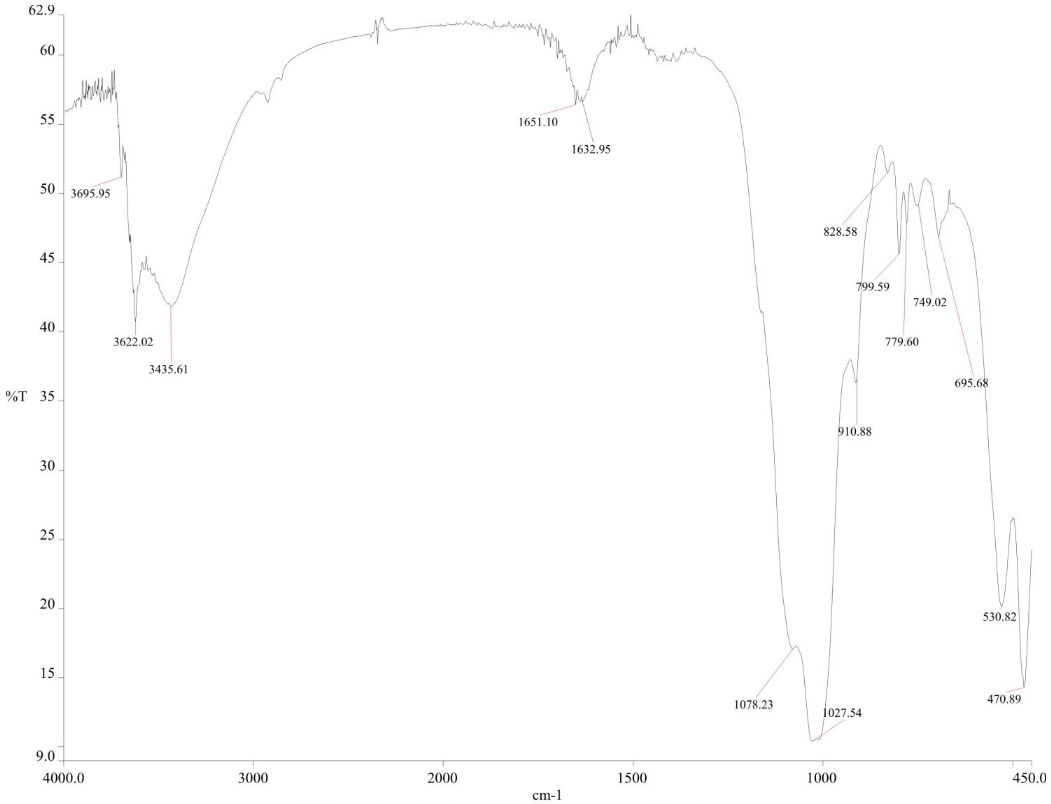
APPENDIX



IR spectra of soil clay of guava orchard.



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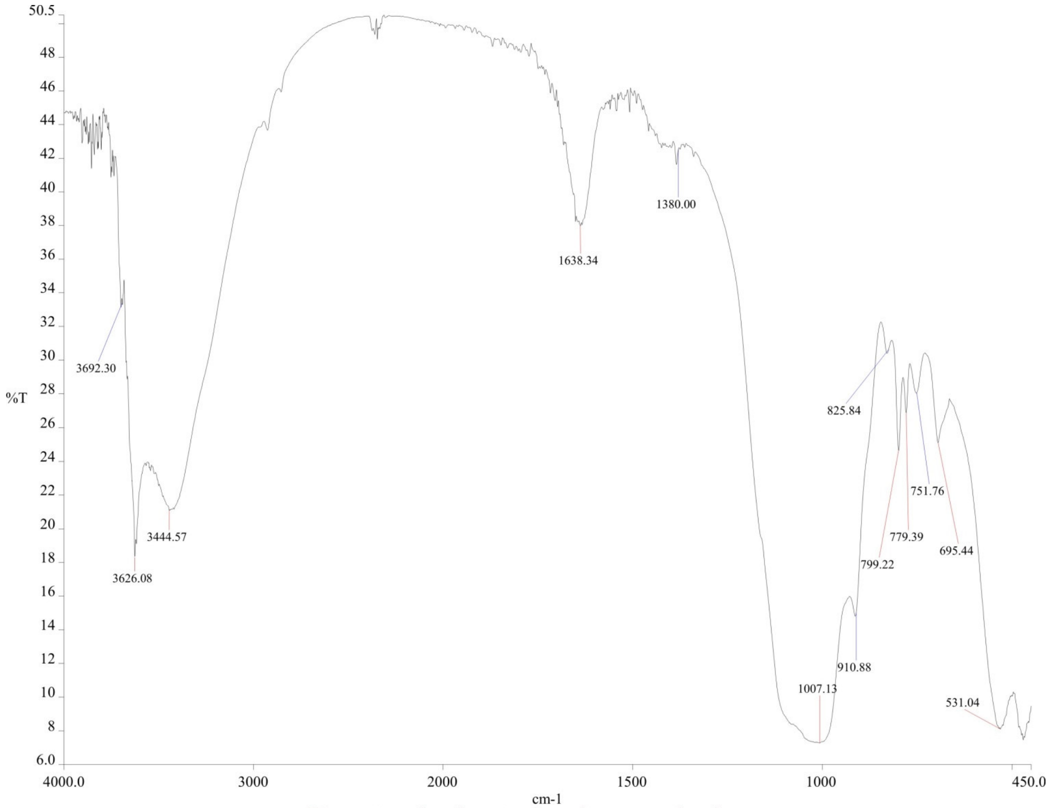

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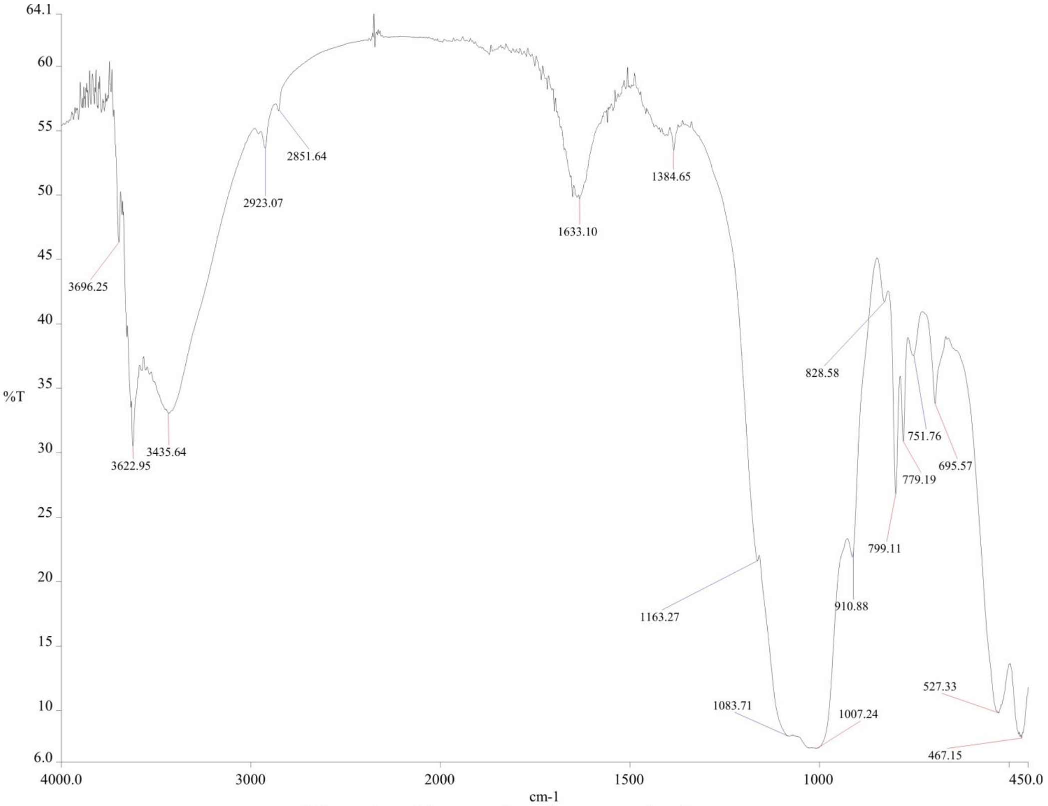
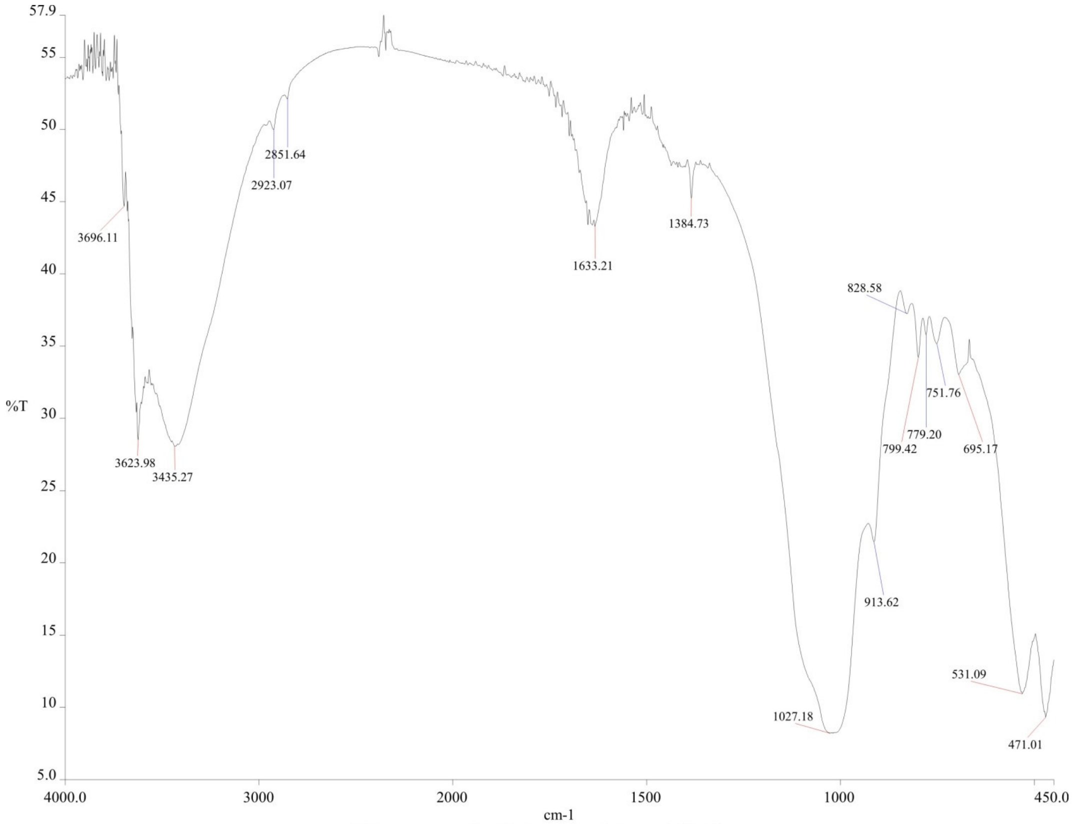
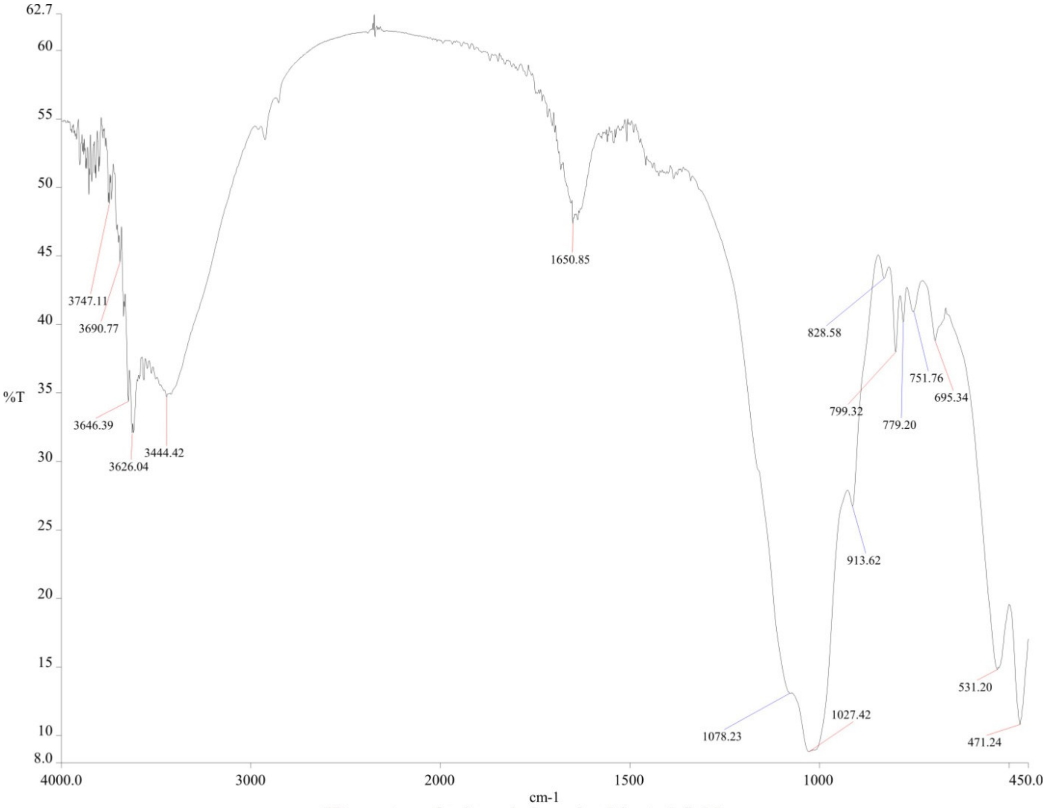


IR spectra of humus clay of guava orchard.



IR spectra of soil clay of cultivated field.



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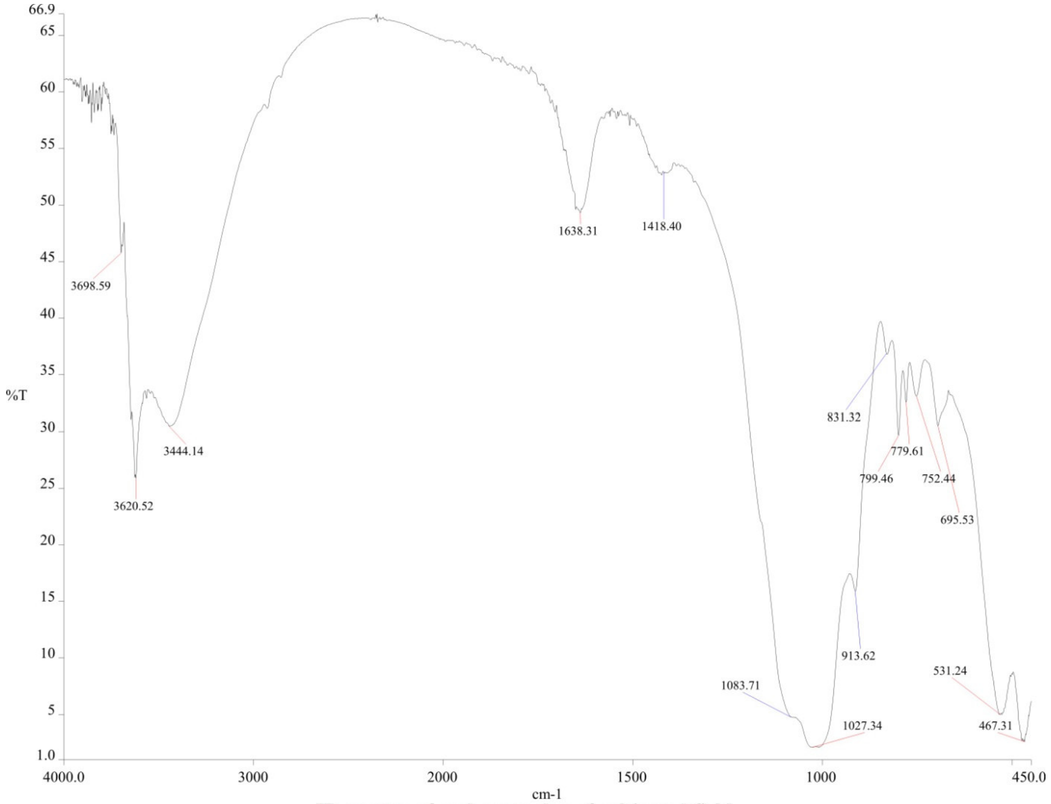

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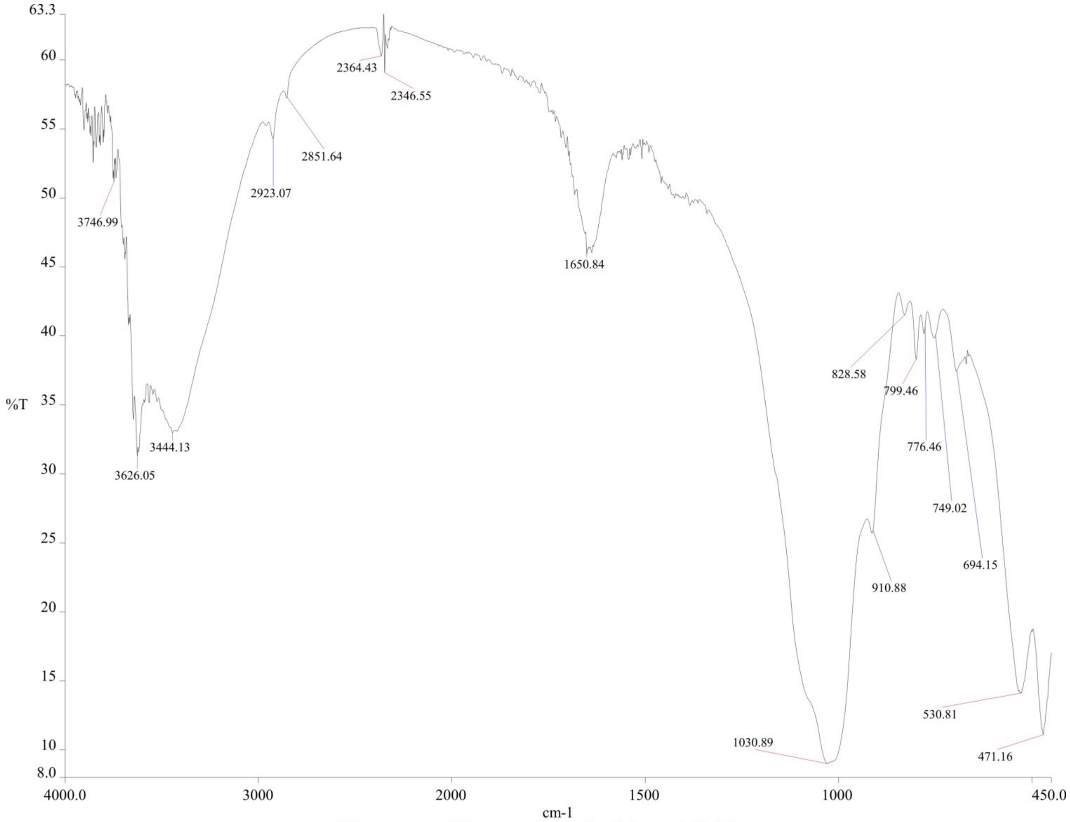

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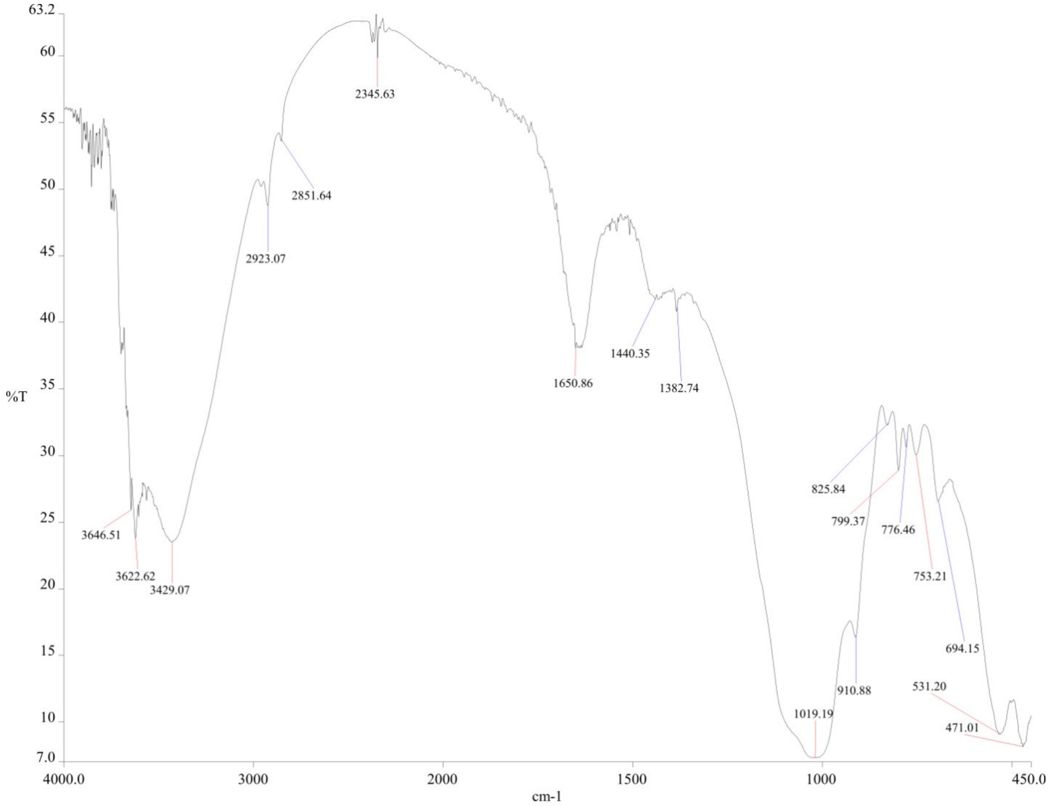
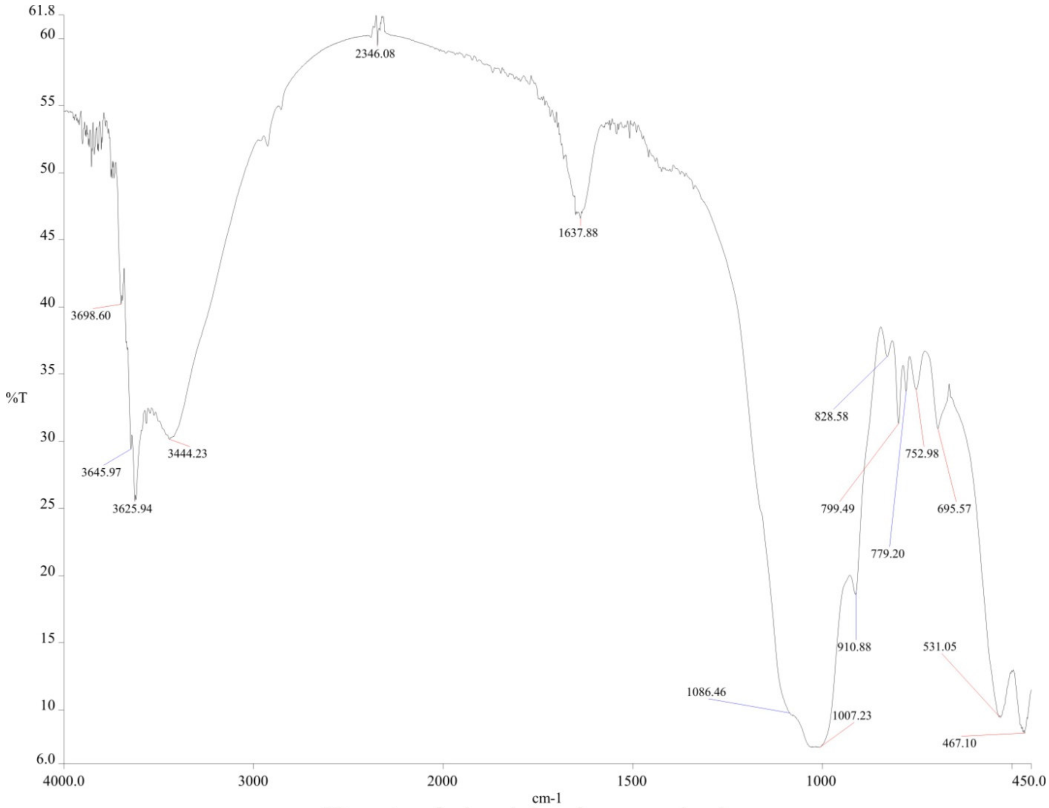
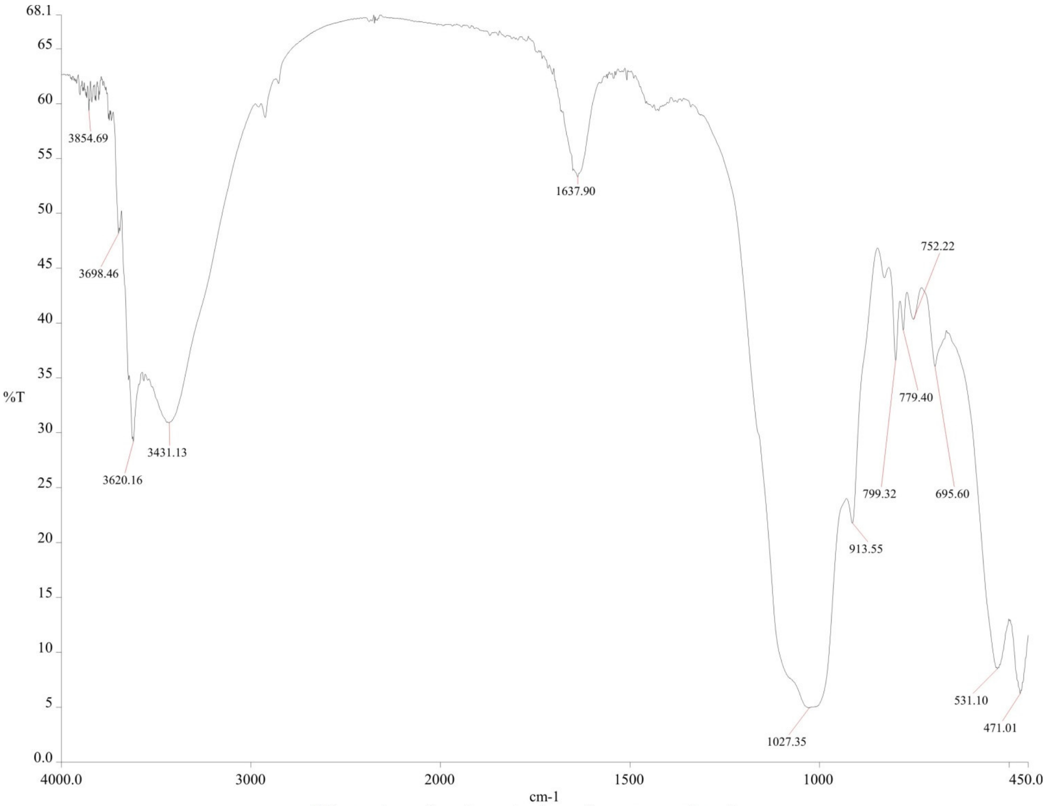


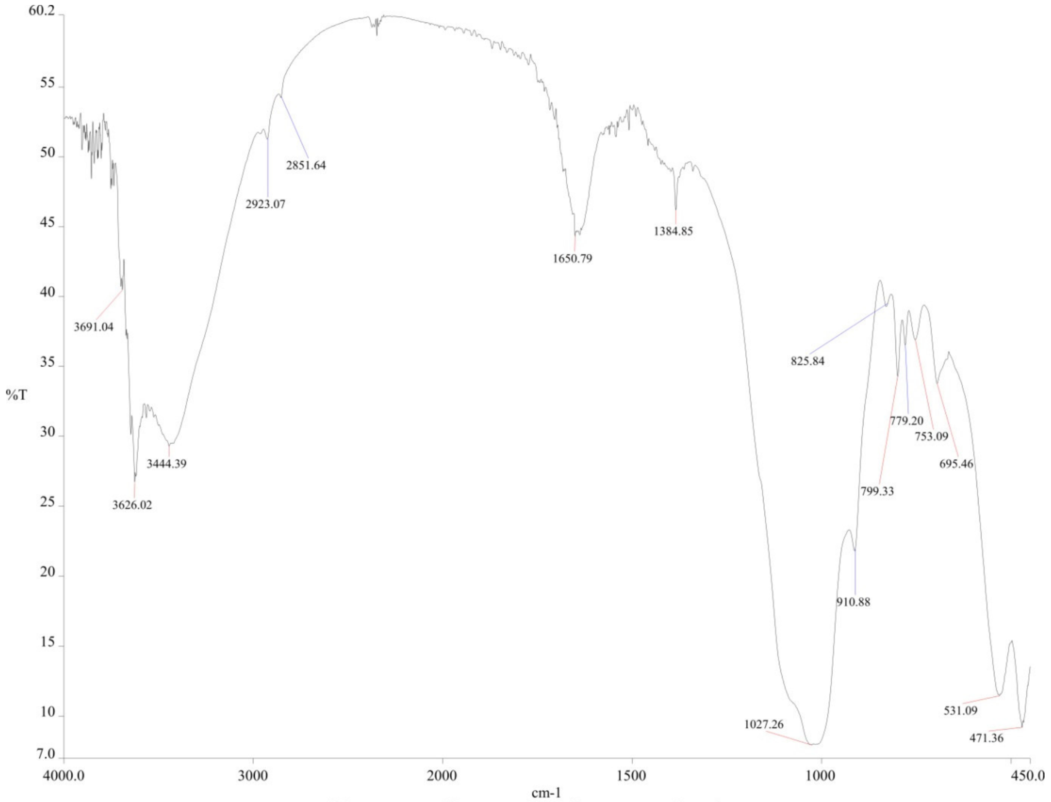
IR spectra of soil clay of mango orchard.



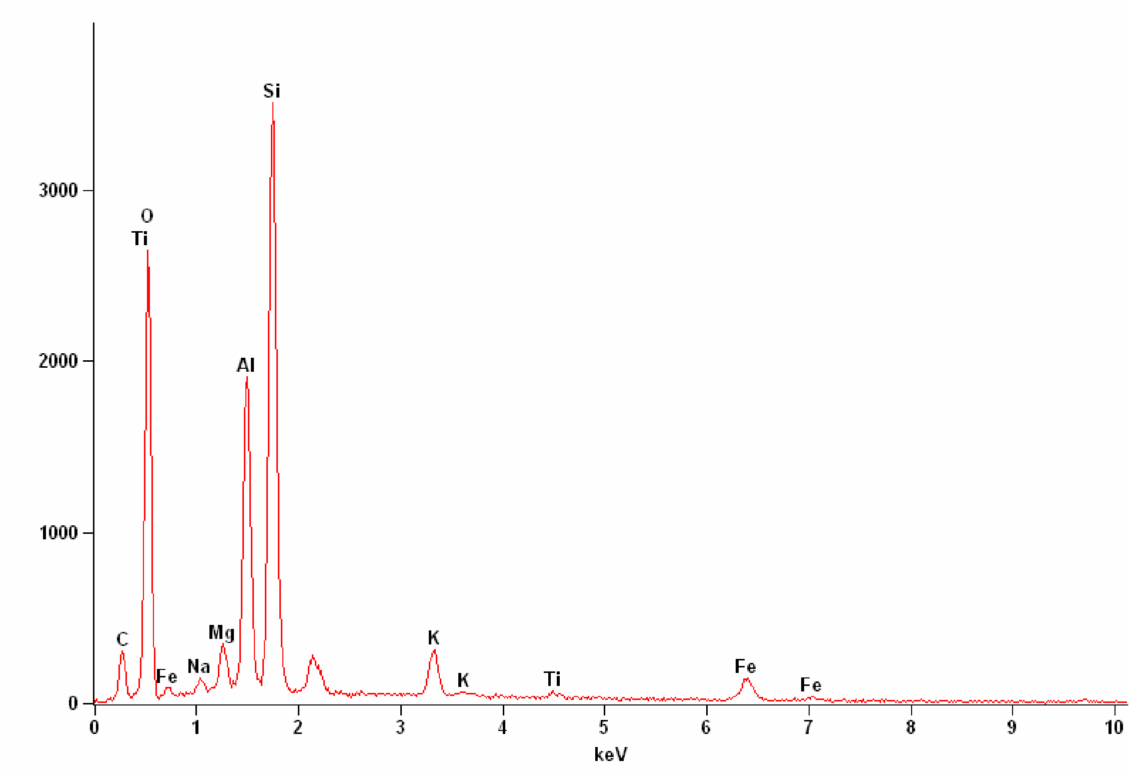
IR spectra of mineral clay of mango orchard.



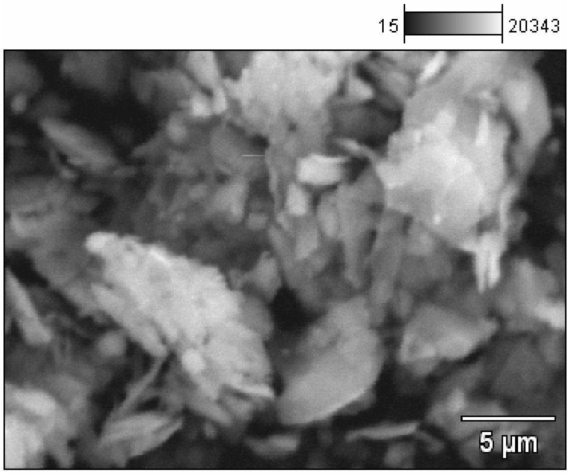
IR spectra of carbonate clay of mango orchard.

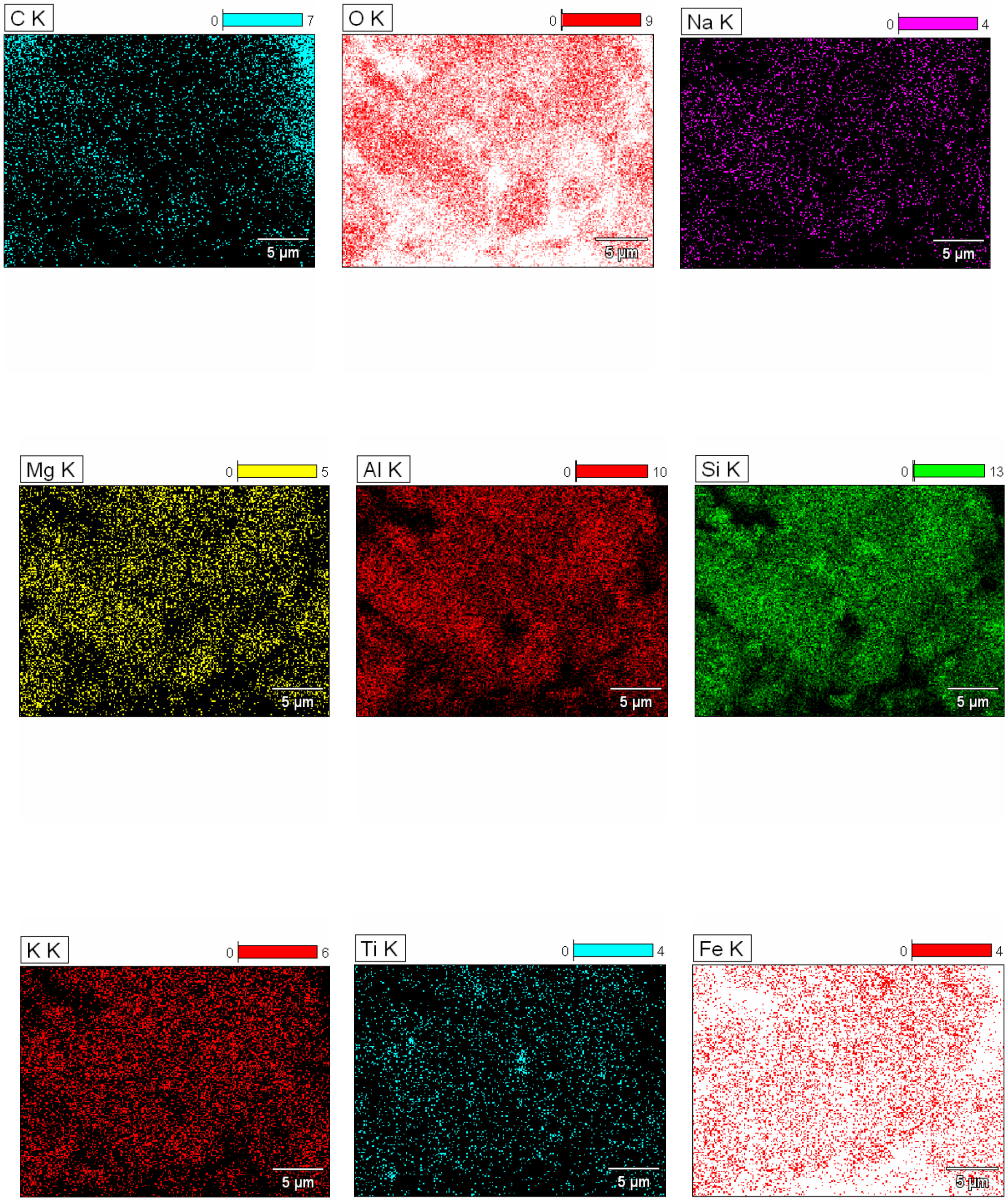


IR spectra of humus clay of mango orchard.



Element	*Atom %*
C	09.78
O	55.56
Na	0.45
Mg	0.73
Al	7.81
Si	18.42
K	1.62
Ti	0.28
Fe	5.35
Total	100.00





EDS spectra, x-ray mapping and elemental distribution of humus clay of cultivated soils (S3)

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