# Water Cycle

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Using UV-Vis differential absorbance spectra of tropical peat water DOM fraction to determine trihalomethanes formation potential and its estimated cytotoxicity

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#### ARTICLEINFO

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#### ABSTRACT

Absorbance differential spectra could be utilized to identify dissolved organic matter (DOM) characteristics such as the disinfection by products (DBPs) formation. This research purposed to establish the relationship between absorbance differential spectra and trihalomethanes-4 (THM4) formation potential, as well as the estimated cytotoxicity of tropical peat water DOM percentage. The ion exchange resin was used to separate the DOM components from peat water. In addition, the UV-Vis spectrum was examined between 200 and 700 nm. The hydrophobic-acid (HPOA) fraction contains the highest concentration of dissolved organic carbon (DOC), THM4 production potential, and calculated cytotoxicity. On the other hand, the hydrophilic-neutral (HPIN) fraction has the lowest potential THM4 production. The UV-Vis absorbance spectra of all DOM fractions showed a comparable peak at 277 nm. The differential spectra of 277 nm ( $\Delta A_{277}$ ) indicated a significant association with DOC concentration (99.6 %), trichloromethane (TCM) creation (86.6 %), total-THM4 (TTHM4) (81.3 %) formation, THM4 estimated cytotoxicity (89.7 %), and a moderate correlation with bromodichloromethane (BDCM) formation (55.6 %). Meanwhile,  $\Delta A_{277}$  had a poor correlation with brominated THM4 formation potential (chlorodibromomethane (CDBM): 2.59, tribromomethane (TBM): 2.78 %). The absorbance differential spectra might be employed as a surrogate measure for the peat water DOM fraction and its precursor properties to form THM4 during the chlorination process, as well as its estimated cytotoxicity.

#### 1. Introduction

Wetland has covered about 5 % of earth and become a major landscape in several regions such as in Southeast Asia countries [1]. Wetland that is covered by organic matter is considered as peatland [2]. The decomposition that occurs on the organic matter is derived from the vegetation, which produces the organic soils that cover the surface [1-3]. Since the peatland has thin organic soil, it has become important terrestrial carbon store and vital element for global carbon soil-atmosphere exchange [4]. The existence of the peatland spreads around the world from Asia, Africa, Europe, and America [5]. Most of the peatlands are lied in the South East Asia region as tropical peatland (56.2 %) [1]. Indonesia has a big portion of tropical peatland especially in Sumatra and Kalimantan Islands [2].

The peatland is not only having big carbon storage, but also has a massive water catchment area [6-8]. The runoff from this area can affect the water quality with high organic matter content, dissolved from peat soils [8]. The decomposition process of peat soils does not only enrich the peat water with humic and fulvic acid that has strong hydrophobicity, but also other hydrophobicity fractions such as transphilic and

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hydrophilic dissolved organic matter [9–11]. Since the tropical peat water has been used as an important water source in the tropical peat-land [2,12], the presence of the DOM fractions may be another challenge in treating tropical peat water.

The characteristic of natural organic matter (NOM) dissolved in tropical peat water has important rules on the formation of THM4 especially trihalomethanes [9,13,14]. The presence of humic and fulvic acid, a hydrophobic-acid (HPOA) DOM has been observed as a major precursor of THM4 formation [15]. This large molecular weight dissolved organic matter has a strong hydrophobic acid character with a massive aromatic carbon chain [16,17]. The HPOA DOM fraction is not the only fraction that can contribute to the formation of THM4 in peat water [9]. In fact, the presence of other fractions such as transphilic (TPH), hydrophilic-charged (HPIC), and hydrophilic-neutral (HPIN) has a big contribution to THM4 formation in certain characteristics and conditions [9]. The precursor characteristic is not only determined by high aromaticity content, but also by other factors such as, molecular weight, compound content, halogen content, and water matrix [15].

Most of the DBPs are formed from the chlorination processes, i.e.; cytotoxic, carcinogenic, mutagenic, neurotoxic, genotoxic, and teratogenic. Since higher concentration of THM4 is formed during the chlorine disinfection process, the presence of THM4 should be aware. Previous research had reported the cytotoxicity of a single compound of DBPs including THM4 [18]. The presence of THM4 in water could trigger several health effects including bladder and cancer [19].

UV-Vis differential absorbance spectra has been widely used to determine the DOM properties in water [20–23], including metal-binding properties [21,24], molecular weight [25], treatment efficiency [26], and DBPs formation [27]. The conventional UV<sub>25</sub>a absorbance method has been used as effective surrogate parameter for predicting the presence of NOM and DBPs. On the other hand, the use of UV-Vis spectral parameters such as spectral slopes, spectral ratio, and differential spectra gains more attention of researcher to determine more specific characteristic of DOM in water. Hopefully, the use of UV-Vis differential absorbance could be determined the THM4 formation potential and estimated cytotoxicity of pear water DOM fraction.

This research was conducted to make a preliminary observation on the calculated cytotoxicity from formed THM4 on the DOM hydrophobicity fraction of peat water. The result of this research could be used as a guide for choosing suitable treatment method for tropical peat water, that could reduce the toxic effect of formed THM4 from the final disinfection process.

#### 2. Materials and methods

#### 2.1. Samples and chemicals

Samples were collected from the Indragiri Hits Resency's peatland in Riau Province, Indonesia. Sigma Aldrich (USA) provided all fractionation resias (Superlite DAX-8, Amberlite XAD-1, and Amberlite IR-958). THM4 standard compounds (Trihalomethanes Calibration Mix-Sigma Aldrich) including chloroform/TCM, bromodichloromethane/BDCM, chlorodibromomethane/CDBM, and bromoform/TBM in methanol. Merck (Germany) that supplied the fractionation chemicals (hydrochloric acld, sodium hydroxide, sodium chloride, sodium sulfite, and methanol), the THM4 extraction reagents (methyl tert-butyl ether and sodium sulfate), sodium sulfite and the phosphate buffer. Pudak Scientific (losal) provided the chlorine solution (sodium hypochlorite 25 %). Every solution was diluted in Milli-Q grade water.

#### 2.2. DOM fractionation and chlorination

The fractionation of peat waters dissolved organic matter (DOM) was carried out in accordance with our prior research [9,10]. To remove suspended particles from peat water, a 0.45 m membrane was used. Samples were adjusted to pH 2 before put into a Superlite DAX-8 resin,

which absorbed the HPOA fraction. The unabsorbed fraction was put into an Amberlite XAD-4 resin, which absorbed the TPH component. The absorbed HPOA and TPH fractions were eluted with sodium hydroxide. The unabsorbed fraction that passed the XAD-4 was put into an anion exchange Amberlite IRA-958 resin that absorbed HPIO fraction. Then, it was eluted with a sodium hydroxide/sodium chloride combination. The XAD-4 effluent that was not retained by any of the resins was the HPIN fraction. Sodium hypochlorite solution with 1 % chlorine content was added to 100 mL of all peat waters DOM fractions and stored for 24 h at pH 7. Each operation was repeated three times to ensure data accuracy.

#### 2.3. Analytical methods

THM4 was analyzed based on EPA 551.1 [28] procedures by using Gas Chromatography (GC) Agilent 7890A that was coupled with Agilent 5975C Mass Selective Detector (MSD) and Agilent 7693 Series Automatic Liquid Sampler. Then, we used Agilent MSD ChemStation soft ware to analyze the data. The DOC was analyzed with a TOC analyzer (Shimadzu TOS-VCSH) utilizing APHA 5310 technique B [29]. Whereas, the UV-Vis Spectrophotometer (Shimadzu DV-1700) was used to determine the UV-Vis absorbance of peat water DOM fractions. Estimated cytotoxicity was calculated based on THM4 formation data by dividing the molar concentration of each reported THM4 (M<sub>THM4</sub>) with the supplied cytotoxicity (LC<sub>50</sub>) values (M<sub>LC50</sub>) exposed to Chinese hamster ovarium (CHO) cell [18] (Eq. (1)).

Estimated cytotoxicity = 
$$\frac{M_{THM4}}{M_{LC50}}$$
 (1)

#### 3. Results and discussions

#### 3.1. Peat water characteristics

Table 1 shows the raw peat water and its DOM fractions characteristics. The DOC content in tropical peat water is very high due to the high concentration of humic acid, as depicted by its high color content and acidic pH. The UV<sub>254</sub> absorbance of peat water is also high due to high DOC concentration. The SUVA value of raw peat water is higher than 4 indicates that the peat water has high hydrophobic DOM [30]. The acidic pH of natural peat water indicates that the DOM is discovered in the form of humic acid [9].

The HPOA fraction has the highest percentage of DOC concentration (63.29 %) followed by TPH (16.57 %), HPIN (11.21 %), and HPIC (6.97 %) fractions. The DOM content in the peat water is also indicated by the UV254 absorbance that is dominated by the HPOA fraction. The SUVA values of HPOA and TPH fractions are higher than 4, indicate strong hydrophobicity domination. On the other hand, since the HPIC and HPIN are hydrophilic fractions, the SUVA values are lower than 2. Even the TPH exhibits a slightly higher  $E_2/E_3$  ratio (absorbance ratio of  $A_{250}/$ 

Table 1
Peat water DOM fractions characteristics

(ETS)	Raw	DOM Fract	DOM Fractions Proofread (ETS)				
		HPOA	TPH	HPIN	HPIC		
pH	5.20	N.A.	N.A.	N.A.	N.A.		
Color	3050	N.A.	N.A.	N.A.	N.A.		
(PtCo)							
DOC (mg/	$52.06 \pm$	$32.95 \pm$	$8.63 \pm$	$5.84 \pm$	$3.63 \pm$		
L)	3.73	3.51	1.52	0.80	0.65		
$UV_{254}$	$3.95 \pm$	$2.40 \pm$	$0.46 \pm$	$0.07 \pm$	$0.06 \pm$		
(cm <sup>-1</sup> )	0.00	0.01	0.005	0.0005	0.0005		
SUVA (L/	$7.58 \pm$	7.28 $\pm$	$5.28 \pm$	$1.14 \pm$	$1.55 \pm$		
mg·m)	0.59	0.82	0.95	0.94	0.35		
$E_2/E_3$	$5.02 \pm$	$5.25 \pm$	$5.21 \pm$	$2.95 \pm$	$3.09 \pm$		
	0.01	0.00	0.00	0.00	0.00		
$E_4/E_6$	$8.06 \pm$	$1.18 \pm$	$1.22 \pm$	$1.30 \pm$	$0.61 \pm$		
	0.01	0.00	0.00	0.00	0.00		

A365) compared to the HPOA percentage, indicating a smaller molecular weight and a higher degree of aromaticity [31,32]. The E2/E3 ratio shows that DOM is independent of chromophoric DOM (C-DOM), decreasing with increasing molecular weight [33]. The HPOA fraction also has higher E<sub>4</sub>/E<sub>6</sub> value (absorbance ratio of A<sub>465</sub>/A<sub>665</sub>) compared to other fractions. Beyond aromaticity, the E4/E6 ratio correlates with molecule size, overall acidity, and carbonyl content [34].

#### 3.2. UV-Vis differential absorbance spectra

Fig. 1 shows the UV-Vis differential absorbance, and differential spectra at 277 nm, of peat waters' DOM fractions. All UV-Vis differential spectra of DOM fraction show similar differential absorbance trend that increase from wavelength of 200 nm-277 nm peak then rapidly decreased reaching the visible light wavelength (Fig. 1a). This differential absorbance trend shows that wavelength of 277 nm (UV277) as the highest absorbance peak among all DOM peat waters' fractions. The HPOA fraction has the highest UV277 differential absorbance due to its content of humic acid. Then, it is followed by HPIC, with nearly similar absorbance values, TPH, and HPIN fractions.

The differential spectra of UV<sub>277</sub> ( $\Delta$ A<sub>277</sub>) can be calculated based on the absorbance trend (Fig. 1b). Conversely with  $\Delta$ A<sub>277</sub> absorbance, the HPOA fraction has the lowest \$\triangle A\_{277}\$ that is followed by TPH, HPIC, and HPIN fractions. Meanwhile, the DOC concentration shows opposite pattern (Table ). HPOA fraction as the highest concentration is followed. lowed with TPH, HPIC and HPIN fractions. This may be happened due to the HPOA fraction that is contained of humic and fulvic acid as major DOM that can be found in natural water. The  $\Delta A_{277}$  is close to  $\Delta A_{272}$ which had been proposed by previous study [35] that associated with the continuous reaction of activated aromatics and highly correlated with THM4 formation.

The UV absorbance of natural water between 250 and 280 nm is commonly used to predict the concentration of aromatic structures, identified as chlorine-reactive site of NOM [35]. The majority of researches have demonstrated a linear relationship between DBP concentrations and absorbance at a single wavelength of 272 nm. with very high determination coefficients (R2) of typically greater than 0.90. This suggests that a significant quantity of precursors, characterized by absorbance at this wavelength, will undergo a reaction to generate an intermediate product, that eventually releasing the observed DBP [35]. The selection of this wavelength is based on the empirical observation of the highest differential absorbance at this specific wavelength [36].

These findings demonstrate some shared properties of water differential spectra. The differential spectra have been used to determine several DOM properties such as DBPs formation [22,27,36,37], metal binding [24,38], ion binding into extracellular polymeric substances [39], chlorite formation and chlorine consumption [23], molecular weight [25,39,40], evolution of charges of peat fulvic and humic acid [41] and NOM removal on coagulation process [42] as shown in Table 2. To the best of our knowledge, all previous studies on UV-Vis differential absorbance spectra were not conducted on correlation between differential absorbance spectra of DOM fractions with THM4 formation in association with its estimated cytotoxicity, especially for high humic content water such as peat water.

Differential absorption refers to a change in UV-Vis absorption at specific wavelength or within specific wavelength range under different conditions [43]. The formation of THM group was successfully identified from river water at  $\Delta A_{272}$  [35,36]. The formation of DBPs from various river water samples were identified at  $\Delta A_{272}$  with correlations of 90-95 %, ion binding from Suwanee River humic acid (SRHA) samples at various differential wavelength with correlations >80 % [44], and chlorite formation from SRHA and Suwanee River fulvic acid (SRFA) samples at  $\Delta A_{316\text{--}400}$  with correlations >90 % [23], and DOM at  $\Delta ln A_{400}$ with a correlation of 99 % [42]. There was a direct correlation between the concentration of substances in water and the magnitude of  $\Delta UV$ -Vis [45,46]. Higher concentrations generally result in larger absorption changes, especially in the UV range. The composition of substances, including specific functional groups (e.g., aromatic rings, phenolic groups, carboxylic acids), can also affect the differences in  $\Delta UV$ -Vis absorption [23,47]. Different components can contribute to specific UV-Vis features, allowing the identification of chemical characteristics.

#### 3.3. THM4 formation potential of peat water DOM fractions

Fig. 2 shows THM4 formation and its estimated cytotoxicity of pear water DOM fraction after 24 h chlorination in pH 7. In the formation of Article

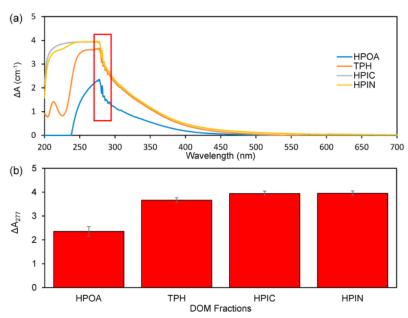


Fig. 1. Differential UV-Vis absorbance (a), and  $\Delta A_{277}$  value (b) of tropical peat water DOM fractions.

Table 2
Available research in correlation between DOM properties and UV-Vis differential absorbance.

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<mark>ential a</mark> bsorbance.	Ar	ticle Error <i>(</i>	ETS)	· vio dinci
Object p. (ETS)	Water source	Δ Wavelength	Correlation	References
Individual DBPs	Tolt River	$\Delta A_{272}$	95 %	[22]
THM THMs and HAAs	Lake Sartori	ΔΑ <sub>272</sub>	98 %	[37] [40]
THMS and HAAS	Alibeykoy raw and fractioned water	ΔA <sub>254</sub>	Low correlation	[40]
Binding of copper	SRFA	ΔA <sub>208</sub> ,	99 %	lışşıng ","
(II) by		$\Delta A_{242}$		
Suwannee River fulvic		ΔΑ <sub>276</sub> ,		
acid (SRFA)		$\Delta A_{314}$ , $\Delta A_{378}$ , and		
(,		ΔA <sub>551</sub>		
Ions binding onto	AerAOB.	$\Delta A_{300}$	98,	[39]
extracellular	AnAOB, Sp. (E	<b>B</b>	97, and 95 %	
polymeric substances in	activated sludge	3)	90	
different mixed	Si da ge			
microbial				
cultures				To mil
THM4 and HAN4	Membrane bioreactor	ΔLnA <sub>350</sub>	99 %	[27]
P	treated water of	ETS)		
	municipal			
	wastewater			
Metal ion binding	(Ji'nan, China) Caffeic acid,	$\Delta A_{240}$	correlated	[38]
wictai foil billuring	ferulic acid,	ΔA <sub>276</sub> ,	correlated	[50]
	sinapic acid,	ΔA <sub>316</sub> , ΔA <sub>385</sub>		
	terephthalic			
	acid, isophthalic			
	acid, esculetin			
	and myricetin			
Metal-DOM	SRHA	$\Delta S_{350-400}$	91-98 %	[24]
interaction Chlorite	SRHA and	ΔΑ <sub>235</sub>	01 00 0/	f0.03
formation and	SRFA and	$\Delta A_{316}$ $\Delta A_{400}$	91–99 % 92–99 %	[23]
ClO2				
consumption				
DBP concentrations	Du Nord River	$\Delta A_{272}$	62–99 %	[36]
Carboxylic-like	SRHA	ΔS <sub>260-270</sub> and	98 %	[21]
groups and		$\Delta S_{350-400}$		
total NOM-				
bound Al (III) ions				
DBF	SRNOM	$\Delta A_{272}$	90 %	[35]
Wei <mark>ght-averaged</mark>	SRHA Sp. (E)	$\Delta A_{200}$	99 %	[25]
molecular weight	SRFA		97 %	
evolution of	Carboxylic and	ΔΑ <sub>280</sub>	98-99 %	[41]
charges of peat	phenolic range	$\Delta A_{350}$		
fulvic and humic acid				
Dissolved organic	Source water	$\Delta ln A_{400}$	99 %	[42]
matter removal	for the Beijing			,
by coagulation	Mega-city			
metal-NOM complexes	Metal-esculetin solution	$\Delta A_{240}$ $\Delta A_{276}$	80 %	[26]
comprexes		AA212 and		
	Missir	$\Delta A_{390}$		
DOC	Tropical peat	$\Delta A_{277}$	81–99 %	This study
concertation, THM4	water			
formation and				
its estimated				
cytotoxicity of				
tropical peat water DOM				
fractions				

SRFA: Suwannee River fulvic acid, AerAOB aerobic ammonium-oxidizing bacteria, AnAOB anaerobic ammonium-oxidizing bacteria, SRHA: Suwannee River humic acid, ARNOM Suwannee River NOM.

chlorinated THM4, TCM dominated almost every fraction of peat water DOM, except HPIC, as can be seen in Fig. 2a. Our result is in line with previous researches [48–50], which stated that TCM is the most formed THM in the chlorination process. The highest TCM formation is in the HPOA, followed by the TPH fraction. The low TCM formation only takes place in the HPIC fraction, which is dominated by brominated THM4. The hydrophobic (HPOA and TPH) has a higher TCM formation potential than the hydrophilic fraction due to higher aromatic compounds that precursor to TCM [51].

The BDCM has the second-highest formation potential. It is higher in TPH, followed by HPDA fraction. Since hydrophilic fraction has higher precursor properties than brominated DBPs [52], the HPIC fraction also has high DBCM formation. Brominated THM4 dominates the formation of THM4 in HPIC fraction, although it is not as high as chlorinated THM4. CDBM has be highest formation in HPIC, followed by TBM as the smallest formed THM4 in each fraction. According previous studies [48, 53], TBM's presence was influenced by bromide ions in water. The formation of brominated DBPs could rule the THM4 formation since the water contained high hydrophilic fractions [52].

HPOA fraction is highly contributed to the formation of total THM4 (t-THM4FP) with 51.9 % of formation followed by TPH fraction at 31 % (Fig. 2b). Consequently, the patterns of TCM and TTHM4 formation are nearly similar. TCM is the major THM4 that formed during the chlorination of water containing high levels of humic substances [50,54,55]. The HPIC fraction has the smallest TTHM4 formation at 5.7 %. The humic and fulvic acids in HPOA fraction are the major precursor to THM4 formation [15]. Similarly, TPH fraction contains β-dicarbonyl acids, were important in the THM4 formation [15]. TPH fraction is defined as a DOM fraction that has intermediate polarity between hydrophobic and hydrophilic DOM and has low aromatic and phenolic carbon compared to HPOA fraction [56]. Also, TPH fraction is defined as low hydrophobic fraction [57], so still has high estimated cytotoxicity. Previous studies reported that the hydrophilic fractions had tryptophan as a significant precursor to THM4 [58,59].

#### 3.4. Estimated cytotoxicity

The calculated cytotoxicity of THM4 shows similar pattern with THM4 formation. Although brominated THM4 has the highest cytotoxicity value compared to chlorinated THM4 [18], the high formation of TCM still dominates the THM4 estimated cytotoxicity (Fig. 2c). The formation of BDCM is the second source of THM4 estimated toxicity in all peat water fractions. Since the HPOA fraction has the highest DOC concentration in peat water, it dominates the estimated THM4 cytotoxicity followed by TPH, HPIC and HPIN fractions (Fig. 2d).

The toxicity of DBPs is determined by the organic matter's precursor characteristics and its concentration in water. DBPs are cytotoxic, genotoxic, carcinogenic, neurotoxic, mutagenic, and teratogenic in the majority of cases [60]. THM4 is one of the two DBPs regulated by the USEPA. The formation of other regulated DBP, haloacetic acids-5 (HAA5) in peat water DOM fraction had been studied previously [9, 10]. However, the data on other DBPs that may more toxic than regulated DBPs are still lacked. In addition, several DBPs are developmentally toxic and have growth inhibition [61,62]. The estimated cytotoxicity in this study was based solely on THM4 formations during the chlorination of in peat waters DOM fractions. It should be noted that the cytotoxicity calculated does not represent the complete amount of toxicity of generated DBPs after chlorine disinfection. Furthermore, the observed DBPs are not the only source of toxicity. More studies should be done to investigate the generation of other DBPs and their toxicity in the chlorination process of tropical peat water DOM fractions.

#### 3.5. THMs formation mechanism

Fig. 3 shows the mechanism of DBPs formation of each DOM example in the presence of bromide. Chlorine initiates the attack on organic

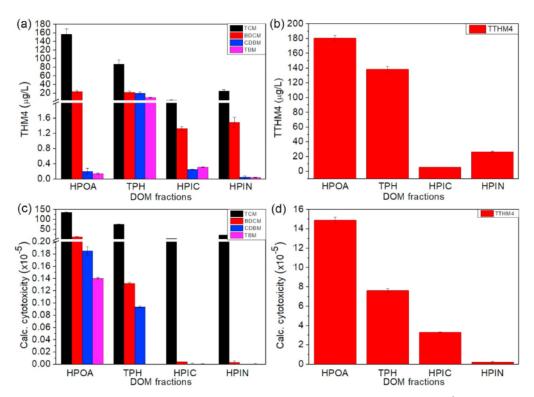


Fig. 2. THM4 formation and of tropical peat water DOM fractions its estimated cytotoxicity: (a) THM4, (b) TTHM4, (c) estimated cytotoxicity of TTHM4.

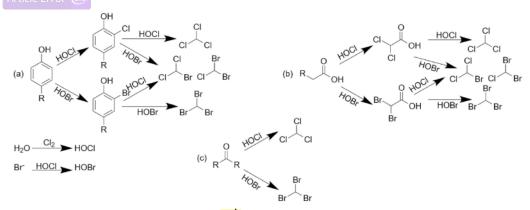


Fig. 3. The interaction between chlorine and DBP precursor: (a) phenolic, (b) carboxyl, and (c) carbonyl compounds.

matter through electrophilic substitution, leading to the formation of chlorinated DBPS, such as THM4 [63]. The presence of bromide in water can react with chlorine to form hypobromous acid. HPOA and TPH fractions contain of high phenolic/aromatic carbon with high molecular weight. A phenolic compound can form haloform such as THM during chlorination process (Fig. 3a). Bromide in water can react with chlorine to generate hypobromous acid and bromate, both of which exhibit higher reactivity compared to chlorine [14]. HPOA fraction in peat water is contained of humic and fulvic acid [9,64]. Humic acids are macromolecular and complex, with core components that are substituted aromatic and aliphatic hydrocarbons with high molecular

weight (2000–5000 Da) [15]. On the other hand, fulvic acid is identified by lower molecular weight and aromaticity compared to humic acids (500–2000 Da) [15].

TPH is a DOM fraction with intermediate polarity between hydrophobic and hydrophilic DOM and low aromatic and phenolic carbon when compared to HPOA [56]. The carboxyl group (COOH) is an example of an HPIC DOM fraction, as it can function as an acid by losing a proton to produce a negatively charged carboxylate ion. Carbonyl (C=O), an uncharged yet polar functional group (containing partial positive and partial negative charges), serves as an example of an HPIN fraction. Carboxyl groups are frequently found in amino acids fatty fused

acids, and other macromolecules, while carbonyls can be found in a wide range of biological components, including proteins, peptides, and carbohydrates [15].

3.6. Correlation between DOC, THM4, and calculated cytotoxicity and differential spectra rticle Error

Fig. 4 shows the correlation between  $\Delta A_{277}$  and DOC concentration, THM4 formation potential and its estimated cytotoxicity of peat waters

DOM fractions.  $\Delta A_{277}$  was highly correlated to each peat water DOM fraction (99.6 %) (Fig. 4a). Moreover,  $\Delta A_{277}$  also shows high correlation with highly chlorinated-THM4 (TCM) (86.6 %) (Fig. 4b) and moderate correlation to BDCM formation (55.6 %) (Fig. 4c). However, the formation of high brominated-THM4 shows remarkably weak correlation with  $\Delta A_{277}$  with 0.25 % and 0.27 % for CDBM (Fig. 4d) and TBM (Fig. 4e). Since the formation of THM dominates the THM4 formation of peat waters DOM fractions, the TTHM4 formation has moderate correlation with  $\Delta A_{277}$  (69.6 %) (Fig. 4f). A

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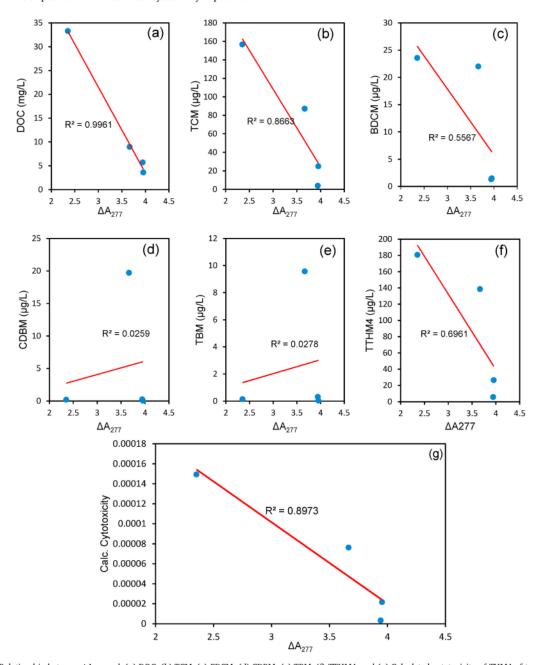


Fig. 4. Relationship between  $\Delta A_{277}$  and: (a) DOC, (b) TCM, (c) BDCM, (d) CDBM, (e) TBM, (f) TTHM4, and (g) Calculated cytotoxicity of THM4 of tropical peat water DOM fractions.

study of Krasner et al. (2022) also reported that correlation of  $\Delta A_{277}$ -TCM was higher than  $\Delta A_{277}$ -BDCM. The BDCM, CBDM, and TBM formation was influenced by the presence of bromide in water [65]. Meanwhile, another previous study found that the high bromide concentration affected the  $\Delta A$ -THM4 relationship [35].

Since the  $\Delta A_{277}$  values of HPIN and HPIC fractions were nearly similar, a perplexing deviation in data was observed for  $\Delta A_{277} \approx 4$  especially in relation to highly correlated parameters such as TCM (Fig. 4b) and cytotoxicity (Fig. 4g) with  $\Delta A_{277}$  value. Although  $\Delta A_{277}$  is moderately correlated to TTHM4, the calculated cytotoxicity of TTHM4 shows strong correlation to  $\Delta A_{277}$  with higher percentage (89.7 %) (Fig. 4g). The primary DBP precursors in natural water are chromophoric DOM with unsaturated moieties that typically have ultraviolet-visible (UV-Vis) absorbance [27]. In addition, the presence of chromophoric DOM tend to form TCM as dominant THM4 during chlorination process [50]. Although the cytotoxicity of TCM is lower than brominated THM4, the high formation of TCM dominates the THM4 cytotoxicity that impacts the  $\Delta A$ -estimated cytotoxicity relationship.

Although the relationship between UV-Vis differential spectra and several peat water DOM fraction characteristics such as DOC concentration. THM4 formation, and THM4 estimated cytotoxicity has been investigated in this study, more precise DOM fraction and raw peat water specifications should be considered for future research. There are several parameters to consider, including the presence of distinct DOM fractions such as hydrophobic base, hydrophilic acid, hydrophilic neutral, and DOM fractions based on molecular weight. In addition, specific experimental condition such as effect of pH, chlorine concentration, chlorine contact time and presence of bromide in water are need to be examined. Also, the formation of other regulated DBPs, HAA5, nitrogenous DBPS such as haloacetonitriles, halonitromethanes, and haloacetamide, and phenolic DBPs should be investigated with regard to their association with UV-Vis differential spectra in order to discover new applications for UV-Vis differential spectra.

#### 3.7. Potential application UV-Vis differential spectra

In tropical regions, the peat water DOM characteristics especially the presence of hydrophobic fractions such as humic acid depicted in its color [66] are influenced by several factors such as season and tides [54]. The conventional water treatment using alum coagulation—sedimentation process could not completely remove the presence of DOM in peat water [9]. The coagulation process is effective in removing the hydrophobic DOM fraction but has limited impact on the removal of hydrophilic fractions [67]. The remaining HPIC and HPIN fractions dissolved in treated water could serve as sources of DBPs precursor [10,15,68]. This study used the peat water DOM fraction differential spectra to determine THM4 formation during chlorination process. By knowing the differential spectra of raw peat water and remaining DOM fraction in treated peat water, the THM4 formation during final chlorination process and its cytotoxicity could be predicted.

#### 4. Conclusion

The UV–Vis differential absorbance spectra of peat water DOM fractions are related to DOC concentration, THM4 formation, and THM4 estimated cytotoxicity. Ion exchange resin was used to separate the DOM fraction into HPOA, TPH, HPIC, and HPIN fractions. The UV–Vis differential spectra of peat water DOM fractions show a significant peak at 277 nm ( $\Delta\Lambda_{277}$ ). The  $\Delta\Lambda_{277}$  shows a strong relationship with DOC concentration, TCM formation, total THM4 formation, and THM4 estimated cytotoxicity, with percentages of 99.6 %, 86.6 %, 81.3 %, and 89.7 %, respectively. Furthermore, the BDCM formation exhibits a moderate correlation with  $\Delta\Lambda_{277}$  (55.6 %). However, the production of highly-brominated THM4 has no relation with  $\Delta\Lambda_{277}$ . Based on our findings, the  $\Delta\Lambda_{277}$  might be employed as a surrogate parameter for

DOM fractions DOC concentration in relation to THM4 and its cytotoxicity during peal water treatment process.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- **Proofread** This part of the sentence contains an error or misspelling that makes your meaning unclear.

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