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Perspective

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Ca²⁺ and SO₄²⁻ interactions with dissolved organic matter: Implications of groundwater quality for CKDu incidence in Sri Lanka

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ABSTRACT

It has recently been proposed that recalcitrant dissolved organic carbon (DOC) in groundwater plays a potent etiological role in the peculiar distribution of chronic kidney disease of unknown etiology (CKDu). This study aims to elucidate the interactions of Ca^{2+} and SO_4^- with a model organic fraction of humic acid (SHA) to determine the possible relationship of CKDu incidence with the DOC in drinking water. XPS and FT-IR methods respectively determined the surface functional groups and chemical composition of protonated dissolved organic carbon (HDOC) in a CKDu high-risk zone (HR) of Sri Lanka and in SHA. Higher surface C composition (87.9%) and lower O (11.4%) were observed for HDOC from the HR region than for SHA (C: 73.8%, O: 24.7%). Aromatic C with less O-containing functional groups was observed in HDOC. The IR band at 1170 cm⁻¹ confirms the formation of organic sulfonate (C-SO3) on SHA. A band at 1213 cm⁻¹ due to organic sulfonate in HDOC from the CKDu HR region was also identified. The IR band at 866 cm⁻¹ evidenced the formation of CaCO3 on SHA above pH 7.4. XPS data confirmed the presence of sulfur oxidation states corresponding to SO_3^{2-} and SO_4^{2-} at 168.9 eV and 170.1 eV binding energies, respectively. The binding energies at 347 eV and 351 eV for Ca $2p_{3/2}$ and Ca $2p_{1/2}$ eV, respectively, confirmed the bidentate complexation of Ca²⁺ with COO⁻ and sulfonate groups on SHA. The organic sulfonate formed is postulated as a uremic toxicant.

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Introduction

The vital role played by the quality of groundwater in the endemic areas of chronic kidney disease with unknown etiology (CKDu) in Sri Lanka has always been considered to be significant (Athuraliya et al., 2003; Wasana et al., 2016). To date, a majority of studies have been based on identifying possible inorganic factors in drinking water to demarcate CKDu regions in Sri Lanka (Chandrajith et al., 2011; Diyabalanage et al., 2017). It is argued that major cations (e.g., Ca²⁺) in hard groundwater, along with other factors, may induce the disease (Wickramarathna et al., 2017). Data related to natural or anthropogenic organic contamination in drinking water as a possible risk factor for CKDu is scarce to date.

Dissolved organic carbon (hereafter DOC) is ubiquitous, and it is a primary carbon source for most biogeochemical processes (Moran, 2000; Shin et al., 2016). The DOC stems from lithogenic and anthropogenic sources. Lithogenic DOCs are enriched with matter from both allochthonous and autochthonous sources. The primary allochthonous sources include decayed terrestrial soil carbon and aquatic vegetation litter (Novak et al., 2019; Ritson et al., 2019; Zhang et al., 2019). Phytoplankton and aquatic benthic macrophytes are the primary autochthonous sources (Ritson et al., 2019). Anthropogenic DOC sources include wastewater effluents, and agricultural and urban runoff (Ignatev and Tuhkanen, 2019). Vegetation litter is recalcitrant to microbial degradation in aquatic systems (Ritson et al., 2019). The DOC is a heterogeneous mixture of a vast array of organic compounds with functional groups of differing reactivities (Wolthoorn et al., 2004). In DOC, fulvic (FA) and humic acids (HA) are highly reactive. However, the division between HA and FA is defined operationally.

DOC contains aliphatic, hetero-aliphatic acetal, aromatic, carboxylic, and carbonyl functional groups (Vasilevich et al., 2018). The dominant surface functional groups are phenolic and carboxylic groups, which have considerable chelating ability with metal ions and mineral surfaces (Weng et al., 2006a, 2006b). When pH > 7, both phenolic and carboxylic groups of humic substances (HS) can de-protonate, resulting in a negative charge that enhances metal ion mobility in aquatic systems (Mladenov et al., 2015). In some groundwater, DOC is mainly aromatic (Mladenov et al., 2015). DOC is a natural surfactant, with hydrophilic and hydrophobic moieties (de Wit et al., 1993).

Protein—like DOC (tyrosine, tryptophan) can be metabolized into uremic toxins by gut microbiota, which causes oxidative stress to the kidney tissues in chronic kidney disease (CKD) (Wang and Zhao, 2018). These uremic toxins are indoxyl sulfate, p—cresylsulfate, and phenyl sulfate (Edamatsu et al., 2018; Wang and Zhao, 2018). The irreversible lethal kidney disease (end—stage disease such as Balkan endemic nephropathy) prevalent among communities in the coastal regions of Texas (USA) correlates well with the DOC in aquifers leached from oxidized lignite at high pH (Chabbi et al., 2006; Ojeda et al., 2018). Our previous study examined the distribution of DOC in shallow groundwater of three CKDu risk zones (high risk, HR; low risk, LR; and no risk, NR) and a control zone (CR) in Sri Lanka (Makehelwala et al., 2019). As shown previously, the groundwater in the HR zone is enriched with highly aromatic, non-biodegradable DOC in 900–1800 Da MW fractions, and the DOC content is also correlated directly with Mg^{2+} , Ca^{2+} , and SO_4^{2-} in groundwater (Makehelwala et al., 2019). The dominant cations and anions in the CKDu prevalence areas are Ca^{2+} and Mg^{2+} , and HCO_3^- , Cl^- , SO_4^{2-} , respectively (Wickramarathna et al., 2017). However, the interactions of DOC, Ca^{2+} , and SO_4^{2-} in shallow groundwater as a CKDu risk factor have not been discussed to date.

We examined the Ca²⁺ complexation with wellcharacterized Sigma humic acid (hereafter SHA) as a function of pH and electrolyte types to elucidate similar interactions between Ca²⁺ and natural DOC extracted from the water in CKDu endemic zones. In groundwater, HS is a mixture of HA and FA fractions (Makehelwala et al., 2019). In CKDu risk zones the DOC concentrations in groundwater are low (3 mg/L); therefore, separate experiments were conducted with well-characterized SHA to ascertain its similarities with protonated DOC (HDOC) in CKDu zones in Sri Lanka. SHA water-soluble fractions were selected for this task. Upon equilibration with water for a prolonged period, the composition of the soluble SHA fraction varies significantly. Hence, control of the equilibration time for organic fraction extraction of humic substances is critical. The water-soluble fractions of SHA in our samples showed a marked resemblance to DOC in the HR zone. Therefore, a large fraction of the experiments was conducted with water-soluble SHA. The DOC and SHA were characterized by vibrational (FT-IR), fluorescence (EEM) and X-ray photoelectron (XPS) spectroscopic data. We also identified the complexation of SHA-Ca²⁺ and the formation mechanisms of sulfonate, which shed light on the investigation of membrane fouling and uremic toxicant formation

Further, in DOC- or SHA-mediated systems, $CaCO_3$ crystallization was observed above pH > 7.4. To our knowledge, consideration of the role played by DOC in the prevalence of CKDu is new. Our results show a formation pathway of uremic toxicants using appropriate spectroscopic methods. Our data also provide a novel method to examine mechanisms of DOCmediated environmental toxicant formation.

1. Materials and methods

1.1. Chemicals

An analytical mixed standard of Ca²⁺ was used for the ICP-OES calibration (GSB, China). CaCl₂ and NaHCO₃ (SCR, Sino-Pharm, China) were used to simulate hard groundwater conditions in the HR zone. The adjustment of pH and background electrolyte concentration was carried out with 0.1 mol/L NaOH, 0.1 mol/L HCl (ACS grade, SCR-SinoPharm, China) and 1 mol/L NaCl and 1 mol/L Na₂SO₄ (SCR-SinoPharm, China) respectively. Humic acid (SHA, Sigma-Aldrich humic acid, USA) was used to prepare simulated DOC solutions. SHA purification was carried out as described by Hering et al. to remove divalent cations and other contaminants (Hering and Morel, 1988). Superlite XAD–8 macro–porous resin and cation exchange resins required for DOC extractions in HR zone

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groundwater were from China. Sodium removal from the natural DOC sample was carried out using a cation exchange resin (protonated DOC, hereafter HDOC) from China. Ultrapure water (0.22 μ m Q-POD, 25°C, 18.2 MΩ, TOC \leq 5 μ g/L, Milli-Q Advantage A10, Merck, Germany) was used for the sample preparation, standard chemical, and working solution preparation.

1.2. Groundwater DOC extraction

As shown in Appendix A Fig. S1, a 200 L shallow groundwater sample was collected within latitude 8°51'03.6" and longitude 80°44'53.3" in Padaviya (i.e. HR zone), Anuradhapura district (NCP), which lies within the dry zone of Sri Lanka, with 1000–1500 mm annual rainfall. The groundwater sample was collected in October, 2018 during the north-east monsoon rain climatic season (September to March 2018). Hydrologically, the selected groundwater source is replenished by a tributary (e.g., local name: MaOya), a branched river flowing along the study area from south to north–east direction. The Padaviya reservoir is the primary irrigation surface water body in the study area. The surface waters are fed continuously by the local precipitation during the north-east monsoon. The aquifers are located in shallow regolith underlain by deep fracture zones in crystalline rocks with a non-continuous single water table (Panabokke, 2007).

The sample preparation and extraction of humic and fulvic fractions were carried out using the method detailed in the International Humic Substances Society (IHSS, 1991). The water sample was collected into pre-acid washed HDPE cans by pumping directly from the source well. Concentrated HCl was added to adjust pH to 2 and the samples were stored until DOC extraction. Adsorption and elution of organic solutes XAD-8 resin was used to determine onto the hydrophobic-hydrophilic fractions of DOC. As specified by (Thurman and Malcolm, 1981), the resin was cleaned with methanol to remove impurities during the manufacturing process followed by washing with hot deionized water (60°C) several times to remove methanol.

Finally, the resin was washed with 0.1 mol/L NaOH to remove organic impurities and rinsed several times with ultra-pure water to remove NaOH traces. The purified XAD-8 resin was packed in a 500 mL bed volume glass column. The sample was passed under gravity flow to retain the HA and FA on the column. The elution was carried out with 600 mL of 0.1 mol/L NaOH. This eluant was acidified (HCl) to pH 2 to avoid oxidation of humic substances. The filtrate was re-concentrated by passing through 50 mL XAD-8 resin and eluted with 0.1 mol/L NaOH. The sodium salt of the DOC was immediately passed through acid-washed (0.1 mol/L HCl) cation-exchange resin (20 mL bed volume) for protonation. The resultant solution was freeze-dried to yield the DOC substrate (hereafter designated as HDOC). The HDOC is a mixture of both humic (low molecular weight) and fulvic acid fractions.

1.3. Water-soluble Sigma humic acid fraction (SHA)

Humic acids are a heterogeneous mixture that contains a large number of organic compounds. Different fractions of

organic compounds can be extracted by equilibrating with water for a specific time to prepare the organic fraction bestmatched to the HDOC in the CKDu HR region. A 0.05 g SHA sample was equilibrated with 100 mL Milli Q water for 12 hr followed by filtering through a 0.22 µm membrane to obtain the water-soluble SHA fraction. Three sets of samples were prepared for this study, (1) 0.5 g/L SHA, 0.002 mol/L NaHCO₃, (2) 0.5 g/L SHA, 0.002 mol/L NaHCO3 and 0.002 mol/L NaCl, (3) 0.5 g/L SHA, 0.002 mol/L NaHCO₃ and 0.002 mol/L Na₂SO₄. High pressure size exclusion chromatography (HPSEC) analysis was performed to identify the AMW of SHA, with a high-performance liquid chromatography (HPLC) system equipped with a dual λ absorbance detector (Waters 2487, USA) and binary HPLC pump (Waters 1525, USA). Samples were filtered through 0.22 µm membrane filters, and 200 µL aliquots were injected into a semi-preparative column (TSK gel G3000SWXL, TOSOH, 7.8 mm ID \times 30 cm, 5 μ m). The mobile phase was 0.015 mol/L phosphate buffer solution (0.00255 mol/L NaH₂PO₄, 0.00245 mol/L Na₂HPO₄, and 0.01 mol/ L NaCl) at pH 6.6. The column calibration was carried out with standard polystyrene sulfonate solution for 1.8-32 kDa MW fractions (Sigma Aldrich, USA). A polystyrene sulfonate standard was used as the apparent molecular weight (AMW) calibration standard (Sigma-Aldrich, USA). The resulting retention time data were converted into AMW at UV 254 nm relative absorbance by using the MW calibration standard. The obtained MW data was analyzed with non-linear curvefitting calculations to identify overlapped peaks using OriginPro 2018 (OriginLab Corporation, USA). The DOC content of the extractant was measured with a TOC analyzer (Shimadzu-V_{CPH}, Japan). The DOC was quantified against a 0-20 mg/L Potassium hydrogen phthalate calibration standard (Sigma-Aldrich, USA) as non-purgeable organic carbon (NPOC).

The fluorescence sub-fractions of DOC and SHA were measured using a fluorescence spectrometer (Hitachi F-7000, Japan) with a xenon lamp and quartz cuvette (1 cm path length). The excitation and emission matrices (EEM) were obtained by scanning emission (EM) wavelength from 220 to 550 nm and excitation (EX) wavelength from 200 to 400 nm with 5 nm sampling intervals for both EX and EM. The scanning speed was adjusted to 12,000 nm/min, and the slit width was 5 nm for both EX and EM. The PMT (photomultiplier tube) voltage was set to 700 V.

1.4. HDOC and SHA surface functional groups

The spectral signatures of DOC in HDOC and SHA were acquired by Fourier transform infrared spectroscopy (ATR–FTIR) (Nicolet–iZ10, Thermo Scientific, USA) in the range of 400–4000 cm⁻¹ and X–ray photoelectron spectroscopy (XPS) (Escalab 250Xi, Thermo Scientific, USA) with a monochromated Al K α X–ray source and a 180 hemispherical analyzer with a three-channel detector. The X–ray photoelectron spectrometer was run in CAE mode. Survey and high–resolution spectra were collected using pass energy of 100.0 eV. The analyzer spot size was 650 µm and the lens was in standard mode. The energy step size was 1.000 eV. Sample palettes for XPS analysis were prepared by mounting the powdered sample into a double tape of ethanol-cleaned foil paper and then applying 2–3 MPa pressure (DTB, FW–5A,

China). Finally, this sample palette was mounted to the analysis cell. The elemental composition was estimated by the software provided with XPS spectrometer using the areas of C 1s, O 1s, and N 1s peaks. The final XPS data were analyzed by peak-fitting with CasaXPS (Casa software, Germany). Non-linear curve-fitting calculations (Origin Pro 2018) resolved the overlapped spectral data for FTIR analysis.

1.5. SHA-Ca complexation study

The SHA and Ca²⁺ complexes were synthesized according to the following procedure. Three samples containing 0.5 g/L SHA were prepared in 0.002 mol/L NaCl, 0.002 mol/L Na₂SO₄, and ultra-pure water. The 0.002 mol/L Ca²⁺ and 0.002 mol/L NaHCO3 were used to simulate the water hardness of the groundwater in the CKDu HR region (86 mg/L Ca²⁺, 61 mg/L SO₄²⁻, 272 mg/L, pH 7.8). The pH adjustments were made either with 0.01 mol/L HCl, or 0.01 mol/L NaOH. Homogeneous solidsolution samples were prepared with a rapid mixer at 200 r/ min. The 10 mL sample aliquots were taken into HDPE centrifuge tubes. The SHA-Ca2+ solutions were mixed overnight at 150 r/min to allow complexation to reach equilibrium. The solid-solution separation was carried out by 0.22 µm filtration. The total calcium concentration was measured by inductively coupled plasma optical emission spectrometry (ICP–OES, Perkin Elmer, Optima 8300, USA). The SHA–Ca²⁺ complexes prepared in different background electrolytes were freeze-dried for ATR-FTIR and XPS analysis.

2. Results and discussion

2.1. Elemental composition and surface functional groups

The XPS spectral data probed the nature of HDOC and SHA regarding the elemental composition and functional groups. The XPS survey spectra of the two samples (SHA and HDOC) show the dominance of carbon (C 1s), oxygen (O 1s), and nitrogen (N 1s) as shown in Appendix A Fig. S2. The amount of sulfur was negligible for both samples, SHA and HDOC. However, a sulfur peak of S 2p at 168.897 eV was observed for HDOC. As shown in Table 1, the surface elemental composition of the HDOC and SHA samples was comparable: C, 87.9% and 73.8%; N, 0.7% and 1.6%; O, 11.4% and 24.7%, respectively. The [O]/[C] atomic ratio accounts for the relative abundance of oxygen–containing functional groups on the surface composition

Table 1 — Surface elemental composition and atomic ratios of HDOC and SHA by XPS analysis.								
Sample ID	Surfa comp	ace elem oosition (Atomic ratios at surface					
	С	Ν	0	O/C	N/C			
HDOC	87.9	0.7	11.4	0.13	0.008			
SHA	73.8	1.6	24.7	0.33	0.021			
SHA' ^a	68.7	1.1	25.9	0.37	0.016			
^a Monteil-Riverta et al., 2000.								

of SHA and other available data (Doskočil et al., 2018), the HDOC in the CKDu HR zone in Sri Lanka contains the lowest proportion of O-derived functional groups (Table 1). Both the bulk and surface O contents of the organic matter were analyzed by an elemental analyzer (Doskočil et al., 2018). The [N]/[C] atomic ratio reflects the relative abundance of nitrogen-containing functional groups on the surface of organic matter. The HDOC sample contained a lower amount of N functional groups on the organic matter when compared with SHA. The N composition of lignite-derived humic acid from coal basins elsewhere are comparable with that of the HDOC (Doskočil et al., 2018). The surface elemental compositions of our SHA and other samples were comparable (Monteil-Rivera et al., 2000) (Table 1). That study also shows that the oxygen content of SHA is higher in the bulk than at the surface (Monteil-Rivera et al., 2000).

As shown in Fig. 1, the de-convolution of C 1s and O 1s XPS spectral signatures was carried out using 30% Lorentzian and 70% Gaussian fitting functions. The same functions were used for both SHA and HDOC. The C 1s components are associated with (1) un-substituted aromatic carbon (C-C/C-H), (2) 01 aliphatic carbon (C-C/C-H), (3) α -carbon (C-C(O)O), (4) alcohol/ether carbon (C–O), (5) ketonic carbon (C=O), and (6) carboxylic carbon (C(O)O), and the O 1s components are associated with (1) ester, carboxyl (O-C), (2) O=, (3) alcohol, acetal, hemiacetal (O-H), and (4) amide, ester, carboxyl (O=C), Fig. 1(a-d). The binding energies and other data of the C- and O-containing functional groups are shown in Table 2. The O 1s peak attributed to O=C due to amide, ester and carboxyl group appears at ~531.8 eV for both SHA and HDOC. The peak attributed to O-H in alcohol, acetal, hemiacetal appears at 532.481 eV for HDOC and 532.566 eV for SHA, respectively. The peak of O-C in ester, carboxyl appears at 532.574 eV for HDOC and 533.452 eV for SHA, with the 0.878 eV binding energy difference being within the experimental limits (Table 2). The O containing groups in organic matter show different relative proportions for HDOC and SHA. Ester and carboxyl O-C groups showed the highest percentage in both HDOC (76.82%) and SHA (65.61%) samples. Both O-H (22.35%) and O=C (10.35%) were higher in SHA than HDOC (Table 2). High proportions of ester-type O-C and relatively low binding energy make HDOC sites favorable for cation exchange. The kinetic energy of the photo-electrons in the XPS beam can increase with negative surface charge on the organic matter due to coulombic repulsion. Accordingly, the binding energy decreases, i.e., $h\nu$ = $E_{K}+$ $E_{B}+$ Ø, the sum of kinetic, and binding energy (Konno, 2016)). Similarly, the C 1s binding energies of α -C-C(O)O (285.83 eV), C(O)O (289.051 eV), and C=O (287.14 eV) are lower in HDOC than in SHA. The highest energy difference is shown in ketone, C=O, which implies the possibility of cation complexation in natural HDOC. The FTIR spectroscopic data corroborate well with XPS in the identification of functional groups in the organic matter.

2.2. FT-IR analysis of HDOC and SHA

The surface functional groups of HDOC in HR zone groundwater and SHA were analyzed by ATR–FTIR spectroscopy. As shown in Fig. 2, the IR spectral signatures of the SHA and HDOC are almost identical. The alkoxy (R–O–R, R–O, R–OH)





groups appear in the 950–1150 cm⁻¹ range. IR bands corresponding to alkene C-H in-plane and out of plane bending vibrations are observed in both samples. A spectral change noted in this region is due to protonation of carboxylate anions in HDOC. The -OH stretching vibrations occur in the region of 2500–3500 cm⁻¹ as a broad peak. As shown in both samples, the band corresponding to -OH overlaps with C-H vibrations, viz. 2850-3050 cm⁻¹. The IR spectral region of 700-1400 cm⁻¹ was analyzed for the identical features of HDOC and SHA under sulfate electrolyte at pH 7. To resolve overlapped peaks, the bands were de-convoluted using the Gaussian spectral function (Fig. 3). As shown in Fig. 3, the

Table 2 – Binding energies, quantitative data for the C 1s and O 1s chemical peaks of HDOC and SHA.							
Sample	HDOC		SHA				
	BE (eV)	RP ^a	BE (eV)	RP			
C 1s							
C–C/C–H (aromatic)	284.69	42.40	284.686	36.45			
C–C/C–H (aliphatic)	285.2	21.55	285.202	29.45			
α-C-C(O)O (α-carbon)	285.83	23.03	285.902	17.08			
C(O)O (carboxylic)	289.051	10.69	289.186	9.28			
C–O (ether/alcohol)	286.58	1.72	286.598	3.67			
C=O (ketone)	287.14	0.61	287.412	4.06			
0 1s							
O—C (ester, carboxyl)	532.574	76.82	533.452	65.61			
0=	536.238	14.03	536.69	1.69			
O-H (alcohol, acetal, hemiacetal)	532.481	7.49	532.566	22.35			
O=C (amide, ester, carboxyl)	531.821	1.66	531.897	10.35			
^a Relative proportion (in %) of each functional group corresponding to fitted curves in Fig. 1.							

sulfate interactions for both SHA and HDOC are almost identical. In the case of HDOC, the IR band at 1213 cm⁻¹ shows the presence of sulfonate intrinsically present in the sample. However, in SHA, due to the addition of SO_4^{2-} , the band at 1170 cm⁻¹ shows the presence of sulfonate.

2.3. SHA: water-soluble humic acid fraction

A water-soluble fraction of the SHA was chosen to match the DOC of groundwater in the CKDu HR zone of Sri Lanka. As shown in Fig. 4, the AMW of the SHA in 0.002 mol/L NaCl, 0.002 mol/L Na₂SO₄ and ultra-pure water at pH 7, were fractionated by HPSEC and EEM methods, and the data were compared with those of DOC. Detailed analysis of AMW fractionation of DOC is given elsewhere (Makehelwala et al., 2019). The SHA in Fig. 4b-c shows a poly-dispersive nature with different electrolytes. Three different groups of molecular fractions, designated as AMW fraction-I (<900 Da: carbohydrates, aldehyde, ketones, alcohols), AMW fraction-II (900-1800 Da: building block of refractory organic substances), and AMW fraction-III (1800-4000 Da: large molecules, polysaccharides, humic substances) were categorized (Wong et al., 2002). In all cases, AMW fraction-II (900-1800 Da) showed different behavior, Fig. 4a-c. The SHA in ultra-pure water did not contain AMW fraction-II (900-1800 Da) (Fig. 4-a). However, AMW contained 1703 Da and 1644 Da, and 1735 Da fractions in NaCl and Na₂SO₄, respectively (Fig. 4b-c). In the presence of different electrolytes, the relative abundance of the building blocks of the AMW fraction-II changes. The highest proportions of the building blocks of refractory organic substances in SHA are shown in Na₂SO₄ solution. Previous data showed that the

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AMW fractionation in DOC contains four types of fraction-I, three types of fraction–II, and three types of fraction–II with more recalcitrant organic substances, 1145 Da, 1329 Da, 1355 Da than other regions in Sri Lanka (Makehelwala et al., 2019). All three samples of SHA contained seven types of fraction-III (>1800 Da) and two AMW fraction-I (<900 Da), Fig. 4a-c. Also, the SHA showed the same AMW fractionation pattern as DOC in CKDu HR zone groundwater (<3100 Da). The EEM spectra of SHA in different background electrolytes and the control sample are shown in Fig. 5a-c. The HA fraction corresponds to the region of (Ex/Em) at ((250-500) nm/ (380-500) nm) and FA to the region of (Ex/Em) at ((200-250) nm/(380-500) nm) (Sun et al., 2018). The distribution of DOC in the CKDu HR zone also showed similar trends as data from SHA, as a mixture of HA and FA (Fig. 5d). Therefore, the SHA was selected as a model substrate to represent DOC in the CKDu HR zone in Sri Lanka.

2.4. SHA and Ca²⁺ complexation study

SHA-Ca²⁺ complexes were synthesized in 0.002 mol/L NaCl, 0.002 mol/L Na₂SO₄, and FT-IR and XPS analyzed ultra-pure water (control sample). Due to moderate to weak bonding between Ca²⁺ and SHA, the molecular configurations of SHA-Ca²⁺ were perturbed in the electrolytes used. Both in NaCl and the control sample (pH 6.2), the C 1s band of SHA-Ca²⁺ de-convolutes into six components (Appendix A Fig. S3), namely (1) un-substituted aromatic carbon (C-C/C-H), (2) aliphatic carbon (C-C/C-H), (3) α -carbon (C-C(O)O), (4) alcohol/ether carbon (C-O), (5) ketonic carbon (C=O), (6) carboxylic carbon (C(O)O). However, in Na₂SO₄, the C 1s band of SHA-Ca²⁺ is de-convoluted into five components. The missing component corresponds to C=O.

Table 3 summarizes the relative proportions (RP%) of C, O, S, Ca in different oxidation states upon calcium ion complexation with the SHA. The complexation of Ca²⁺ occurs via O functional groups on SHA while the other C-derived functional groups (aromatic, aliphatic) do not change significantly. This is significantly different from the relative proportions of α -C. The RP% of α -C is the highest in Na₂SO₄, and the values were comparable in the NaCl and control samples. This is due to strong SO_4^{2-} binding on SHA via α -C. The carboxylic (C(O)O) RP% is lower in NaCl than in Na₂SO₄. No peak is discerned for C=O in Na₂SO₄, and the RP% is lower in NaCl and the control sample. These observations evidence the formation of SHA-Ca²⁺ complexes via carboxylic -O and =Ogroups. However, the ketone group on SHA disappears as sulfate interacts with α -C on SHA with higher RP.

As shown in Appendix A Fig. S3, the O 1s band of SHA–Ca²⁺ complexes (at pH 6) is de-convoluted into three components: (1) O=, (2) O-C (ester, carboxyl), (3) O=C (amide, ester, carboxyl). There are significant changes in oxygen-containing groups on the SHA surface after Ca²⁺ complexation. The RP% of the O-C (ester/carboxyl) significantly decreases in all samples and to some extent in Na_2SO_4 (Table 3). The comparative binding energies of O-C against SHA are 0.248 eV, 0.524 eV, and 0.079 eV in NaCl, Na₂SO₄, and control samples, respectively. The binding energy differences of 0.2-0.3 eV are mostly insignificant (Konno, 2016). Therefore, this effect is the highest in the presence of Na_2SO_4 . The E_B (Binding energy) difference for O = (0.637 eV), O = C (0.41 eV)and RP% is higher in Na₂SO₄ than other samples. All these



Fig. 3 - FT-IR spectroscopic data for (a) HDOC, (b) SHA and sulfate complexation at pH 7. The black dashed line shows the raw data; red and green lines show the fitted data of different spectra.

observations indicate that the highest effect on SHA occurs in the presence of sulfate.

The presence of S in two oxidation states warrants further discussion. They are significantly different from the RP% of α -C-C(O)O and C=O. The RP% of α -C in Na₂SO₄ and NaCl follows the order, RP^{SO2-}_{4 α-C} > RP^{Cl-}_{α-C}. Also, the RP% in the

O-C (ester) functional group is the lowest in Na_2SO_4 . This denotes the binding of SO_4^{2-} via α -C (ester) on SHA. Two sulfur oxidation states are observed on the SHA surface as the characteristic peaks S 2p_{3/2} and 2p_{1/2} (Appendix A Fig. S3). The binding energies of the doublets are 168.9 eV and 170.1 eV for SO_3^{2-} and SO_4^{2-} respectively. The corresponding binding energies reported in the literature for SO₂ sorption on TiO₂ are lower than our values (Baltrusaitis et al., 2011). The humic substances found in several locations in a European basin were reported to have sulfone groups in their structure (Doskočil et al., 2018). They further identified three structural groups of sulfur at 163.8 eV (sulfide, thiophene), 165.9 eV (sulfoxide), and 168.0 eV (sulfone) (Doskočil et al., 2018). Due to their similar E_B, sulfide and thiophene peaks are often overlapped. In the present study, we observed a sulfur peak at 168.9 eV (sulfone) due to sulfate reduction during interactions between SHA and SO₄²⁻.

As shown in Appendix A Fig. S4 and Table 3, calcium shows two characteristic peaks with binding energies of 347 eV and 351 eV due to Ca $2p_{3/2}$ and Ca $2p_{1/2}$ electrons, respectively. In earlier work, the same characteristic peaks were observed at 347.8 eV and 351.4 eV (He et al., 2017). In our experiments, we found the same values for Ca²⁺ and SHA in Na_2SO_4 . However, when Ca^{2+} and SHA are in NaCl or the control sample, the E_B is reduced, indicating metal complexation via COO⁻ (Table 3). The same explanation can be given for the observed reduction of the RP% of α -C and C=O (ketone) groups upon SHA–Ca²⁺ complexation. This observation implies that two energy levels of Ca²⁺ exist to accept electrons from coordinated oxygen, which appear as two peaks. The data confirm the formation of SHA-Ca²⁺ bidentate surface complexes via carboxylic groups in the NaCl and control samples. However, the enhanced binding energy observed in Na₂SO₄ represents positive charge on the SHA surface due to Ca²⁺ bidentate complexation via O on surface-bound sulfone and ester groups. The disappearance of C=O (ketone) in the Na₂SO₄ sample is related to the delocalization of double bonding with Ca²⁺ bidentate bonding. Previously, the FT-IR spectroscopic data showed the formation of sulfonate groups at 1170 cm^{-1} (Fig. 3). Therefore, XPS and FI-TR results corroborated the identification of the sulfur binding position as the SHA ester (O=C-O-) group.



Fig. 4 – Apparent molecular weight data determined by HPLC–SEC in the solutions of (a) SHA (b) SHA in 0.002 mol/L NaCl (c) SHA in 0.002 mol/L Na₂SO₄. The black dashed line shows the raw data, the red line shows the curve fitted data, and the green line shows the fitted individual spectra.

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Fig. 5 – Excitation emission spectroscopy study of (a) SHA (b) SHA in 0.002 mol/L NaCl (c) SHA in 0.002 mol/L Na₂SO₄ (d) HR zone groundwater in Sri Lanka. EEM peaks for HA (Ex (250–500)/Em (380–500) nm) and FA (Ex (200–250)/Em (380–500) nm).

2.5. FT-IR spectroscopy study of Ca-SHA complexation

Appendix A Fig. S5 shows the complexation data for Ca²⁺ and SHA in different electrolyte matrixes and the control

conducted in the pH range 3.5–9.5 at 298 K. In different electrolyte matrixes, Ca^{2+} exhibits a varying degree of complexation affinity on SHA surface sites. Fig. 6 shows the FT-IR of SHA– Ca^{2+} complexes as a function of pH and the background

Table 3 – Binding energies, quantitative data for C 1s, O 1s, S 2p and Ca 2p chemical peaks under the for Ca^{2+} and SHA complexation at pH6 in different electrolytes, NaCl, Na_2SO_4 and control sample (SHA).

Sample	Nac	21	Na ₂ SO ₄		SHA		
	BE (eV)	RP ^a	BE (eV)	RP	BE (eV)	RP	
C 1s							
C–C/C–H (aromatic)	284.687	36.31	284.508	33.30	284.665	55.08	
C—C/C—H (aliphatic)	284.926	46.01	285.046	29.54	285.213	19.14	
α-C-C(O)O (α-carbon)	285.12	5.34	285.544	24.51	286.065	9.97	
C(O)O (carboxylic)	288.778	3.78	288.54	8.98	288.528	11.86	
C–O (ether/alcohol)	286.497	8.19	286.359	3.66	286.759	3.10	
C=O (ketone)	287.809	0.38	nd ^b	nd	287.279	0.85	
O 1s							
O—C (ester, carboxyl)	533.204	36.33	532.928	7.02	533.373	25.31	
0=	536.548	3.08	536.053	19.64	537.13	11.52	
O—H (alcohol, acetal, hemiacetal)							
O=C (amide, ester, carboxyl)	531.902	60.59	531.487	73.34	531.959	63.17	
Ca 2p							
Ca 2p _{3/2}	347.679	64.39	347.818	65.26	347.73	63.25	
Ca 2p _{1/2}	351.236	35.61	351.442	34.74	351.3	36.75	
S 2p	Na_2SO_4						
	pH 6		pH 7				
SO ₃ ^{2–} (sulfone)	168.906	59.9 ^a	168.939	63.83			
SO4-	170.081	40.21	170.141	36.17			
^a Relative proportion (in %) of each functional group corresponding to fitted curves in Appendix A Fig. S3, Fig. S4; ^b Not detected.							

electrolyte type in the range 400–2000 cm⁻¹. The spectrum of SHA has peaks at 1007 cm⁻¹, 1030 cm⁻¹, 1084 cm⁻¹, and 1115 cm⁻¹, which are characteristic of alkoxy groups (R–OR, R–O, R–OH) and (possibly) carbohydrates. The bands at 1217 cm⁻¹, 1706 cm⁻¹ (C=O stretching), and 1775 cm⁻¹ are due to –COOH groups, and the bands at 1379 cm⁻¹ (anti--symmetric stretching), 1430 cm⁻¹, 1600 cm⁻¹ are due to the –COO⁻ functional groups of pure SHA (Ait Baddi et al., 2004; van Zandvoort et al., 2015). When the pH was increased from acidic to alkaline, the intensity of the peaks due to –COOH diminished or disappeared in NaCl and the control for SHA. The peaks corresponding to –COOH groups in SHA completely disappeared in Na₂SO₄.

SHA in both NaCl and the control showed a linear trend in Ca²⁺ complexation capacity at pH 3.5 to 6.3 (Appendix A Fig. S5). The complexation of SHA–Ca²⁺ in Na₂SO₄ is linear from pH 3.5 to 7.4 (Appendix A Fig. S5). This can be elucidated by FTIR spectroscopic data as well. For all samples, a new peak appears at 866 cm^{-1} which is attributed to CaCO_3 crystallization or SHA-Ca²⁺ complexation. In agreement with Zhenze Li, the CaCO₃ peak emerges at slightly alkaline pH (>pH 7.4) and DOC-Ca²⁺ complexation occurred at very high organic content (Li, 2014). This phenomenon is more significant in SHA samples in NaCl electrolyte than in Na₂SO₄. In the control sample, the SHA-[Ca²⁺] complex vs. pH curve shows a gradual decrease due to $CaCO_3$ precipitation at > pH 7.4 (Appendix A Fig. S5). The rapid increment of Ca^{2+} complexation with SHA slows down at > pH 7.4 in the NaCl electrolyte, which indicates the adsorption of CaCO₃ on SHA. Crystallization of CaCO₃ is pH-dependent, but the groundwater pH of the CKDu HR zone is greater than 7 (Makehelwala et al., 2019). Therefore, CaCO₃ precipitation and complexation with HA/FA can occur concurrently.

As shown in Fig. 6, the observed FT-IR peaks are similar for SHA in both the NaCl and control samples. The peak ascribed to the substituted aromatic/alkene C–H bending vibrations at 914 cm⁻¹ is more prominent in NaCl and control samples than in Na₂SO₄. This is due to sulfate interactions with SHA. In all cases, the peaks ascribed to COO⁻ groups on the SHA surface are shifted from 1430 to 1390 cm⁻¹ and 1600 to 1566 cm⁻¹, which indicate Ca²⁺ complexation with SHA through the carboxylate anion (COO⁻).

For SHA in Na_2SO_4 , the peaks at 1007 cm⁻¹ and 1030 cm⁻¹ due to alkoxy group -O- stretching vibrations in different

environments shift to 1011 cm^{-1} and 1033 cm^{-1} ; a new peak appeared at 997 cm⁻¹ ascribed to Ca²⁺ complexation with -Oon SHA; the peaks at 1084 cm⁻¹ and 1115 cm⁻¹ shift to higher wavenumbers at 1106 cm⁻¹ and 1133 cm⁻¹ respectively with enhanced absorbance values. The formation of sulfonates is ascribed to the presence of peaks in the 1200–1100 ${
m cm}^{-1}$ range. In Fig. 3b, the new peak appearing at 1170 cm⁻¹ is ascribed to the sulfone group on SHA. Therefore, this indicates the bonding of sulfate with alkoxy (ester) groups on SHA. Our FTIR data show low proportions of Ca²⁺ complexation with SHA in Na₂SO₄ due to sulfate and SHA interactions through alkoxy groups. At acidic pH (pH 3.4 and 5.2), the peak at cm^{-1} due to $-\text{COO}^-$ disappeared. Also, the COO $^-$ peak shifted to lower wavenumbers than in other samples, indicating the formation of Ca²⁺ complexes with SHA through carboxylates. Therefore, Ca²⁺ non-competitively interacts with COO⁻ on SHA in NaCl. In Na₂SO₄, both Ca^{2+} and SO_4^{2-} compete for -O on ester (alkoxy) groups. Firstly, sulfate forms a bond with -O on ester groups, as sulfate has an improper geometry p-orbital with weak π bonding to -O and it prefers to form proper geometrical bonding with adjacent -0 (0=0) (Magnusson, 1990). Therefore, the organic sulfonate $(C-SO_3^-)$ on SHA occurs in reduced form in sulfate electrolyte. Afterward, Ca^{2+} forms complexes with ester =0 and newly appeared sulfonate =0 via bidentate bonding.

2.6. Implications for CKDu root cause study in Sri Lanka

To date, over thirty hypotheses have been put forward for the etiology of CKDu (Sri Lanka). Most of these hypotheses have focused on the effect of inorganic contaminants in drinking water for the peculiar distribution of CKDu in the dry zone of Sri Lanka. For the first time, the potent role played by natural organic matter as a precursor or direct disease—causing agent for the prevalence of CKDu is proposed. We have also noted the precursive action of natural organic matter in the formation of potent uremic toxins.

Balkan endemic nephropathy in the USA was statistically correlated with natural organic matter in the water of aquifers leached from oxidized lignite (Chabbi et al., 2006; Ojeda et al., 2018). Sri Lanka is a tropical country. CKDu is highly prevalent among males in agricultural farming communities in the dry zone of Sri Lanka. Progressive kidney failure has occurred



Fig. 6 – The FT-IR study of Ca^{2+} complexation with SHA of control sample, with NaCl and Na_2SO_4 as electrolyte at different pH (3.5–9.4). The IR spectrum observed at 400–2000 cm⁻¹ region. The red line represents the SHA. The CaCO₃ precipitation started to appear at > pH 7.4 and calcium speciation to the DOC-Ca complex occurred at very high organic content (Li, 2014).

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among sugarcane workers in Central America due to dehydration in hot and humid environments (García-Trabanino et al., 2015). These environmental conditions (temperature 33–36°C at noon) are almost the same as those in the CKDu HR regions in the dry zone of Sri Lanka. Occupational heat stress related to chronic dehydration may develop into CKDu globally as a result of global warming (Nerbass et al., 2017). The paddy farmers in Sri Lanka have used untreated groundwater for drinking during the last decades. The DOC in aquaculture plays a major role as regulating high temperatures in water (Caplanne and Laurion, 2008). Therefore, body temperature enhancement effects in the paddy farmers in Sri Lanka can occur during farming, which leads to heat stress.

Previously, protein-like tryptophan and tyrosine were identified in HR CKDu regions in Sri Lanka (Makehelwala et al., 2019). These compounds can be metabolized to uremic toxins, indoxyl sulfate, and p-cresylsulfate by gut microbiota, which cause oxidative stress in CKD (Edamatsu et al., 2018; Wang and Zhao, 2018). Phenyl sulfate is also a uremic toxin, as it decreases glutathione levels in renal porcine tubular cells, which causes oxidative stress (Edamatsu et al., 2018). The CKDu in Sri Lanka is known as 99% of specific biopsy-proven primary tubule interstitial disease (Gifford et al., 2017). The sulfone groups on the SHA surface with low MW are a precursor for the formation of uremic toxins to generate oxidative stress in kidneys. Ca²⁺ plays an essential role in dissolved organic matter to accelerate microbial activities (Zhao et al., 2015). Therefore, the removal of these complexes from CKDu endemic groundwater bodies is essential. Further research into SHA–SO₃–Ca²⁺ complexes as uremic toxins is warranted in CKDu root cause identification.

2.7. Drawbacks and future directions

Due to the heterogeneous nature of the SHA and HDOC, their water-soluble molecular fractions do vary with the equilibration time, which sometimes yields non-reproducible data. However, such a situation can be minimized by careful regulation of the experimental parameters under controlled environmental conditions. Indeed, we attempted to quantify the carbon-containing functional groups of both HDOC and SHA by liquid phase ¹H and ¹³C NMR, without success. Hence, investigation with solid-phase NMR is recommended, and the pertinent data will be reported in the future. SHA–Ca²⁺/HDOC complexation requires complementary data obtained by measuring free Ca²⁺ activities using ion-selective electrodes calibrated in suitable metal ion buffers. It is also pertinent to examine Ca²⁺ and low-molecular-weight fraction DOC (<1000 Da) in the fate determination of metal-organic complexes in natural waters. Further investigation into dissolution mechanisms of CaCO3 in different electrolyte matrixes common in CKDu endemic areas is also an essential research question.

3. Conclusions

Water-extracted SHA fractions can be used as analogs to examine the effect of HDOC in CKDu zones. The HDOC in HR CKDu regions contains higher proportions of surface aromatic and aliphatic groups than SHA. Ca²⁺ complexation by SHA follows the order: $Ca^{2+}_{control} > Ca^{2+}_{NaCl} > Ca^{2+}_{Na2SO4}$. When pH > 7.4, Ca^{2+} removal occurs via $CaCO_3$ precipitation, especially in NaCl and water mediums. In the presence of SO_4^{2-} , the CaCO₃ formation is somewhat reduced. Competition between Ca^{2+} and SO_4^{2-} for complexation with -O in the ester sites of SHA was noted. The improper *p*-orbital with weak π bonding in sulfate forms proper geometrical p-orbital bonding with adjacent -O on SHA as reduced organic sulfonate (C-SO3). Ca2+ complexation occurs through =O on ester and =O on sulfonate groups, where Ca²⁺ non-competitively formed complexes through $-COO^{-}$ of SHA in the NaCl or control sample. Ca²⁺ forms bidentate complexes with COO- or sulfonates in SHA. The DOC-sulfone-Ca²⁺ complexes may act as a uremic toxicant. The presence of Ca²⁺ enhances the microbial activity in CKDu endemic regions in Sri Lanka. In search of CKDu root causes, the precursor role played by natural organic matter in uremic toxicant formation requires further research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.09.018.

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