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# Characterization of dissolved organic carbon in shallow groundwater of chronic kidney disease affected regions in Sri Lanka



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# HIGHLIGHTS

- DOC in shallow groundwater of CKDu areas was characterized in dry and wet seasons.
- Less oxidizable and higher aromatic fulvic acid fraction was observed in HR zone.
- Autochthonous carbon source occurred in all groundwater DOC due to FI > 1.8.
- 900–1800 Da MW fraction obtained as building block of refractory organic substances.
- Significant correlations observed among DOC, PCP, Ca, sulfate, total Fe for HR.

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# ABSTRACT

Number of Chronic Kidney Disease patients with no identifiable cause (Chronic Kidney Disease Unknown Aetiology, CKDu) are escalating in the North Central Province (NCP) of Sri Lanka. This study examined distribution of dissolved organic carbon (DOC) in shallow groundwater of three CKDu risk zones (high risk, HR; low risk, LR and no risk, NR) and a control zone (CR) from wet to dry seasons. The interactions of DOC with calcium and magnesium ions and metabolites of selected pesticides were also examined. The lowest  $\binom{COD}{DOC}$  values of 0.60  $\pm$  0.19, 0.58  $\pm$  0.17 were reported in the DOC of the HR water in the wet and dry seasons, respectively, and this DOC fraction encompasses organic compounds with the lowest labile C with the highest aromaticity. Four distinct fluorescence DOC fractions in the HR water were identified with fulvic acid component associated as dominant non-labile C fraction. The essential building blocks of non-labile C were concentrated into molecular weight (MW) fraction II (900–1800 Da). The DOC source in all groundwater was identified as autochthonous (fluorescence index > 1.8). In the HR water, pentachlorophenol (PCP) was also detected in appreciable quantities. The factor loadings based on principal component analysis (PCA) showed a positive correlation between DOC and sulfate, calcium, total iron, PCP in the HR groundwater. Accordingly, it can be inferred that divalent cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>) abundant in the HR groundwater interact with phenolate and carboxylate functional groups in DOC at alkaline pH.

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# 1. Introduction

A deadly disease, CKDu firstly reported almost three decades before, is prevalent among the farming community in Sri Lanka. Although the exact disease etiology is controversial to date, the vital role played by "the water quality factor/s" is shown repeatedly. Contamination of hard groundwater by multiple inorganic species seems to aggravate the incidence of the disease (Chandrajith et al., 2011b; Wasana et al., 2016; Wasana et al., 2017). In endemic areas, the levels of Cd and As in groundwater are low, and the concentration of these chemical species in biological tissues of CKDu patients relates negative exposure (Diyabalanage et al., 2017; Rango et al., 2015). In most cases, presence of elevated levels of fluoride and hardness (commonly [Ca<sup>2+</sup>] > [Mg<sup>2</sup> <sup>+</sup>] with major anions,  $[HCO_3^-] > [Cl^-] > [SO_4^{2-}])$  in water seems to aggravate the prevalence of CKDu (Wickramarathna et al., 2017). Hitherto less attention has been paid to the possible role of DOC and its interactions with di- or trivalent ions in hard groundwater sampled from the CKDu endemic zones.

In hard water, DOC possesses both hydrophilic and hydrophobic moieties that enhance both the binding ability of cations in hard water and pesticide solubility (Indarawis and Boyer, 2012; Mazzei and Piccolo, 2012). Although not listed as a priority contaminant by regulatory agencies, water with high hardness is unpalatable. Most of the CKDu zones in Sri Lanka are recognized as hydro–geochemical zones with high hardness (Chandrajith et al., 2011a). The provision of safe, palatable water is an essential remedial measure for disease prevention. In CKDu zones, to control high fluoride and total dissolved solids (TDS) in drinking water, reverse osmosis (RO) treatment plants are currently operational, e.g., to date over 400 RO drinking water plants are in use. It is noted that the DOC in water is a typical membrane foulant and its mitigation should seriously be considered in operation and maintenance of RO drinking water plants (Lee et al., 2009b; Liikanen et al., 2005).

The DOC is a ubiquitous organic matter fraction in aquatic ecosystems that passes through a 0.45 µm filter and is a mixture of fulvic acid (FA, low MW), humic acid (HA, high MW) and humin (often called humus). The HA and FA fractions are collectively called humic substances (HSs). HSs are negatively charged amorphous substrates with reactive carboxylic and phenolic functional groups which can form chemical bonds between particle ligand and mineral ions (Weng et al., 2006). The water soluble FA contains aliphatic, hetero-aliphatic, acetal, aromatic, carboxylic and carbonyl functional groups in their structure (Wolthoorn et al., 2004). Aged Bangladesh groundwater DOC contained more aromatic than carboxylic functional groups (Mladenov et al., 2015). The nature of functional groups, pH, specific ion concentration and background ionic strength influences the metal binding affinity of HSs (Nederlof et al., 1993). Mechanistic interpretation of charging of HS is challenging due to their heterogeneity with complexing sites and multiple reactive ligands (de Wit et al., 1993a). One or more factors such as polyelectrolyte nature, conformational changes, polydispersity, or electrostatic effects are often neglected to reduce the complexity in interpreting surface charging of heterogenic HSs. Proton binding affinity depends on the concentration of the background electrolyte where higher ionic strengths give thin and low ionic strength gives thick diffuse double layer due to screening of electrostatic effect. Humic substances can be considered as spheres or cylinders by considering their structural properties; higher MW particles are coiled by H-bonding to form spheres, and low MWs particles are uncoiled to make the cylindrical structures (Nederlof et al., 1993). The Langmuir-Freundlich calculations by proton titration method denoted the greater heterogeneity for cylindrical HSs than spheres (de Wit et al., 1993b). In this work, we assumed FA as rigid spheres to account for chemical heterogeneity.

The HSs–metal sorption mechanism via distinct organic functional groups has been described by the NICA–Donnan model (Milne et al., 2003). Previous studies have shown that Cd<sup>2+</sup> and Ca<sup>2+</sup> can compete with protons for carboxylic and phenolic groups on HS. However,

calcium ions do not compete with protons towards phenolic groups as they tend to release protons at alkaline pH (Milne et al., 1995). The low MW FA can be formed strong electrostatic interactions with mineral iron surfaces (i.e. Goethite) and arsenate in groundwater and also, it can be desorbed from mineral surface to water with bound arsenate at low pH (Weng et al., 2009). The reduced proportions of quinone moieties on FA of aged groundwater can be complexed with microbiologically reduced iron (II) to enhance arsenic mobility (Wacławek et al., 2017).

The urine samples of CKDu patients in endemic areas have been reported pesticide residue levels above reference value for 2,4dichlorophenoxyacetic acid (2,4–D) (3.5%) and PCP (1.7%), respectively (WHO, 2012). Propanil (PRP) was the second most used pesticide in Sri Lanka until 2013 (Pesticide registrar office data, Sri Lanka, 2015). Presently, these pesticides are banned within Sri Lanka due to their nephrotoxicity, but by-products can remain in groundwater as they can be more persistent than the parent compounds (Carena et al., 2017; Milan et al., 2012). In 2015, the Stockholm Convention classified PCP and its allied salts/esters as a persistent organic pollutant (POP). Bioconcentration of hydrophobic contaminants showed a distinct effect on their solubility in the presence of DOC by Van der Waal's interactions (Haitzer et al., 1999). The PCP is very stable in environment due to its symmetrical structural properties. Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are thought to be impurities or by-products of organochlorine pesticides (i.e. PCP) which is extremely carcinogenic POPs into the environment (Hrabák et al., 2016). The photo-degradation products of PCP in water found as de-chlorinated products, acetic acid, oxalic acid, and chloride ion which were dependent on solvent polarity where it required more extended UV irradiation to degrade PCP in organic solvents (Espino 2008). However, the relationship between pesticides and DOC in groundwater of the CKDu affected area in Sri Lanka has not yet been established.

This study focused on the fraction identification and comparison of chemical properties to ions binding ability of DOC in shallow groundwater in CKDu endemic and non–endemic zones in Sri Lanka. Selected pesticides, their residues, and inorganic ions were analyzed to identify their possible correlations with the DOC by a multivariate statistical analysis method. Finally, this information was used to identify the degree of source water quality required to supply safe drinking water to CKDu prevalent zones by the nano–membrane filtration (NF) technology. This research will shed light in identifying external nephrotoxic micro–contaminants in CKDu endemic zones for future research.

#### 2. Materials and methods

#### 2.1. Study area

The North Central Province of Sri Lanka is in the dry climatic zone with a 1000–1500 mm annual rainfall and 27 °C to 33 °C mean monthly temperature. CKDu was firstly observed between 2000 and 2002 among the farming community and exhibited different risk factors from CKD (Athuraliya et al., 2008). For this study, groundwater samples were collected from 50 locations in Anuradhapura district (NCP, Sri Lanka) within latitude 7°54′03.3″ and 8°53′38.4″, longitude 80°14′23.5″ and 80°48′48.2″ (Fig. S1). These locations from the same soil and weathered rock profile which has been categorized as "shallow groundwater". Nine tenths of Sri Lanka consist of Precambrian metamorphic rocks metamorphosed under granulitic and amphibolite farcies (Cooray, 1967). The study locations were situated in hornblende biotite gneiss and charnokite terrains overlaying predominantly with reddish brown earth soils (Fig. S1).

According to CKDu epidemiologic data, sampling locations were categorized as three risk zones: HR, LR, NR zones (Table S1). Shallow groundwater samples were collected from Kandy (7°16'38.3″ and 7°18'39.6″, longitude 80°41'59.6″ and 80°43'55.7″) area as a CR zone where none of the CKDu patients were reported with the same climatic conditions. The physico-chemical conditions of the CR zone are essentially the same. In this area of the tropics two climatic seasons are apparent, wet and dry. Accordingly, water samples were collected in both zones in (a) wet season (December 2016), (b) dry season (May 2017).

# 2.2. Chemicals

Analytical standards of 2,4-D, PCP, PRP, 3,4-dichloroaniline (3,4-DCA), 4,5-dichlorocatecol (4,5-DCC) were used for pesticides analysis (AccuStandards, Inc., USA). The HPLC grade methanol (MeOH, 99.9%), dichloromethane (DCM, 99.9%), acetonitrile (ACN, 99.9%), formic acid (98%) and mix standard were used for ion chromatography analysis (J and K Scientific Ltd., China). Potassium hydrogen phthalate standard was used for the DOC determination (Sigma-Aldrich, USA). The low-level  $(0-5 \text{ mg L}^{-1})$  testing vials were used for COD analysis (HACH, USA). Polystyrene sulfonate standard was used as apparent molecular weight (AMW) calibration standard (Sigma-Aldrich, USA). Solid phase extraction (SPE) (Oasis® HLB,  $60 \text{ mg/3 cm}^3$ ) cartridges and vacuum manifold set were used for large volume pesticide extraction (Waters, USA). Ultrapure water  $(18.2 \text{ M}\Omega \text{ cm}, 25 ^{\circ}\text{C})$  was used for the standard chemical and working solution preparations.

#### 2.3. Groundwater sample collection and preparation

Groundwater samples (2 L) were collected into well cleaned HDPE bottles and concentrated by the SPE-HLB method in the vicinity at the laboratory in Sri Lanka. A groundwater sample was allowed to pass through the Oasis®HLB extraction cartridge at a flow rate of 5 mL min<sup>-1</sup>. The HLB cartridges were pre-conditioned with 3 mL of methanol, 3 mL of ultrapure water, 3 mL of acidic water (pH 2.5 by H<sub>3</sub>PO<sub>4</sub>) before the sample was pumped. The cartridges were dried under ambient environmental conditions. The air-dried samples concentrated by HLB cartridge yielded an excellent 96%-99% recovery within of 2-4 weeks. The sample elution was carried out at the laboratory in China (Research Center for Eco Environmental Sciences, Chinese Academy of Sciences, Beijing) by  $2 \times 500 \,\mu$ L dichloromethane, followed by  $2 \times 500 \,\mu$ L methanol for better elution. The eluates were evaporated under nitrogen gas at room temperature. Then the sample was re-dissolved with 1 mL 40% ( $\nu/\nu$ ) methanol/ water and filtered through 0.22 µm polyethersulphone membrane filters (ANPEL Laboratory technologies, Shanghai) for LC-MC/MC analysis. Separate 100 mL groundwater samples were collected into HDPE bottles and filtered through 0.45 µm polyethersulphone membrane filters (ANPEL Laboratory technologies, Shanghai) within 24 h. These small sized samples were stored at 4 °C for 1-2 weeks before dispatch to China for further chemical analysis. The onsite pH measurements were made by Orion pH meter combined with Ross Ultra Triode electrode (Fisher scientific, Canada).

### 2.4. Analytical methods

#### 2.4.1. DOC and COD<sub>Mn</sub> analysis

The DOC was quantified against  $0-10 \text{ mg L}^{-1}$  Potassium hydrogen phthalate calibration standard as non-purgeable organic carbon (NPOC) with the TOC-V<sub>CPH</sub> analyzer (Shimadzu Japan). Measured DOC concentrations were calculated using a calibration curve ranging from 0 to 20 mg  $L^{-1}$ . Low level (0.5–5.00 mg  $L^{-1}$ ) Chemical Oxygen Demand measurements were carried out according to the manganese-III digest (Acidic permanganate index) method (COD<sub>Mn</sub>) by UV-visible spectrophotometer (DR 6000, HACH, USA).

#### 2.4.2. Ultraviolet spectroscopy and SUVA

The UV-visible spectrometer equipped with a quartz cell (1 cm path length) was used to estimate absorbance at 254 nm (Shimadzu  $UV_{254}$ , Japan). The specific ultra-violet absorbance (SUVA,  $L mg^{-1} m^{-1}$ ) was calculated as Eq. 1:

$$SUVA = \frac{UV_{254} (cm^{-1})}{DOC (mg L^{-1})} \times 100$$
(1)

#### 2.4.3. Inorganic parameters

Inorganic anions (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Br<sup>-</sup>) were measured by ion chromatography (DIONEX, ICS-1000, Thermo Scientific, USA) equipped with separator column, chemical suppressor and conductivity cell. Ion analyses were performed using suppressed conductivity detection. The eluent was a mixture of 4.5 mM Na<sub>2</sub>CO<sub>3</sub> and 0.8 mM NaHCO<sub>3</sub> at 2 mL min<sup>-1</sup> flow-rate, sampling volume was 20 µL. The inorganic cations  $(Ca^{2+}, Mg^{2+}, Na^+, K^+)$  were measured by an inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer, Optima 8300, USA) and total Fe was measured by an ICP-MS (Agilent technologies, USA).

#### 2.4.4. Pesticide analysis

Mixed standard solutions for HPLC-MS/MS analysis contain 1-500  $\mu g L^{-1}$  of the target compounds which was volume up with 40% ( $\nu/\nu$ ) methanol/water solution. The selected pesticides were analyzed by an HPLC (Agilent Technologies, 1200 Infinity series, USA) coupled with LC/MS detector (Agilent Technologies, 6460 Triple Quadrapole, USA) and pneumatically assisted electrospray interface. The HPLC-MS/MS consisted with Acquity UPLC C–18 column (2.1  $\times$  100 mm ID) with 1.7 µm particle size (Waters, Ireland). Separation module and an ionization interface were operated in negative ionization mode for 2,4-D, PCP herbicides and 4.5-DCC by-product, and positive ionization mode for PRP herbicide and its 3,4-DCA by-product, respectively. The mass spectrometer was operated in multiple reactions monitoring (MRM) mode. The optimized analytical parameters used in the HPLC-MS/MS have shown in Table S2. Two of the compounds were analyzed under negative mode (ESI-) and three compounds analyzed as positive mode (ESI +). Under the selected conditions the molecules were ionized as [M + H]<sup>+</sup> or [M-H]<sup>-</sup>. The composition of mobile phases used in chromatographic separation was Solvent A (0.1% formic acid in water) and Solvent B (acetonitrile) at a constant flow rate of 0.3 mL min<sup>-1</sup>. The gradient was programmed to increase Solvent B from 40 to 100% in 8 min, and returning to the initial condition (40% B) in 1 min. These conditions were maintained for 9 min.

#### 2.4.5. Fluorescence spectral analysis (3D-EEM)

Fluorescence spectra for sub fractions of DOC were measured using a fluorescence spectrometer (Hitachi F-7000, Tokyo, Japan) with a xenon lamp and a quartz cuvette (1 cm path length). The excitation emission matrices (EEM) were obtained by scanning emission (EM) wavelength from 220 nm to 550 nm and excitation (EX) wavelength from 200 nm to 400 nm with 5 nm sampling intervals for both EX and EM. The scanning speed was adjusted to 12,000 nm min<sup>-1</sup> and slit width was 5 nm for both EX and EM. PMT (Photomultiplier tube) voltage was set to 700 V. The fluorescence index (FI) is denoted as Eq. (2):

$$FI = \frac{I_{EM} (450 \text{ nm} : 370 \text{ nm})}{I_{EM} (500 \text{ nm} : 370 \text{ nm})}$$
(2)

where the FI is given as the ratio of the emission intensities measured at 450 nm and at 500 nm respectively with an excitation of 370 nm (Klapper et al., 2002).

### 2.4.6. High pressure size-exclusion chromatography (HPSEC)

Size exclusion chromatography analysis was performed to identify AMW of DOC molecules with an HLPC system equipped with Dual  $\lambda$  Absorbance Detector (Waters 2487) and binary HPLC pump (Waters 1525, USA). Samples were filtered through 0.22 µm membrane filters and 2 mL aliquots were injected into a semi–preparative column (TSK gel G3000SWXL, TOSOH, 7.8 mm ID  $\times$  30 cm, 5 µm). The mobile phase was a 0.015 M phosphate buffer solution (0.00255 M NaH<sub>2</sub>PO<sub>4</sub>, 0.00245 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.01 M 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). The resulted retention time data were converted into AMW at UV 254 nm relative absorbance by using MW calibration standard. The raw MW data was analyzed with non–linear curve fitting calculations to identify overlapped peaks using Origin 8.

# 2.4.7. Statistical analysis

Data were treated using principle component analysis to identify possible correlation patterns of DOC with other pesticides, metabolites and inorganic ions. The data sets of the HR, LR, NR and CR zones were analyzed using statistical software (Canoco5 and IBM–SPSS). Ordinary PCA (variable centered) on log transformed data to carry equal weights for standardization. The PCA results were presented in combined biplots of different variables and sampling locations. Sampling locations are shown as points while the variables are represented by arrows running from the origin of the plot to the position of the variable scores. The direction of an arrow represents the direction of increasing variable concentration across the sampling location and length represents the strength of the increase. The angle between pairs of arrows indicates the approximate correlation between variables.

## 3. Results and discussion

### 3.1. Consistency of DOC in three zones

Table 1 shows the variations of water DOC,  $COD_{Mn}$ , FI, and  $(\frac{COD_{Mn}}{DOC})$  values in HR, LR, NR, and CR zones with climatic conditions. In CKDu zones, namely HR, LR and NR, the DOC concentration varied between 1.35 and 2.08 mg L<sup>-1</sup> showing a monotonous behavior. However, in the CR zone, the mean DOC content varied within a narrow interval between 1.37 and 1.62 mg L<sup>-1</sup>.

The COD<sub>Mn</sub> value represents the C fraction digestible by MnO<sub>4</sub><sup>-</sup> is used to ascertain labile C ( $C_{oxi}^{Risk Zone}$ , chemically and biologically active form) (Auerswald and Weigand, 1996; Tian et al., 2008). The seasonal variation of the mean  $(\frac{COD_{MR}}{DOC})$  ratio in groundwater increased when moving from HR (0.02) to CR (0.48). When compared to NR zone, the  $(\frac{COD_{MR}}{DOC})$  ratio in the CR zone showed a four folds increase. The lowest seasonal variation of the  $(\frac{COD_{MR}}{DOC})$  ratio was recorded in HR zone groundwater.

Accordingly, the  $(\frac{COD_{MID}}{DOC})$  ratio varied as  $C_{oxi}^{HR} < C_{oxi}^{LR} < C_{oxi}^{NR} < C_{oxi}^{CR}$ . In dry climatic season, the temperature conditions (e.g.  $32 \pm 3$  °C) are favorable for enhance bio degradation, but the lowest  $(\frac{COD_{MID}}{DOC})$  ratio was observed in the HR zone. When compared to other CKDu risk zones, the lowest seasonal variation of COD<sub>MID</sub> was present in HR groundwater indicating less oxidizable DOC fractions in HR zone. The recalcitrant DOC was obtained elsewhere, in the alkylebenzene sulfonate contaminated groundwater zone while labile DOC obtained in the protists contaminated groundwater in an oxygen–depleted sandy aquifer in Cape Cod, MA (DOC < 3.6 mg L<sup>-1</sup>) (Kinner et al., 2002). To further examine the "C factor" on groundwater of CKDu endemic area, a detailed DOC characterization was carried out.

#### 3.2. Characterization of DOC

## 3.2.1. UV<sub>254</sub> and 3D-EEM

The UV absorption band at 254 nm is result due to excitation of the conjugated  $\pi$  electrons on unsaturated double bond (>C==C<) which is a measure of DOC aromaticity (Babaei et al., 1997; Weishaar et al., 2003). The highest SUVA at 254 nm value of 3.29 L mg<sup>-1</sup> m<sup>-1</sup> was recorded in HR groundwater measured during the wet climatic season. As shown in Table 1, the SUVA measured in groundwater decreased in order as HR > LR > NR > CR during the wet climatic season.

Fig. 1 shows the four main EEM peaks observed at  $\frac{Ex(215-229)}{Em(278-299)}$  (Peak a) for protein–like (tyrosine);  $\frac{Ex(239)}{Em(346)}$  (Peak b) for protein–like (trypto– phan);  $\frac{Ex(230-249)}{Em(403-414)}$  (Peak c) for fulvic acid–like;  $\frac{Ex(268-278)}{Em(289-294)}$  (Peak d) for soluble microbial by-product-like spectrum. When compared to CR in NCP groundwater, the FA fraction was dominant and showed a long resident time. The EX/EM wavelengths for measured fulvic acid-like substances in groundwater are specified as 249/414, 234/409, 244/403 for HR, LR and NR, CR respectively (Table 2). In dry climatic season, these peaks shift to the low wavelength (blue shift) and no corresponding peaks had appeared in FA of CR. For both climatic seasons, the order of the wave lengths for peak c was  $\lambda_{HR} > \lambda_{LR}$  and  $_{NR} > \lambda_{CR}$ . For HR, the intensity of peak c was increased as a function of climatic seasons. This variation was minimal for LR or NR zones. Such a variation cannot be seen in the CR, i.e. all spectra formed as without dilution of the original sample. As shown in Table 2, the fluorescence intensities of the peak a and d decreased in the HR groundwater collected from wet to dry climatic season. The intensity of the peak *a* increased for LR, NR and CR zones which indicate enhanced microbial activity. When compared to CR zone, it can be inferred that the FA fractions in groundwater of HR zone were non-biodegradable. The dominant HS of the groundwater in HR zone was FA enriched with non-labile C and high aromaticity.

#### Table 1

Mean values of DOC, COD, UV-visible and fluorescence characterization for different locations of NCP groundwater.

Item	No of samples	$DOC (mg L^{-1})$	$\text{COD}_{Mn} (\text{mg L}^{-1})$	$COD_{Mn}/DOC$ ratio	UV-visible spectroscopy		Fluorescence spectroscopy
					$UVA_{254}  (cm^{-1})^a$	$SUVA_{254,} (L mg^{-1} m^{-1})^b$	FI <sup>c</sup>
HR zone $\pm$ S.D. <sup>d</sup> , a <sup>e</sup>	18	$1.68\pm0.69$	$1.12\pm0.42$	$0.60\pm0.19$	$0.031 \pm 0.023$	$3.29\pm7.44$	$2.12\pm0.16$
b <sup>f</sup>	24	$2.02\pm1.60$	$1.26 \pm 1.23$	$0.58\pm0.17$	$0.044\pm0.055$	$1.91 \pm 0.45$	$1.98 \pm 0.11$
LR zone $\pm$ S.D., a	15	$2.08\pm0.74$	$1.32\pm0.58$	$0.65\pm0.13$	$0.034\pm0.021$	$1.49\pm0.42$	$2.11\pm0.14$
b	11	$1.35\pm0.17$	$0.92\pm0.24$	$0.70\pm0.19$	$0.022\pm0.004$	$1.66 \pm 0.21$	$2.01\pm0.08$
NR zone $\pm$ S.D., a	14	$1.54\pm0.28$	$0.95\pm0.22$	$0.61\pm0.11$	$0.019\pm0.007$	$1.25 \pm 0.33$	$2.09 \pm 0.10$
b	9	$1.87\pm0.36$	$1.43\pm0.69$	$0.73\pm0.23$	$0.037\pm0.016$	$1.92\pm0.54$	$2.06\pm0.02$
Control zone $\pm$ S.D., a	5	$1.37\pm0.28$	$0.59\pm0.31$	$0.41\pm0.17$	$0.014\pm0.002$	$1.05 \pm 0.10$	$2.29\pm0.00$
b	5	$1.62\pm0.65$	$1.49\pm0.85$	$0.89\pm0.35$	$0.016\pm0.018$	$0.78\pm0.61$	$2.09\pm0.05$
Total no of samples, a	52						
h	49						

<sup>a</sup> Ultraviolet absorption at 254 nm.

<sup>b</sup> Specific ultraviolet absorption at 254 nm (UVA<sub>254</sub>  $m^{-1}$ /DOC mg  $L^{-1}$ ).

<sup>c</sup> Fluorescence index.

<sup>d</sup> Standard deviation.

e Wet season.

f Dry season.



Fig. 1. Excitation emission spectroscopy study of NCP groundwater, (1) HR zone (2) LR and NR zone (3) CR zone for (a) wet season (b) dry season. Peak a for protein–like (tyrosine), peak b for protein–like (tryptophan), peak c for fulvic acid–like and peak d for soluble microbial by–products–like DOC fractions.

The elemental composition of HSs from different parts of the world has been shown remarkably low variations with respect to C, H, O, N content suggesting that refractory organic C production is irrespective to the different environmental conditions, but it relies on C source (Rice and MacCarthy, 1991). The FI can serve as simple index to identify the source of the HSs in aquatic environment, and is defined as ratio of emission intensity at a wavelength of 450 nm to that at 500 nm, obtained with an excitation of 370 nm which correlates well with the relative contribution of microbial versus higher plant organic matter (Cory and McKnight, 2005). The previous study has been showed that FI has a value of ~1.4 for terrestrially derived FA and a value of ~1.9 for microbially derived FA (McKnight et al., 2001). In this study, the value of FI for all zones in both dry and wet seasons showed a similar trend

(Table 1). Autochthonous (microbial) organic precursors are presented as HS in all samples (FI > 1.8). It is necessary to gain an idea of the molecular size of DOC fractions to identify suitable membrane filters for effective groundwater treatment. Therefore, molecular weight fraction identification of DOC was carried out for all regions.

#### 3.2.2. Molecular weight fraction of DOC

Fig. 2 shows the chromatograms of the fractioned DOC in groundwater sampled during wet climatic season at  $UV_{254nm}$  measured by HP– SEC. The DOC in HR zone groundwater contained ten MW fractions, but in LR/NR and CR groundwater contained only seven MW fractions which indicates poly–dispersive nature of DOC particles. The MW fraction distributions were ranged between 270 and 3100 Da. FA is soluble

# Table 2

The florescence intensities of DOC at wet and dry seasons in NCP groundwater.

	HR, a.u <sup>a</sup>		LR and NR, a.u		CR, a.u	
	Wet	Dry	Wet	Dry	Wet	Dry
Peak a <sup>b</sup> Peak b <sup>c</sup> Peak c <sup>e</sup> Peak d <sup>f</sup>	2558 (294/220) nd <sup>d</sup> 2549 (414/249) 4069 (294/268)	2245 (294/215) nd 2637 (409/244) 3466 (294/278)	1241 (289/224) nd 646 (409/234) 2844 (294/278)	2226 (294/215) nd 688 (404/230) 2525 (294/273)	401 (299/299) 505 (346/239) 344 (403/244) 1858 (294/278)	1755 (278/229) nd nd 3258 (289/273)

<sup>a</sup> Atomic unit.

<sup>b</sup> Protein-like (tyrosine).

<sup>c</sup> Protein-like (tryptophan).

<sup>d</sup> No peak detected.

<sup>e</sup> Fulvic acid-like.

<sup>f</sup> Soluble microbial by-product-like.

in alkaline pH. The less poly–dispersed, small size FA molecules can have more water soluble functional groups on their surface than large HA (Beckett et al., 1987).

For further analyses, these chromatograms were divided into three groups; namely SEC fraction-I (<900 Da), SEC fraction-II (900-1800 Da) and SEC fraction-III (1800-4000 Da). As shown in Table 3, the SEC fraction-I was assigned to low molecular weight molecules such as carbohydrates, aldehydes, ketones, alcohols, and the SEC fraction-II was assigned to building block of refractory (non-biodegradable) organic substances, while the SEC fraction-III was assigned to large molecules such as polysaccharides and humic substances (Wong et al., 2002). It implies the same set of EEM data in Fig. 1(1-a), S2-a and S2-b for HR, LR/NR and CR, respectively. The SEC fraction-I shows the microbial by-products and protein-like (tyrosine and tryptophan) molecules as low MW for HR, LR/NR and CR (Table 3). There was less amount of SEC fraction-I organic moieties in the CR zone when compared to the HR, LR/NR zones. The more organic moieties present in the SEC fraction-II is considered favorable for the formation of recalcitrant/refractory DOC in the HR zone. Finally, we observed large organic moieties in the SEC fraction-III as fulvic acid (Table 3, Fig. 1(1-a), S2-a, S2-b). The observed SUVA value demonstrated the highest hydrophobicity of organic moieties in the HR zone. Therefore, as mentioned in Section 3.1, it is implied that FA in the HR zone acted as a refractory organic substance (recalcitrant). Hydrophobic organic acids (including humic substances) possess high UV absorptivity whereas hydrophilic organic acids show low UV absorptivity. As shown in Fig. 2 and Table 3 the hydrophobicity of FA varied as HR > LR and NR > CR.

#### 3.3. Selected herbicide data

In this study, three herbicides 2,4–D, PCP, PRP and two degraded byproducts as 3,4–DCA, 4,5–DCC were chosen (Table 4). Within study regions, the herbicides were selected according to their usage, environmental persistence and CKDu prevalence (WHO, 2012). The HPLC– MS/MS conditions were optimized as in Table S2.

The 2,4–D was only detected in the groundwater sampled during wet season. The 4,5–DCC, the degraded by–product of PCP was not observed in both seasons. However, PCP, PRP and 3,4–DCA were observed in both seasons. The PCP is environmentally persistent in CKDu endemic area. As shown in Table 4, the order of hydrophobicity depends on the octanol–water partition coefficient (log  $K_{ow}$ ) as PCP > 3,4–DCA > PRP > 2,4–D. Therefore, high toxicity can be observed during bio–accumulation of PCP due to its highest lipophilic properties. Under slightly alkaline pH (~7.5) conditions prevails in NCP groundwater, PCP and FA can be hydrolyzed into phenolate and carboxylate ion. Therefore, at these stringent conditions, FA and PCP interactions are not likely due to their strong electrostatic repulsions. However, the mobility of trace elements may enhance as a result of iron–FA complexation (Wacławek et al., 2017).

The conversion of PRP into 3,4–DCA occurs via biodegradation (Carena et al., 2017). We have observed the 3,4–DCA in all groundwater

samples, presumably as the result of PRP conversion. DOC in calcareous soil favors enhanced absorption of 3,4–DCA via FA derived phenolate ion in alkaline pH (González-Pradas et al., 2005).

# 3.4. Correlation of DOC with calcium, sulfate and pesticides

Variation of  $Ca^{2+}$ ,  $SO_4^{2-}$ , DOC and pesticide show a complex behavior that requires multivariate statistical approach. Principle component analysis was therefore used to identify possible correlations.

Principal component comparison of groundwater parameters in HR zone is shown in Fig. 3(a and e). The sum of the eigenvalues (PC1-PC4) is equal to one. For all data sets examined, the eigenvalue of PC1 and PC2 explained a significant variance. Therefore, these components were used for further analysis. The percentage variance for PC1 in the dry season is 96.93% and 42.71% for the wet season in the HR zone; 98.9% and 61.32% in the LR zone; 98.50% and 47.15% in the NR zone; 98.02% and 69.42% in the CR zone. In the HR zone, as shown in Fig. 3(a and e), significant positive correlations exist between DOC and Mg<sup>2</sup>  $Ca^{2+}$ , total Fe, PCP,  $SO_4^{2-}$  for both climatic seasons. Fig. 3(b and f) shows a negative correlation with  $SO_4^{2-}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ , total Fe, PCP for LR zone in the wet climatic season; a positive correlation between DOC and  $Mg^{2+}$ ,  $SO_4^{2-}$ ,  $Ca^{2+}$ , PCP and a negative correlation of DOC with total Fe in the groundwater collected during the dry climatic season. Based on the data shown in Fig. 3(c and g), the following correlation can further be derived for the NR zone. DOC in groundwater collected during the wet climatic season is shown a positive correlation with  $SO_4^{2-}$  and a negative correlation with  $Ca^{2+}$ ,  $Mg^{2+}$ , total Fe, PCP. In the dry climatic season, DOC is shown a positive correlation with total Fe,  $Mg^{2+}$ ,  $SO_4^{2-}$ ,  $Ca^{2+}$  and a negative correlation with PCP. In CR, DOC correlated positively with PCP and negatively with  $Ca^{2+}$ ,  $Mg^{2+}$ , total Fe,  $SO_4^{2-}$  in the wet climatic season. However, during the dry season, the correlation patterns are reversed as in Fig. 3(d and h).

As shown in the data (Table S3) during the wet climatic season, the  $SO_4^{2-}$  concentration in water was higher in HR, LR, NR zone than in CR zone. The SO<sub>4</sub><sup>2-</sup> abundant in HR, LR, and NR zones governs oxic conditions in groundwater and it is an electron acceptor after depletion of dissolved oxygen. Similarly  $HCO_3^-$  and  $CO_3^{2-}$  common in hard groundwater terrains act as a competitor electron acceptor to  $SO_4^{2-}$  under anaerobic conditions (Wacławek et al., 2017). A correlation between DOC and  $SO_4^{2-}$  is denoted the sulfur containing organic matter in groundwater of the HR, LR and NR zones, and no sulfur containing DOC in the CR zone. The low  $SO_4^{2-}$  concentration appeared at the HR groundwater sampled during the wet season implied the dilution by precipitation. Magnusson et al. (1990) explained that the  $SO_4^{2-}$  tetrahedral structural chemistry; improper geometrical d-orbital on sulfur can make weak  $\pi$ boding with oxygen (=S=0). This weak bonding can produce lonepair electrons on oxygen. Therefore, oxygen prefers to forms  $\pi$  bonding with adjacent oxygen with proper *p*-orbital geometry ( $\cdots 0=0$ ) (Magnusson, 1990). It is easy to remove 0=0 on  $SO_4^{2-}$  tetrahedral structure when the interaction of  $SO_4^{2-}$  into DOC via sulfur at alkaline pH. This sulfur ( $=C-O-SO_2$ ) can be screened the interaction of



Fig. 2. Molecular weight data by HPLC-SEC in NCP of Sri Lanka, (a) HR groundwater, (b) LR and NR groundwater, (c) CR groundwater.

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 Table 3

 Molecular weight fraction and DOC, SUVA values of three selected groundwater sampling locations in wet season.

Zone	MW, Da		DOC,	SUVA <sub>254</sub> , L		
	Fraction-I (<900)	Fraction-II (900–1800)	Fraction-III (1800–4000)	mg L <sup>-1</sup>	mg <sup>-1</sup> m <sup>-1</sup>	
HR	535 616 690 800	1145 1329 1355	1988 2563 3100	2.66	3.64	
LR and NR	270 351 592 700	1102 1703	2274	2.63	1.71	
CR	478 699	1083 1242	1834 2387 2842	1.71	0.53	

divalent cation  $(Ca^{2+}, Mg^{2+})$  onto FA in groundwater (author's unpublished data).

For the HR zone as shown in Fig. 3, DOC is correlated with  $Ca^{2+}$  in groundwater sampled in both climatic seasons. For other zones namely LR or NR, similar correlations were noted only in groundwater sampled during the dry season. In dry climatic season samples,  $SO_4^{2-}$  correlates with DOC. The presence of elevated  $Ca^{2+}$  content implied the screening effect of  $SO_4^{2-}$  on FA–Ca interactions. Calcium species in water are precipitated on the membrane as a foulant (Lee et al., 2009b). In the presence of DOC, calcium ions are soluble in the solution (Zhao et al., 2015). Further studies into the DOC and calcium fouling mechanism are required for efficient operations of NF drinking water plants.

Meanwhile, the correlation between DOC and total Fe was observed in the HR zone for groundwater sampled in both climatic seasons; such correlations are only visible for NR and CR groundwater sampled during dry season and no such relationships are noted in LR zone groundwater (Fig. 3). The dominant dissolved iron species in groundwater at pH ~7.5 in the HR zone are  $Fe(OH)_2^+$ ,  $Fe(OH)^{2+}$ ,  $Fe_2(OH)_2^{4+}$ ,  $Fe_3(OH)_5^{4+}$  and  $Fe^2$ + rather than precipitated  $Fe(OH)_3$  (Lee et al., 2009a). This dissolved iron species can form bonds with negatively charged FA. Soluble ferric ions on groundwater tend to form stable complex ions with sulfate due to their favorable energy for molecular bonding (Zhang et al., 2018).

#### 3.5. Correlation of DOC with PCP and 3,4-DCA

As shown in Fig. 3, strong correlation exists between DOC and PCP in the HR zone groundwater. The SUVA value is proportional to the degree of FA aromaticity. Highly aromatic DOC (or FA) is correlated well with the PCP content found both in the HR and LR zones and their relevant molecular interactions are postulated as largely hydrophobic. In the NR zone, no PCP correlation exists in highly aromatic DOC in groundwater sampled during dry climatic season due to less amount of FA when compared to the higher amounts of protein-like (tyrosine) and soluble microbial by-products in the groundwater (Fig. 1(2-a) and (2-b)). In the CR zone, EEM spectral data suggest low proportions of proteinlike tyrosine which implies groundwater enriched with higher aromatic DOC (or FA) that favors PCP and DOC interactions. The correlation of PCP and Fe was observed only in the samples collected during the wet climatic season for all regions. Both in HR and CR zones, the correlations between DOC-Fe and Fe-PCP are noted. However, no EEM peaks correspond to FA appeared in groundwater collected from CR zone in dry climatic season. Therefore, the DOC-Fe-PCP interactions are likely to occur only in the HR zone. Further clarifications required to identify the sorption mechanism/s of PCP onto FA.

#### 3.6. Implications to membrane filtration

Pressure–driven membrane filtration technology offers a promising physical method for water treatment. Among other factors membrane fouling seriously imparts the effectiveness of this technology. DOC, mainly fulvic and humic acids moieties are common precursors of organic membrane fouling (Zhu et al., 2018). The fouling occurs by blocking pores of membranes via both hydrophobic attractions and inorganic scale formation. Due to comparable sizes of pore spaces and the fulvic assemblages, membrane blocking occurs at the nanoscale.

#### Table 4

Mean values of pesticides, by-products detected in groundwater and physico-chemical properties of herbicides used in North Central Province.

	2,4-D	РСР	PRP	3,4-DCA	Reference
High risk zone $\pm$ SD, ng/L (wet) <sup>a</sup>	$0.05\pm0.13$	$2.76\pm4.59$	$0.90 \pm 2.74$	$0.55\pm0.81$	This study
Dry <sup>b</sup>	nd	$2.11 \pm 6.84$	$0.36 \pm 0.08$	$0.63\pm0.48$	This study
Low risk zone $\pm$ SD, ng/L (wet)	$0.001\pm0.01$	$1.54 \pm 5.17$	$0.37 \pm 1.22$	$0.92\pm0.85$	This study
Dry	nd	$0.02\pm0.07$	$0.33 \pm 0.02$	$0.28\pm0.23$	This study
No risk zone $\pm$ SD, ng/L (wet)	nd	$2.51 \pm 7.27$	$0.25 \pm 0.31$	$1.09 \pm 1.38$	This study
Dry	nd	$0.26\pm0.73$	$0.33 \pm 0.02$	$0.71\pm0.50$	This study
Control zone $\pm$ SD, ng/L (wet)	nd	$0.58\pm0.80$	$0.32 \pm 0.01$	$2.47 \pm 0.70$	This study
Dry	nd	$1.59 \pm 2.47$	$0.15 \pm 0.23$	$2.17 \pm 2.18$	This study
Detection frequency in urine	33%	-	-	-	(WHO, 2012)
CKDu cases above ref. limit	3.5%	1.7%	-	-	(WHO, 2012)
Solubility in water at 20 °C, mg $L^{-1}$	24,300	1000	95	580	(Lewis et al., 2016) <sup>c</sup>
Log K <sub>ow</sub> at pH 7, 20 °C <sup>d</sup>	-0.82	3.32	2.29	2.69	(Lewis et al., 2016)
pK <sub>a</sub> at 25 °C <sup>e</sup>	3.40	4.73	19.1	2.97	(Lewis et al., 2016)
Aqueous hydrolysis at pH 7, 20 °C	Stable	Stable	Stable	Stable	(Lewis et al., 2016)

<sup>a</sup> Wet season.

<sup>b</sup> Dry season.

<sup>d</sup> Octanol-water partition coefficient.

<sup>e</sup> Acid dissociation constant.

**Fig. 3.** PCA for groundwater quality comparison in NCP and Control region for two seasons, (a) HR zone–wet season (Number of samples, N = 18), (b) LR zone–wet season (N = 15), (c) NR zone–wet season (N = 14), (d) CR zone–wet season (N = 5), (e) HR zone–dry season (N = 24), (f) LR zone–dry season (N = 11), (g) NR zone–dry season (N = 9), (h) CR zone–dry season (N = 5). Correlation of groundwater quality factors (red arrows). Abbreviations: Ca = Ca<sup>2+</sup>; Mg = Mg<sup>2+</sup>; Na = Na<sup>+</sup>; K=K<sup>+</sup>; Cl = Cl<sup>-</sup>; Br = Br<sup>-</sup>; PRP = propanil, PCP = pentachlorophenol; DCA = 3,4–dichloronaline; COD = chemical oxygen demand; DOC = dissolve organic carbon; TIC = total inorganic carbon; TC = total carbon; UVA = ultraviolet absorbance at 254 nm; SUVA = specific UV absorbance at 254 nm; ORP = oxidation reduction potential. (For interpretation of the references to color in this figure legend, the reference to the web version of this article.)

<sup>&</sup>lt;sup>c</sup> Lewis, K.A., Tzilivakis, J., Warner, D.J. and Green, A. (2016) An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment: An International Journal 22(4), 1050–1064. https://doi.org/10.1080/10807039.2015.1133242.





Besides, fulvic acids and other DOC are carbon sources for the microbes that initiate biological membrane fouling (Beyer et al., 2014). In hard water, membrane blocking occurs via scale formation by CaCO<sub>3</sub> and CaSO<sub>4</sub> precipitates (Sari and Chellam, 2017). Fulvic acids form complexes with divalent cations which inhibit CaSO<sub>4</sub> and CaCO<sub>3</sub> precipitation that reduces inorganic scaling. The divalent cation rejection was high presence of both DOC and CaSO<sub>4</sub> as a formation of a very compact organic foulant layer with the higher ionic strength in membrane filtration (Sari and Chellam, 2017). Further investigations into the role of DOC–metal complexes on membrane fouling are in forefront research.

#### 4. Conclusions

For the first time in Sri Lanka, the DOC in groundwater sampled from CKDu endemic areas were characterized. The  $(\frac{COD_{MR}}{DOC})$  ratio of the HR zone was not changed during two climatic seasons due to the low labile carbon content in shallow groundwater. Hydrophobicity of the DOC was examined by SUVA value. Highest aromatic DOC was observed in the HR zone groundwater. According to the 3D-EEM spectroscopic data, the dominant HS fraction in groundwater of CKDu endemic area was FA. Based on FI data, irrespective of the climatic zonation, the DOC precursors were derived from autochthonous carbon sources. The DOC fraction was classified into three groups: Fraction–I (MW < 900 Da), Fraction-II (900-1800 Da), and Fraction-III (1800-4000 Da). Fraction-II is denoted as the building blocks of non-biodegradable organic substances which are mostly enriched in the HR zone groundwater. It was implied the polydispersity with recalcitrant carbon in the HR zone. PCA was carried out to identify multivariate correlations of DOC with inorganic ions and pesticides. In both climatic seasons, a positive correlation was observed for DOC and  $SO_4^{2-}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , total Fe, PCP in the HR zone. The low  $SO_4^{2-}$  and high  $Ca^{2+}$  concentrations in groundwater of the LR, NR and CR zones implied screening of Ca<sup>2+</sup> and DOC (or FA) interactions by sulfur moieties on FA ( $\equiv$ C-OSO<sub>2</sub>). In the HR zone, highly aromatic DOC is believed to interact with PCP as weak physical bonding. Correlations of DOC with Fe and PCP with Fe have pointed to interactions among DOC-Fe-PCP. The correlation study of DOC with  $Ca^{2+}$  and  $SO_4^{2-}$  are important for the forefront research on membrane fouling mechanism.

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# Appendix A. Supplementary data

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