Sepiolite and sepiolite-bound humic acid interactions in alkaline media and the mechanism of the formation of sepiolite-humic acid complexes

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Abstract: The surface-controlled reactions and interfaces of clays and/or clay minerals are strongly determined by the adsorption of dissolved humic substances which have surface active characters. Consequently, this may lead to an improved understanding of organo-clay complexes for remediation purposes of polluted areas. The interactions between sepiolite and sepiolite-bound humic acid (HA), and its influence on the mechanism of the formation of sepiolite-humic acid complexes were examined by characterizing the structure of sepiolite through UV/Vis spectroscopy, field emission scanning electron microscopy (SEM+FEG), specific surface area, pore size and volume, and Fourier transform infrared spectroscopy (FTIR). The UV-VIS spectral results of the raw sepiolite showed that there is no correlation between UV₂₅₄ and the amount of humic substance released from sepiolite is dependent on increasing extraction time. The low values of humification index of the sepiolite confirmed the presence of HA with higher, and similar molecular weight, particle size, humification degree and aromaticity. Hence, it can be concluded that HA groups are presumably associated with the large quantity of silanol groups located on the sepiolite external surface preferentially through H-bonds and/or surface complexation interactions between the functional groups in HA and dissolved metal cations (M^{n+}). This finding is in good agreement with results from FTIR and SEM-FEG investigations. EDAX analysis of the HA-extracted sepiolite illustrated a strong decrease in Mg^{2+} , Ca^{2+} and K^{+} content indicating that electrically charged sites on HA may form metal complexes of high stability through chelation.

Keywords: Sepiolite; sepiolite-bound humic; humic acid complexes; extraction; mechanism

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1. Introduction

Humic materials are the most widely-spread natural complexing ligands occurring in nature. One of the main functions of dissolved humic materials in the environment is to remove toxic metals, anthropogenic organic chemicals, hydrophobic (including PAHs) and other pollutants from water (Peña-Méndez et al., 2005; Shin et al. 1999; Radian and Mishael, 2012). The higher solubility and mobility of the pollutants in the presence of dissolved humic materials due to their ability to reduce surface tension of water, result in their binding to dissolved humic materials. This can not only affect their transport and aqueous concentration but it can also influence their chemical degradation, photolysis, volatilization, toxicity, and bioavailability (Chiou et al. 1986; Spitzzy and Leenheer, 1991; Rav-Acha, and Rebhun, 1992; Kordel et al. 1997; Frimmel, 1998; Laor et al., 1998; Frimmel and Christman, 1998; Frimmel et al., 2002). It has also been known that dissolved humic substances, such as humic acid (HA, insoluble at acidic pH) and fulvic acid (FA, water soluble at acidic to alkaline pH), are complex and heterogeneous mixtures of polydispersed materials, negatively charged due to the presence various chemically reactive functional groups, including carboxylic ($-\text{COOH}$) and phenolic $-\text{OH}$ groups on aromatic and aliphatic chain, with pH-dependent properties (Gu et al., 1994; Vermeer et al., 1998; Vermeer and Koopal, 1998; Chen et al., 2007). FA has a higher amount of carboxylic and phenolic groups (higher molar mass), contains more carbon, and is less soluble compared to HA, which consists of a more aromatic structure Stevenson, 1994). The major reason for the importance of FA and HA in soil/clay chemistry, sediment biogeochemistry and environmental reactivity is the presence and position of the functional groups (particularly carboxyl and phenolic hydroxyl groups), which make FA and HA effective in cation exchange and complexation reactions (Tan, 1986; Simpson et al., 2006).

Among the inorganic substances that constitute the soils, clays and metal oxide minerals are the most abundant, and their surface-controlled reactions and interfaces are strongly determined by the adsorption of dissolved humic substances which have surface active characters. As a consequence, this characteristic could improve the understanding of organo-clay complexes for the purpose of contaminated soil remediation. Some of the dominants factors influencing the nature of complexation between dissolved humic substances and clays are (i) the nature of the clay mineral (surface chemistry, specific surface area, pores), (ii) environmental conditions such as pH, ionic strength, composition of cations, anions, etc., and (iii) the nature the dissolved humic substances that are often interdependent (Theng, 2012). Researchers have extensively investigated the influence of the change in solution conditions (e.g. nature of exchangeable cations, medium pH and ionic strength, molecular weight of humic substances) on dissolved humic substances (FA/HA) adsorption on clay mineral surfaces (Mortland, 1970; Greenland, 1971; Baham and Sposito, 1994; Schlautman and Morgan, 1994; Arnarson and Keil, 2000; Satterberg et al., 2003; Feng et al., 2005). It is apparent that while the process of selective adsorption of dissolved humic substances onto clay surfaces is generally accepted, there is considerable variation due to the heterogeneities and complexity of humic substances in their composition and conformation that are reported to be preferentially sorbed (Nayak et al., 1990; Murphy et al., 1994; Collins et al., 1995; Vermeer and Koopal, 1998; Vermeer et al., 1998; Namjesnik-Dejanovic et al., 2000; Khalaf et al., 2003; Wang and Xing, 2005; Feng et al., 2006; Chilom and Rice, 2009). Furthermore,

adsorption of FA/HA on minerals is dependent on clay-polyvalent ion like Ca^{2+} , Mg^{2+} , Fe^{3+} , Al^{3+} , and on the surface properties of the mineral (Varadachari et al., 1991; Varadachari et al., 1994; Varadachari et al., 1995; Kretzschmar et al., 1997; Tombácz et al., 1998; Furukawa, 2000; Specht et al. 2000; Chorover and Amistadi, 2001; Książopolska, 2001; Balcke et al., 2002; Kaiser and Guggenberger, 2003; Feng et al., 2005; Majzik and Tombácz, 2007; Martinez et al., 2010; Ksiezopolska and Pazus, 2011; Zhang et al., 2012; Ding et al., 2013). As a result, the differences in type of clay minerals would lead to different combinations of adsorption mechanisms (Evans and Russell, 1959; Werhaw, 1986; Theng and Tate, 1989; Wershaw and Pinckney, 1989; Zhou et al., 1994; Ohashi and Nakazawa, 1996; Wattel-Koekkoek et al., 2001; Wattel-Koekkoek, 2002; Saada et al., 2003; Tombácz et al, 2004; Majzik and Tombácz, 2007; Sposito, 2008). Most of these studies have focused on the interaction between dissolved humic substances and the crystalline clay minerals such as kaolinite (typical two-sheet phyllosilicates), vermiculite (the expanding three-sheet phyllosilicates), smectite (strongly expanding three-sheet phyllosilicates), chlorites (four-sheet silicates). These are bound to the soils but also to the layer-fibrous structure clay minerals such as the palygorskite and sepiolite including dissolved humic substances. However very little work has been published regarding their relevance to many aspects of surface and environmental chemistry. An attempt was made by Benli (2014) to present the effects of humic acid release from sepiolite on its interfacial and rheological properties of alkaline dispersions. The results observed that highly alkaline pH caused an increase in the total amounts of HA and free ions in relation to the formation/precipitation of both hydrophobic HA aggregate and HA-Mg^{2+} complexes which have a spherical shape in the size range of $18\text{--}63 \pm 8$ nm at pH 11.5. Based on these findings, alkaline environment also results in significant changes in the surface properties of sepiolite from the anisotropic to the higher hydrophobic level like natural hydrophobic minerals, e.g. talc. In an early technical note (Singer and Huang, 1989) it was shown that the effect of pH and heat activation on the adsorption behavior of humic acid was obtained from a typical Cryoboralf on the sepiolite used from Vallecas and the palygorskite from Florida. Humate adsorption on both palygorskite and sepiolite at pH 6.5 was found to increase with increased initial humate concentration, and decrease with preheating the clay samples and then considerably dispersing in the humate solutions. Golden et al. (1985) also investigated the instability of palygorskite and sepiolite taken from the same region under slightly alkaline conditions (0-8 mmole NaOH) to determine the transformation of palygorskite and sepiolite to smectite under laboratory conditions. In a similar manner, the study about the behaviour and stability of the Vallecas sepiolite in aqueous solutions of neutral and weak alkaline (0.04 N NaOH) conditions at room temperature have been conducted by Martinez-Ramirez et al. (1996) Collectively, these two studies indicate that palygorskite treatment for 24 days in these media yielded a smectite, and that after 24-28 days in similar conditions, the sepiolite is destroyed gradually, e.g. produced small surface structural changes. Currently, there is no published research on the structural and functional characterization of sepiolite-complexed dissolved organic matter and the corresponding interaction mechanisms of humic substances with sepiolite surfaces in order to better understand the adsorption and colloidal-rheological environmental applications of sepiolite.

The clay minerals palygorskite and sepiolite occur in many arid and semi-arid soils (Kadir et al., 2002). They are both fibrous in form, a characteristic dictated by their chain-type (linear) structure and are predominantly silico-magnesian containing varying amounts of Al^{3+} , K^+ , Ca^{2+} , Fe^{2+} , Fe^{3+} ions. They contain hydroxyls, zeolitic and bound water. Sepiolite and organo sepiolite have a significant potential as alternative adsorbents. This is due to their inherent strong sorption and complexation ability (mainly due to their high surface area, to their structural channels running parallel to the fibre length, and to their pores) toward various environmental pollutants of concern. This coupled with their environmental compatibility and cost effectiveness makes them ideal alternative adsorption materials for a wide range of industrial and pharmaceutical wastewater treatment applications.

The purpose of the present study is to develop an understanding of the interactions between sepiolite and humic acid bound to sepiolite, and their impact on the mechanism of sepiolite-humic acid complexes formation with a view to optimize the adsorption capability of the system. Two different approaches have been used to study the sepiolite-complexed organic matter. One involves the gradual removal by extraction of the humus substances from the sepiolite and replacing it by humic acid and the other is to understand the relations between surface complexation, and their nature in the sepiolite-organic matter system.

2. Materials and methods

2.1. Materials

Sepiolite used in the experiments are natural, non-swelling fibrous clay from Sivrihisar-Kurtşeyh occurring at the upper Sakarya Section of Central Anatolian Neogene Basins southeast of Eskişehir, Turkey.

Inorganic chemicals such as HCl and NaOH used for adjusting pH and zeta potential measurements were obtained from Fisher Scientific, and all solutions were prepared using de-ionized water with a conductivity of 0.067 $\mu S/cm$.

2.2. Methods

Raw sepiolite was comminuted by two stage crushing in laboratory jaw and roll crusher to prepare samples less than 2 mm as a starting material, and it was then dry ground in a vibrating ball mill for short grinding time at 25 min to avoid the structural deformation (Vučelić et al., 2002; Kojdecki et al., 2005). Particle size distribution of ground samples was analyzed by a technique based on laser light scattering using MALVERN Mastersizer 2000.

The density of both sepiolite samples was measured using a Quanta- chrome Ultrapycnometer 1000, and bulk density was measured with Haver bulk density tester according to EN 1097-3 standard (1998). The BET surface area, pore volume and average pore diameter of sepiolites before and after extraction experiments were determined using the Qantachrome instrument using nitrogen gas adsorption at $-196^{\circ}C$. Before each measurement all samples were outgassed for 10 h by heating $50^{\circ}C$ (Sarıkaya, 1981) under vacuum.

Chemical analysis of the sepiolite was determined with an X-ray fluorescence (XRF) spectrometer (Rigaku ZSX Primus II XRF). X-ray powder diffraction patterns (XRD) of the

tested sepiolite samples were measured by Panalytical X Pert Pro MPD. Total organic carbon (TOC) measurement of the original sepiolite samples was carried out at Istanbul Technical University EMCOL Labs in Turkey. A JEOL JSM-7100F field emission scanning electron microscope (SEM-FEG) equipped with a Link System energy dispersive X-ray (EDX) microanalyser was used to observe the textural features of natural and humic acid extracted sepiolite. Fourier transform infrared spectra (FTIR) were recorded on the Agilent Cary 640 FTIR high performance spectrometer using attenuated total reflectance (ATR) and diffuse reflection (DR) for rapid characterization of sepiolite samples. The spectra were measured in the range from 4000 to 400 cm^{-1} (32 scans, 4 cm^{-1} resolutions).

The zeta potentials of sepiolite samples were determined at $23 \pm 5^\circ\text{C}$ by Zetasizer Nano Z (Malvern Instruments) which uses micro-electrophoresis/electrophoretic light scattering technology. Zeta potential measurements were carried out as a function of the pH of natural sepiolite suspensions. In this procedure, the sepiolite suspensions with a minimum solid concentration of 0.1 g/l to avoid particle interaction ($10^{-2}\%$ mass according to Malvern's recommendation) were conditioned at 500 rpm. The pH value of each suspension was adjusted by adding either NaOH or HCl. The pH of the solution was measured using the combination HI 1131 pH electrode with Ag/AgCl reference cell (HI 2210 pH meter, Hanna Instruments). After conditioning for 10 min followed by centrifuging for another 10 min at 3000 rpm an aliquot was taken from the supernatant and used to measure the zeta potential. The average of ten measurements was taken to represent the measured potential. The mean relative standard deviation (σ) of the values reported usually did not exceed 5.

To separate organic matter from the inorganic, humic acid was isolated from air dried sepiolite according to the procedure recommended by the International Humic Substances Society (Swift, 1996). Briefly, 10 g of airdry sepiolite were mixed vigorously on a stir plate (300 rpm) under N_2 in 100 ml of 0.1 M NaOH for different times (max. 4 h) at room temperature. Conducting extraction under N_2 is necessary to avoid redox processes, to which quinone and phenol systems and their derivatives present in the aromatic core of humic acid particles are highly sensitive (Ksiezopolska and Pazus, 2011). The alkaline solution was separated from the residue by centrifugation (11,000 rpm for 20 min). At the end of this time the supernatant was filtered through 0.45 μm syringe membrane filter (Millipore), and the concentration of humic acid remaining in the aqueous phase was measured immediately to avoid the ageing effect occurred in the presence of oxygen according to Pokorna et al. (2001) by double beam UV-visible spectrophotometer (Biochrom Libra S60) at 250, 254, 265, 350, 365, 400, 450, 465, 550, 600, 665, 700 nm wavelengths using 10 mm path length quartz cell. Since humic substances usually yield uncharacteristic spectra in the UV-visible, these data were used for the determination of the E_4/E_6 (absorbances at 465 nm and 665 nm), the E_2/E_3 ratio (absorbances at 250 nm and 365 nm), the absorptivity ϵ_{272} (absorbance at 272 nm) and the band half-width of the electronic transition absorbance bands Δ_{ET} (absorbance at 280 and 350). All these data give information about changes in aromaticity, hydrophobic content, biodegradability, and functionality of humic substances (Pokorna et al., 2001; Beznosikov and Lodygin, 2009) and whether certain fractions of the humic substance are isolated preferentially from the sepiolite. Total organic carbon (TOC) measurement of the liquid samples was performed by accredited testing laboratory of Thames Water Utilities Ltd. in the UK. A Perkin-Elmer Optima 5300DV inductively coupled plasma optical emission

spectrometry (ICP-OES) was used to analyse for the metal ion Mg^{2+} in the supernatant. Considering the alkaline nature of the supernatant, a drop of ICP quality nitric acid from Fluka ($\geq 69\%$) was added to the supernatant before analysis so as to avoid hydrolysis and/or precipitation of the metal ions. The alkaline modified sepiolite samples were washed 12-14 times with deionized water to remove any unbound organics, re-centrifuged and decanted until their pH reached to the initial pH. Finally, they were dried at 50°C overnight and analyzed for the FTIR, SEM, and zeta potential.

3. Results

3.1. Sepiolite characterization

The chemical and physical characteristics of raw sepiolite are shown in Tables 1. Sepiolite used in this study is very low in aluminum and iron. Sepiolite, ground to $\leq 120.2\ \mu\text{m}$, produced an average particle size (d_{50}) of $19.2\ \mu\text{m}$, whereas its density and bulk density were $2.33\ \text{g/cm}^3$ and $304.2\ \text{g/L}$, respectively. Organic matter is also present in almost every sepiolite sample. The total organic and inorganic carbon ratios of raw sepiolite were 1.21% and 1.43, respectively (Table 1).

The X-ray diffraction (XRD) pattern belonging to raw sepiolite is given in Figure 1. The sample mineralogical composition showed clearly that the major component was sepiolite, followed by dolomite which its presence was confirmed through the bands at $1427\ \text{cm}^{-1}$ and $728\ \text{cm}^{-1}$ in the FTIR spectra of raw sepiolite (Eren et al., 2010; Lescano et al., 2014). From the diffractogram of sepiolite, the presence of peaks distinctive for sepiolite and dolomite are obvious, indicating poor crystallinity. Moreover, the FTIR spectrum of raw sepiolite (Figure 2) ranges from very to intermediately crystalline sepiolite due to the absence of the band at $3720\ \text{cm}^{-1}$ assigned to the OH stretching vibration of the silanol (Si-OH) groups. The $3640\ \text{cm}^{-1}$ band was suggested to arise from limited trioctahedral substitutions, the $3620\ \text{cm}^{-1}$ band from structural MgOH dioctahedral stretching modes and by the presence of the very small band of the triple bridge group Mg_3OH in the octahedral sheet at $3685\ \text{cm}^{-1}$, attributed to structural hydroxyls (Martienssen, 2009). Based on the analysis of FTIR spectra as well as XRD data (Figure 1) and the relatively high CaO content 6.01% in Table 1, the sepiolite sample studied is shown to consist mainly of a mclay fraction and impurities such as organic matter and dolomite generally accepted as representing a moderate quality.

The structure and morphology of sepiolite determines the presence of a large number of terminal silica tetrahedra on the ribbon at the external surfaces (Serratos, 1979). Silanol groups are formed as a result of broken Si-O-Si bonds at external surfaces, balancing their residual charge by accepting either a proton or a hydroxyl group to form Si-OH groups which are capable of reacting with certain organic substances by formation of different bondings and by modifying the structure and surface of the sepiolite. The abundance of Si-OH groups in sepiolite determined using FTIR are shown in Figure 2. The bands in the $1200\text{--}400\ \text{cm}^{-1}$ range represent the lattice vibrations of sepiolite. The bands of Si-O combination stretch at 1209 , 1010 , and $975\ \text{cm}^{-1}$ form as a result of the Si-O vibrations (Sevim et al., 2011). The bands at 495 and $508\ \text{cm}^{-1}$ can be attributed to an O-Si-O bending, while that at 616 and $650\ \text{cm}^{-1}$ results from an Mg-OH bending mode vibrations. The C-O stretching vibrations

obtained at 1456 and 881 cm^{-1} are the characteristics of carbonate, which is in agreement with the results of chemical analysis and XRD patterns. A band of the triple bridge group Mg_3OH at 3686 cm^{-1} is a characteristic IR spectra of sepiolite attributed to structural hydroxyls. On the other hand, the bands recorded at 3560 cm^{-1} and 3238 cm^{-1} are respectively assigned to the OH stretching, representing the zeolitic water in the channels and bound water coordinated to magnesium in the octahedral sheet. The OH-bending mode at 1656 cm^{-1} is also due to the $-\text{OH}$ bending vibrations of zeolitic water (Frost et al., 2001).

The nitrogen adsorption/desorption isotherm at -196°C and cumulative pore volume coupled with log-differential pore volume distribution curves are shown in Figure 3. The adsorption isotherm of natural sepiolite is of the type II according to The International Union of Pure and Applied Chemistry (IUPAC) classification and appears in the shape of a hysteresis to a certain degree (Broekhoff, 1979; Sing et al., 1985; Shields et al., 2004; Alothman, 2012). The visual inspection of isotherm shapes indicates that the porous network of the sepiolite sample presents micropores along with mesopores and macropores. The total amount of nitrogen adsorbed at high relative pressure is 55%. This result indicates higher porosity, yielding a greater available volume for nitrogen adsorption. The saturation plateau is not parallel to the relative pressure axis, indicating that the sepiolite contain a large portion of mesopores. This is confirmed by the cumulative pore volume and log-differential pore volume distribution curves shown in Figure 4. The natural sepiolite has a BET surface area of 358 m^2/g , a pore diameter between 7.26 ~ 251.16 Å, and its pore volume with pore diameter of 7.26~19.69 Å is about 13.44%. Moreover, a very sharp peak at pore half-width around 8 Å obtained from the log-differential pore volume distribution curve spanning the interval 7-251 Å (Figure 4), related to the entrance of N_2 inside the intracrystalline tunnels, is an indication of uniform micropores and multimodal pore size distribution in the sepiolite. The average pore diameter related to micro-mesopores, determined from specific micro-mesopore volume and specific surface area, was about 47.2 Å.

The textural features observed by field emission scanning electron microscopy (SEM-FEG) confirm that the natural sepiolite studied present a characteristic fibrous morphology (length, width, fibre curliness, fibre arrangement, and porosity) as illustrated in Figure 5. The fibres are composed of tablets and needle-like structures arranged in parallel (Suárez and García-Romero, 2012), and consist of intermediates between 1 and 10 μm of length that are much longer than their widths. Semi-quantitative EDX analysis has shown that natural sepiolite is composed mainly of Si and Mg and smaller amounts of Ca and Al.

Electrokinetic surface-charge measurements have long been recognized as extremely useful tools for the interfacial characterization of colloidal systems. These measurements are increasingly successful in characterising solid surfaces. Sepiolite has negatively charged sites on its planar surface, and the amphoteric surface hydroxyl groups (Si-OH) located onto the channel sides. Certain isomorphic substitutions in the tetrahedral or octahedral sheets, such as Al^{3+} instead of Si^{4+} (Shuali et al., 2011) or Mg^{2+} by Al^{3+} (Ruiz-Hitzky, 2011), are responsible for the exchangeable cations that are needed to compensate for the electrical charge and constitute the charged adsorption sites (P sites), similar to smectites and other layered charged silicates. The silanol groups at external surfaces of sepiolite can be protonated and deprotonated under acid and alkaline pH conditions, respectively. At the point of zero charge

(p.z.c.), the surface is essentially uncharged, while at pH values above and below the p.z.c, it will be negatively and positively charged, respectively.

The zeta potential profiles of raw sepiolite given in Figure 6 as a function of suspension pH measured over the range 2–10 indicates that the changes of the zeta potential curves for raw sepiolite follow the trend from positive to negative with increasing pH. Positive zeta potentials are observed for the sepiolite sample in strong acidic solutions ($\text{pH} \leq 3.22$). Raw sepiolite carry an apparent negative zeta potential in the majority of the tested pH range; its p.z.c value was 3.22, which is comparable to published data (Çelik, 2004; Sabah et al., 2007; Chen et al., 2012). The resulting zeta potential was -15.8 ± 3.87 mV for raw sepiolite at $\text{pH}=7.26$ which was the natural pH of suspension. It is widely recognized (Frenkel et al., 1992; Tarchitzky et al., 1993; Vdović, 2001) that the negative zeta potential of mineral particles in natural environments is usually the result of the organic matter such as dissolved humic substances present in the clays. Acting as a coating and/or incorporated in the structure, dissolved organic matter renders the surface charge of particles uniformly negative regardless of their composition and various functional groups attached to aliphatic-aromatic backbone structure which are highly sensitive to different chemical conditions of solution. Kretzschmar et al. (1997) have reported that sorption of humic acid to kaolinite should therefore continuously shift the electrophoretic mobility of the clay particle to more negative values, and contributes towards the lowering of p.z.c of kaolinite from 4.8 to 2.0.

3.2. Characterization of sepiolite-bound humic acid (HA)

HAs generally show strong absorbance in the UV–Vis range (from 190 to 800 nm), particularly in the UV region, because of the presence of aromatic chromophores and/or other organic compounds. Accordingly, the UV-Vis spectra of the three natural organic matter fractions showed a generally decreased absorptivity (or optical density) as the wavelength increased (Chen et al., 2002; Nadi, 2012). Since HAs usually yield uncharacteristic spectra in the UV-visible, soil scientists have used the ratio of optical densities or absorbance of their dilute, aqueous solutions at 465 and 665 nm (E_4/E_6) for characterization purposes of HAs. Higher molecular weight and higher degree of condensation of the aromatic rings will be indicated by lower E_4/E_6 ratio value. Humic acids have a lower E_4/E_6 ratio, more humification or ageing. This ratio is independent of the concentration of the HA but is characteristic of different natural organic matter fractions or HAs obtained from different sources. A low E_4/E_6 or E_2/E_3 ratio for soil HA may be largely attributed to the absorption by aromatic C=C functional groups (Pokorná et al., 2001; McDonald et al., 2004; Helms et al., 2008; Yang and Xing, 2009).

Classical parameters such as the absorbance ratio E_4/E_6 and the $\Delta \log K$ coefficient, the so called index of humification degree, correlates also with the average molecular weight and size of humic substances (Chen et al., 1977; Giovanela et al., 2010). Though, other ratios like E_{350}/E_{450} or E_{350}/E_{550} or E_{250}/E_{365} can be used with the same purpose (Burba et al., 1995; Enev et al., 2014). In the present study, the variation range of the absorbance ratios E_4/E_6 from 20 to 240 min, of the extracted humic substances, are given in Table 2. As can be observed, the values for the E_4/E_6 ratio of humic substances extracted from the raw sepiolite were low and changed in a narrow range (3.19-3.28) during the increasing extraction time. Kononova

(1982) suggests values of the relationship E_4/E_6 for humic acid smaller than 5.0 and between 6.0 and 8.0 for fulvic acids (Cunha et al., 2009).

4. Discussion

The results obtained intend to shed some light on the interactions between sepiolite and humic acid bound to sepiolite, and its influence on the mechanism of the formation of sepiolite-HS complexes. Two basically different approaches have been used to study the sepiolite-complexed organic matter. One involves the gradual removal of the HAs from the sepiolite, and comparative investigation of the residual samples; and the other is to study the relations between surface complexation, and surface charge modification in sepiolite-organic matter system.

As expected, and in agreement with previously published data (Österberg et al., 1987; Giovanela et al., 2010), in all the spectra obtained in the UV-Vis region are typical of HAs and followed the featureless, monotonic increase in intensity almost consistently from 700 to 250 nm (Figure 7). However, little structural information can be gathered from such spectra, but through examination of the values of the different indexes (E_{465}/E_{665} , $\log E_{400} - \log E_{600}$, and E_{450}/E_{700}) calculated from the UV-Vis spectra, some qualitative estimations about the HAs can be made.

Summers et al. (1987) and Nadi (2012) also reported a strong correlation between UV absorbance at 254 nm (UV_{254}) and dissolved organic carbon (DOC). In contrast, there was not a correlation between UV_{254} and the amount of organic carbon released from sepiolite depending on increased extraction time. This was confirmed by the subtraction $E_{450} - E_{700}$ which expresses the relative amount of extracted HA (Dick and Burbab, 1999), as a function of time, indicating that HA extraction reached its maximum within the first 20 min and then no significant change was observed thereafter (Table 2).

The values obtained for the E_4/E_6 ratios were around 3.0 for the humic acid of the raw sepiolite. As a consequence, the low values of humification index of the raw sepiolite confirmed the presence of HA with higher, and similar molecular weight, particle size, humification degree and aromaticity. The similarity of the $\Delta \log K$ values used as indexes for the degree of humification of HA extracted from the raw sepiolite are in agreement with the results deduced from the above. Based on these findings, no structural modification of HA was observed during this extraction period. In addition, the $\Delta \log K$ can also be used to classify the humic acid, in general, which can be grouped into four major types: A, B, R_p , and P types. In our case (Table 2), all samples obtained from different extraction time were classified as humic acid of type B according to Kumada (1988).

In general, it appears that several bonding mechanism may be operating in the formation of clay-humus complexes. However, the relative contributions of the various mechanisms are not clear. Moreover, the predominant bonding mechanisms are likely to differ for different clay minerals. For instance, raw sepiolite contains the lowest proportion of the total organic carbon (TOC), as seen as from Table 1 and Figure 8, much of which is humic acid whose direct bonding to exposed crystal and/or the octahedra at the edges of sepiolite is not significant. Considering the rapid extraction of humic acid with 0.1M NaOH in the first 20 min and the little amount of released humic acid, change depends on the increasing extraction

time, and no detectable structural alteration of sepiolite occurred in the absence of humic substance (Figure 8-a). It can be concluded that humic acid containing many functional groups was presumably associated with the large quantity of silanol groups (in contrast to other clay mineral groups, such as smectites and vermiculites). These are located on the external surface at the edges of the structural channels of sepiolite preferentially through H-bonds and/or surface complexation interactions between the functional groups in humic acid and dissolved metal cations (M^{n+}). It has been deduced that the binding of humic acid is controlled by i) the sepiolite surface active groups on the external surfaces in the form of surface-bonded HAs, and ii) the mono-, di-, and multivalent cations like K, Ca^{2+} , Mg^{2+} and Al^{3+} and their potential factors which determine the relative complex-promoting abilities of the cation, which are in good agreement with results of SEM investigations (Figure 10).

In the alkaline extraction, the pH-dependent charge of the mineral surface as well of the HS, is converted to its negative form, and HS solubilization occurs due to repulsion between equally negative charged surfaces and formation of soluble HS salts. By this extraction, mainly H-bonding and surface complexation interactions between HS and mineral surfaces are broken up (Stevenson, 1994).

Figure 9 shows the FTIR spectra of the raw sepiolite and the samples before and after extraction in 0.1 M NaOH solution for 20, 60, 120 and 240 min. The main features of the sepiolite spectral data ranging from 4000 to 3500 cm^{-1} , indicate main vibrational frequencies at 3686 and 3560 cm^{-1} corresponding to Mg–OH group and bound water coordinated to magnesium in the octahedral sheet, respectively. The band characteristic of OH stretching from coordinated water that appears at 3560 cm^{-1} , resulted in a broadening but its frequency did not change significantly even at increasing extraction times. On the other hand, treatment with increasing extraction time gradually diminished the intensity of the vibration bands of the OH groups corresponding to the coordination of water molecules that vibrate at 3685 cm^{-1} . These are placed in the most external part of the sepiolite and joined to the changes in sepiolite surface, indicating that small changes are being produced on the external clay structure. Special attention should be given to bands located at 3720 cm^{-1} which is assigned to the OH stretching vibrations of silanol groups (Si-OH) located at the external surface of the silicates. Perturbations in the OH stretching vibrations of the silanol groups is often used to prove the existence of interactions between adsorbed organic species and the sepiolite surface (Aranda et al., 2008; Wicklein et al., 2010). In our study, the bands observed near 3720 cm^{-1} depend on the time and are produced most probably by free $Mg(OH)_2$ (Ahlrichs et al., 1975). Consequently, these bands of HA-extracted sepiolite with increasing extraction time from 20 to 240 min showed no significant change in comparison to the raw sepiolite (Figure 8), indicating that its release from sepiolite did not alter the structure.

It is well known from the literature that in strongly alkaline media, Si tetrahedra of clay minerals undergo partial or total dissolution process, the Si-O-Si groups break and fixation of OH takes place simultaneously, and the maximum quantities of polyvalent ions such as Ca^{2+} , Mg^{2+} , Fe^{2+} etc. released from the clay minerals (Martínez-Ramírez et al., 1996; Miura et al., 2012). The existence of these phenomena has been confirmed by SEM-EDAX analysis in spite of a widening of the band assigned to the Si-OH vibrations which cannot be observed in the FTIR spectra of the sepiolite after extraction. SEM and EDAX results obtained at 240 min (Figure 10) able to separate organic matter from the inorganic one according to the procedure

recommended by the International Humic Substances (Swift, 1996) have shown that the quantity of mono and polyvalent ions like K^+ , Ca^{2+} , Mg^{2+} and Al^{3+} containing raw sepiolite decreased greatly and that of elemental oxygen increased after extraction depend on the high concentration of OH^- ions in strong alkaline media. This helps breaking the sepiolite-humic acid metal bridges, suggesting that the humic acid strongly bonded through the soft and hard cations to basal surfaces of sepiolite and exchangeable cations, have an important role in sepiolite-HS complexation. It has been established by Varadachari et al. (1994) that humic acid is bonded through cations to the external surfaces and edges of illite and kaolinite. The exchangeable-cation-humic acid linkage is the strongest in illite compared with montmorillonite or kaolinite. Interaction of humic acid with montmorillonite disrupt its stacking arrangement and swelling favours complexation. In goethite and hematite, both cation bridging and surface coordination are involved in bonding (Varadachari et al., 1991; Varadachari et al., 1995; Varadachari et al., 1997; Varadachari et al., 2000). Moreover, the SEM image of HA-extracted sepiolite exhibited more uniform and regular structure than that of original sepiolite, and its large surfaces have been replaced by more clean and smooth surfaces (Figure 10). These changes in morphology would also result in changes in the physical and physico-chemical properties of the sepiolite, such as changes in color, particle size, stability, viscosity, surface area and porosity of the raw sepiolite. The semi-quantitative EDAX analyses of the raw and HA-extracted sepiolite showed that the percentages of Si, Al, Mg, Ca and monovalent cation K in the solid residues were significantly reduced with increasing extraction time from 20 min to 240 min. Decreasing the percentage of Si from 39.80% to 12.73% depending on the extraction time may explain the Si tetrahedra of sepiolite undergoing partial or total dissolution in strongly alkaline media (Martínez-Ramírez et al., 1996). In the EDAX analysis of the HA-extracted sepiolite, a strong decrease in the Mg, Ca and K contents can be observed, indicating that electrically charged sites (mainly carboxyl groups) on HA may form metal complexes of high stability through chelation. This promotes the dissolution of metals from the sepiolite framework and makes up largely for the content of oxygen-containing functional groups, such as COOH, phenolic OH, and C=O groups of various types in solution. Tan (1975) inferred similarly from changes in the infrared spectra of humic (and fulvic) acids which had previously been in contact with either kaolinite or montmorillonite.

Figure 11 presents the amounts of Mg^{2+} ions dissolved and of TOC released which increased slightly and almost linearly with time up to 240 min. This finding would again suggest that mineral dissolution was not limited by the rate of metal cation diffusion out of the sepiolite structure, indicating that the greater the affinity of the metal cation for HA, the easier the dissolution of the cation from sepiolite surfaces.

The macromolecular configuration of humic acid strongly depends on pH and acidic functional groups on HAs (mainly carboxylic and phenolic) determine the binding characteristics of HAs for proton and other polyvalent cations. Senesi and Brunetti (1996) have reported that with increasing composting time and maturity of humic acid, there is loss of aliphatic groups richer in aromatic groups as more stable humic acid are formed. It is proposed that humic acid behave as "negatively charged polyelectrolytes" or "flexible linear colloids" at $pH > 3$ due to dissociation of carboxyl groups ($3 < pH < 9$) and phenolic hydroxyl groups ($pH > 9$) (Tate et al., 1980). At least some components of HA are surface-active, and

these components have been shown to form micelles in concentrated, alkaline aqueous solutions (Chilom and Rice, 2009). Our results indicate that the HA extracted from sepiolite at pH>11 has a high molecular weight and a high humification degree and are aromatic. This forms around sepiolite grains the shorter and stiffer (more aromatic) chain-like humic acid segments to form structured pseudomicellar domains rather than aggregated structure at high pH as suggested by Ohashi and Nakazawa (1996) and Yates III et al. (1997) These structures are however transitional in nature, disintegrate as the metal ions gradually migrate to location where they form stable inner-sphere complexes with functional groups on the humic acid molecule (Engelbreton and Von Wandruszka, 1998). Their formation and hydrodynamic sizes are strongly influenced by ionic size and metal ions (Von Wandruszka et al., 1997), especially Mg^{2+} and Ca^{2+} ions, pH and ionic strength. It is worth noting that although such structures of HAs could be responsible for the thermodynamically stable sepiolite suspensions (inhibited gel formation) in natural system as represented in Figure 12 and their sizes determined by Benli (2014), were also directly observed by high-resolution AFM images, were also changed in the range of $18-63 \pm 8$ nm at pH 11.5. In an early review (Frenkel et al., 1992; Tarchitzky et al., 1993), it was shown that humic and fulvic acids were added to reference clay suspensions, which always increased colloidal stability. The above results are consistent with the BET-surface area measurement data. To get an insight into the surface area and average pore diameter, the BET surface area measurements were performed on the original and HA-extracted sepiolite, and the results are illustrated in Table 3. It is clear that there is a strong relation between extracted HA, HA colloids, specific surface area, and microporosity in sepiolite.

Sepiolite BET measurements following upon extraction of organic matter showed that the specific surface area and pore volume of the HA extracted sepiolite are smaller than those of the raw sepiolite. After 20 min, the specific surface area and pore volume of the HA extracted sepiolite strongly decreased from $358 \text{ m}^2/\text{g}$ to $125 \text{ m}^2/\text{g}$ and from 0.559 to $0.290 \text{ cm}^3/\text{g}$ then they re-increased from $125 \text{ m}^2/\text{g}$ to $247 \text{ m}^2/\text{g}$ and to $243 \text{ m}^2/\text{g}$, and from 0.290 to $0.315 \text{ cm}^3/\text{g}$ and to $0.380 \text{ cm}^3/\text{g}$, respectively, until they reached equilibrium. Sorption of HA molecules onto sepiolite surfaces at low extraction time effectively reduces the specific surface area mainly due to preferential sorption at reactive sites such as channels, rough surfaces, or micropores. Singer and Huang (1989) reported that the possibility that part of the humate had also penetrated the sepiolite channel surfaces cannot be entirely ruled out. This does not necessarily mean that HA molecules and their metal complexes are sorbed within the micropores. Because of the small size of micropores (<2 nm), penetration of HA molecules with a radius of gyration of 2.1 nm (Österberg et al., 1993) and their metal complexes which have the size range of $18-63 \pm 8$ nm at pH 11.5 (Benli, 2014), seems unlikely. A more reasonable explanation is sorption at the mouths of such pores or their blocking of sepiolite's micro/meso pores for larger structured pseudomicellar domains by organic macromolecules or both. This overall relationship implies that pore blockage is due to organic matter, although the blockage may occur because of either pore filling or simple coverage of the pore openings. However, the BET surface area value (approximately equal to the BET value of sepiolite extracted at 20 min) obtained by subtracting the BET surface area value of extracted sepiolite at 240 min ($243 \text{ m}^2/\text{g}$) from the value of raw sepiolite ($358 \text{ m}^2/\text{g}$) leads us to believe

that some micropores are still filled by organic matter at the initial stage of HA extraction. Nevertheless, the uniformly reincrease in specific surface area and pore volume (e.g. decreasing pore diameter) of HA extracted sepiolite (Table 3) and the decrease in the amounts of polyvalent ions such as Mg^{2+} , Ca^{2+} , etc. released from the sepiolite (Figure 10) with increasing extraction times from 60 to 240 min suggested and confirmed that the large soluble HA molecules and polyvalent metal ions build up pseudomicelle (=HA macromolecules) under high alkaline environment based on the increased extraction time. This will favor their transfer from the sepiolite surfaces and/or the mouths of pores to the solution phase, resulting in the opening of closed micro/mesopores and increase of specific surface area and pore volume. Another possible explanation is that the HA macromolecules are sterically hindered from entering the channels of the sepiolite.

5. Conclusions

The UV-VIS spectral results of B-Sep showed that there is no correlation between UV_{254} and the amount of organic carbon released from sepiolite with increasing extraction time. This was confirmed by the subtraction $E_{450}-E_{700}$ which expresses the relative amount of extracted humic acid (HA), as a function of time, indicating the HA extraction reached its maximum fast within the first 20 min with no significant change afterwards.

The values for the E_4/E_6 ratio of humic substances extracted from the raw sepiolite were low and changed in a narrow range (3.19-3.28) during the increasing extraction time. The low values of humification index of B-Sep confirmed the presence of HA with higher, and similar molecular weight, particle size, humification degree and aromaticity.

The rapid extraction of humic acid and the small amount released had no apparent detectable structural alteration of the sepiolite. It can be concluded that humic acid containing many functional groups was presumably associated with a large quantity of silanol groups located on the external surface at the edges of structural channels of sepiolite preferentially through H-bonds and/or surface complexation interactions between the functional groups in humic acid and dissolved metal cations (M^{n+}), which are in good agreement with results of FTIR and SEM investigations.

SEM-EDAX analysis of the HA-extracted sepiolite showed a strong decrease in the Mg^{2+} , Ca^{2+} and K^+ contents. This indicates that electrically charged sites (mainly carboxyl groups) on HA may form metal complexes of high stability through chelation which promotes the dissolution of metals from sepiolite framework. These are made up largely of oxygen-containing functional groups, such as COOH, phenolic OH, and C=O groups of various types in solution.

HA extracted from sepiolite at $pH > 11$ has a high molecular weight and a high humification degree and aromatic and forms around sepiolite grains the shorter and stiffer (more aromatic) chain-like humic acid segments to form structured pseudomicellar domains rather than aggregates structure at high pH which could be responsible for the thermodynamically stable sepiolite suspensions (inhibited gel formation) in natural system.

Overall the results showed that to get an insight into the surface area and average pore diameter, there is a strong relation between extracted HA from sepiolite, HA colloids and specific surface area, microporosity in sepiolite.

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Table 1. Characteristics of the raw sepiolite.

Composition	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅	SrO ₂	SO ₃	F	LOI
%-M	46.40	0.93	0.38	6.01	24.10	0.25	0.54	0.06	0.01	0.08	0.08	1.51	19.67
Color	brown												
Moisture (%)	10												
Density (g/cm ³)	2.33												
Apparent bulk density (g/l)	304.2												
pH of the point of zero charge (p.z.c.)	3.22												
BET Surface area (m ² /g)	358												
Mean pore diameter (Å)	47.2												
Particle size													
% less than 120.2 µm	100												
% less than 19.2 µm	50												
Total organic carbon (TOC) (%)	1.21												
Total inorganic carbon (TIC) (%)	1.43												

Table 2. UV-Vis absorbance parameters from 20 to 240 min of the HA extracted from raw sepiolite.

		UV Absorbance													
Wavelength (nm)		250	254	265	350	365	400	450	465	550	600	665	700	E ₄₆₅ /E ₆₆₅	E ₄₅₀ -E ₇₀₀
Time (min)	240	0.707	0.684	0.639	0.267	0.233	0.171	0.112	0.099	0.054	0.041	0.031	0.028	3.19	0.62
	120	0.729	0.707	0.664	0.280	0.245	0.179	0.118	0.105	0.057	0.042	0.032	0.029	3.28	0.63
	60	0.709	0.687	0.646	0.277	0.243	0.180	0.119	0.106	0.059	0.044	0.033	0.030	3.21	0.61
	20	0.689	0.670	0.632	0.273	0.240	0.177	0.118	0.105	0.058	0.043	0.032	0.029	3.28	0.61

$$E_{465}/E_{665} = \text{Abs}_{465} / \text{Abs}_{665}$$

$$\Delta \log K = \log \text{Abs}_{400} - \log \text{Abs}_{600}$$

$$E_{450}-E_{700} = \text{Abs}_{450} / \text{Abs}_{700}$$

Table 3. Some physico-chemical characteristics of the raw sepiolite before and after extraction depend on the extraction time.

Extraction Time	BET Surface Area (m ² /g)		Pore Diameter (Å)		Pore Volume (cm ³ /g)	
	Before extraction	After extraction	Before extraction	After extraction	Before extraction	After extraction
20		125		56.56		0.290
60		247		36.84		0.315
120	358	245	47.20	35.69	0.559	0.378
240		243		35.52		0.380

Fig. 1. X-Ray diffractograms of raw sepiolite.

Fig. 2. FTIR spectra of raw sepiolite.

Fig. 3. N_2 adsorption and desorption isotherms at -196°C for raw sepiolite.

Fig. 4. Cumulative pore volume and pore-size distribution curves for raw sepiolite.

Fig. 5. SEM image and semi-quantitative (EDX) analysis of raw sepiolite.

Fig. 6. The variation of zeta potential of raw sepiolite with pH values.

Fig. 7. UV-vis absorbance of humic substances extracted from raw sepiolite.

(a)

(b)

Fig. 8. Qualitative comparison of sepiolite organic matter (a) obtained in different extraction times with the reference organic matter (b) extraction test (UBC, 2015).

Fig. 9. FTIR spectra of the raw and humic acid extracted sepiolite as a function of extraction time.

Fig. 10. SEM images and the semiquantitative EDAX analyses of the raw sepiolite (a) and HA-extracted sepiolite at 20 min (b) and 240 min (b), respectively.

Fig. 11. Kinetic of Mg dissolution from sepiolite and released TOC as a function of extraction time ($pH > 11$).

Fig. 12. HA extracted sepiolite suspension after 1 month (a) and 6 months (b) (Extraction time, 240 min; pH , 11.98).