

# Study of clay–organic complexes

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**The union of clay minerals and organic substances in soil to form clay–organic complex is a synthesis as vital to continuance of life and as less understood as photosynthesis. The X-ray spectromicroscopy technique has enabled the identification of spatial variability of organic matter forms. Synchrotron-based near-edge X-ray spectromicroscopy of thin sections of entire and intact free organo-mineral microaggregates are able to demonstrate on spatial scales below 50 nm resolution. The zonal concept of clay–organic interactions offers a new basis for understanding and predicting the retention of organic compounds, including contaminants, in soils and sediments. Combination of atomic/molecular structural data and energy derivatives opens up new insights into binding sites and transport forms between biological and/or xenobiotic substances and soil organic matter. Therefore, understanding the mechanism/processes of formation and properties of clay–organic complexes may facilitate development of agricultural management systems that increase long-term C stability or sequestration in soils.**

**Keywords:** Clay–organic complex, molecular modeling, spatial complexity, X-ray microscopy, zonal model.

ORGANIC substances/molecules react with clay particles and form complexes with varying stability and properties. Clay–organic complexes enriched in top soils are the main reservoirs of plant nutrients. Wattel-Koekkoek and Buurman<sup>1</sup> define clay–organic complex as ‘clay-associated organic matter as all organic matter present in the clay-sized fraction, both free organic particles and organic matter bound to minerals’. But, in general terminology it may be defined that clay-bound organic matter (OM) is the OM present in the <2 µm minerals or entrapped in <2 µm microaggregates. The fertility and ecological properties on the one hand, and the substantial analytical difficulties in the direct observation of such chemically complex environmental particles on the other, have stimulated recent research in this field<sup>2</sup>. Specific compounds bearing certain chemical functional groups may be responsible for binding to mineral surfaces<sup>3</sup> and form very stable organic carbon pools with long turnover

times, whereas others may be present as particulate OM within pores of aggregates and can be rapidly mobilized, for example by tillage.

## Complex formation between clays and organic substances

The concept of clay–organic complex formation in soil is interesting, one of the processes of linking OM to clay particle (C) through polyvalent cations and represented as  $[(C-P-OM)_x]_y$ , where  $x$  and  $y$  are whole integers depending upon the size of primary clay particles. In addition to this, several other modes of interaction like water bridging, ligand exchange, hydrogen bond, etc. also help in formation of complexes between inorganic mineral constituents and organic molecules<sup>4</sup>.

The types and amount of layer silicates, intercalation of OM, content of pedogenic oxides, soil properties, vegetation, etc. are decisive in the formation of clay–organic bonds. Fractions rich in kaolinite often showed less carbon contents<sup>5</sup>, the smectite-rich fractions contain organic carbon within a wide range<sup>6</sup> while the fractions dominated by allophane have the largest carbon contents. Kang and Xing<sup>7</sup> reported the sorption of relatively low molecular fractions of humic acid (HA) on goethite surface leading to fractionation. However on the contrary, Genz *et al.*<sup>8</sup> observed a thermodynamically favourable sorption of relatively higher molecular weight fraction of soil organic matter (SOM) on granular ferric hydroxides, while kinetic studies revealed sorption of low-molecular weight fractions.

Various theories on the reaction sites of clay minerals with humic substances have been proposed<sup>4</sup>, however, evidence for the retention of humic substances between the layers of expanding layer silicates in field condition is limited, and comes generally from soils in acidic environments<sup>9,10</sup>. The smectites exhibit inter-layer space of  $c$ -axis spacing of 1.77 nm or approximately 1.8 nm on Mg saturation and glycerol solvation. However, smectites can exhibit much larger  $c$ -axis spacing on solvation. Barrer and Millington<sup>11</sup> report  $c$ -axis spacing of 2 nm on solvation of montmorillonite with dodecylammonium salt. Wolfe *et al.*<sup>12</sup> report  $c$ -axis spacing of 2–2.76 nm on solvation of alkylammonium bentonites with butanol. The  $c$ -axis spacing of montmorillonite becomes infinitely

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large on unrestricted solvation in water (free-swell test). The properties of external surfaces are much more important for the binding of organic molecules than the inner-particle crystal structure of clay minerals. Non-crystalline components of the mineral phase contribute significantly to the enrichment of OM in organic–mineral fractions<sup>13–15</sup>.

Study of complex formation between clay and organic substance has been approached from essentially two directions. The first involves isolation and separation of complex from soils based on size and density followed by their characterization<sup>16–20</sup>. Alternatively, the interaction process may be studied by allowing organic substance and their fraction to react with known clay species or vice-versa and examining the resulting complexes<sup>21–23</sup>. Since the reactants here are relatively well characterized and the experimental conditions can be readily controlled, this approach yields more meaningful information on probable bonding mechanisms.

### Composition of organic substances in clay–organic complex

#### <sup>13</sup>C nuclear magnetic resonance spectroscopy (<sup>13</sup>C-NMR)

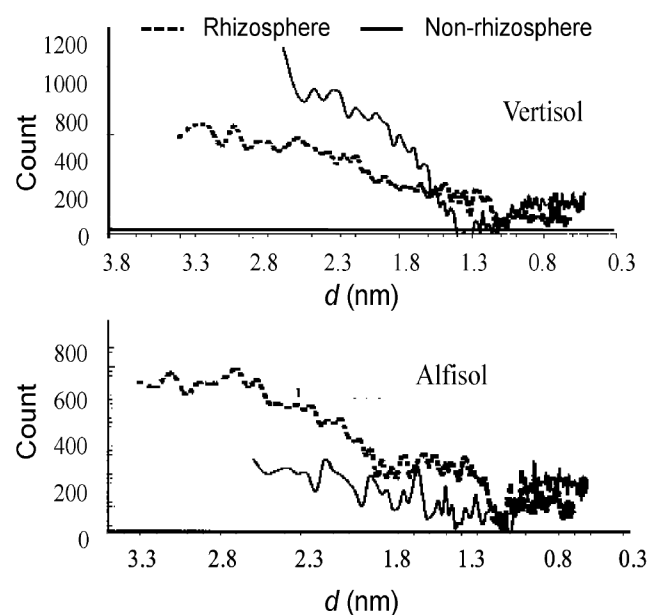
The application of <sup>13</sup>C-NMR enables an overview of the abundance of C species in particle-size fractions without pretreatment<sup>24,25</sup>. Fractions of >20 µm contained more O-alkyl-C than clay, especially in two histosols (size ranging up to 4000 µm). The largest proportions of alkyl C were observed in an alfisol and a histosol (75–4000 µm)<sup>26</sup>. The proportions of aromatic C increased with increasing particle size. Clay and fine silt fractions from a podzol (Aeh horizon) were especially enriched in aromatic carbon<sup>27</sup>, whereas these C species were often low in histosols. The C bound in carboxyl groups seemed to be scarcely influenced by particle size. The limitations associated with the technique were highlighted by Randall *et al.*<sup>28</sup>.

#### Analytical pyrolysis

The combination of pyrolysis and field ionization mass spectrometry (Py-FIMS) and curie-point pyrolysis gas chromatography/mass spectrometry (Py-GC/MS), has been used to study organic–mineral interactions in particle-size and density fractions of soils and SOM fractions under different land uses<sup>29–30</sup>. On an average, about 62% of C and 81% of N was pyrolysed from such samples. One of the great advantages of Py-FIMS over other methods lies in the temperature-dependent volatilization of organic molecules which gives an indication of the stability of the chemical bonds involved<sup>31,32</sup>.

#### Differential X-ray diffraction (DXRD) analysis

Conventional X-ray diffraction (XRD) diagram is not sufficient for semi-quantification of clay minerals and their intergrades based on peak area measurement because of overlapping of adjacent broad peaks. Moreover, humus material being amorphous does not show any peak in the conventional XRD and hence its presence and stability cannot be studied by this technique<sup>33</sup>. To overcome these problems to study clay–organic complex by XRD, an attempt has been made for profile fitting by differential XRD (DXRD), which can also detect presence of clay–organic complex and its stability. Area under DXRD curve shows the amount of dissolved humus as a function of basal space of clay minerals (Figure 1). Studies show that more humus has been removed from interlayer position of rhizosphere sample (in the range of basal spacing of 33–13 Å) compared to non-rhizosphere in case of alfisol, while in vertisols more humus has been removed from interlayer position of non-rhizosphere sample<sup>34</sup>. This indicates that complexation of humus in the interlayer position due to expansion of smectite mineral and formation of smectite–kaolinite layer in rhizosphere sample so, clay–organic complex formed in rhizosphere sample of vertisols were more stable than that of alfisols. The clay mineralogical suits of vertisols were dominantly of smectite with appreciable amounts of kaolinite and mica (smectite and interstratified minerals 54%, kaolinite 26% and mica 20%), whereas the alfisols contained mica (mica 50%, kaolinite 34% and smectite/interstratified minerals 16%).



**Figure 1.** Differential X-ray diffraction (DXRD) pattern of clay–organic complex isolated from rhizosphere and non-rhizosphere of vertisol and alfisols (Source: ref. 34).

### Scanning electron microscopy analysis

The scanning electron microscopy (SEM) in conjunction with XRD, chemical, biochemical and spectroscopic analysis has revealed the existence of two distinct types of clay-associated humic substances. The first type of humic substance exists as a diffuse filamentous form that covers surfaces of smectite and protoillite particles in medium and fine clay fractions, having low C/N ratio (10), high levels of extractable biomolecules (primarily amino acids and fatty acids), and relatively strong  $^{13}\text{C}$ -MAS-NMR peaks in the aliphatic region<sup>35,36</sup>. The second type of humic substance exists as discrete high density metal-humic complexes, having higher C/N ratio (17), lower levels of extractable biomolecules, and produces relatively stronger  $^{13}\text{C}$ -MAS-NMR peaks in the carboxyl, O-alkyl, and alkene-aromatic regions<sup>36</sup>. The DRIFT (diffuse reflectance infrared Fourier transform spectroscopy),  $^{13}\text{C}$ -NMR and elemental analysis techniques showed that aliphatic moieties of HA preferentially sorbed on kaolinite and montmorillonite, while carboxylic functional groups play a significant role in sorption of HA on goethite<sup>37</sup>. The  $^{13}\text{C}$ -MAS-NMR spectra of organo-mineral aggregates of chernozem in Northeast China indicated that the contents of aromatic carbon in clay, silt, fine sand and coarse sand were 25.6%, 28.19%, 17.22% and 26.32% respectively<sup>38</sup>.

### Near-edge X-ray fine structure spectroscopy

X-ray spectromicroscopy (XSM) has enabled the identification of spatial variability of OM forms, but was limited to extracted soil particles and individual micropores within aggregates. But, synchrotron-based near-edge X-ray fine structure (NEXAFS) offered valuable insights into organic carbon forms associated with clay-organic complex, individual particles extracted from soil and individual pores within microaggregates on spatial scales below 50 nm resolution<sup>39-43</sup>. Synchrotron-based scanning transmission X-ray microscopy (STXM) coupled with near-edge X-ray absorption fine structure (C 1s-NEXAFS) spectroscopy was ably used to investigate the nanoscale physical assemblage and C chemistry of 150  $\mu\text{m}$  microaggregates, extract microvariation to map C molecular composition with high spatial resolutions (up to 50 nm), resolve the nanoscale biogeocomplexity of unaltered soil microaggregates and also C, mineral and pore assemblage features to resolve without altering the physical structure of the microaggregate. This helped in construction of thickness maps<sup>40</sup>, which represent cluster spectra of C forms and those that define regions of mineral and pore space. Lehmann *et al.*<sup>44</sup> support observations of OM stabilization on surface microfilms and in pore structures of the organo-mineral assemblage. Ade and Stoll<sup>45</sup> reviewed the potentiality of NEXAF technique

for the characterization of organic and inorganic materials and also discussed the perspectives on future developments.

### Conceptual models of clay-organic complexes

The conceptual models assume that SOM consists of a heterogeneous mixture of compounds that display a range of amphiphilic or surfactant-like properties, and are capable of self-organization in aqueous solution. Bilayer model of clay-organic interactions<sup>46</sup>, sharply contrasted with the traditional view of clay-organic interactions<sup>47</sup>, which were visualized as associations of large, multifunctional polymers with mineral surfaces via broad range of bonding mechanisms<sup>47,48</sup>. Further, bilayer model postulated that decayed organic materials are often bound to clay surfaces by amino acids or proteins.

The zonal model was developed by Kleber *et al.*<sup>3</sup> based on the amphiphilicity of SOM fragments, and the intimate involvement of proteinaceous compounds in organo-mineral associations. According to this model, the variety of surface types in soils is represented by a low charge smectite, a hydroxylated Fe-oxide coating, and a hydrophobic kaolinite siloxane surface. Zonal concept<sup>3</sup> proposes that the self-assembly of SOM on clay mineral surfaces creates a layered or zonal structure with regular features. Spectroscopic evidence suggests that the decomposing organic residues in soils can be viewed as a mixture of largely amphiphilic fragments<sup>49-52</sup>. The amphiphilicity of many of these molecular fragments, and the polarity of the aqueous soil solution in which they are contained, cause them to self-assemble into micellar associations when suspended at high concentrations, and into layered or zonal structures when they come in contact with mineral surfaces.

### Results in support of conceptual models

Assuming such a zonal structure, Kleber *et al.*<sup>3</sup> enumerated a number of phenomena observed in soils and sediments, including: decrease of C/N ratio with decreasing particle size and with increasing particle density<sup>52</sup>, decrease of  $^{14}\text{C}$  content with decreasing C content in heavy fractions<sup>52</sup>, preferential sorption of large, hydrophobic organic compounds in sorption experiments with soils and in sediments<sup>49</sup>, existence of at least two divergent C and N pools in heavy fractions: an older, more stable pool of C and N, and a more recent, fast cycling pool of C and N<sup>53</sup>, mineral-associated OM accumulation in A horizons beyond levels consistent with monolayer coverage<sup>50</sup>, correlation of concentrations of poorly crystalline mineral phases with amounts of oxidation-resistant SOM<sup>28</sup> and seasonal changes in water repellency in soils<sup>52</sup>.

Stabilization of OM in soils is predominantly due to its interaction with mineral phases<sup>54</sup>. Interaction occurs with polar organic functional groups of amphiphiles via ligand exchange with singly coordinated mineral hydroxyls to form stable inner-sphere complexes, proteinaceous materials adsorbed on charged surfaces ('contact' zone) and association of hydrophobic substances with noncharged mineral surfaces ('zone of hydrophobic interactions')<sup>3</sup>. Sollins *et al.*<sup>55</sup> showed (in an Oregon forest andic soil) that C, N and lignin phenol concentrations all decreased and <sup>14</sup>C mean residence time increased with particle density. Kögel-Knabner *et al.*<sup>56</sup> also showed the importance of the mineral fraction in OM protection. In coarse-textured soils, the biochemical protection of SOM was the primary mechanism controlling SOM dynamics<sup>57</sup>. On the contrary, soils with high clay content, stabilization by physical and chemical mechanisms, including aggregation, sorption on mineral surfaces, and entrapment within fine pores, will be the primary control over C accumulation<sup>58–60</sup>. According to the model proposed by Kleber *et al.*<sup>3</sup>, the formation of particularly strong organo-mineral associations appears to be favoured in the so-called contact zone by situations where proteinaceous materials unfold upon adsorption, thus increasing adhesive strength by adding hydrophobic interactions to electrostatic binding.

### Molecular models of clay–organic complexes

Molecular model structures of clay–organic complexes help in better understanding of humic properties, such as association/dissociation processes, binding and trapping of biological and anthropogenic materials, and environmental functions. Molecular modelling of humic/xenobiotic complexes<sup>15</sup> showed the potential of this method to contribute to a better understanding of interactive processes on an atomic and molecular basis. Computer-based modelling using software like ChemPlus 3.1 for quantitative structure–activity relationships offered the possibility of determining molecular properties of humic substances, dissolved OM (DOM) in water and SOM in soil fractions and whole soils and the steps are explained by Schulten and Leinweber<sup>61</sup> as given below. In a first step, comprehensive investigations combining geochemical, wet-chemical, biochemical, and spectroscopic, thermal, agricultural and ecological data with analytical pyrolysis resulted in a preliminary C skeleton of HAs<sup>62</sup>. In the second step of the model's evolution, a 2-D HA structure including functional groups such as COOH, aliphatic and aromatic OH, ketones, O and N heterocyclic compounds and nitriles etc., was proposed<sup>63</sup> and converted to a 3-D model using Hyper-Chem. The third step consisted of molecular modelling of HA oligomers and polymers in the range of 10–15 HA units<sup>15</sup>. In a fourth step, techniques of molecular modelling were employed to

perform molecular-mechanics calculations and geometrical optimization of HAs and xenobiotics in water<sup>15,64–66</sup>.

### Stabilization of carbon in clay–organic complex

Stabilization of carbon has great importance for biogeochemical cycles in an ecosystem on a global scale<sup>67</sup>. Stabilization of carbon in soil is mainly achieved through two processes, clay–organic interactions and occlusion of these aggregates by clay particles<sup>68,69</sup> but to what extent they contribute is still unknown. The conceptual model<sup>50</sup> integrates all three processes of OM stabilization in soils namely selective preservation of recalcitrant compounds, spatial inaccessibility to decomposer organisms, and interactions of OM with minerals and metal ions. The model concept relates the diverse stabilization mechanisms to active, intermediate and passive pools. Biogenic aggregation preserves OM in the intermediate pool and is limited to topsoil horizons<sup>70</sup>. Spatial inaccessibility due to the occlusion of OM in clay microstructures and due to the formation of hydrophobic surfaces stabilizes OM in the passive pool. The importance of clay–organic interactions for OM stabilization in the passive pool is more and increases with soil depth. Tillage increases the importance of clay–organic interactions or OM stabilization, and in Ap horizons with high microbial activity and C turnover, clay–organic interactions can contribute to OM stabilization in the intermediate pool. Effect of increasing concentrations of greenhouse gases in the atmosphere on global climate has increased interest in the soil C cycle and increasing soil C sequestration. The transformation of plant residues into stabilized clay–humic complexes is a key process influencing both soil quality and whether a soil is a net sink or source of C to the atmosphere. During the post-harvest incubation, <sup>14</sup>C specific activity (Bq g<sup>−1</sup> C) increased from 0 to day 360 in all particle-size fractions<sup>33</sup> and which provided the direct evidence for the movement of new C from plant residue into the clay associated humic complexes.

### SOM content in Indian soil and carbon retention

SOM not only affects sustainability of agricultural ecosystems, but is also extremely important in maintaining overall quality of environment as soil contains a significant part of global carbon stock. Studies indicate that addition of organics, conservation tillage and inclusion of legumes have considerably improved soil aggregation and enhances C and N sequestration under different cropping systems<sup>71–76</sup>. Indian dryland soils are low in organic C but have potential to sequester, harnessing through appropriate production systems and management practices<sup>77,78</sup>.

Total soil organic carbon pool in soils of India is estimated at 21 Pg to 30 cm depth and 63 Pg to 150 cm

depth. The SOC pool in soils of India is 2.2% of the world pool for 1 m depth and 2.6% to 2 m depth<sup>79</sup>. Carbon sequestration potential of Indian soils is estimated to be 39.3–49.3 Tg C/year (mean of 43.3 Tg C/year), which can be significant towards reducing the net emission from fossil fuel combustion in India. Retention of carbon in soils improves agronomic/biomass productivity, these options also improve water quality and mitigate climate change by decreasing the rate of enrichment of atmospheric CO<sub>2</sub>. Realization of this vast potential, which is in the interest of the nation, requires adoption of recommended management practices including the use of mulch farming and conservation tillage, integrated nutrient management and manuring, agroforestry systems, restoration of eroded and salinized soils, and conversion of agriculturally marginal lands into restorative land uses<sup>80</sup>.

Detailed studies on both the synthetic<sup>81–84</sup> and naturally occurring clay–organic complexes (under different soils of India)<sup>85–87</sup> lead to the development of a comprehensive picture of interactions of soil minerals with organic substances; carbon retention in soils to a varying quantity. These concepts have been developed based on the data generated through an in-depth study of crystal structures, chemical reactions, high precision analytical and instrumentation techniques<sup>34,81–87</sup>.

## Conclusion

Two-dimensional microscale and nanoscale observations of the carbon distribution in clay–organic complex through STXM, FTIR and NEAXAF provided new insights into the mechanisms of clay–organic complex formation. Knowledge of physical, chemical and biological properties of organic, mineral particles enables the development of conceptual models and their role in SOM turnover. Zonal concept elucidates the structure and dynamics of clay–organic associations in natural systems and opens up a new area towards improved understanding of organic C and N dynamics in soils and may also facilitate the modelling of the fate and transport of contaminants.

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