# UNIVERSITY<sup>OF</sup> BIRMINGHAM University of Birmingham Research at Birmingham

## Characterisation and Performance of three Kenaf coagulation products under different operating conditions

Okoro, Benjamin; Sharifi, Soroosh; Jesson, Mike; Bridgeman, John; Moruzzi, Rodrigo

DOI: 10.1016/j.watres.2020.116517

*License:* Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version Peer reviewed version

Citation for published version (Harvard):

Okoro, B, Sharifi, S, Jesson, M, Bridgeman, J & Moruzzi, R 2021, 'Characterisation and Performance of three Kenaf coagulation products under different operating conditions', *Water Research*, vol. 188, 116517. https://doi.org/10.1016/j.watres.2020.116517

Link to publication on Research at Birmingham portal

#### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.

•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.

•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?) •Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

#### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

1	<b>Title</b> :

2	Characterisation and Performance of three Kenaf coagulation products under different
3	operating conditions
4	
5	Authors:
6	Benjamin U. Okoro <sup>1</sup> , Soroosh Sharifi <sup>1</sup> , Mike Jesson <sup>1</sup> , John Bridgeman <sup>2</sup> , Rodrigo Moruzzi <sup>3</sup>
7	
8	Affilations
9	1: University of Birmingham, Department of Civil Engineering, Edgbaston, Birmingham,
10	United Kingdom
11	2: University of Bradford, Faculty of Engineering and Bioinformatics, Bradford, United
12	Kingdom
13	3: Universidade Estadual Paulista (UNESP), Instituto de Geociências e Ciências Exatas, Rio
14	Claro, Sao Paulo, Brazil.
15	
16	Corresponding author:
17	Benjamin U. Okoro
18	Email:buo727@bham.ac.uk
19	
20	

### 22 Abstract

23 The Sustainable Development Goal (SDG) 6.1, established by the United Nations General 24 Assembly in 2015, targets universal and equitable access to safe and affordable drinking water 25 for all by 2030. An essential factor in achieving this goal is the harnessing of "green" 26 coagulants - naturally occurring, environmentally friendly materials which are effective 27 coagulants for use in water treatment, with good availability in developing countries, inherent 28 renewable properties and ease of biodegradation. In order to gain from these benefits, it is 29 essential to fully understand how such coagulants may best be utilised, particularly concerning 30 their practical application in developing countries. In this study, three different plant-based 31 coagulation products (PCPs), namely Hexane (HxKP), saline (StKP) and crude (CrKP) extracts 32 of Kenaf plant seed (Hibiscus cannabinus, a species of the Hibiscus plant), were applied to high 33 (HTW), medium (MTW) and low (LTW) turbidity water in order to determine their 34 performance and coagulation ability. The ability of the three Kenaf coagulant products (KCPs) 35 to remove hydrophobic fractions of natural organic matter (NOM) was measured. The impact 36 of KCPs on the treated water organic matter content (a known disinfection by-product (DBP) 37 precursor) was examined using known surrogates of natural organic matter (NOM) i.e. the 38 dissolved organic carbon (DOC), ultraviolet absorbance at 254 (UV<sub>254</sub>) and specific ultraviolet 39 absorbance (SUVA<sub>254</sub>). Results obtained quantify the implications of using these coagulants during the water disinfection process. A parametric study, measuring the effect of different 40 41 operating parameters, such as untreated water turbidity, pH, dosages, retention time, and KCP 42 storage time, was completed. Turbidity removal performance for HxKP and StKP was very good with > 90% removal recorded for HTW and MTW, respectively, at pH seven within 2 43 44 hours retention time. Images obtained from scanning electron microscopy (SEM) analysis

45 revealed a high likelihood of the coagulation mechanism of KCPs to be adsorption-interparticle 46 bridging brought about by their flake-like structures and surfaces charges. Varying pH had no 47 measurable influence on the coagulation performance of the KCPs. Comparing their efficiency 48 with Moringa Oleifera (MO, a previously researched PCP) and alum showed that HxKP had a 49 negligibly different particle removal as MO. StKP turbidity removal performance was below 50 HxKP by 1% for HTW and LTW and 2% for MTW but performed higher than the CrKP by 51 5% and 7% in HTW and MTW, respectively. The optimum dosage of HxKP and StKP reduced 52 DBP surrogate values, indicating that its precursor is also minimized, although a slight shift 53 from this optimum dosage showed a significant rise in their concentration thus signifying a 54 potential increase in DBPs during disinfection.

55 Keywords: Turbidity; Coagulation-flocculation; Molecular interaction; Plant-based
56 coagulants; Water treatment

57 List of Abbreviations: Chemical coagulation products (CCPs); Chromatographically purified 58 Kenaf protein (ChrKP); Crude extracted products (CrKP); Disinfectant by-product products 59 (DBP); Dissolved organic carbon (DOC); Electric double layer (EDL); Electrophoretic 60 mobility (EM); Energy dispersive analysis of X-rays (EDAX); Fourier Transform Infra-Red 61 analysis (FT-IR); Fruit seed extract (FSE); Hexane extracted products (HxKP); Hexane leaf 62 extract (HLE); High turbidity water (HTW); Humic acid (HA); Isoelectric point (IEP); Kaolin 63 model water (KMW); Kenaf coagulation products (KCPs); Low turbidity water (LTW); 64 Medium turbidity water (MTW); Moringa oleifera (MO); Natural organic matter (NOM); Plant 65 coagulation products (PCPs); Salt extracted products (StKP); Scanning Electron Microscopy 66 (SEM); Specific ultraviolet absorbance at 254nm (SUVA<sub>254</sub>); Sustainable development goal 67 (SDG); Trihalomethanes (THMs); Ultraviolet light (UV) absorbance at 254 nm (UV<sub>254</sub>); United Nations International Children's Emergency Fund (UNICEF); Water Kenaf product 68 69 (WKP); World health organisation (WHO); Zeta potential (ZP);

70

#### 71 **1. Introduction**

72 Unimproved water sources, especially in sub-Saharan Africa and Oceania, remain a threat to 73 the realisation of the United Nations Sustainable Development Goal (SDG) 6.1 of safe and 74 affordable water for all by 2030 (WHO and UNICEF, 2019). Eight out of ten people in rural 75 neighbourhoods in the sub-Saharan Africa region lack even essential water services, with the 76 majority depending on surface water or unimproved water sources (WHO and UNICEF, 2017; WHO and UNICEF, 2019). Most surface water sources like rivers, streams and ponds are 77 78 polluted and unfit for drinking due to natural and anthropogenic influences such as unregulated 79 industrial discharges (Ezeabasili et al., 2014), climate change and the drought-induced 80 migration of livestock to water sources servicing rural populations (Bello, 2013). Most of the 81 polluted rivers, streams and ponds contain high concentrations of natural organic matter 82 (NOM) including humic acid (HA), adding taste, odour and colour to them (Ezeabasili et al., 83 2014). The presence of NOM in drinking water makes water unpotable due to several hygiene 84 and health reasons, with one of the most important being the formation of toxic chemical 85 species during the disinfection process. During disinfection of NOM enriched waters, disinfection by-products (DBPs), e.g. trihalomethanes (THMs) and haloacetic acids, are 86 87 formed, which are reported to be harmful to health owing to their lethal, carcinogenic and 88 mutagenic potentials (Brown et al., 2015; Niu et al., 2015; Gough et al., 2014; Bongiovani et 89 al., 2015). Removing NOM in water should be to strict standards in order for this undesirable 90 consequence of disinfection to be avoided. However, water quality compliance in most 91 developing countries is severely lacking due to the lack of infrastructure and government 92 commitment to water supply, thus diminishing access to primary and improved service delivery 93 with a resultant negative impact on the population's health.

94 Chemical coagulation is a well-established technique used at the start of a water treatment 95 process. Most of the commercially available coagulation products are chemical-based (hence chemical coagulation products, CCPs) such as iron (FeCl<sub>3</sub>) and aluminium salts (Al<sub>2</sub>SO<sub>4</sub>) 96 97 (Sharp et al., 2006; Guo et al., 2015). Studies have associated the use of CCPs such as alum 98 with Alzheimer's disease (Flaten, 2001; Exley, 2017) and neurological syndromes (Zatta et al., 99 2003), casting doubt on their safe and sustainable use as a coagulant. Several chemical-based 100 household water treatment products currently in the market are not affordable, especially to 101 those in rural communities, due to the high costs of procurement (WHO, 2019), while other 102 CCPs are only effective for the treatment of low turbidity water, limiting their application. 103 Further; these CCPs produce high sludge volume, have a reduced sludge recyclability rate and 104 a high carbon footprint during their production (Villanueva et al., 2004). Consequently, an 105 alternative, for use in combination with or as a replacement for CCPs, is desirable in order to 106 overcome or reduce these limitations.

107 The use of plant coagulation products (PCPs) in place of CCPs is not only efficient in terms of 108 cost but also means access for all since these plants are widely grown and have a good 109 adaptation to different soils. Several studies have investigated the potential of using PCPs in 110 the treatment of water in developing countries, with research examining the use of Moringa 111 Oleifera (MO) (Ndabigengesere and Subba Narasiah, 1998; Camacho et al., 2017; WHO and 112 UNICEF, 2019) and extracts from members of the Hibiscus (Jones and Bridgeman, 2019) and 113 Cactaceae (Oladoja et al., 2017) families. These PCPs have been shown to have antibacterial 114 abilities (Jones and Bridgeman, 2017), and the active coagulation components, e.g. the 115 carbohydrates and protein, are rich in nutrients and have no known health impact in humans. 116 Sludge generated by these PCPs are biodegradable and also of reduced volume 117 (Ndabigengesere and Narasiah, 2010). Chemical coagulants such as alum perform better at lower pH values thereby making the water acidic and requiring adjustment to make it potable, 118

whereas pH change seldom occurs when using PCPs, enhancing their suitability as water treatment materials. When used as an aid to coagulation with alum, PCPs have the advantage of improving the effectiveness of alum by forming larger and denser flocs and thereby reducing the quantity of alum needed. For example, the use of Hibiscus leaf extract (HLE) as a coagulant aid is reported to improve turbidity removal from 45 to 72% (Awang and Aziz, 2012).

124 Kenaf is from the Malvaceae family, which is known for its tall, woody nature, and is grown 125 widely in the tropical and subtropical regions of many countries. Kenaf varieties obtained from 126 different parts of the world are also rich in oil, fatty acid (palmitic, linoleic and oleic acid 127 (Mohamed et al., 1995)), and other important bioactive chemical components. The seed also 128 contains phospholipids, sterols, and other nutritional components (Mohamed et al., 1995; 129 Nyam et al., 2009). Just like most plant seeds, Kenaf predominantly contains proteins 130 (Ndabigengesere and Narasiah, 1996; Ghebremichael et al., 2005) although other compounds 131 such as phenolic acids (Nyam et al., 2009) and carbohydrates (Nyam et al., 2009; Mariod et 132 al., 2010) have been found and may also contribute to their coagulative behaviour (Oladoja, 133 2015). The active agents in Kenaf crude seed were reported to be anionic proteins with a 134 molecular weight between 10 – 100 kDa (Mariod et al., 2010; Jones, 2016). Jones (2016) also 135 reported that a thermo-resistant protein exists with a molecular weight of 6 kDa, and that it 136 possesses good coagulation potential.

These compounds can be extracted using different techniques to produce Kenaf coagulation products (KCPs) with different concentrations of these coagulation compounds. (Jones, 2016) study is the only known work that used different KCPs, i.e. water extracted (WKP) and chromatographically purified Kenaf protein (ChrKP) as a coagulant-flocculant, and tested for their coagulation/flocculation potentials. Jones' tests used Kaolin model water (KMW) seeded with chemical compounds present in organic materials. Jones' report showed that ChrKP gave the best turbidity removal of 92% in 100NTU KMW, while the WKP gave 90%, 79% and 73% 144 for 200NTU, 100NTU and 50NTU KMW, respectively. Despite attaining high turbidity 145 removal, ChrKP and WKP could not attain the WHO minimum turbidity standard of 4NTU, 146 thereby, making the water unsuitable for use. KCPs derived from the salting-in process (StKP) 147 (Ndabigengesere and Narasiah, 1996; Okuda et al., 1999), solvent purification (HxKP) (Dos 148 Santos et al., 2016) and the protein fractionation process (Mariod et al., 2010; Dos Santos et 149 al., 2016) have not previously been examined for their potential use in water treatment. Some 150 KCPs such as ChrKP involve expensive extraction processes which may be costly to acquire 151 by poor households. However, simpler extracts like the WKP, involve very little or no cost of 152 preparation and can easily be used by villagers and commoners. Determining the best of these 153 KCPs is arguably a balance between having the highest performance and the lowest processing 154 cost and time.

155 Limited information is available on the morphology of these KCPs, their coagulation 156 mechanism, and their performance, despite the increasing interest in this crop in academia and 157 industry (Yang et al., 2019). To produce potable water, disinfection (usually using chlorine) is 158 required after coagulation/flocculation, yet there is currently no study on DBP formation using 159 KCPs. Therefore, a comprehensive study would be beneficial to understand the effect the 160 different KCPs on the DBP formation in the treated water.



161

(a)

7

162 Fig. 1. Kenaf (*Hibiscus cannabinus*) plant showing (a) plant flower (b) raw, grinded and163 delipidated kenaf seed derived from Kenaf plant

164 Inspired by recent results on the use of PCPs in water treatment, this study evaluates the performance of three KCPs (Fig. 1), i.e. Crude Kenaf (CrKP), Salted Kenaf (StKP) and Hexane 165 166 (solvent) extracted Kenaf (HxKP) in turbidity removal from low (LTW, 30NTU), medium 167 (MTW, 150NTU) and high turbidity water (HTW, 500NTU). It provides a better understanding 168 of the three KCPs' performance and examines the coagulation mechanism involved under a 169 range of pHs, contact times and dosages, which is lacking in published literature. This 170 introduction is followed by a description of the methodology applied (Section 2), a detailed 171 discussion of the key results (Section 3) and finally the important conclusions which have been 172 drawn from this work.

#### 173 **2.** Materials and methods

174 Coagulation/flocculation experiments were performed using three water samples to examine 175 the performance of three KCPs extracted from Kenaf seeds. Water quality indicators including 176 pH, turbidity, dissolved organic carbon (DOC), 254nm light absorbing compounds (UV<sub>254</sub>), 177 and specific absorbance (SUVA<sub>254</sub>) were studied to understand the KCPs influence during the 178 water treatment process. Zeta potential, Fourier Transform Infra-Red analysis (FT-IR) and 179 Scanning Electron Microscopy (SEM) were used to determine the morphology and 180 characteristics of the KCPs studied.

#### 181 2.1 Materials

Analytical grade chemicals and reagents used included sodium chloride (NaCl-Fisher product #: 10428420) for improving protein solubility and hexane (Fisher product #: 10735141) as the delipidating solvent. Milli-Q water (18 M $\Omega$ ·cm) was used for the preparation of all solutions. The dried *Hibiscus cannabinus* (Kenaf) seeds were obtained from Yola Market located in Yola, Adamawa State, Nigeria, located between the geographic coordinates  $9^{\circ}13'48''$  North latitude and  $12^{\circ}27'36''$  East longitude. The Kenaf seeds from this source are typical of Kenaf worldwide. 2% w/v of Aluminium sulphate Al<sub>2</sub> (SO<sub>4</sub>)<sub>3</sub> (Fisher product #: 10233850) was prepared and used as a comparative coagulant.

#### 190 **2.2 Natural and composite water samples**

191 Samples were collected from the Bournbrook river, Birmingham, UK, between November 192 2018 and April 2019. The river traverses both rural and urban catchments and is recharged by 193 runoff from surrounding areas and combined sewer overflows (Carstea et al., 2009). Turbidity 194 values studied in this work reflected the range of turbidity experienced in developing counties 195 like sub-Saharan Africa and are consistent with previous research (Pritchard et al., 2010). Low 196 turbidity water (LTW) and medium turbidity water (MTW) were collected from two points 197 along the river course, with high turbidity water (HTW) being derived by adding river-bottom 198 sediment to MTW. Only samples with turbidity within 10% of the target values of 30NTU, 199 150NTU and 500NTU were used. Turbidity values were measured by a calibrated Hach 2100N 200 Turbidimeter. Water samples were collected in a set of 10-litre plastic containers and were 201 stored at 4°C after collection until use to minimise sample perturbation; water was removed 202 from storage no more than 8h before sample analyses in order to preserve water quality and 203 prevent ingrowth of organic materials. Bottom sediments were collected using a metal scoop, 204 carefully transferred to a clean container and then washed through a 65µm sieve to eliminate 205 debris.

#### 206 2.3 Preparation of Kenaf Coagulant Products (KCPs)

The samples were processed based on the modified procedure reported by Jones and Bridgeman (2016). Seeds were stored in a dry environment at room temperature. Milli-Q water was used to rinse the seeds to remove all impurities, after which the seeds were dried for 24hours, ground for 3 minutes and passed through a 300µm sieve. The powder obtained was termed Crude Kenaf, CrKP, and was stored for further use. To obtain a 5% weight to volume (w/v) suspension of sodium chloride (NaCl), 58.44g of 1.0M (NaCl) in 1000 ml of Milli-Q water was added to the CrKP, followed by thorough mixing for 30 minutes using a magnetic stirrer (Stuart Scientific, UK). The obtained solution was filtered through a Whatman No. 3 filter paper to eliminate residue, and then the filtrate was dried. The residue obtained was referred to as Salted Kenaf product, StKP.

217 The unwanted compounds that might reduce the coagulation performance, such as the fatty 218 acid present in the seeds, were removed using an electro-thermal Soxhlet apparatus. 219 Approximately 20g of seed powder was placed in the apparatus' thimble before heating hexane 220 up to 80°C for 8 hours with each intermediate cycle taking approximately 2-3 minutes (Muyibi 221 and Alfugara, 2010). The residue collected from the thimble was dried at room temperature, 222 and then 1g was mixed with 100 mL of saline solution (NaCl: 1 mol/L) which was stirred for 223 30 min, filtered and dried and stored for further use. Hexane delipidated flour was called 224 Hexane Product (HxKP).

#### 225 **2.4 Coagulation/ Flocculation Experiments**

226 Coagulation/flocculation tests were conducted using each of the KCPs. For each jar test, 1 litre 227 of untreated water was used in the simulated coagulation/flocculation process using a variable 228 speed, 2-blade impeller with square section (Phipps and Bird Jar tester), in a 1L capacity 229 unbaffled beaker. The beakers had no stators to suppress vortices and maximize energy transfer 230 to the fluid (Hocking et al., 1999). Most containers used by rural and poor households are 231 expected to be unbaffled so the absence of stators in jar test beakers is arguably more 232 representative of real-world application. Similar to the procedure of Antov et al. (2010), the 233 samples in the beakers were stirred at 200 rpm for 1.5 min. After rapid mixing, the mixing 234 speed was then reduced to 30 rpm for 15min and, at this stage, zeta potential measurements 235 were taken. After the slow mix stage, water samples were drawn from 3cm below water surface 236 using a syringe into a 30mL cuvette and turbidity values were taken 10 mins, 20 mins, 50 mins, 237 70 mins, 120 mins and 24hrs (1440mins) after the sample was taken. The different settling 238 times used gave an insight into the settling behaviour of the suspended particles. Samples were 239 also collected at 48 hours (2880 mins) to monitor the organic matter content in treated water. 240 A control assay using only untreated water was also used to evaluate the effect of unaided 241 sedimentation on particle removal and the overall treatment process. The turbidity removal 242 percentage was calculated using:

243 % turbidity removal 
$$= \frac{T_{initial} - T_{residual}}{T_{initial}} \times 100$$
 Eq. (1)

where  $T_{initial}$  is the turbidity of the untreated water and  $T_{residual}$  denotes the turbidity of the treated water at the end of the settling period. Total suspended solid (TSS) concentration was approximately 2.3 times of turbidity value of the untreated water (Tchobanoglous, 2014). Dosage optimisation tests were conducted to examine the performance of HxKP, StKP and CrKP in treating high, mid and low turbidity river water. Different protein dosages for the KCPs were chosen to investigate the impact of coagulant dosage on the water types and these results are provided in Section 3.3.1.

#### 251 2.5 KCP and Water Characterization

The surface morphologies of KCPs give an understanding of their role in the coagulation/flocculation process by providing insight into their adsorption-bridging behaviour and the nature of their surface. The functional groups and chemical fingerprint on the surface of the KCPs were determined using a Perkin Elmer Fourier Transform Infrared spectrometer (FT-IR) (Yu and Irudayaraj, 2005). Also, the surface morphology and elemental analysis of the KCPs were examined using a Hitachi TM3030 Plus scanning electron microscope (SEM),
equipped with energy dispersive analysis of X-rays (EDAX).

259 Water quality variables examined included turbidity, UV<sub>254</sub> absorbing compounds and DOC. 260 The method of Lowry et al. (1951) was used for soluble protein determination by taking 261 absorbance at 660nm, and all experiments were replicated thrice to rule out random or 262 experimental error. Turbidity measurements conformed with the Standard Method for 263 Examination of Water and Wastewater (Rice et al., 2012), and were made using a 2100N Hach 264 turbidity meter by placing a 30 ml unfiltered (untreated or treated) sample in a pre-rinsed vial. 265 Instrument calibration was done using the StablCal Calibration Set for the 2100N turbidity 266 meter, obtainable from Hach. pH readings were obtained using Therm-Scientific Orion 3 Star 267 according to the Standard Method procedures (Rice et al., 2012). Also, the zeta potential was 268 measured using a Zetasizer Nano ZSP (Malvern instrument, UK) with a disposable 269 polycarbonate folded capillary cell with gold plated electrodes (DTS 1070). The zeta potential 270 test uses the electrostatic forces of repulsion between particles to determine the surface charge 271 of the particles. UV<sub>254</sub> absorbing compounds were measured using a Varian Cary 50 Probe. 272 The SUVA<sub>254</sub> value indicating the composition of natural organic matter in water is given by 273 the ratio of  $UV_{254}$  to DOC:

274 
$$SUVA_{254} = \frac{UV_{254}}{DOC} \times 100$$
 Eq. (2)

275 **3. Results and discussions** 

#### 276 **3.1 Properties of river water**

#### 277 Characteristics of the untreated water samples are shown in

Table 1. Medium and high turbidity samples had high values of UV<sub>254</sub> and SUVA<sub>254</sub>, which
reflect organic material compounds present in water. SUVA<sub>254</sub> values greater than 4 indicate

280 compounds that are hydrophobic and aromatic, while SUVA254 values less than 3 show that 281 the organic materials are mostly hydrophilic (Matilainen et al., 2011). Organic materials with 282 high SUVA<sub>254</sub> values have higher molecular weights (mW). They may require more advance 283 treatment procedures and also have a high chlorine demand and total disinfectant byproduct formation potential. Untreated low turbidity water gave the lowest SUVA254 and DOC values 284 285 signifying a reduced concentration of hydrophobic NOM. Also, the zeta potential values 286 obtained from all waters showed that suspended particles in water are negatively charged, 287 which agrees with previous reports (Jarvis et al., 2005). These similar charges on particles 288 make them disperse and agglomeration resistant.

Quality Variable	Water A - HTW	Water B - MTW	Water C - LTW	
Turbidity (NTU)	$500 \pm 2$	$150 \pm 7$	$32 \pm 3$	
UV <sub>254nm</sub> (cm <sup>-1</sup> )	$1.8 \pm 0.12$	$0.7\pm0.01$	$0.13 \pm 0.02$	
SUVA <sub>254</sub> (L/mg m)	14.1	10.2	2.5	
DOC (mg/L)	$12.8\pm0.6$	$7.0\ \pm 0.4$	$5.2\pm0.2$	
pН	$7.6\pm0.3$	$7.3\pm0.0$	$7.2\pm0.2$	
Zeta potential (mV)	$-16.3 \pm 0.6$	$-16.6 \pm 1.2$	$-16.9 \pm 1.2$	

289 Table 1. Characteristics of untreated water.

\*Result shown are in mean concentration  $\pm$  standard deviation (SD)

#### 290 **3.2 KCP characterisation**

The protein content of the StKP and HxKP products were found to be higher than the CrKP (Table 2; zeta potential shown in the table is discussed in Section 3.3.3). The low solubility of the protein in the crude extract could be due to shielding of the coagulating proteins by the fatty layer and other compounds present. Camacho *et al.* (2017) made similar observations using *Moringa Oleifera* (MO) in treating cyanobacteria rich water. Research conducted by Jones

296 (2016) revealed protein concentration in Hibiscus esculentus (Okra) salt extract to be 1018 297 mg/L and 264 mg/L for Okra water extract. This variation in protein concentration is due to 298 the nature of the solvent used in the extraction process. During extraction, water was observed 299 to be a weak and mild solvent for protein extraction due to its low ionic strength, whereas NaCl 300 solution was very effective in this regard due to the salting-in process (Ndabigengesere and 301 Subba Narasiah, 1998). This salting-in process helps stabilise the protein molecules by causing 302 a reduction of the electrostatic energy between the protein molecules, thus, improving their 303 solubility.

КСР	Protein content	Zeta potential	pН
	(mg/L)	(mV)	
CrKP	$667 \pm 0.1$	$-21 \pm 0.1$	$7.1 \pm 0.0$
StKP	$1307\pm0.2$	-15 ± 1.0	$6.3 \pm 0.1$
HxKP	$1030\pm0.1$	$-17 \pm 0.3$	$6.8 \pm 0.2$

304 Table 2. Protein Characterization of KCPs.

Result shown in mean concentration  $\pm$  standard deviation (SD)

305 The SEM images of the CrKP, StKP and HxKP (Fig. 2a-c) reveal a heterogeneous flake-like 306 structure. At a magnification of x500, it was observed that the CrKP had interconnected 307 channels with narrow pores and flake-like structures, suspected to be the active binding sites. 308 Results show a highly carbonaceous and fibrous structure of seed. Elemental composition of 309 KCPs obtained by EDAX revealed an irregular shaped structure and highly carbonaceous 310 material. Chemical analysis showed the presence of inorganic elements in trace amounts. From 311 the mapping image and cross-sectional layout of the elemental profile, it was observed that Carbon (C) appears to be evenly distributed in the sample with a few dense spots noted (not 312 313 shown) which confirms the high carbon content (73%) of the KCP. Inorganic elements detected



315 HxKP can be confirmed by the presence of a higher concentration of NaCl in both products.

in trace amounts were K, Ca, Mg, Na, P, S, Al and Cl. Higher performance of the StKP and

316

314

Fig. 2. SEM imagery and EDAX elemental analysis of the (a) Crude Kenaf, CrKP (b) Salted
Kenaf, StKP and (c) Hexane Kenaf product, HxKP



320 Fig. 3 FT-IR spectra of crude, salt and hexane KCPs.

321 The FT-IR analytical spectrum depicted in Fig. 3 shows various functional groups present in the investigated KCPs. The bands between 3150cm<sup>-1</sup> and 3500cm<sup>-1</sup> correspond to hydroxyl (-322 323 OH stretching vibration mode) and amine group (-NH stretching) which are present in fatty 324 acids, proteins and carbohydrates (Nidheesh et al., 2017). Bands corresponding to peaks 2925cm<sup>-1</sup> and 2851cm<sup>-1</sup> are attributed to asymmetric and symmetric stretching of C-H-CH<sub>2</sub> – 325 326 an aliphatic compound, present in organic compounds such as fatty acids (Araújo et al., 2010). 327 Significant differences exist between the FT-IR spectra of the three KCPs, especially in C-H-CH<sub>2</sub> stretching vibration group, which show a higher peak in CrKP and StKP compared to 328 329 HxKP. The difference could be due to the delipidating process, which resulted in the elimination of most of the fatty compounds present. Bands at 1721-1580cm<sup>-1</sup> reflect the 330 331 presence of carboxylic acid C=O and amides groups, respectively. Bands 1420-1460, and the 332 peak at approximately 1510, correspond to C=C aromatic group. These groups are present in 333 lignin, cellulose and hemicellulose (Meneghel et al., 2013). The peak at approximately 1329

cm<sup>-1</sup> indicates the presence of a C=O bond and N-H vibrational mode, which extends to 334 335 primary and secondary amides (Reddy et al., 2011), therefore, confirming the presence of protein in KCPs. Presence of phenols C-O is indicated by the band at 1237-1243 cm<sup>-1</sup> 336 (Meneghel *et al.*, 2013). Spectra show a strong C-O band in 1055-1063  $\text{cm}^{-1}$  which confirms 337 the presence of alcohols, ethers and carbohydrate (Musikavong and Wattanachira, 2013). 338 339 Different functional groups like C-O-C stretching illustrate the presence of polysaccharides and -OH bending bonds in the spectral region between 1015 and 800 cm<sup>-1</sup> (Kwaambwa and 340 341 Maikokera, 2008). These bands confirm the presence of coagulating compounds in KCPs.

#### 342 **3.3** Evaluation of the coagulation/flocculation process

#### 343 **3.3.1** Effect of water turbidity and coagulant dosage

344 The optimum dosage for the turbidity experiment was derived by using different protein 345 concentration shown in Table 3. The protein concentration was analysed using the Lowry 346 method of protein estimation (Lowry et al., 1951) for the LTW and MTW ranging between 13 347 mg/L-100 mg/L, 26mg/L-196mg/L and 21 mg/L-154 mg/L for CrKP, StKP and HxKP, 348 respectively. After the jar test experiment, the minimum residual turbidity (RT) values for all 349 the water types were noted, and the dosages corresponding to these RTs were selected as the 350 optimum dosages which were used for subsequent experiment. The optimum CrKP, StKP and 351 HxKP dosages used in the HTW were selected from their estimated protein concentrations 352 ranging between 33-534 mg/L, 65-1046 mg/L and 51-824 mg/L respectively. The optimum 353 dosages derived for the high turbidity water (500 NTU) experiments were 824 mg/L for HxKP, 354 915 mg/L for StKP and 67 mg/L for CrKP. For medium turbidity (150NTU) water tested, the 355 optimum dosages were 82 mg/L, 196 mg/L and 67 mg/L for HxKP, StKP and CrKP while for 356 low turbidity water, optimum dosages obtained were 21 mg/L, 26 mg/L and 13 mg/L for HxKP,

- 357 StKP and CrKP, respectively. Derivation of the optimum KCPs dosages are not discussed in
- 358 detail, only a summary of their performance is provided

Water type	KCPs	mg polymer/g of TSS (*)					
LTW	CrKP	0.18 (13)	0.36 (27)	0.54 (40)	0.73 (52)	0.91 (67)	1.36 (100)
	StKP	0.36 (26)	0.71 (52)	1.07 (78)	1.42 (105)	1.78 (131)	2.67 (196)
	HxKP	0.28 (21)	0.56 (41)	0.84 (62)	1.12 (82)	1.40 (103)	2.10 (154)
	CrKP	0.04 (13)	0.08 (27)	0.12 (40)	0.15 (52)	0.19 (67)	0.29 (100)
MTW	StKP	0.08 (26)	0.15 (52)	0.23 (78)	0.30 (105)	0.38 (131)	0.57 (196)
	HxKP	0.06 (21)	0.12 (41)	0.18 (62)	0.24 (82)	0.30 (103)	0.45 (154)
	Equivalent weight						
	measured in the Jar test	20	40	60	80	100	150
	experiment (mg/L)						
HTW	CrKP	0.03 (33)	0.06 (67)	0.12 (133)	0.23 (267)	0.41 (467)	0.46 (534)
	StKP	0.06 (65)	0.11 (131)	0.23 (262)	0.45 (523)	0.80 (915)	0.91 (1046)
	HxKP	0.04 (51)	0.09 (103)	0.18 (206)	0.36 (412)	0.63 (721)	0.72 (824)
	Equivalent weight						
	measured in the Jar test	50	100	200	400	700	800
	experiment (mg/L)						

Table 3. Protein dosage used for optimisation experiment tests in LTW, MTW and HTW.

360 **\*** V

\* Values enclosed in brackets are protein concentration (mg/L) estimated using Lowry method.

Table 4 shows the optimum polymer concentration/ g of suspended solid, obtained for the coagulation/flocculation experiments. The optimum dosages for KCPs varied from 0.04 to 0.8 mg polymer/ g TSS, with the CrKP having the least dosage. It is interesting to note that using dosages above the optimum CrKP dosage of 0.18 polymer/g TSS, diminished the particle destabilising ability, and this is attributed to high dissolved organic carbon content caused by its insoluble non-coagulating molecules. Conversely, the HxKP and StKP had a higher dosage range owing to their additional treatment such as delipidation and salting-in, which improvedthe polymer-particle interaction.

369

Table 4. Optimum dosage of KCPs for treating High, Medium and Low turbidity water at pH

371 7.

	LTW	MTW	HTW
КСР	mg p	oolymer/g of TS	S (*)
CrKP	0.18 (13)	0.04 (13)	0.06 (67)
StKP	0.36 (26)	0.57 (196)	0.80 (915)
HxKP	0.28 (21)	0.24 (82)	0.72 (824)

<sup>372</sup> \* Values enclosed in bracket are protein concentration (mg/L) estimated using Lowry method.

373 Table 5 Residual turbidity (RT) NTU at different settling times for HxKP, StKP and CrKP in

374 (a) LTW (b) MTW and (c) HTW. Experiment pH = 7

	LTW	MTW	HTW	LTW	MTW	HTW	LTW	MTW	HTW
KCPs		10mins			20mins			50mins	
CrKP	$13 \pm 0.15$	$43 \pm 1$	$48\pm0.78$	$11 \pm 0.15$	$40\pm0.56$	$42\pm0.15$	$8\pm0.05$	$25\pm0.21$	$35\pm0.15$
StKP	$12\pm0.31$	$16\pm0.3$	$15\pm0.42$	$11\pm0.23$	$12\pm0.17$	$13\pm0.25$	$9\pm0.14$	$10\pm0.16$	$12\pm0.11$
HxKP	$14 \pm 0.15$	$20\pm0.31$	$30\pm2.8$	$12\pm0.1$	$15\pm0.13$	$20\pm0.12$	$9\pm0.1$	$13\pm0.24$	$9\pm0.11$
		70mins			120mins			1440mins	_
CrKP	$7\pm0.08$	$19\pm0.1$	$34\pm0.27$	6 ± 0.18	$15\pm0.21$	$32\pm0.13$	$3\pm0.06$	$9\pm0.06$	$26\pm0.25$
StKP	8 ± 0.15	$9\pm0.04$	$12\pm0.1$	6 ± 0.17	$8\pm0.01$	$11\pm0.03$	$4\pm0.09$	$6\pm0.16$	$7\pm0.14$
HxKP	$7\pm0.31$	$7\pm0.05$	$7\pm0.01$	$5 \pm 0.15$	$6\pm0.04$	$5\pm0.01$	$3\pm0.08$	$4\pm0.03$	$3\pm0.03$

375 Result shown in mean concentration  $\pm$  standard deviation (SD)

376 The values displayed in Table 5 shows that the minimum turbidity removal recorded for low

and medium turbidity waters after about 70 mins settling period were 77%, i.e. attained 7 NTU

residual turbidity (RT) and 87% (RT of 7 NTU), respectively. The turbidity removal using the 378 379 KCPs optimum values are shown in sedimentation curves given in Fig. 5. Comparing Fig. 4 a-380 c revealed that at 70min settling time, the HTW had the highest floc sedimentation rate, followed by medium and low turbidity waters. The floc settling velocity thresholds at 10min 381 was 3 mm/min. Subsequent retention times of 20, 50, 70 120 and 1440mins gave average 382 383 settling velocity of 1.5, 0.6, 0.43, 0.25 and 0.02 mm/min respectively. Settling velocity explains 384 the average sedimentation rate of colloidal particles in the polymer-particle suspension. The 385 settling velocity for all KCPs in the LTW was similar and varied little, compared to the MTW 386 and HTW, and this was due to particle concentration in suspension.

From the figures, particle destabilisation was lowest at the highest settling velocity of 3 mm/min. The influence of mixing after the flocculation process is seen in the high settling velocity at the start of residence (settling) time (10 min). As the residence time increases, the settling velocity reduces from 3 mm/min to 0.02 mm/min due to the reduction in the interaction of the flocculated particle. The CrKP gave the least residual turbidity for all the settling velocities at optimum dosages. The low performance of CrKP implies that it had a lower destabilisation power than the HxKP and StKP, especially in the MTW and HTW.



Fig. 4 Settling velocity distribution curve for optimum dosage of KCPs in (a) LTW (b) MTW
and (c) HTW. Experimental pH = 7

397 From Table 5, the least turbidity removal recorded for optimum dosages of HxKP in LTW, 398 MTW and HTW were 57% (RT of 14 NTU), 87% (RT of 20 NTU) and 94% (RT of 30 NTU) 399 respectively after 10min of settlinge time. This is expected as most of the flocs formed only 400 began settling 10min post-flocculation period. Turbidity removal recorded for all the samples 401 exceeded 60% with the majority attaining 90% removal after settling for 10 mins. The high 402 sedimentation rate recorded was due to the rapid flocs settlement after the coagulation-403 flocculation process using KCPs. Also, the high concentration of particles in the HTW might 404 have led to increased particle interaction and collision as they settle. Comparing the results 405 obtained after 70min settling time with that of WHO (2012) revealed that the treated water 406 exceeded the maximum allowable limit (MAL) of 4NTU.

407 Further evaluation of turbidity removal revealed that using optimum CrKP dosage of 66 mg/L 408 in HTW gave a removal of 93% (RT of 34 NTU) after 70 mins settling period. Both the 409 optimum value of the HxKP and StKP exceeded the turbidity removal of CrKP, by 6% and 5% 410 respectively. This reduced performance of the CrKP is likely caused by fatty substances and 411 the low concentration of coagulation agents in the KCP. The delipidating process of the HxKP involved oil extraction from the seeds with hexane, an organic solvent which aided the removal 412 413 of most of the fat and oil present (Camacho et al., 2017). The seed oil is said to form a barrier 414 around the PCPs, thus preventing contact between them and the particles in solution (Camacho 415 et al., 2017). Improved performance of the salt extraction process is similar to reports of Okuda 416 et al. (1999) showing higher protein dissociation and increased solubility, thus demonstrating 417 that HxKP and StKP can significantly reduce the suspended particles in water.

418



Fig. 5. Coagulation/flocculation assay showing turbidity removal percentage at different
settling times for HxKP, StKP and CrKP in (a) LTW (b) MTW and (c) HTW. Experiment pH
= 7

Prolonged settling time significantly influenced the final turbidity of the coagulated water. In the turbidity tests with prolonged settling periods (greater than 720 mins), turbidity was successfully reduced to 3 NTU for StKP. Similarly, for MTW, the turbidity value for HxKP and StKP at 720mins settling time were both 3 NTU, while 4 NTU was obtained when StKP was used in LTW. The best performance recorded at the 24-hour settling period revealed turbidity reduction of 99% (26 NTU), 97% (9 NTU) and 90% (3 NTU) for the HTW, MTW 429 and LTW samples, respectively. From the shape of the curve in Fig. 5a and Fig. 5b, it is 430 believed that clumping of particles and subsequent sedimentation continued after the slow stir 431 period. This continuous but gradual particle settlement could be attributed to the continuous 432 solubilisation of protein as experienced in another research using MO (Baptista *et al.*, 2015).

433

### **3.3.2** Contribution of KCP dosage to organic load of treated water

434 To complement the turbidity results and provide insight on the effects of KCPs on coagulated 435 water, NOM surrogates such as the DOC, SUVA254 and UV254 were analysed to determine the 436 concentration of dissolved organic matter, which is a known precursor to DBP. The analysis 437 for UV<sub>254</sub> absorbing compounds provided information on the aromatic (double-bonded ring 438 structures) organic matter present (Matilainen et al., 2011). The optimum KCP dosages found 439 for turbidity also apply for the NOM surrogates, and were used for these tests. The UV<sub>254</sub> results 440 revealed significant removal across the various sample waters. As shown in Fig. 6a-c, the use 441 of hexane and salted KCPs gave higher removal than their crude form-CrKP. It can also be 442 seen that the HxKP gave the best UV<sub>254</sub> removal (93%) compared to the StKP (91%) and CrKP 443 (90%). The optimum protein concentration in the StKP coagulant was higher than the HxKP 444 and CrKP (Table 4), but despite this high protein content, removal of 254nm light absorbing 445 compounds was below that for HxKP, indicating a higher concentration of suspended and 446 dissolved compounds in the StKP itself. A similar trend exists for the optimum CrKP used, 447 which gave a higher UV<sub>254</sub> absorbance value than the HxKP and StKP. This is expected since 448 CrKP contains the highest concentration of suspended solids.



449

Fig. 6. Coagulation/ flocculation assay showing UV<sub>254</sub> removal efficiency at different settling
times for HxKP, StKP and CrKP in (a) LTW (b) MTW and (c) HTW. Experiment pH = 7.
Error bars signify standard deviation of triplicate measurements.

SUVA<sub>254</sub> values obtained in river water revealed high concentrations of organic matter content, predominantly of hydrophobic origin. Fig. 7 shows the DOC and SUVA<sub>254</sub> values over a range of KCP dosages. Based on the DOC analysis, the increase in KCP dosage resulted in a linear increase in DOC value specifically for the dose range 400 mg/L to 800mg/L, whereas the SUVA<sub>254</sub> analysis showed that KCP dosages above the optimum level led to an increase in values for the specific light-absorbing compounds at 254nm. For HTW, using optimum dosages of HxKP, StKP and CrKP slightly reduced the DOC after a 70min settling time, while 460 a value above the optimum range caused an increase in DOC concentration. The lowest DOC 461 concentration of 23mg/ L was recorded for CrKP dosage of 534 mg/L while similar dosages 462 for HxKP and StKP gave DOC values of 14 mg/L and 17 mg/L respectively, indicating that 463 the crude extract performed poorly relative to the other KCPs. Treated water TOC 464 concentration above the USEPA guideline value of 2mg/L is presumed to favour chlorinated 465 by-products formation (USEPA, 2010), the KCPs DOC were above this range, indicating that 466 an additional treatment process would be required to make water potable. Also, KCPs 467 contribution to the DOC of treated water confirms that the chemical components in the seed 468 can decrease water quality. This is probably due to the high dosages required for KCPs when 469 applied as primary coagulant, and could be potentially resolved using KCPs as coagulant aids 470 (lower dosages) instead. Similar reports exist confirming PCPs contribution to DOC of treated 471 water (Feihrmanna et al., 2017) and this is worrisome as the continued presence of these 472 compounds in treated water could encourage DBP formation on disinfection.

473 The SUVA<sub>254</sub> values derived when optimum dosages of HxKP, StKP and CrKP were used in 474 HTW were 1.1 L/mg.m, 1.1 L/mg.m and 2.2 L/mg.m. Comparing these results with the 475 SUVA<sub>254</sub> value of untreated water (14 L/mg.m) in Table 1 shows a SUVA<sub>254</sub> reduction of 92% 476 for both HxKP and StKP and 84% for CrKP. All SUVA254 values obtained were below 4, 477 except for a CrKP dosage of 467 mg/L. SUVA254 values below 4 reflect acceptable extent of 478 hydrophobic and non-humic hydrophilic compounds in the water while a value above 4 implies 479 that the system may require additional treatment processes to dispose of the organic load 480 (Matilainen et al., 2011). The reduction of SUVA254 by the HxKP and StKP was because of 481 the delipidating and salting-in process, which do not occur for the CrKP. The delipidating 482 process possibly reduced the lipids content which is responsible for forming an oil or emulsion 483 coat around the coagulating agents, leading to a reduction of reactive surfaces for the 484 coagulation/flocculation processes. Examination of the FT-IR spectra of CrKP, StKP and HxKP in Fig. 3, shows reduction (especially for the HxKP) in bands between 3150cm<sup>-1</sup> and
3500cm<sup>-1</sup>. These bands correspond to the hydroxyl group (-OH stretching vibration mode),
which are present in fatty acids (Nidheesh *et al.*, 2017). The delipidating process possibly
removed the lipophilic compounds from the seeds responsible for sheathing protein in solution.



490 Fig. 7. DOC and SUVA<sub>254</sub> values for different KCP Dosage in HTW using HxKP, StKP and 491 CrKP. Conditions: settling period = 70 mins, pH = 7. Error bars signify standard deviation of 492 triplicate measurements

493 Allowing the treated water stand for 24-hours (1440 mins) and 48-hours (2880 mins) showed 494 a significant difference in DOC and turbidity level. In order to avoid odour issues, previous 495 studies recommended a 24 h storage duration for PCPs (Jahn et al., 1986; Jones and Bridgeman, 496 2016). In this study, it was observed that the optimum dosage of HxKP and StKP produced no 497 odour at 48 h storage period. In many practical cases, the 48h residence time is likely to be 498 used by households in rural areas, especially communities located several kilometres from 499 untreated water sources. This long storage time may require chlorine disinfection to make water 500 safe and free from objectionable odours caused by microbial decomposition of organic 501 materials.

#### 502 **3.3.3 KCPs Coagulation Mechanism**

503 The particle-polymer interaction shown in Fig. 8a, is grouped into three regions. The first 504 region (zone 1) illustrates the behaviour of KCPs below their optimum dosage and gradual 505 destabilisation of suspended particles. Particle destabilisation is indicated by a decrease in the 506 particle electrophoretic mobility (EM) for StKP and HxKP respectively. For the CrKP, an 507 initial increase in the EM signifies poor floc formation as illustrated by lowest residual 508 turbidity. In zone 2, the negative mobility of the suspended particles in all water increased to 509 the prominent point of inflection. This increase, which is similar to the previous zone, reflects 510 low turbidity removal. Following the inflection, the negative mobility of the particles decreased 511 (zone 3) up to the optimum dosage of the HxKP, slightly increased for the StKP, while the 512 CrKP remained unchanged. A change is seen in the residual turbidity for StKP and HxKP, 513 respectively. However, CrKP dosages caused the resuspension of particles. Fig. 8a gives the 514 zeta potential of the KCPs across a range of dosages. The use of different KCPs dosages gave 515 significantly different zeta potential values. Two-way analysis of variance showed that there 516 was a significant difference (p < 0.05) between the zeta potential of HxKP, StKP and CrKP (F 517 = 44.91, p = 0.00). This change in zeta potential value provides evidence of particles 518 destabilisation and restabilisation for the range of dosages used. Different particle stability 519 reported may be because of a range of reasons including molecules in KCPs (polymer), the 520 influence of their polymer preparation technique such as salting-in, and the untreated water 521 chemistry such as the valency of ions present.



Fig. 8. (a) Zeta potential, electrophoretic mobility and residual turbidity – dosages profile of
KCPs in HTW; pH = 7 (b) Zeta potential, electrophoretic mobility and residual turbidity – pH
profile of KCPs in HTW using optimum dosages Conditions: settling period = 70 mins; error
bars signify standard deviation of triplicate measurements. IEP curve for medium ionic strength
water solution (0.5M) is shown by the dotted lines; solid lines depicts HTW. Z1, Z2, Z3 depicts
zone1, 2 and 3.

Acidic and basic pH influences the magnitude of the zeta potential (ZP) by making it more positive or negative (Bhattacharjee, 2016). The ZP magnitude gave useful information on the surface electrical properties of the polymer-particle suspension. Fig. 8b shows the isoelectric point (IEP) of KCPs at their optimum dosage. IEP was approximately at 1.5, confirming that KCPs are anionic and that negative charges predominate on their surface. Around the IEP, the 534 suspension is unstable, and the chances of particles clumping together is high. Acidity causes 535 the KCPs to be hydrolysed into a cationic polymer, favouring reaction with negatively charged 536 NOM. Consequently, the energy barrier between the two surfaces is overcome, resulting in floc 537 formation. Based on residual turbidity of the KCPs, it is hard to rule out adsorption of anionic 538 polyelectrolytes to the surfaces of negatively charged particles. Adsorption was facilitated by 539 increase in ionic strength of the suspension by the salting-in process during KCPs preparation, 540 which increases the sodium chloride concentration of the suspension, leading to increased ionic 541 strength. This rise in ionic strength has previously been reported to improve particle 542 destabilisation (Oladoja et al., 2017).

543 It is also worth noting that cations such as calcium, magnesium, potassium and sodium are 544 present in natural water samples. The EDAX analysis indicated in Fig. 9, provides evidence of divalent ( $Ca^{2+}$ ,  $Mg^{2+}$ ) and possibly trivalent cations ( $Fe^{3+}$ ,  $Al^{3+}$ ) in the water. At a low pH,  $Ca^{2+}$ 545 preferential binds with the carboxylic groups while Zn<sup>2+</sup> prefers the amine group (Adusei-546 547 Gyamfi et al., 2019). It is assumed that other cations in the suspension bounded to various functional groups present such as the hydroxyl, carboxyl, amine and methoxy groups as 548 549 previously observed in the FT-IR spectra (Fig. 3), which have undergone the hydrolysis 550 process. Their dissociation minimised the negative charge density on the KCPs, causing 551 attraction of negatively charged particles and later production of several attractive and 552 repulsive interactions such as polymer-polymer and polymer-particle interaction. Similar 553 reaction involving low calcium concentration assisted in flocculating negatively charged 554 particles by linking anionic sites on polymer and particles by a 'calcium bridging' process 555 (Gregory, 2013). Based on this observation, the turbidity removal by the HxKP and StKP was 556 thought to be because of complexation of functional groups on their surface with ligands 557 possessing opposite charges. It is assumed that an increase in ionic strength caused 558 compression of the electric double layer (EDL) and led to a decrease of the zeta potential of

- the water. Conversely, the water treated using CrKP, had a lower ionic strength which slightly
- 560 decreased the particle's zeta potential causing only a partial destabilisation of particles.



561 562

563 564

565 566

Fig. 9. SEM imagery and EDAX elemental analysis of sludge of (a) CrKP treated water (b)
StKP treated water (c) HxKP treated water

569 Furthermore, the proximity of KCPs active sites to colloid particles can also result in stronger 570 bridge assembly, causing friction due to their proximity, and this must have led to the bridging-571 out coagulation recorded especially for HxKP and StKP. Obstruction of some active binding 572 sites by non-coagulating molecules and poor protein solubility is likely to have reduced the 573 effectiveness of the CrKP. Besides, the optimum dosages used in water increased with the 574 particle concentration, which supports the adsorption phenomenon. KCPs have low to medium 575 weight ranging from 10 – 100 kDa (Mariod et al., 2010; Jones, 2016), which further confirms 576 their ability to promote particle-polymer interactions. However, considering the performance 577 recorded for the KCPs, it was evident that electrostatic repulsion, which is associated with the 578 charge neutralisation process, was not the dominant coagulation mechanism. Although there 579 was a slight decrease in EM and the zeta potential value, these values remained negative 580 indicating that the polycations were insufficient in removing the negative charge. It is also 581 unlikely that sweep flocculation took place since EM remained negative. So, based on the 582 strength of above observation, the KCPs coagulation mechanism is a combination of particle 583 electric double layer compression by polymeric compounds facilitated by ligand complexation 584 or particle adsorption on one or more sites through charge-charge interaction, hydrogen 585 bonding and van der Waals forces of dispersion.

#### 586 4. Conclusions

587 This study examined the use of Kenaf Coagulation Products (KCPs, an extract of Kenaf plant 588 seeds) produced using three methods: crude extract (CrKP), salted extract (StKP) and hexane 589 (delipidated) extract (HxKP). The structural characteristics of CrKP, StKP and HxKP and their 590 performance in terms of turbidity removal, dissolved organic carbon (DOC) concentration, 591 specific absorbance (SUVA<sub>254</sub>) value in high (HTW), medium (MTW) and low (LTW) 592 turbidity water have been measured. Results obtained from the SUVA<sub>254</sub>, DOC, FT-IR, SEM– 593 EDAX analysis, IEP and pH values can be summarised as follows:

595

594

• Soluble protein concentration of KCPs, determined by Lowry's method, varied and was of the order HxKP> StKP > CrKP. Variation in concentration was due to salting-in

effect which aided in the dissolution of active coagulation components, and delipidatingeffect on seed which removed the poorly coagulating compounds.

- StKP and HxKP were found to be effective for turbidity treatment of both MTW and
  HTW (>90%) whereas performance in LTW was below 80%. For all waters tested,
  HxKP gave the highest turbidity followed by StKP then CrKP. HTW and MTW
  required higher KCPs dosages than the LTW.
- SUVA<sub>254</sub> values showed that pre-treatment of water with HxKP and StKP significantly
   removes the hydrophobic fraction of NOM in water, thus, reducing the potential for
   THMs formation during disinfection. A linear relationship existed between the KCPs
   dosage and DOC, implying that the addition of organic matter from the KCPs occurs
   as dosage increases. The study shows the benefit of optimum dosage selection in
   controlling DBPs precursor concentration in water.
- Particle bridging facilitated by the adsorption process was the destabilisation
   mechanism of KCPs, and their performance was only slightly affected by the pH of
   water. Also, the IEP of KCPs was found to be approximately 1.5, signifying that
   surfaces are predominantly negatively charged.
- The FT-IR and SEM EDAX studies indicated the bonding mechanism of the KCPs.
   The bonding between suspended particles and KCPs was mainly by the shielding and
   attachment using their flake-like structures of the KCPs.

By comparing both the qualitative and quantitative analysis, the KCPs studied can be used only as a pre-treatment coagulant where there is no suitable alternative coagulant for effective treatment of LTW, MTW and HTW. Irrespective of the untreated water turbidity, using dosages outside of the optimum dosage range can affect treatment performance. Further research effort should focus on getting high-performing low-cost purified KCP to provide users and scientific community with information on their treatment efficiency and their range of application. KCPs contributes differently to the organic matter load of the treated water and their performance also depends on the untreated water turbidity and settling duration. High organic load recorded for the KCPs treated water especially for CrKP, makes them potential precursors for DBPs formation on disinfection. Since, no studies currently exist profiling KCPs ability to form DBP under typical exposure conditions, new studies would help to address and overcome this knowledge gap and also identify the safest conditions needed for their use.

#### 627 CRediT authorship contribution statement

Benjamin U Okoro: Methodology, Software, Formal analysis, Investigation, Data curation,
Writing - original draft.

630 Soroosh Sharifi and Mike Jesson: Conceptualization, supervision, writing - review and editing

631 John Bridgeman and Rodrigo Moruzzi: Conceptualization, writing – review and editing.

#### 632 Acknowledgements

The first author would like to thank the University of Birmingham Global Challenges PhD
Scholarship (GCS) and the University of Birmingham School of Engineering for funding this
work.

#### 636 References

## 637 ADUSEI-GYAMFI, J., OUDDANE, B., RIETVELD, L., CORNARD, J.-P. & CRIQUET, J.

638 2019. Natural organic matter-cations complexation and its impact on water treatment:
639 A critical review. *Water Research*, 160, 130-147.

640 ANTOV, M. G., ŠĆIBAN, M. B. & PETROVIĆ, N. J. 2010. Proteins from common bean

641 (Phaseolus vulgaris) seed as a natural coagulant for potential application in water
642 turbidity removal. *Bioresource technology*, 101, 2167-2172.

- 643 ARAÚJO, C. S., ALVES, V. N., REZENDE, H. C., ALMEIDA, I. L., DE ASSUNCAO, R.,
- 644 TARLEY, C. R., SEGATELLI, M. G. & COELHO, N. M. M. 2010. Characterization
- and use of Moringa oleifera seeds as biosorbent for removing metal ions from aqueous
  effluents. *Water Science and Technology*, 62, 2198-2203.
- 647 AWANG, N. A. & AZIZ, H. A. 2012. Hibiscus rosa-sinensis leaf extract as coagulant aid in
  648 leachate treatment. *Applied Water Science*, 2, 293-298.
- 649 BAPTISTA, A. T. A., COLDEBELLA, P. F., CARDINES, P. H. F., GOMES, R. G., VIEIRA,
- 650 M. F., BERGAMASCO, R. & VIEIRA, A. M. S. 2015. Coagulation-flocculation
- process with ultrafiltered saline extract of Moringa oleifera for the treatment of surface
  water. *Chemical Engineering Journal*, 276, 166-173.
- BELLO, A. U. 2013. Herdsmen and farmers conflicts in North-Eastern Nigeria: Causes,
   repercussions and resolutions. *Academic Journal of Interdisciplinary Studies*, 2, 129.
- BHATTACHARJEE, S. 2016. DLS and zeta potential What they are and what they are not?
   *Journal of Controlled Release*, 235, 337-351.
- 657 CAMACHO, F. P., SOUSA, V. S., BERGAMASCO, R. & RIBAU TEIXEIRA, M. 2017. The
- use of Moringa oleifera as a natural coagulant in surface water treatment. *Chemical Engineering Journal*, 313, 226-237.
- 660 CARSTEA, E. M., BAKER, A., PAVELESCU, G. & BOOMER, I. 2009. Continuous
  661 fluorescence assessment of organic matter variability on the Bournbrook River,
  662 Birmingham, UK. *Hydrological Processes: An International Journal*, 23, 1937-1946.
- 663 DOS SANTOS, T., BONGIOVANI, M. C., SILVA, M., NISHI, L., COLDEBELLA, P.,
- VIEIRA, M. & BERGAMASCO, R. 2016. Trihalomethanes minimization in drinking
  water by coagulation/flocculation/sedimentation with natural coagulant Moringa
  oleifera Lam and activated carbon filtration. *Can. J. Chem. Eng.*, 94, 1277-1284.

- 667 EXLEY, C. J. J. O. A. S. D. R. 2017. Aluminum should now be considered a primary 668 etiological factor in Alzheimer's disease. 1, 23-25.
- 669 EZEABASILI, A., OKORO, B. & OKONKWO, A. 2014. Assessment of Water Supply Quality
- 670 in Awka, Anambra State, Nigeria. *AFRREV STECH: An International Journal of*671 *Science and Technology*, 3, 81-93.
- 672 FEIHRMANNA, A. C., BAPTISTAA, A. T. A., LAZARIB, J. P., SILVAB, M. O., VIEIRAB,
- M. F. & VIEIRA, A. M. S. 2017. Evaluation of Coagulation/ Floculation Process for
  Water Treatment using Defatted Cake from Moringa oleifera. 57.
- FLATEN, T. P. J. B. R. B. 2001. Aluminium as a risk factor in Alzheimer's disease, with
  emphasis on drinking water. 55, 187-196.
- 677 GHEBREMICHAEL, K. A., GUNARATNA, K. R., HENRIKSSON, H., BRUMER, H. &
- DALHAMMAR, G. 2005. A simple purification and activity assay of the coagulant
  protein from Moringa oleifera seed. *Water Research*, 39, 2338-2344.
- 680 GREGORY, J. 2013. Flocculation fundamentals. *Encyclopedia of colloid and interface* 681 science. Springer, Berlin, 459-491.
- 682 HOCKING, M., KLIMCHUK, K. & LOWEN, S. 1999. Polymeric flocculants and flocculation.
- JAHN, S., MUSNAD, H. A. & BURGSTALLER, H. 1986. The tree that purifies water:
  cultivating multipurpose Moringaceae in the Sudan. *Unasylva*, 38, 23-28.
- 685 JARVIS, P., JEFFERSON, B. & PARSONS, S. A. 2005. How the Natural Organic Matter to
- 686 Coagulant Ratio Impacts on Floc Structural Properties. *Environmental Science &* 687 *Technology*, 39, 8919-8924.
- JONES, A. N. 2016. Investigating the potential of Hibiscus seed species as alternative water
   treatment material to the traditional chemicals.

- JONES, A. N. & BRIDGEMAN, J. 2016. An assessment of the use of native and denatured
  forms of okra seed proteins as coagulants in drinking water treatment. *J Water Health*,
  14, 768-779.
- JONES, A. N. & BRIDGEMAN, J. 2017. Disinfection ability of hibiscus seeds in water
   treatment. *Proceedings of the Institution of Civil Engineers Water Management*, 1-7.
- 695 JONES, A. N. & BRIDGEMAN, J. 2019. A fluorescence-based assessment of the fate of
- 696 organic matter in water treated using crude/purified Hibiscus seeds as coagulant in
  697 drinking water treatment. *Science of The Total Environment*, 646, 1-10.
- KWAAMBWA, H. & MAIKOKERA, R. 2008. Infrared and circular dichroism spectroscopic
  characterisation of secondary structure components of a water treatment coagulant
  protein extracted from Moringa oleifera seeds. *Colloids and Surfaces B: Biointerfaces*,
  64, 118-125.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. 1951. Protein
  measurement with the Folin phenol reagent. *Journal of biological chemistry*, 193, 265275.
- MARIOD, A. A., FATHY, S. F. & ISMAIL, M. 2010. Preparation and characterisation of
   protein concentrates from defatted kenaf seed. *Food Chemistry*, 123, 747-752.
- MATILAINEN, A., GJESSING, E. T., LAHTINEN, T., HED, L., BHATNAGAR, A. &
  SILLANPÄÄ, M. 2011. An overview of the methods used in the characterisation of
  natural organic matter (NOM) in relation to drinking water treatment. *Chemosphere*,
  83, 1431-1442.
- 711 MENEGHEL, A. P., GONÇALVES JR, A. C., STREY, L., RUBIO, F., SCHWANTES, D. &
- CASARIN, J. 2013. Biosorption and removal of chromium from water by using
  moringa seed cake (Moringa oleifera Lam.). *Química Nova*, 36, 1104-1110.

714	MOHAMED, A., BHARDWAJ, H., HAMAMA, A. & WEBBER, C. 1995. Chemical
715	composition of kenaf (Hibiscus cannabinus L.) seed oil. Industrial Crops and Products,
716	4, 157-165.

- MUSIKAVONG, C. & WATTANACHIRA, S. 2013. Identification of dissolved organic
   matter in raw water supply from reservoirs and canals as precursors to trihalomethanes
   formation. *Journal of Environmental Science and Health, Part A*, 48, 760-771.
- MUYIBI, S. & ALFUGARA, A. 2010. Treatment of surface water with Moringa Oleifera seed
   extract and alum a comparative study using a pilot scale water treatment plant.
   *International Journal of Environmental Studies*, 60, 617-626.
- NDABIGENGESERE, A. & NARASIAH, K. S. 1996. Influence of Operating Parameters on
   Turbidity Removal by Coagulation with Moringa Oleifera Seeds. *Environmental Technology*, 17, 1103-1112.
- 726 NDABIGENGESERE, A. & NARASIAH, K. S. 2010. Use of Moringa Oleifera Seeds as a
- Primary Coagulant in Wastewater Treatment. *Environmental Technology*, 19, 789-800.
- NDABIGENGESERE, A. & SUBBA NARASIAH, K. 1998. Quality of water treated by
   coagulation using Moringa oleifera seeds. *Water Research*, 32, 781-791.
- 730 NIDHEESH, P. V., THOMAS, P., NAIR, K. A., JOJU, J., ASWATHY, P., JINISHA, R.,

VARGHESE, G. K. & GANDHIMATHI, R. 2017. Potential Use of Hibiscus RosaSinensis Leaf Extract for the Destabilization of Turbid Water. *Water Air and Soil Pollution*, 228, 1-9.

NYAM, K. L., TAN, C. P., LAI, O. M., LONG, K. & CHE MAN, Y. B. 2009. Physicochemical
properties and bioactive compounds of selected seed oils. *LWT - Food Science and Technology*, 42, 1396-1403.

- OKUDA, T., BAES, A. U., NISHIJIMA, W. & OKADA, M. 1999. Improvement of extraction
  method of coagulation active components from Moringa oleifera seed. *Water Research*,
  33, 3373-3378.
- OLADOJA, N. A. 2015. Headway on natural polymeric coagulants in water and wastewater
  treatment operations. *Journal of Water Process Engineering*, 6, 174-192.
- 742 OLADOJA, N. A., SALIU, T. D., OLOLADE, I. A., ANTHONY, E. T. & BELLO, G. A. 2017.
- A new indigenous green option for turbidity removal from aqueous system. *Separation and Purification Technology*, 186, 166-174.
- 745 PRITCHARD, M., CRAVEN, T., MKANDAWIRE, T., EDMONDSON, A. & O'NEILL, J.
- 2010. A comparison between Moringa oleifera and chemical coagulants in the
  purification of drinking water–An alternative sustainable solution for developing
  countries. *Physics and Chemistry of the Earth, Parts A/B/C*, 35, 798-805.
- 749 REDDY, D. H. K., RAMANA, D., SESHAIAH, K. & REDDY, A. 2011. Biosorption of Ni
- (II) from aqueous phase by Moringa oleifera bark, a low cost biosorbent. *Desalination*,
  268, 150-157.
- RICE, E. W., BAIRD, R. B., EATON, A. D. & CLESCERI, L. S. 2012. Standard methods for *the examination of water and wastewater*, American Public Health Association
  Washington, DC.
- 755 TCHOBANOGLOUS, G. 2014. Wastewater engineering : treatment and reuse. Metcalf &
  756 Eddy
- revised by George Tchobanoglous, Franklin L. Burton, H. David Stensel. *In:* BURTON, F. L.,
  STENSEL, H. D., METCALF, EDDY & AECOM (eds.) 5th edition. ed. New York:
  New York : McGraw-Hill.
- 760 USEPA 2010. Comprehensive Disinfectants and Disinfection Byproducts Rules (Stage 1 and
  761 Stage 2): Quick Reference Guide.

- 762 VILLANUEVA, C. M., CANTOR, K. P., CORDIER, S., JAAKKOLA, J. J., KING, W. D.,
- LYNCH, C. F., PORRU, S. & KOGEVINAS, M. 2004. Disinfection byproducts and
  bladder cancer: a pooled analysis. *Epidemiology*, 15, 357-367.
- 765 WHO 2012. Guidelines for drinking-water quality fourth edition.
- WHO 2019. Results of round II of the WHO international scheme to evaluate household water
   treatment technologies.
- WHO & UNICEF 2017. Progress on drinking water, sanitation and hygiene: 2017 update and
   SDG baselines, World Health Organization.
- WHO & UNICEF 2019. *Progress on household drinking water, sanitation and hygiene I 2000-*2017, World Health Organization.
- YANG, M., LIBERATORE, H. K. & ZHANG, X. 2019. Current methods for analyzing
  drinking water disinfection byproducts. *Current Opinion in Environmental Science* & *Health*.
- 775 YU, C. & IRUDAYARAJ, J. 2005. Spectroscopic characterization of microorganisms by
- Fourier transform infrared microspectroscopy. *Biopolymers: Original Research on Biomolecules*, 77, 368-377.
- ZATTA, P., LUCCHINI, R., VAN RENSBURG, S. J. & TAYLOR, A. J. B. R. B. 2003. The
   role of metals in neurodegenerative processes: aluminum, manganese, and zinc. 62, 15-
- 780 28.
- 781