A fluorescence-based assessment of the fate of organic matter in water treated using crude/purified Hibiscus seeds as coagulant in drinking water treatment

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Abstract

This study used fluorescence excitation-emission matrices (EEMs) analysis to investigate the characteristics of natural organic matter (NOM) in treated water using okra crude extract (OCE), sabdariffa crude extract (SCE) and kenaf crude extract (KCE) as coagulants. In addition, an assessment of the impact of purified okra protein (POP), purified sabdariffa protein (PSP) and purified kenaf protein (PKP) was undertaken. The performance evaluation of these coagulants in terms of increase or decrease in dissolved organic carbon (DOC) was compared with Peak T fluorescence intensity observed at excitation wavelength 220-230 nm, and emission wavelength 340-360 nm. Fluorescence analysis of water treated with the crude extracts identified the removal of DOC in peaks A and C region whereas the increase in DOC from the protein was predominantly found in peaks T and B region. Furthermore, it was observed that the purified proteins were noted to be capable of reducing the DOC

concentration in raw water where all fluorophores were not detected. The application of OCE, SCE and KCE yielded an increase in DOC of 65, 61 and 55% respectively, corresponding to increases of 65, 29 and 54% in peak T fluorescence intensities, at 100 mg/l dose. Furthermore, DOC concentration was reduced by 25, 24 and 18% using POP, PSP and PKP respectively as coagulants with corresponding decreases in fluorescence intensity of 46%, 44 and 36% in POP, PSP and PKP, at a lower dose of 0.1 mg/l. Therefore, it is clear that Peak T fluorescence intensity could be used to characterise organic matter in treated water using natural extracts to assess final water quality.

Keywords: Fluorescence intensity; Hibiscus seed; water treatment; extracts; proteins

1.0 Introduction

Organic matter (OM) mainly originate from multiple biological degradations of plants and animal products (Pernitsky and Eng, 2004, Thurman, 2012). Collectively, these substances are known as natural organic matter (NOM), and many of these compounds exist in solution (Gregory, 2005). NOM in water is measured as total organic carbon (TOC), with the soluble fraction (that which can pass through a 0.45μm filter membrane) measured as dissolved organic carbon (DOC), (Bolto, 1995). Organic compounds with varying characteristics are found globally in many water bodies, especially in surface waters such as in lakes, streams, ponds and rivers. NOM may consist of molecular weight (MW) substances, and many functional groups (Pernitsky and Eng, 2004), where the low MW compounds are challenging to remove via simple coagulation, flocculation and clarification processes (Bolto, 1995). The presence of NOM in natural water can cause bad odour, taste, colour, and bacterial re-growth problems (Yan et al., 2006, Bolto and Gregory, 2007), and disinfection by-product (DBPs) formation when in contact with disinfectants (Bridgeman et al., 2011, Liu et al., 2014).

NOM found in water consists of both hydrophilic and hydrophobic components (Matilainen et al., 2011). Hydrophilic are compounds such as protein, gums, starch and many synthetic polymers which remain in solution and are difficult to remove (Matilainen et al., 2010, Matilainen et al., 2011, Wu et al., 2003).

Much of the NOM in water, such as humic substances, can be regarded as hydrophilic, as dissolved components (Gregory, 2005), and is characterised by brownish colouration, and as suspended materials (colloids). The specific surface area of colloids and the existence of a surface charge on the colloids explain the prevalence of negatively charged surface forces over volume forces, which stabilise the systems and negate any possibility of elimination by natural settling (Matilainen et al., 2011).

Therefore, NOM in drinking water should be removed to improve water quality. Moreover, since the prevalence of NOM in water can affect its removal efficiency, a suitable characterisation method of NOM would enhance the performance of water treatment process. Recently, however, there has been an increase in interest in the use of fluorescence spectroscopy to characterise NOM in drinking water treatment. Fluorescence spectroscopy is a robust technique, simple and efficient in providing an accurate evaluation of organic compound removal in water treatment (Bieroza et al., 2009b). It also offers potential for online monitoring of DBPs formation in water treatment processes (Bieroza et al., 2009b). Several studies have used fluorescence excitation-emission matrix (EEMs) to assess NOM removal in drinking water (Bieroza et al., 2009a, Carstea et al., 2010). Similarly, the use of fluorescence EEMs to monitor river contamination by tissue mill and landfill leachate have been reported elsewhere (Baker, 2002, Baker, 2005). EEM data present a unique overlap of fluorescence intensities over different excitation and emission wavelengths (Bridgeman et al., 2011). Within the fluorescence EEM, the presence of organic matter can be visualised as

peaks, and these peaks were classified by Coble (1996) as; peaks A and C (humic and fulvic-like substances) while peak T and B (tryptophan and tyrosine-like proteins) obtain at shorter emission wavelengths. Bieroza et al. (2009b) showed in a study that the combination of peak C emission wavelength and peak T fluorescence intensity might be used as an indicator of TOC removal. Conversely, in the coagulation unit, Gone et al. (2009) and Markechová et al. (2013) observed that peak T fluorescence intensity was least well-removed compared to that of peaks A and C in raw water treated using aluminium sulphate (AS), and can be used to assess residual DOC post-coagulation.

Coagulation process is the most important unit process employed to facilitate suspended colloids and NOM removal from drinking water (Jarvis et al., 2005) by changing the surface chemistry of the particles. It is the most widely used principle in traditional water works where other unit processes are highly dependent upon it for effective performance. Aluminium and iron salts are the two most used coagulants in this regard (Duan and Gregory, 2003, Ghebremichael et al., 2005). However, economic constraints mean that the cost of importing these chemicals is a major challenge for developing countries (Diaz, 1999, Ghebremichael et al., 2006). As such this has rendered many communities unable to access clean drinking water, especially those living in rural areas. Thus, there is an urgent need for the production of an affordable alternative material for water treatment in developing countries. Consequently, in order to make water supply available for people in rural areas, there has been increased interest in the study of natural extracts in water treatment to augment the use of synthetic chemicals. *Moringa oleifera* (MO) is reported to be the most studied natural plant material, performing the dual functions of coagulant and disinfectant in water treatment (Jahn and Dirar, 1979, Madsen et al., 1987, Ghebremichael et al., 2006). Additionally, a few other naturally-occurring materials of plants origin have been tested in this regard, such as *Cactus latifaria* (Diaz, 1999, Zhang et al., 2006), Common beans (Sciban

et al., 2006), Mustard seeds (Bodlund et al., 2014). Furthermore, Hibiscus plants have also been tested in drinking water treatment. Al—Samawi and Shokralla (1996) used okra seed pod in conjunction with aluminium sulphate (AS) to treat 3000 NTU synthetic water and reported a 97.1% reduction in turbidity and a corresponding reduction of over 50% AS volume. Others have tested the potential of okra mucilage in the treatment of water and tannery effluent (Agarwal et al., 2001, Anastasakis et al., 2009). Similarly, Jones and Bridgeman (2016b) investigated the floc strength of three Hibiscus species, components *viz*. okra, sabdariffa and kenaf as primary coagulants and as coagulant aids in water treatment, demonstrating a significant increase in floc strength and size. Furthermore, Jones and Bridgeman (2016a) revealed partial inactivation of *E-coli* and *faecal coliform* in water using crude Hibiscus extracts while *total coliform* remains largely unaffected due to the presence of multiple microbes. Conversely, purified Hibiscus proteins achieved 100% inactivation of *E-coli, faecal* and *total coliform* bacteria after one-hr post-coagulation. Although, the inactivation impact of Hibiscus seed on faecal coliform and E-coli bacteria has been reported previously Jones and Bridgeman (2016a), it has no health effects on human beings when consumed. It is noteworthy that Hibiscus seeds are currently a primary source of protein and food in many developing countries. Additionally, Hibiscus seeds have been used in folk medicine for the treatment of several ailments, hence it is considered safe for human consumption.

Kenaf-derived activated carbon has also been studied in the treatment of water contaminated with heavy metals (Chowdhury et al., 2012). Unfortunately, one of the greatest challenges of using natural extract in water treatment is the continuous increase in organic loads in the clarified water (Ndabigengesere and Narasiah, 1998, Ghebremichael et al., 2006), resulting in changes in colour, taste, and odour. Additionally, organic compounds from the seed can react with the disinfection chemicals such as chlorine leading DBPs formation, thereby rendering

120 the treated water unfit for human consumption. More importantly, natural extract contains
121 numerous organic compounds such as tryptophan. Study has shown that *E-coli bacteria* has
122 the ability to produce an indole odour from tryptophan(WHO, 2008) which may affect
123 human health. Similarly, the presence of other organic compounds in water could cause a
124 change in taste and colour. To address this problem, Okuda et al. (2001) and Ghebremichael
125 et al. (2005) purified the coagulant protein in MO to reduce the impact of NOM in the final
126 water. Similarly, Sciban et al. (2006) isolated the proteins in common bean and observed a
127 reduced DOC concentration in treated water. However, most of these studies measured the
128 organic compounds in terms of DOC in water.

129 Several characterisations tools are used to identify and monitor NOM compounds in water.
130 Bridgeman et al., (2011) divided these into four tiers of analysis, *viz*, preliminary
131 characterisation, size characterisation, chemical identification and behaviour and spectral
132 signature. Preliminary characterisation, which focuses on dissolved OM components for
133 isolation, includes the following analyses; DOC and TOC, ultraviolet absorbance and
134 suspended solids concentration. However, there are several other sophisticated laboratory-
135 based analytical techniques (e.g. high performance size exclusion chromatography, gas-
136 chromatography mass spectrometry (GC-MS) and resin extraction) for differentiating the
137 physiochemical properties of the various components (Bridgeman et al., 2011) although these
138 processes have limitations to properly characterise the various NOM fractions in the system.
139 The use of optical techniques to monitor wastewater quality and treatment processes has also
140 been studied previously, particularly UV-vis absorbance spectroscopy (Henderson et al.,
141 2009).

142 **Table 1 Advantages (+) and drawbacks (-) of fluorescence measurement and other known protocols**

Fluorescence spectroscopy	Other protocols for analysing NOM in water
1. (+) Rapid assessment of water and	9. (-) Assessment takes a longer time and

	wastewater OM. Sensitive in characterising aquatic OM. 2. (+) Incorporates an on-line monitoring tool. 3. (+) Requires small sample volume. 4. (+) Minimal sample preparation is required 5. (+) Provides substantial information on the composition of OM present. 6. (-) OM characterisation is based on many parameters describing absorption and emission energy. 7. (-) Fluorescence quenching can affect fluorescence measurement. 8. (-) Inner filtering effect can impact the result which requires correction prior to measurement.	routine measurement are conducted with limited value in terms of OM characteristics. 10. (-) Measurement is off-line 11. (-) Requires extensive sample preparation 12. (-) Only a limited OM fraction can be fractionated 13. (-) Large sample volume is needed 14. (-) Limited information on OM composition is made available. 15. (+) Quenching has no effect on the measured OM value. 16. (+) No known effect of inner filtering on the measured values.

Nevertheless, despite the advances made to analyse and characterise NOM in water, there remain some advantages and limitations of these processes as shown in Table 1.

The aim of this study was to demonstrate the application of fluorescence EEMs in a water treatment context where Hibiscus seeds were used as coagulants in order to identify fluorescence dissolved organic matter (fDOM) in clarified water, and to compare this with against the traditional DOC analysis which only provides information on dissolved components of NOM in water. In this way, new information could be used to provide a better understanding of the characteristics of NOM in the final water to address the main challenge of using natural extract in water treatment. Additionally, the work reported here seeks to understand the relationship between fluorescence intensity and residual DOC concentration in both raw and treated water. To achieve this objective, for the first time fluorescence fingerprints were used to assess the efficacy of crude and purified Hibiscus seeds for water treatment.

2.0 Materials and methods

2.1 Seeds collection and preparation

All the seed samples were purchased from Marama, a local market in Nigeria. For proper assessment, the seeds were harvested from mature dried plants. The seed kernels were manually removed from the seedpod and capsules, followed by washing with laboratory tap water to remove contaminants that may affect the quality of seeds. The seed was dried and ground into a fine powder for 2 minutes using a Tema laboratory disc mill. The ground seed powders were then sieved in a set of sieves arranged in descending order. The powder retained in the 212 μm, and 300 μm sieve sizes were combined, and thoroughly mixed and then used in the preparation of the extracts.

2.2 Chemicals and reagents

Analytical grade sodium chloride (NaCl) (Fisher Scientific, UK), sodium phosphate monobasic monohydrate (Sigma-Aldrich, Germany), 98% hexane and sodium phosphate dibasic (Sigma-Aldrich, UK) were used in the study. All suspensions were prepared using Deionized (DI) water.

2.3 Preparation and extraction of crude seed coagulants

The crude seed extract (CSEs) were prepared from the ground seed powders following (Jones and Bridgeman, 2016a). Briefly, 1.0 M NaCl solutions were added to the seed powder to make 2% (w/v) suspension. The suspension was vigorously mixed using a magnetic stirrer for 15min then centrifuged at 4500 rpm for 10 minutes using a Heraeus Megafuge16 (Thermo Scientific, Germany). The suspension was decanted and then filtered through a Whatman No. 42 filter paper. The filtrates were termed crude extracts and used as coagulants in a series of jar test experiments.

2.4 Preparation and protein purification processes

2.4.1 Lipid extraction

The combined seed powders obtained in (section 2.1) were defatted using high-grade hexane in a Soxhlet extractor. 20 grammes of the ground powder was extracted in the extraction thimble of the apparatus. For efficient extraction, two litres of solvent volume (high-grade hexane) were heated to 60 °C. The process was run continuously for 8 hrs with each complete cycle taking between 2 to 3 minutes. The residue from the extraction thimble was dried overnight at room temperature (19±2°C), the dried residue was then ground into a fine powder using pestle and mortar. The ground oil-free powder was then employed in the subsequent protein purification process.

2.4.2 Protein purification

Protein purification was conducted according to (Jones and Bridgeman, 2016b) where a 1 ml HiTrap Q HP anionic ion exchange column, (GE Healthcare, Sweden) was used for the purification of the proteins. The column connected to a pump (Watson-Marlow Breeder pump 323, UK), and the pump head adjusted to a flow rate of 1 ml per minute. The preservatives were washed with 10 ml of DI water, followed by ten column volumes (CV) of 1 M NaCl dissolved in the phosphate buffer. The column was then equilibrated with the phosphate buffer 10 CV before loading the protein. 5 grams of the oil-free powder was dissolved in 0.1 M phosphate buffer and mixed thoroughly for one hour using a magnetic stirrer. The mixture was centrifuged at 20,000 rpm at 4°C for 40 minutes before decanting the supernatant. The supernatant was injected using a peristaltic pump onto the ion exchange column to separate the protein of interest from the contaminants.

The sample was loaded at a flow rate of 1 ml per minute, where the protein of interest was bound to the Column matrix throughout the loading process. The weakly bound contaminants were washed away with the equilibrating (initial) buffer using 10 CV. The proteins of interest were eluted, beginning with, 0.3, 0.5 and 1.0 M of NaCl phosphate buffers and the various fractions collected. The collected fractions were analysed and coagulation performance conducted using a standard jar tester (Phipps and Bird, 7790-900B USA). Protein concentration in both the CSEs and the purified protein samples were obtained following (Bradford, 1976) method.

2.5 Collection of water sample

River water sample was collected in the Bourn Brook river adjacent to the University of Birmingham train station in a set of one-litre (1 L) sterilised Plastic containers. Water sample were incubated at 4°C for 2 hrs before conducting any test to avoid sample deterioration before analysis. Prior to the test and after the test, water samples were filtered through a 0.45 µm Millipore cellulose membrane filter using a vacuum pump and then the filtered samples were brought to instrument temperature of 20°C for fluorescence spectroscopy analysis using EEMs and DOC measurement to reflect ambient water temperature in developing countries. Previous work (Bieroza et al. (2009b) has shown that degradation of samples was insignificant for these storage conditions.

2.6 Assessment of coagulation compound in crude and purified samples

The coagulant compound in the purified Hibiscus seeds was assessed using a 2.5 ml cuvette in a spectrophotometer (Varian Cary 50 probe UV-visible, Australia). Absorbance was measured at a wavelength of 280 nm because protein absorbs light at this wavelength.

225 Similarly, coagulation assay was conducted using the purified samples and absorbance was
226 measured at a wavelength of 600 nm. A 2.90 ml synthetic kaolin water sample was injected
227 in a 2.5 ml SM plastic cuvette UV grade with 0.1 ml of the purified sample to make 2.5 ml
228 mixture. The content was shaken and allowed to stand undisturbed for 45 minutes, and
229 sample absorbance was measured at 600 nm using the spectrophotometer before and after the
230 test. The difference between the initial and the final absorbance measurements gave
231 indication of whether an active coagulation compounds were present in the protein sample.
232 This process is rapid since it eliminates the preparation of a large sample volume and samples
233 can be screened easily and quickly.

234 2.7 Jar test experiments

235 Jar tests were conducted using a conventional apparatus (Phipps and Bird, 7790-900B, USA)
236 comprising six 1L beakers following (Jones and Bridgeman, 2016a) to evaluate the optimum
237 coagulant dose for the DOC and fluorescence measurements. Briefly, the coagulant was
238 added into the beakers during rapid mixing at 200 rpm for 1 min. The mixing speed was then
239 reduced to 30 rpm for 30 min to simulate the flocculation process. The suspension was then
240 allowed to stand undisturbed for 1 hour to facilitate settlement. The long sedimentation time
241 was adopted in order to assess the effectiveness of the process and to see whether the
242 requirement to filter might be avoided after prolonged settlement for people in rural areas. A
243 10 ml treated water sample was drawn via syringe 2cm from the top surface of the water in
244 the beakers. Both initial and final water turbidity were then measured using a turbidity meter
245 (HI 93703, Hanna). The preliminary jar test results were obtained and used as optimum doses
246 in the subsequent experiments.

247 2.8 TOC/DOC measurement

248 Measurement of DOC was performed in water before and after treatment with crude and
249 purified protein samples. Measurement was conducted following Bieroza et al. (2009b) using
250 TOC analyser (Shimadzu TOC-V-CSH), where the study adopted the non-purgeable organic
251 carbon (NPOC) method of DOC determination. Prior to combustion, water samples were
252 sparged with 2 M hydrochloric acid to eliminate inorganic carbon. The mean of three NPOC
253 results was computed, analysed and the typical error being < 10%. All experimental
254 measurements were conducted at room temperature (19 ± 2ºC).

255 2.9 Fluorescence excitation-emission

256 Fluorescence spectroscopy was used to assess water samples before and after treatment with
257 seed extract samples. Fluorescence analysis has been reported in many studies aimed at the
258 characterization of natural organic matter in water (Baker and Inverarity, 2004, Bieroza et al.,
259 2009b, Sanchez et al., 2013). Fluorescence-EEMs were produced in this study following
260 (Bieroza et al., 2009b) using a Varian Cary Eclipse spectrofluorometer at detector scanning
261 wavelength ranges from 200-400 nm (excitation wavelength) and 280-500 nm (emission
262 wavelength), at increments of 5 nm and 2 nm for excitation and emission respectively, with
263 slits width of 5 nm. Instrument stability was checked by recording the Raman values
264 (excitation wavelength 348 nm, emission wavelength 395 nm) before each set of
265 measurements. The Raman value was 10.61 compared with the most recent measurement of
266 10.57 on the instrument. After each test, the cuvette was rinsed thoroughly ten times with de-
267 ionised water and rinsed again with the next sample to be measured at least twice to avoid
268 contamination.

269 **3.0 Results**

270 3.1 Coagulant protein spectra in purified Hibiscus suspensions

Figure 1 presents the fluorescence peaks of compounds unbound to the matrix and the eluted proteins suspension obtained from Hibiscus seeds. The fluorescence EEMs of the coagulant protein suspension present the likely spectra of the coagulant protein in different Hibiscus seed species. Figures (1a, d, and g) present the various peaks in the unbound compounds as observed in the weakly bound POP, PSP and PKP respectively. The location and shapes of the peaks are similar to each other indicating that all the seeds belong to the same plant genus. The dominance of peaks T and B in all the contaminants revealed that they contain high protein contents. Figures (1b and c, e and f, then h and i) are the matrices of eluted fractions of okra, sabdariffa and kenaf proteins with 0.3 and 0.5 M NaCl solutions. Fractions eluted with the 0.3 M NaCl concentration (Figures 1b, e and h) contain coagulant protein compounds as revealed from preliminary jar test results. Peaks T_1 and T_2 are visible in all the samples after protein purification. Additionally, samples eluted with 0.5 M NaCl (Figures 1c, and i) solution showed no visible fluorophore signal and did not coagulate particles in water when tested. However, the fluorophore observed in the region of peak T_1 (Figure 1f) eluted with 0.5 M NaCl solution did not coagulate when it was also tested for coagulation potential.

Under-coagulation condition with the 0.3 M suspensions, the Peak falls below detection limits after the process, indicating its binding and adsorption ability with the NOM as seen in Figure 3b, c and d.

3.2 Characterisation of NOM in water using fluorescence-EEMs

In order to obtain a broader understanding of the character and impact of NOM in treated water, the relationship between DOC removal and fluorescence EEMs data was analysed in water after jar test experiments. The fluorescence-EEM technique was employed for the assessment of DOC removal in treated water using either CSE or purified coagulant proteins obtained from Hibiscus seeds. In all cases, the relationship between fluorescence intensity

295 and residual DOC concentration in treated water was also investigated. Furthermore, recently,
296 several studies have extensively investigated fluorescence fingerprints of OM obtained from
297 EEMs data to locate fluorescence peaks and their intensities in raw and treated waters (Baker,
298 2005, Bieroza et al., 2009b, Zhu et al., 2014, Carstea et al., 2014).

299 The fluorescence peaks nomenclature reported in this work have been adopted from other
300 studies (Bridgeman et al., 2011, Markechová et al., 2013) as in Table 2 while fluorescence
301 major peaks as revealed are presented together with their intensities in Table 3.

302

303 **Table 2 Fluorescence EEMs peaks intensities from (Bridgeman et al., 2011).**

Peaks description		Excitation wavelength (nm)	Emission wavelength (nm)
Humic substances	A	237-260	400-500
Humic substances	C	300-370	400-500
(Highly coloured)	C_1	320-340	410-430
	C_2	370-390	460-480
Tyrosine-like protein	B_1	225-237	309-321
	B_2	275	310
Tryptophan-like protein	T_1	275	340
	T_2	225-237	340-381
Humic (marine)	M	290-310	370-410

304

305 3.3 Fluorescence EEMs of OM in water treated using CSEs

306 The results showing fluorescence peaks and their intensities are presented in Table 3, and
307 fluorescence EEMs are shown in Figures 2 and 3. 10, 25, 50, 75 and 100 mg/l of each of the
308 extract was used in the coagulation test based on a preliminary test to identify the optimum
309 dose for coagulation. A visual observation of the EEMs of Bourn Brook raw water in Figure
310 2a reveals its OM composition. Three fluorescence peaks, (T, B and A) are visible in the
311 water sample. The three fluorescence peaks observed in this study are the most commonly
312 identified fluorophores in a water sample (Baker et al., 2008, Gone et al., 2010, Markechová
313 et al., 2013). It is clear that there are several fluorophores signal seen in this region, one of
314 which may be from protein material. However, in water treated using seed extracts,
315 additional protein from the seed may fluoresces in the region of peaks T and B.

316 The fluorescence signatures of the treated water with OCE, SCE and KCE (Figures 2b, 2c
317 and 2d) show significant fluorophore presence compared to raw water post-coagulation. Most
318 notably, the shape and location of peaks were similar for OCE and SCE-treated waters. Peak
319 T_1 and T_2 fluorescence were more dominant in clarified water than in raw water sample with
320 evidence also of peak B_2 fluorophore presence. The opposite result was observed in OCE and
321 KCE treated water, where higher fluorescence intensities were noted as shown in Table 3.
322 Figures 2b and 2c show visible fluorescence signal of peaks T_1 and T_2, with no evidence of
323 peak C at the end of the treatment. Many related studies have often linked peak T to sewage
324 pollution and regarded it as an indication of microbial activity in water (Baker, 2002, Baker
325 et al., 2008). This study observed an increased fluorescence signal in the region of protein-
326 like peaks (T and B) from the seeds as demonstrated in (Figure 1) because these seeds are
327 sources of proteins. While peak T has been related to microbial presence (Baker et al., 2008)
328 and can be used to monitor contamination in water (Henderson et al., 2009), in this case, peak
329 T was as a result of protein addition from the seeds (Jones and Bridgeman, 2015), (1.0 mg/ml

330 in OCE and 0.9 mg/ml in SCE). The amount of protein used in the coagulation process was
331 5.0 mg in OCE and 4.6 mg in SCE respectively out of the 50 mg/l dose applied in the study.

332

333 Figure 2d, showing treated water using KCE clearly identifies peak C, a humic-like substance
334 with high fluorescence intensity visible in clarified water which was not detected in the raw
335 water. Table 3 shows high peaks T_1 and B_1 intensities in OCE-treated water followed by
336 KCE-treated water. However, the intensity of peak T_2 in KCE-treated water was higher as
337 shown in table 3, its finger print was lower than that in OCE-treated water when the results
338 are compared in Figures 2b and 2d. The only possible explanation for this, could be due to an
339 overlap from other NOM constituents in the water detected at this particular wavelength.

340

341 **Table 3 Major fluorescence peaks emission wavelength and their intensities before and after treatment using both crude extracts and purified proteins.**

Samples	Peak T_1			Peak T_2			Peak B_1			Peak B_2			Peak A			Peak C		
	ex (nm)	em (nm)	Int (au)	Ex (nm)	em (nm)	int (au)	ex (nm)	Em (nm)	Int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)
Raw water	230	348	238	285	360	60	220	302	122	275	275	34	230	411	147	335	413	52
Treated CE																		
▪ OCE	220	350	671	280	352	156	265	310	217	280	310	80	220	410	295	320	428	73
▪ SCE	225	348	333	280	352	145	225	306	164	280	310	73	220	411	165	320	411	57
▪ KCE	225	342	516	280	352	250	225	310	211	280	310	87	220	411	191	320	410	60
Treated PP																		
▪ POP	230	354	129	285	360	40	220	304	87	275	302	32	220	421	125	320	418	45
▪ PSP	230	354	133	285	360	44	220	304	74	275	304	33	220	418	146	320	426	50
▪ PKP	225	356	141	285	360	43	220	302	91	275	304	37	220	410	130	320	421	44

342 3.4 Fluorescence EEMs of OM in treated water with purified proteins

343 Typical fluorescence EEMs Figures (3a, b, c and d) indicate the OM composition in raw and
344 clarified water before and after treatment with POP, PSP and PKP. In this study, the protein
345 fraction eluted with 0.3M NaCl solution was used because of its coagulation potential as
346 observed in a coagulation activity assay and from preliminary jar test experimental results.
347 The amount of protein in each sample was quantified to be 1.2 mg/ml in POP, 1.2 mg/ml in
348 PSP and 1.1 mg/ml in PKP respectively. The DOC results show that the 0.1 mg/l dose
349 provided greater performance regarding DOC removal. Therefore, fluorescence fingerprints
350 of all treated water using 0.1 mg/l coagulant dose were assessed (Figure 3). Furthermore, the
351 impact of two coagulant doses, 0.3 and 0.5 mg/l were considered on residual DOC
352 concentration and data regarding their fluorescence intensities are presented in Table 3. The
353 percentage removal of DOC and percentage decrease in fluorescence intensity was compared
354 at the end of the treatment. After using the 0.5 mg/l dose of PKP in the coagulation process,
355 the result indicated no single observed effect on treated water DOC; the concentration
356 remained largely unchanged with no adverse impact on DOC concentration. Gone et al.
357 (2009) reported that the decrease in peaks T, A and C fluorescence intensities and
358 fluorescence-inferred DOC removal in raw and treated water could be employed as a useful
359 tool to predict DOC removal whereas Hudson et al. (2008) suggested that fluorescence
360 analysis of tryptophan-like protein could reveal the presence of biodegradable organics in
361 water as it relates to biological activity. Additionally, the quality of water is a function of
362 both organic and inorganic constituents; the inorganics often including nitrates and
363 phosphates from agricultural practice, ammonia from sewage discharges, or naturally
364 occurring arsenic. However, the work reported here focused only on fluorescent organic
365 matter. It is noteworthy that fluorescence motoring of the inorganic water constituents was
366 not undertaken due to its limited implication on the objective of the study.

The results show peaks T and A became indistinct after the treatment in all samples, whereas peak B, a tyrosine-like protein, was the least eliminated, and its presence was still visible post-coagulation. Additionally, while the crude extracts have shown high fluorophores in the region of tryptophan-like peaks, the purified proteins revealed its potential to eliminate both the tryptophan-like proteins and humic substances, Peak T and Peak A respectively.

The raw water sample peaks were detected at these centres with the following $\lambda_{excitation}/\lambda_{emission}$ wavelength and fluorescence intensity: peak T (230/348 nm and 238 au.), Peak B (220/302 nm and 122 au) and peak A (230 /411 nm and 147 au.). However, one important contrasting feature associated with the clarified water sample is that it is characterised by an increase in emission wavelength with reduced fluorescence intensity compared with the raw water fluorescence peaks. Figures 3b and 3c show the observed fluorescence peaks of water treated with POP and PSP to be similar even after visual examination, at the following $\lambda_{excitation}/\lambda_{emission}$ wavelengths and intensities, peak T (230/354 nm and 129−133 au.), peak B (220/304−308 nm, and 87−91 au.) and peak A (220−225/421 nm and 125 au.). Figure 3d shows the fluorescence fingerprints in PKP treated water. The various peaks were found at $\lambda_{excitation}/\lambda_{emission}$ wavelength and intensity as follows; peak T (220/356 nm and 141 au.), peak B (225/302 nm and 91 au.) and peak A (235/410 nm and 130 au.) accordingly. The significance of the different peak intensities in both raw and treated water as depicted by the EEMs fingerprints clearly show the character of DOM in the system.
.

Once more, to offer a better understanding of the relationship between DOC removal and reduced peak T fluorescence intensity in the clarified water, the percentage removals of these two parameters were calculated. For both cases, percentage removals of both DOC

concentration and fluorescence intensity were observed to be appreciably higher in treated water using 0.1 mg/l dose of each protein as seen in Table 3. POP samples achieved 25%, 22% and 3% DOC removal while the decrease in OM fluorescence intensity was 46%, 42% and 43% using 0.1, 0.3 and 0.5mg/l doses respectively. Similarly, the percentage DOC removal with PSP was observed to be 24%, 10% and 3% which correspond to 44%, 43% and 42% decrease in fluorescence intensity after the coagulation process. Additionally, equal percentage removal of DOC was observed with the 0.5 mg/l dose in both POP and PSP treated water and their performance on fluorescence reduction equivalent. As expected, however, the lowest percentage DOC removal was observed in PKP treated water. The results show that the 0.1 mg/l dose achieved 18% and 3% with 0.3 mg/l whereas 0% DOC removal was recorded with the 0.5 mg/l dose. Under the same condition, the corresponding percentage decrease in OM fluorescence intensity was 41%, 35%, and 36% respectively.

The overall performance shows that the highest proportion of DOC removal was recorded with the lowest coagulant dose of 0.1 mg/l. In comparison, the maximum decrease in OM fluorescence intensity between the raw and clarified water also occurred with 0.1 mg/l, even though, the performance margin was small across the different doses. For instance, when the percentage DOC removal was zero percentage in PKP clarified water, the decrease in OM fluorescence intensity was 36% while with 3% DOC removal the reduction in fluorescence intensity was 35% under the same experimental condition. A similar scenario was also noted when the percentage removal in DOC was 22% and 3% in POP, the corresponding decrease in fluorescence intensity was 42% and 43%, giving little or no clear relationship. The results show that while the decrease in fluorescence intensity was a measurement of all the OM composition in the water, DOC removal measured only a fraction of TOC in the final water. Hence, the correlation between the two parameters were performed, even though the data

points were few (i.e. 4 sets only). Furthermore, an increase in DOC concentration resulted in increased fluorescence intensity as seen with the crude samples.

Table 4 Percentage reduction and standard deviation of DOC and fluorescence intensity in water treated with POP, PSP and PKP.

	DOC (%) Removal			Fluorescence Intensity(%) Removal		
Dose (mg/l)	POP	PSP	PKP	POP	PSP	PKP
0.1	25	24	18	46	44	41
0.3	22	10	3	42	43	35
0.5	3	3	0	43	42	36
Standard Deviation	9.7	8.7	7.9	1.7	0.8	2.6

While the results for the maximum reduction of fluorescence intensities (Table 4) were observed to be 46, 44 and 41% using POP, PSP and PKP, the accuracy of the reduction in fluorescence intensity in the treated water were 46±0.98, 44±0.46 and 41±1.50% respectively in POP, PSP and PKP. However, the closeness of the results to zero fluorescence intensity (complete reduction) was 54% in POP, 56% in PSP whereas PSK was found to be 59%.

Furthermore, the relationships between peak T fluorescence intensity and DOC concentration was correlated. A strong positive correlation coefficient of 0.76 was found to exist between the variables due to increase in DOC concentration resulting in increased peak T fluorescence intensity after treatment using crude samples. Such relationship demonstrated a significant ($p<0.05$) change in DOC concentration and peak T intensity in raw and final water. Similarly,

when the purified proteins were used, a very strong correlation coefficient of 0.98 was found between the reduced DOC concentration and reduction in peak T fluorescence intensity in the treated water. It was also observed here that the relation was significant ($p<0.05$) because a marginal reduction in DOC concentration resulted in reduced peak T intensity.

It is noteworthy that with 50 mg/l dose (Table 5), the treated water pH remains largely unaffected from 7.6 to 7.4 in OCE and 7.2 in (SCE and KCE) clarified water while turbidity removal was 81, 77 and 73% respectively, from 8.4 NTU. Similarly, it was observed that final water pH was broadly unchanged at 7.4, 7.3 and 7.1 in POP, PSP and PKP treated water with corresponding turbidity reduction of 92, 90 and 86% respectively, using 0.1 mg/l coagulant dose.

Table 5 Raw and final water characteristics using crude and purified Hibiscus seeds.

		0.1 (mg/l) Dose			50 (mg/l) Dose		
Parameters	Raw water	POP	PSP	PKP	OCE	SCE	KCE
pH	7.6	7.4	7.2	7.2	7.4	7.3	7.1
Turbidity (NTU)	8.4	0.67	0.84	1.18	1.60	1.93	2.27
Turb removal (%)	n/a	92	90	86	81	77	73

Table 6 present the results for DOC addition in treated water using coagulant dose range between zero as control, and 10 and 100 mg/l. At the end of the coagulation process, the 100 mg/l dose increased the DOC contents to 19, 15 and 17 mg/l from 6.7 mg/l while DOC was 17.3, 12.8 and 15.8 mg/l in OCE, SCE and KCE treated water in the 75 mg/l dose. Similarly, in the 50 mg/l dose, DOC concentration was 13.9, 10.4 and 12.6 mg/l whereas at 25 mg/l,

DOC concentration increased to 10, 8 and 8.6 mg/l in OCE, SCE and KCE respectively. However, in the 10 mg/l dose, maximum DOC concentration was 9.1 mg/l in OCE treated water while in SCE and KCE final water, DOC concentration was 7.2 and 7.6 mg/l respectively. In addition, turbidity removal efficiency was almost similar between 50 and 100 mg/l dosages, approximately between 80.1 and 81% with POP, 75.6 and 77% with PSP and 71.2 and 73% with PKP (results not in table). Thus, the adoption of 100 mg/l as optimum dose (with high DOC addition) because the objective of study is look at the impact of DOC addition in the treated water using the crude extracts.

Table 6 Concentration of DOC in treated water using OCE, *SCE and KCE in water treatment.*

	DOC (mg/l)		
Dose (mg/l)	OCE	SCE	KCE
0	6.7	6.7	6.7
10	9.1	7.2	7.6
25	10.0	8.0	8.6
50	13.9	10.4	12.6
75	17.8	12.8	15.8
100	15.0	17.0	19.0

4.0 Discussion

The EEMs of the purified coagulant protein suspension present the likely spectra of the coagulant protein in Hibiscus seeds. After purification, the results show the most likely

462 coagulant proteins (active compound causing coagulation activity) in Hibiscus seeds is found
463 in tryptophan-like region; Peaks T_1 and T_2 in all the suspensions. Even though there was a
464 trace of peak T_1 in PSP eluted with 0.5 M NaCl solution, the tryptophan-like protein found
465 here did not show any coagulation potential. It is clear that not all proteins in peak T region
466 are coagulant proteins because the contaminant fraction also shows high fluorophores signal
467 in the region of peak T than peaks (B, A and C), yet, it did not coagulate particles when it
468 was tested in the coagulation assay. After the coagulation process, Peak T falls below the
469 laser detection limit, indicating the fluorophores' binding ability to the particles which settled
470 out with the colloids. Residual tryptophan is reported to have higher adsorption ability than
471 tyrosine (Chen and Kenny, 2007). Using EEMs, Ghebremichael et al. (2009) showed that the
472 coagulant protein in MO is a tyrosine-like protein, while in this work the coagulant protein
473 was observed in the region of tryptophan-like protein. Previously, it has been reported
474 elsewhere that MO consists of two small MW cationic coagulant proteins (Broin et al., 2002,
475 Ghebremichael et al., 2005) whereas the Hibiscus protein was seen to consist of a single band
476 of anionic coagulant protein. The difference between the character and chemical composition
477 of the two plants may have been the main reason for the difference in coagulation behaviour
478 of their proteins which require detail investigation.

479 Assessment of the impact of NOM in treated water was performed using fluorescence
480 matrices. Fluorescence EEM's of raw water and treated water (with either crude extracts or
481 purified proteins) show some clear, distinct features. The dominance of Peaks, T and B in
482 clarified water treated using crude samples was as a result of proteins addition from the seed
483 extracts. Kwaambwa and Maikokera (2007) had shown a direct relationship between
484 fluorescence intensity and concentration in MO protein. The high fluorescence intensities of
485 the protein-like Peaks caused by the extracts could give rise to deterioration in water quality
486 (Ndabigengesere and Narasiah, 1998). Previously, Baker (2002) and Baker et al. (2008)

related the presence of Peak T to microbial activity. Hence, the high Peak T signal in water treated with the crude extracts could significantly encourage microbial activity as substrates for bacterial growth and could result in the production of taste, colour and odour in the clarified water. As revealed in the crude extract and purified suspension spectra (Figure 1), and in water treated with the crude sample, coagulant and non-coagulant proteins were dominant in the region of tryptophan-like peaks. Water treated using CSEs deteriorated in quality 48-hr post-treatment (Ndabigengesere and Narasiah, 1998) because NOM contaminants, such as protein in the extract, could support the growth of *E-coli* and other bacteria(WHO, 2008), resulting in the production of an indole odour. Furthermore, water treated with crude extracts may render it unfit for human consumption especially if the treated water is proposed to be disinfected with chlorine, as chlorine can react with NOM in the water to produce carcinogenic DBPs (e.g. trihalomethanes and haloacetic acids). Although, the inactivation impact of Hibiscus seed on faecal coliform and E-coli bacteria has been reported previously (Jones and Bridgeman, 2016a) it is not toxic to human beings after consumption. It is noteworthy that Hibiscus seeds are currently a primary source of protein and food in many developing countries. Additionally, Hibiscus seeds have been used in folk medicine for the treatment of several ailments, hence it is considered safe for human consumption. Previously, Jones and Bridgeman (2016a), Henderson et al. (2009) postulated that Peak T could be used to monitor contamination in water, thus it is clear that fluorescence could further provide us with a better understanding of the quality of water treated with natural seed extract. The relationship between DOC concentration and fluorescence intensity show an important correlation between the two parameters which indicates possible use of fluorescence to assess the character of organic matters in water against the traditional DOC analyses.

511 The impact of protein purification was clearly seen to be beneficial. Water treated using the
512 purified proteins showed a significant decrease in fluorescence intensities and DOC
513 concentration due to organic compounds removal in the seeds. It was evident from the fDOM
514 and DOC results that the protein purification produced coagulant proteins that did not release
515 organic loads in the final water which are usually the main challenge of using natural extracts
516 in water treatment, causing deterioration in water quality with storage time. Furthermore,
517 EEM spectra and fluorescence intensities, and then DOC measurement showed a reduction in
518 initial NOM in water treated with the purified samples. With the reduced DOC value of 5.1
519 mg/l in water treated using purified proteins, if disinfected with chlorine, this concentration
520 could still be a potential precursor for THMs and haloacetonitrile (HAN) formation but not
521 nitrogenous DBPs (N-DBPs) as revealed by Chen and Westerhoff, (2010). Conversely, crude
522 extracts contains several other compounds other than the coagulant protein of interest, hence,
523 the increase in fluorescence intensity and DOC concentration in clarified water is an
524 indication of organic loads addition which could lead to change in taste, odour and colour
525 (Ndabigengesere and Narasiah, 1998, Ghebremichael et al., 2006). This situation renders the
526 application of crude extract in large scale water treatment difficult whereas the use of purified
527 proteins in water treatment seems to be feasible and sustainable, especially in tropics where
528 the seeds are widely available. However, the pH of the treated water remains unaltered due to
529 protein's buffering ability, eliminating the requirement for the procurement of pH adjustment
530 chemicals. Although, Ndabigengesere and Narasiah (1998) reported poor performance of
531 CSE in treating low turbidity water, all the samples including the crude forms achieved the
532 WHO standard of < 5NTU.

533 **5.0 Conclusions**

- The work reported here has shown that Peak T fluorescence intensity could be a useful tool to identify the presence of organic compounds in water and to evaluate NOM characteristics in water treated using natural plant seeds.
- An increase or decrease in fluorescence intensity is a clear indication of NOM addition or removal as observed in fluorescence peak signal in water treated with crude extracts and purified protein. Crude extracts cause an increased DOC concentration while purified protein resulted in reduced DOC in final water.
- Protein purification improves the performance of Hibiscus seeds as potential water treatment candidates for DOC removal in water at a lower coagulant dose due to increased adsorption capacity. Additionally, the reduced DOC concentration in final water could eliminate the issue of deterioration in treated water quality. However, further studies should be conducted using lower coagulant doses other than 0.1mg/l to investigate its OM removal potential.
- The coagulant protein in Hibiscus plant was observed in the tryptophan-like region when eluted with low, 0.3 M ionic strength, salt solution dissolved in a phosphate buffer. However, the high 1.0M NaCl concentration used in extracting the crude sample requires further study to assess its impact on water quality. Additionally, the presence of phosphate in treated water should be evaluated for possible biofilm formation.
- The main disadvantage of treating water with the CSEs is the addition of organic loads into the final water which could be a potential DBP precursor if the treated water is subsequently disinfected with chlorine.. The use of fluorescence EEMs in this work has demonstrated the importance of protein purification to improve treated water quality using natural plant seeds devoid of NOM that could pose a challenge to consumers.

- It is recommended that water treated with crude salt extract be desalted to avoid change in taste as the current study did not assess the quality of water treated with desalted coagulant.

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Table

Click here to download Table: Table 1 Advantages.docx

Table 1 Advantages (+) and drawbacks (-) of fluorescence measurement and other known protocols

Fluorescence spectroscopy	Other protocols for analysing NOM in water
1. (+) Rapid assessment of water and wastewater OM. Sensitive in characterising aquatic OM. 2. (+) Incorporates an on-line monitoring tool. 3. (+) Requires small sample volume. 4. (+) Minimal sample preparation is required 5. (+) Provides substantial information on the composition of OM present. 6. (-) OM characterisation is based on many parameters describing absorption and emission energy. 7. (-) Fluorescence quenching can affect fluorescence measurement. 8. (-) Inner filtering effect can impact the result which requires correction prior to measurement.	9. (-) Assessment takes a longer time and routine measurement are conducted with limited value in terms of OM characteristics. 10. (-) Measurement is off-line 11. (-) Requires extensive sample preparation 12. (-) Only a limited OM fraction can be fractionated 13. (-) Large sample volume is needed 14. (-) Limited information on OM composition is made available. 15. (+) Quenching has no effect on the measured OM value. 16. (+) No known effect of inner filtering on the measured values.

Table 2 Fluorescence EEMs peaks intensities from (Bridgeman et al., 2011).

Peaks description		Excitation wavelength (nm)	Emission wavelength (nm)
Humic substances	A	237-260	400-500
Humic substances	C	300-370	400-500
(Highly coloured)	C_1	320-340	410-430
	C_2	370-390	460-480
Tyrosine-like protein	B_1	225-237	309-321
	B_2	275	310
Tryptophan-like protein	T_1	275	340
	T_2	225-237	340-381
Humic (marine)	M	290-310	370-410

Table

Click here to download Table: Table 3 Major fluorescence peaks emission wavelength .docx

Table 3 Major fluorescence peaks emission wavelength and their intensities before and after treatment using both crude extracts and purified proteins.

Samples	Peak T_1			Peak T_2			Peak B_1			Peak B_2			Peak A			Peak C		
	ex (nm)	em (nm)	Int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	Em (nm)	Int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)
Raw water	230	348	238	285	360	60	220	302	122	275	275	34	230	411	147	335	413	52
Treated CE																		
▪ OCE	220	350	671	280	352	156	265	310	217	280	310	80	220	410	295	320	428	73
▪ SCE	225	348	333	280	352	145	225	306	164	280	310	73	220	411	165	320	411	57
▪ KCE	225	342	516	280	352	250	225	310	211	280	310	87	220	411	191	320	410	60
Treated PP																		
▪ POP	230	354	129	285	360	40	220	304	87	275	302	32	220	421	125	320	418	45
▪ PSP	230	354	133	285	360	44	220	304	74	275	304	33	220	418	146	320	426	50
▪ PKP	225	356	141	285	360	43	220	302	91	275	304	37	220	410	130	320	421	44

Table

Click here to download Table: Table 5 Raw and final water characteristic.docx

Table 5 Raw and final water characteristics using crude and purified Hibiscus seeds.

		0.123	(mg/l)	Dose	50	(mg/l)	Dose
Parameters	Raw water	POP	PSP	PKP	OCE	SCE	KCE
Ph	7.6	7.4	7.2	7.2	7.4	7.3	7.1
Turbidity (NTU)	8.4	0.67	0.84	1.18	1.60	1.93	2.27
Percentage rem (%)	n/a	92	90	86	81	77	73

Table *4 Percentage reduction and standard deviation of DOC and fluorescence intensity in water treated with POP, PSP and PKP.*

	DOC (%) Removal			Fluorescence Intensity(%) Removal		
Dose (mg/l)	POP	PSP	PKP	POP	PSP	PKP
0.1	25	24	18	46	44	41
0.3	22	10	3	42	43	35
0.5	3	3	0	43	42	36
Standard Deviation	9.7	8.7	7.9	1.7	0.8	2.6

Table

Click here to download Table: Table 6 concentration of DOC in treated water.docx

Table 6 Concentration of DOC in treated water using OCE, SCE and KCE in water treatment.

	DOC (mg/l)		
Dose (mg/l)	OCE	SCE	KCE
0	6.7	6.7	6.7
10	9.1	7.2	7.6
25	10.0	8.0	8.6
50	13.9	10.4	12.6
75	17.8	12.8	15.8
100	15.0	17.0	19.0

Figure

Click here to download Figure: Fig. 1 docx.docx

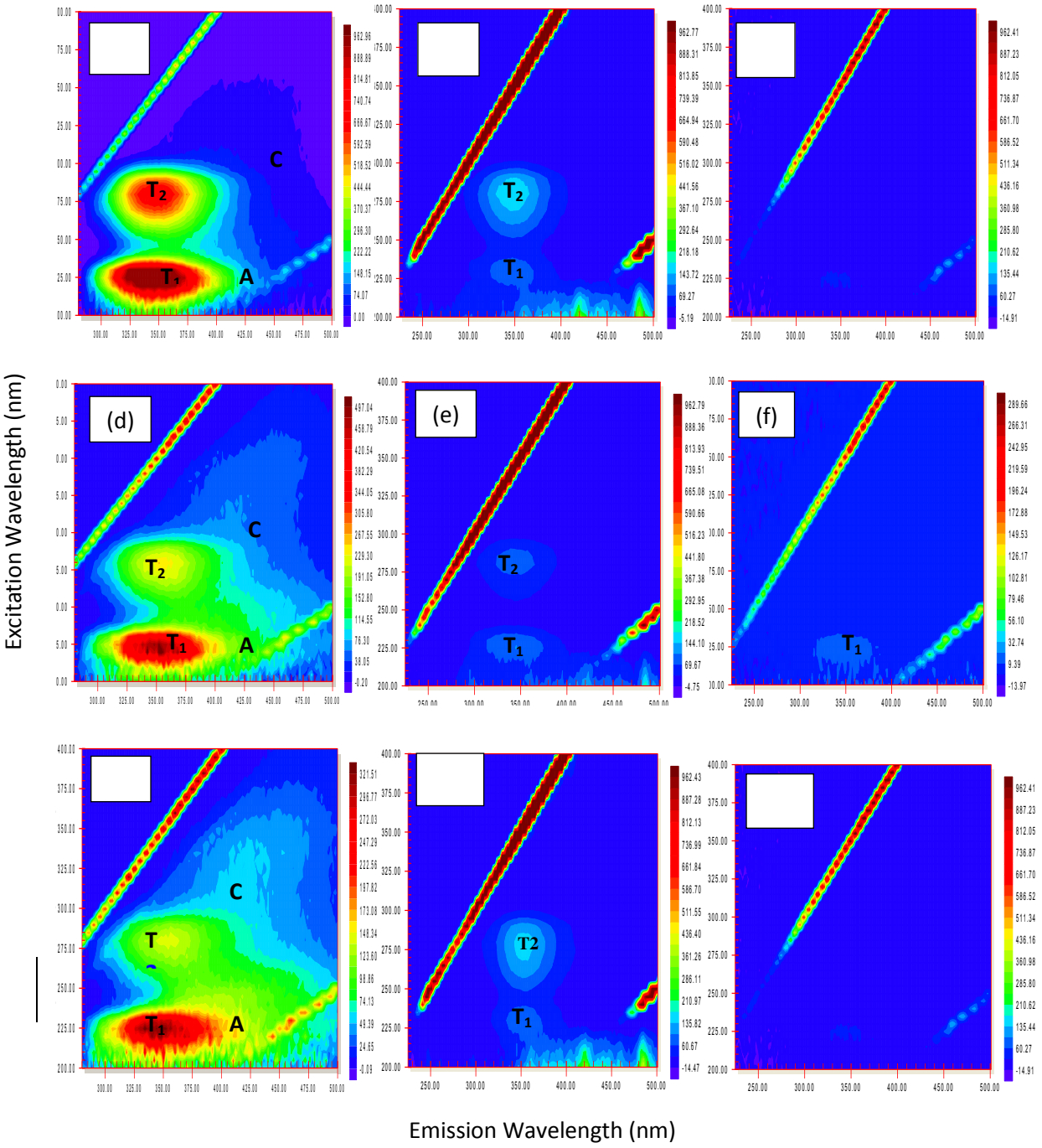


Fig. 1 EEMs spectra of purified proteins (a) POP unabsorbed (b) POP - 0.3M (c) POP-0.5M (d) PSP unabsorbed (e) PSP - 0.3M (f) PSP - 0.5M (g) PKP unabsorbed (h) PKP - 0.3M (i) PKP - 0.5M NaCl.

Figure

Click here to download Figure: Fig 2 Fluorescence.docx

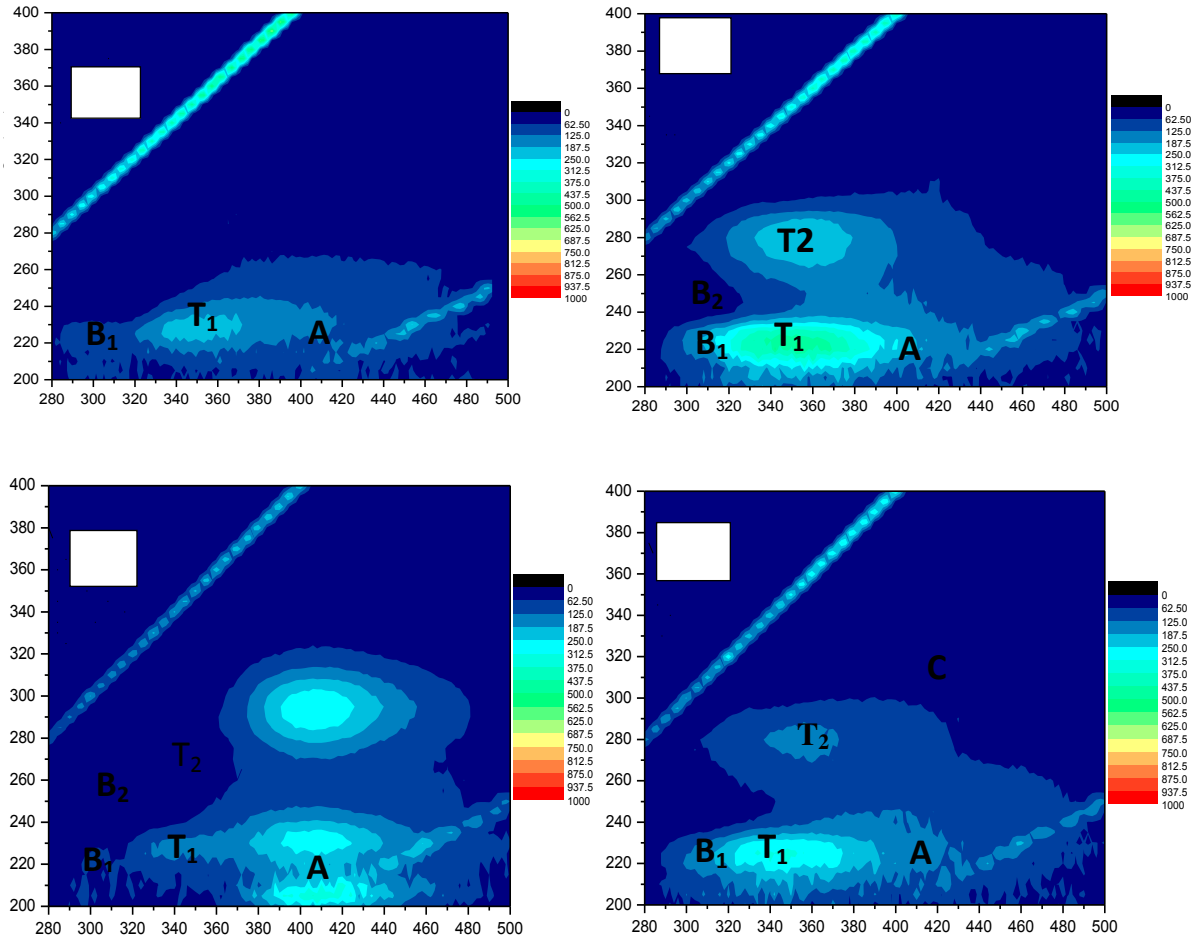
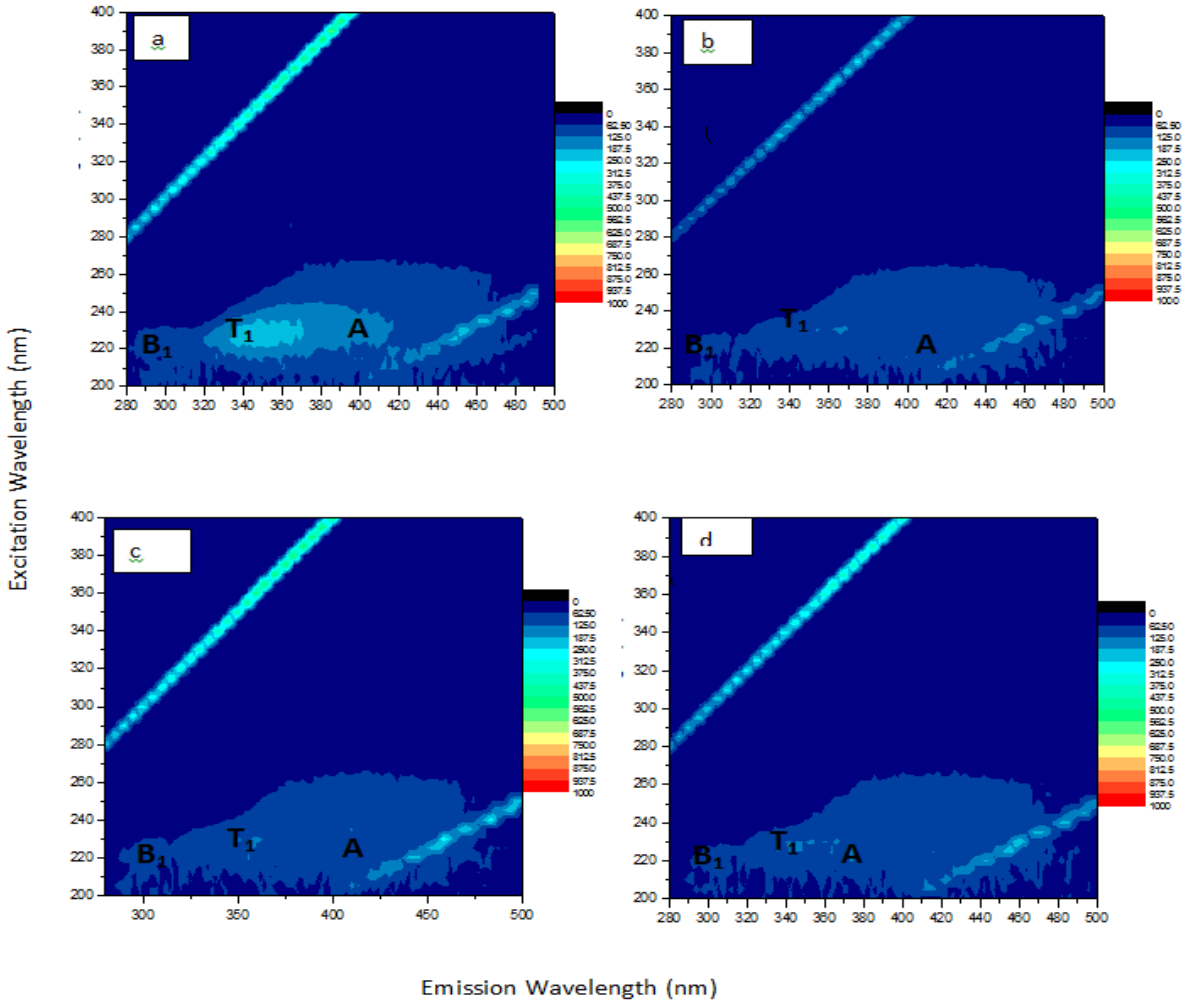


Fig 2 Fluorescence-EEMs of raw water (a), OCE-treated water (b), SCE-treated water (c) and KCE-treated water (d).

Figure

Click here to download Figure: Fig. 3 Fluorescence EEMS of.docx



Fluorescence EEMS of (a) raw water peaks (b), POP treated-water (c), PSP treated-water (d) PKP-treated water.