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Performance of *Moringa oleifera* seeds protein and *Moringa oleifera* seeds protein-polyaluminum chloride composite coagulant in removing organic matter and antibiotic resistant bacteria from hospital wastewater

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ABSTRACT

The aims of this study were focused on the preparation of the *Moringa oleifera* seeds protein-polyaluminum chloride composite coagulant that improves the coagulation process of organic matters and pathogenic bacteria. The performance of *M. oleifera* seeds protein (MOP) and the composite coagulant (MOP-PACl) was investigated using synthetic water and hospital wastewater. Electrophoresis SDS-PAGE, Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM) were performed to characterize MOP. Electrophoresis analysis showed that MOP was a dimeric protein with mean molecular weight of 37.5 kDa. Jar-test results revealed that 64 % of turbidity, 38.36 % of COD, 16.54 % UV_{254}, 74.28 % against *E. coli*, 76.36 % against *V. cholerae* and 90 % against *P. aeruginosa* strains were removed from hospital wastewater using MOP at dose of 320 mg/L. Composite coagulant MOP-PACl significantly improved the quality of treated water with removal efficiency of 86.11 % turbidity, 60.12 % COD, 58.82 % UV_{254}, 79.11 % against *E. coli*, 98.66 % against *V. cholerae* and 100 % against *P. aeruginosa* strains with Al:MOP ratio of 0.54 and aluminum dosage in MOP-PACl coagulant of 4.32 mg/L. In addition, the antimicrobial investigation performed with MOP showed inhibition zone of 15 mm against *E. coli* strain and 16 mm against *P. aeruginosa* strain whereas 20 mm against *P. aeruginosa* and 16 mm against *E. coli* were obtained using MOP-PACl at Al:MOP ratio of 0.27. The results highlighted that MOP and MOP-PACl composite coagulant could be successfully used as coagulant for removing the pollutants from hospital wastewater.

1. Introduction

Hospital centers generate large quantities of wastewater containing numerous pollutants such as organic matter, pathogenic microorganisms, heavy metal, dyes and drug residues or pharmaceutical personal care products (PPCPs) [1,2]. In most hospital centers in Benin, hospital wastewaters (HW) were not usually treated; they are often released in septic tanks, which may lead to the groundwater contamination and bring about the human disease [3]. So, to minimize the risks to human health that represent the HW management, numerous methods such as ozonation [4], membrane technology [5], adsorption [6] and coagulation process [7] were used for their purification. Among of these methods, coagulation process is preferentially applied to remove water pollutants because of its high effectiveness and easiness application on wide range of water types [8,9]. The most coagulants are aluminum, ferric salts and polyaluminum chloride (PACl). The use of chemical coagulants, however, affects the water pH and generates significant amounts of non-biodegradable sludge and metal residues in the treated water. Thus, the natural coagulants were extracted from seeds and leafs of plants such as *Moringa oleifera* [10], *Dolichos lablab* [11], *Strychnos potatorum* [12], *Jatropha curcas* [13], *Cocos nucifera* [14]. *M. oleifera* seeds contain a cationic coagulating protein of low molecular weight and isoelectric point between 10 and 11 [15]. The effectiveness of *M. oleifera* seeds protein has been studied for several pollutants removal such as organic matters [16], microalgae [17], dyes [18], heavy metal [19], pharmaceutical residues [20] and pathogenic bacteria [21,22] from wastewater. Use of *M. oleifera* seeds for water treatment has also several advantages, the seeds are non-toxic and have little effect on

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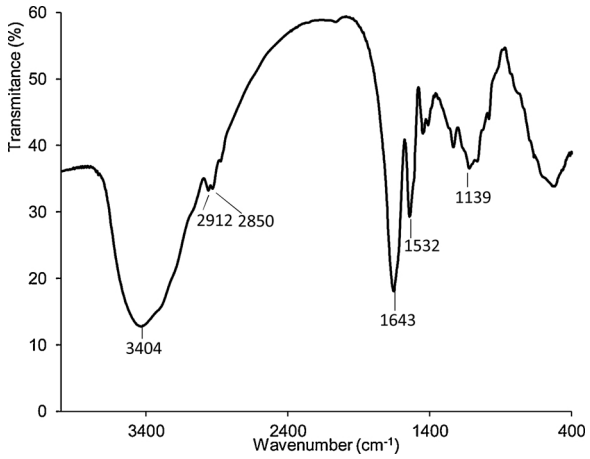

Fig. 1. FTIR spectrum of MOP.

water pH, conductivity and alkalinity and act a low volume of biodegradable sludge [15]. Moreover, the increasing of synthetic compounds presence in wastewater, leads to a decline in the coagulating potential of natural coagulants in their removing. So, natural coagulants were used as coagulating aid with aluminum and ferric salts [23,24]. Compared with chemical coagulants, metal-based coagulant in combination with natural coagulant can bring lower of the treatment cost and significantly improve the treated water quality. However, the investigation of *M. oleifera* seeds protein-PACl composite coagulant in the coagulation behavior of the hospital wastewater is limitedly reported.

This work investigates the removal of turbidity, COD, UV_{254} and pathogenic bacteria from hospital wastewater by *M. oleifera* seeds protein used as single coagulant and then as based composite coagulant with polyaluminum chloride. The extracted protein was characterized by Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM) and Electrophoresis SDS-PAGE. The effects of pH and coagulant dose were performed to explain the mechanism of coagulation process. Finally, the antibacterial properties of MOP and MOP-PACl coagulant were also examined against *Pseudomonas aeruginosa* and *Escherichia coli* strains.

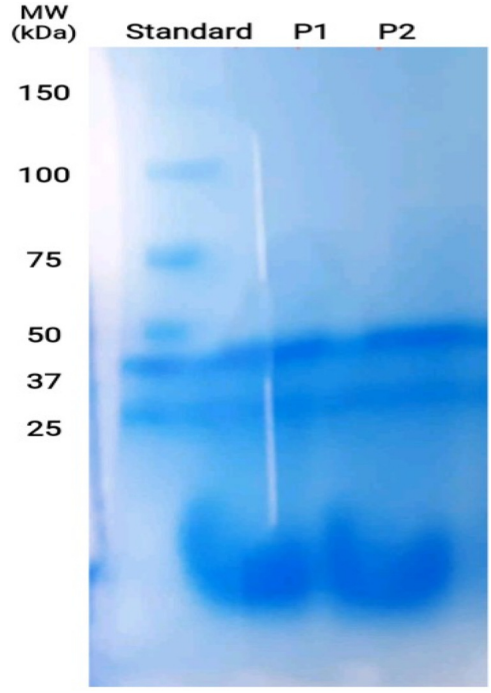

Fig. 3. Electrophoresis image of MOP.

2. Material and methods

2.1. Reagents

All reagents used in this study were analytical grade. n-Hexane, aluminum chloride ($AlCl_3.6H_2O$), NaOH, ferrous ammonium sulphate ($FeSO_4(NH_4)_2SO_4.6H_2O$), potassium dichromate ($K_2Cr_2O_7$), sodium chloride (NaCl), potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$), sodium bicarbonate ($NaHCO_3$), silver sulphate (Ag_2SO_4), Slanetz and Barthley agar, Thiosulfate-Citrate-Bile-Saccharose (TCBS) agar, Chromogenic coliform agar, and kaolinite. The humic acid was extracted from the collected peat from Nokoue Lake in Cotonou, Benin by the IHSS method [25].

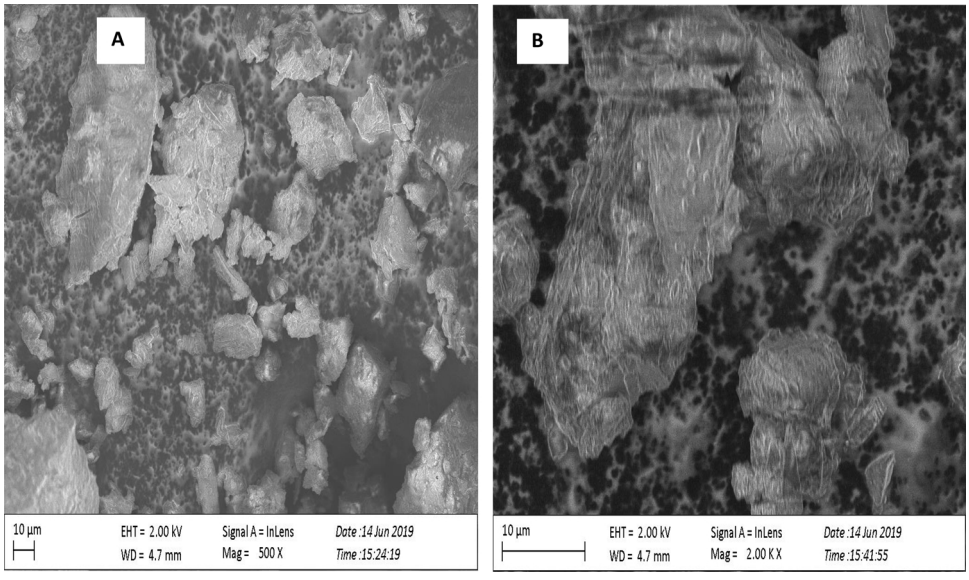

Fig. 2. SEM image of MOP obtained with two magnifications: 500X (A); 2000X (B).

Table 1
Quality of water samples.

Parameters	Synthetic water		Wastewater	Standard
	pH = 6	pH = 8		
Temperature (°C)	–	–	29.8 ± 1.48	< 30
pH	6.04 ± 0.20	8.03 ± 0.22	7.58 ± 0.24	5.5-8.5
Disolved oxygen (mg/L)	–	–	0.27 ± 0.04	> 5
Conductivity (μS/cm)	213 ± 11.31	191 ± 7.07	1261.3 ± 123.6	< 2,700
Turbidity (NTU)	36.75 ± 1.52	36.78 ± 1.73	14.99 ± 0.18	–
Nitrates (mg/L)	51.75 ± 15.51	45.8 ± 5.94	2.70 ± 0.37	< 1
Nitrites (mg/L)	2.13 ± 0.03	1.46 ± 0.76	0.20 ± 0.09	< 1
COD (mg/L-O_2)	278.88 ± 5.88	307 ± 33.94	283.04 ± 41.41	< 90
BOD_5 (mg/L-O_2)	–	–	132.21 ± 7.37	< 30
UV_{254}	0.31 ± 0.08	0.35 ± 0.04	0.28 ± 0.04	–
Vibrio cholerae (CFU/100 mL)	–	–	$5.9010^5 \pm 0.3810^5$	0
Salmonella spp (CFU/100 mL)	–	–	$5.3010^4 \pm 0.1210^4$	0
Escherichia coli (CFU/100 mL)	–	–	$1.0910^6 \pm 0.2610^6$	–
Staphylococcus aureus (CFU/100 mL)	–	–	$6.2010^5 \pm 0.2410^5$	–
Pseudomonas aeruginosa (CFU/100 mL)	–	–	$1.2010^5 \pm 0.2810^5$	–
Fecal coliforms (CFU/100 mL)	–	–	$2.0910^6 \pm 0.4410^6$	10^3
Fecal streptococci (CFU/100 mL)	–	–	$1.7110^5 \pm 0.3210^5$	–

2.2. Extraction of Moringa oleifera seeds protein and preparation of composite coagulant

M. oleifera seeds used in this study were harvested in Natitingou (Benin Republic). The protein was extracted as previously reported by Okuda et al. [26] with slight modification. The seeds are shelled, dried at room temperature for 72 h, crushed and then defatted through Soxhlet extraction with n-hexane during 2 h. 20 g of defatted seeds were put in 1 L of NaCl 1 M solution and the mixture was stirred for 2 h and then settling down for 24 h. The collected supernatant was filtered through Whatman N°1 filter paper and the protein was precipitated by adding 100 mL of 96 % ethanol at 100 mL of filtrate. The resulting sediment was collected, washed and oven dried at 60 °C for 2 h and then labeled as *M. oleifera* seeds protein (MOP). The MOP stock solution was prepared by dissolving 0.5 g of MOP in 500 mL of distilled water, and the solution was stirred for 6 h. MOP-polyaluminum chloride (MOP-PACl) composite coagulant was prepared as previously described by Ng et al. [27]. The solution of polyaluminum chloride (PACl) was prepared using a base titration method with [OH]:[Al] ratio of 2.25. 500 mL of $AlCl_3.6H_2O$ 0.20 M solution was titrated at room temperature by 500 mL of NaOH 0.45 M solution under vigorous stirring for 24 h, then, the resulting solution was settled down for 72 h. The final concentration of Al in the PACl solution was 0.1 M and the final pH of the solution was 4.05. The MOP-PACl composite coagulant was prepared by titrating a fixed volume of MOP stock solution with different volume of PACl stock solution under vigorous stirring and at room temperature to reach a aluminum to MOP ratio (Al:MOP) of 0.135, 0.27 and 0.54 (w/w ratio). The low values of Al:MOP ratios were chosen in our study to minimize the Al level in resulting sludge and treated water.

2.3. Characterization of Moringa oleifera seeds protein

The *M. oleifera* seeds protein extracted was characterized by some analytical methods. The functional groups of MOP were determined by Fourier Transform Infrared (FTIR) spectroscopy. The spectrum was recorded under 4 cm^{-1} of resolution with wavenumber between 400 and 4000 cm^{-1} on Perkin Elmer 100 series spectrometer. The concentration of total protein content MOP was quantified by the Biuret method [28]. The morphology characteristic of MOP was observed by Scanning Electron Microscopy (SEM) on SEM-FEG154OXB microscope. The molecular weight of MOP was evaluated by Electrophoresis SDS-PAGE with 10 % of polyacrylamide gel and 100 μg of MOP treated by β-mercaptoethanol.

2.4. Water samples

Hospital wastewater (HS) samples were collected in September 2018 from Departmental Hospital Center (CHD) of Atakora in Natitingou, wastewater collector. The CHD is a public hospital with about 100 beds and located at 1°22′41″ East longitude and 10°16′42″ North latitude. The wastewater samples were collected in 500 mL of borosilicate flasks previously sterilized at 121 °C using Certoclav brand autoclave during 20 min. The collected wastewater samples were then stored at 4 °C and brought at laboratory for analysis. The stock solution of humic acid (HA) was prepared by dissolving 0.10 g of HA in 100 mL of NaOH 0.10 M solution. The stock suspension of kaolinite was prepared by adding 1 g of kaolinite in 1 L of distilled water, then, the mixture was stirred for 1 h and then settled down during 12 h. The stock solutions of 0.1 M $NaHCO_3$, 0.1 M $NaNO_2$ and 0.1 M KNO_3 were prepared by dissolving the corresponding amount of each salt in 100 mL of distilled water. Preparation of synthetic water (SW) stock was carried out by diluting HA stock solution by stock solutions of $NaHCO_3$, $NaNO_2$ and KNO_3 and the supernatant from the stock suspension of kaolinite. The desired final chemical oxygen demand (COD), turbidity, NO_2^- and NO_3^- ions concentration were obtained by diluting the previous SW stock by the tap water. The pH of resulting SW samples was adjusted to 6.0 and 8.0 using 0.10 M HCl or 0.10 M NaOH solutions.

2.5. Analytical methods

Physicochemical parameters of water samples such as pH, turbidity, dissolved oxygen, electrical conductivity, and temperature were measured in-situ using HANNA HI 991001 pH-meter, HANNA HI 93703C turbidimeter, WTWOXI 3310 oximeter, HANNA HI 99300 conductivity meter and thermometer respectively. Chemical oxygen demand (COD) was determined by reflux method using potassium dichromate as strong oxidant. After organic matters degradation, the remaining unreduced $K_2Cr_2O_7$ was titrated with ferrous ammonium sulphate. The biological oxygen demand (BOD_5) was measured by incubation of water sample at 20 °C during 5 days. The quantification of NO_3^- and NO_2^- ions concentration was performed by colorimetry UV/Visible using VWR 1600 PC spectrophotometer with sulfanilic acid at 435 nm wavelength and sodium salicylate at 416 nm wavelength respectively. Absorbance at 254 nm (UV_{254}) was used as an indicator for aromatic organic matter. It was measured using the water sample filtered through a 0.45 μm cellulose acetate membrane (Sartorius). The bacteria such as *Echerichia coli*, fecal coliform, *Vibrio cholera*, *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella spp* and fecal streptococci strains were

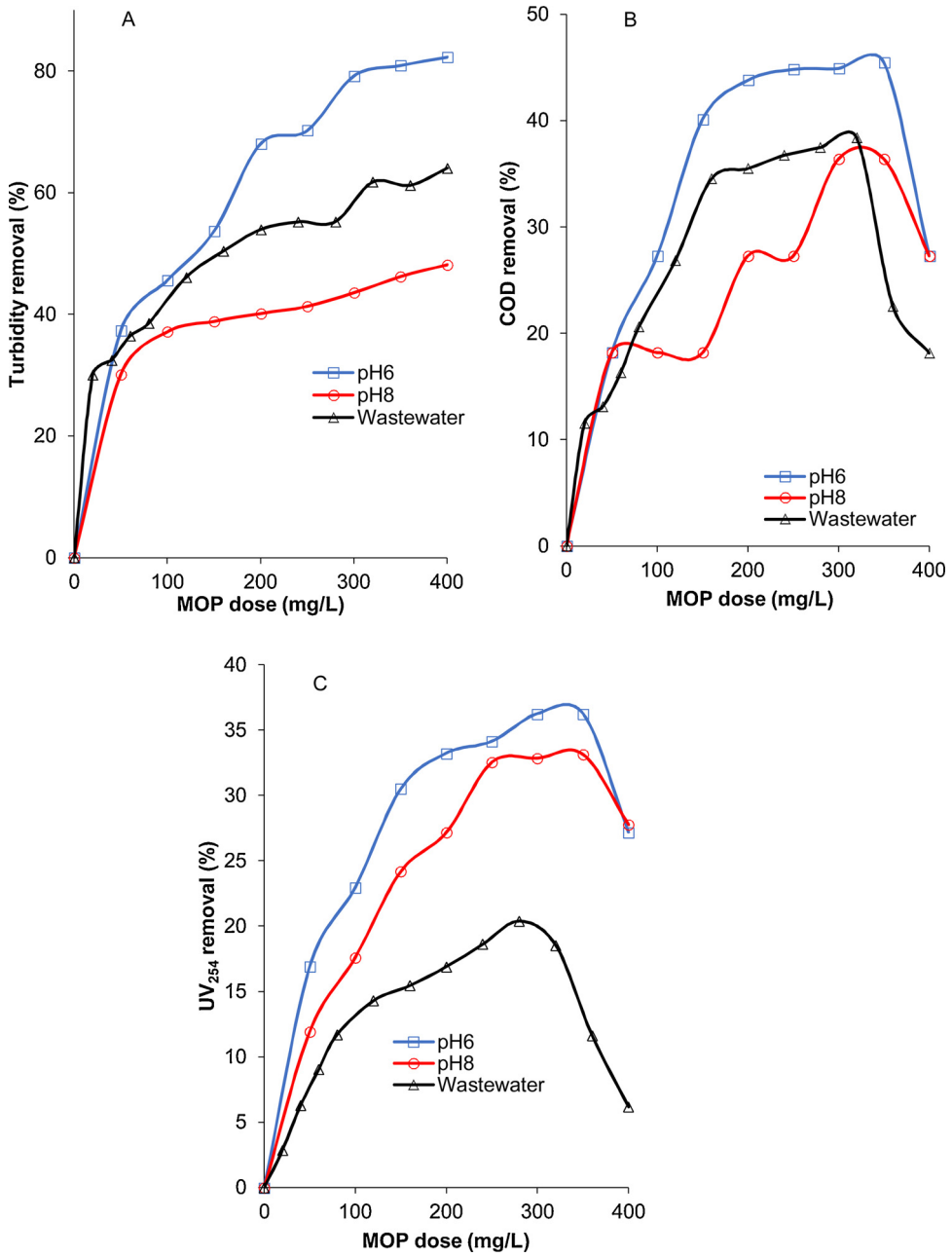

Fig. 4. Effect of MOP: Turbidity removal (A); COD removal (B); UV_{254} removal (C).

isolated through membrane filtration technique using Chromogenic coliform agar, Thiosulfate-Citrate-Bile-Saccharose (TCBS), Cetrimide, Slanetz, Bartley, Chapman and Hektoen as culture media. Gram negative bacteria were identified with API 20E gallery. The antibacterial properties of MOP and MOP-PACl composite coagulant were investigated against *P. aeruginosa* and *E. coli* strains isolated from HS samples according to the standard Kirby-Bauer disc diffusion method reported by Katata-Seru et al. [24]. The bacteria strains were suspended in 1 mL of NaCl (0.9 %) and then swabbed on the Muller Hinton Agar (MHA) plates using sterile cotton swab. The sterile Wattman filter paper discs at mean diameter of 6 mm were impregnated with solutions of MOP and MOP-PACl. The discs were gently pressed in Muller Hinton Agar plates and incubated in inverted position for 24 h at 37 °C temperature. Finally, the average diameter of inhibition zone was measured. The standard antibiotics such as Oxytetracycline, Metronidazole, Erythromycin, Ampicillin, Cotrimaxazole, Amikacin and Ciprofloxacin were used as control.

2.6. Jar-test procedures

The jar-test experiments were carried out on a FC6S six-paddle stirrer flocculator (VELP Scientifica, Italy). 1 L of water sample was stirring rapidly at 200 rpm for 1 min, then, a certain amount of MOP or MOP-PACl coagulant was pipetted into water sample. Then, the mixture continued to stirred rapidly at 200 rpm further 3 min and then, the speed of the mixer was reduced to 45 rpm for another 30 min and followed by 60 min of settling. As soon as the settling period was ended, the water sample was collected at 20 mm below top clarified water surface using a sterile syringe for analyzing the following parameters: turbidity, chemical oxygen demand, UV_{254}, *E. coli*, *V. cholera* and *P. aeruginosa*. The removal efficiency (A%) of each parameter was calculated by the following expression:

$$A = \frac{N_i - N_f}{N_i} * 100 \quad (1)$$

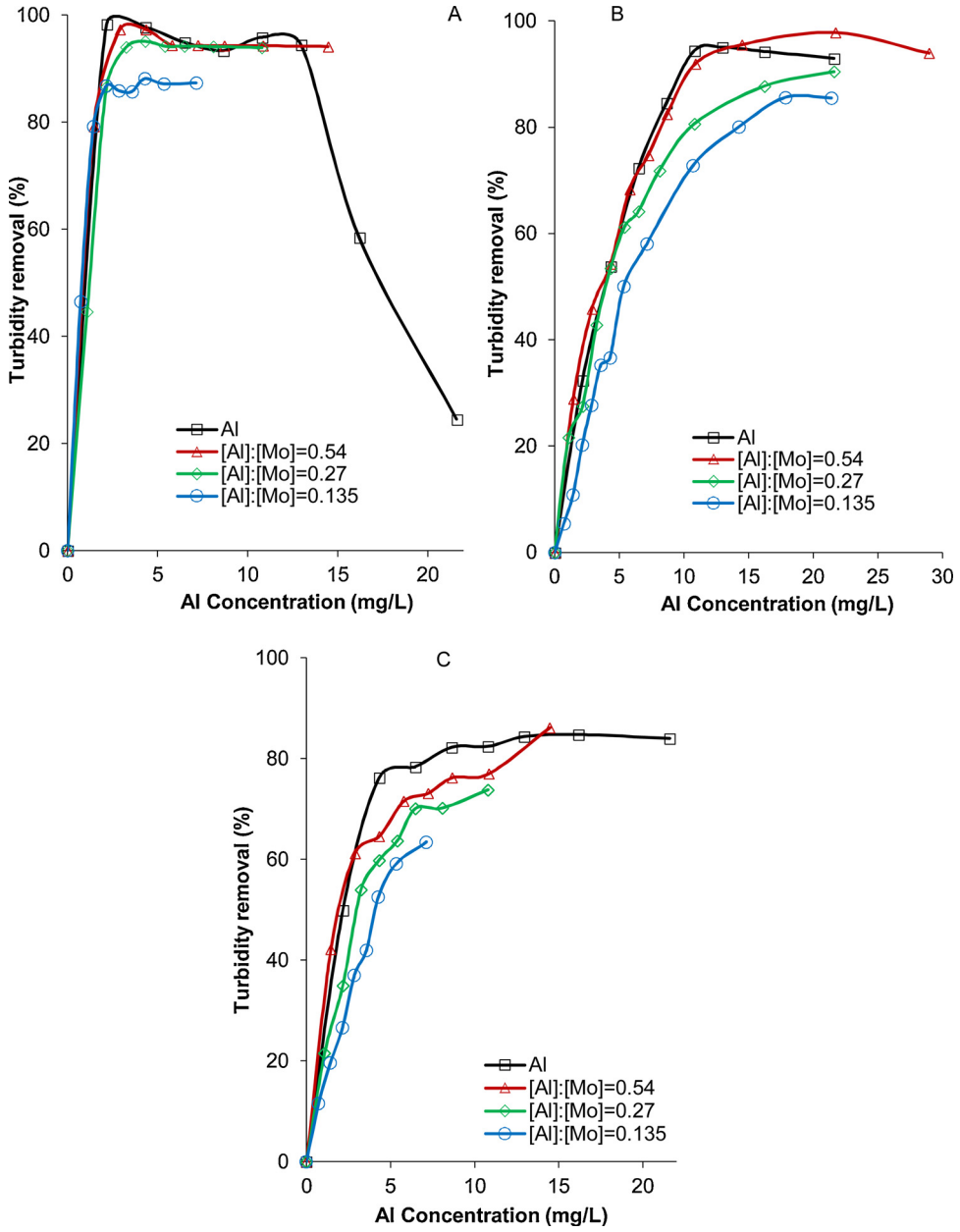

Fig. 5. Turbidity removal: Synthetic water at pH = 6 (A); Synthetic water at pH = 8 (B); Hospital wastewater (C).

where N_i and N_f are the initial and the final value measured of the parameter before and after the coagulation/flocculation process.

2.7. Statistical analysis

Each trial was replicated three times. The results presented are the average of the three trials. Statistical analysis was performed using the Graph Pad Prism 7 software at 5 % significance.

3. Results and discussion

3.1. Characteristics of Moringa oleifera seeds protein

Fourier Transform Infrared spectroscopy of extracted protein was performed to identify the different functional groups on MOP surface. From FTIR spectrum shown in Fig. 1, it is seen a broad absorption band at 3404 cm^{-1} corresponding to the stretching vibration of O—H in alcohol. The peaks at 2912 cm^{-1} and 2850 cm^{-1} are attributed to the C–H aliphatic vibration [29]. The absorption peaks observed at 1643 cm^{-1} and 1532 cm^{-1} are assigned to the C═O vibration in amide I and amide II, respectively which confirms the structure of protein in the *M. oleifera* seeds [30]. The peaks at 1139 cm^{-1}, 760 cm^{-1} are characteristics to the absorption of α-glycosidic and the stretching vibration of C—O—C which are the typical bands of polysaccharide [31,32].

The morphology of MOP was observed by Scanning Electron Microscopy. The images obtained for two magnifications 500X and 2000X are shown in Fig. 2. It is observed that MOP exhibited a heterogeneous morphology characterized by a rougher surface with blocks of irregular shapes and several pores of varying sizes. The presence of these pores may facilitate the removal of wastewater pollutants by adsorption.

The total protein content MOP was quantified by the Biuret method and was 2932.5 ± 17.67 mg L^{-1}. The molecular size of protein was determined by electrophoresis SDS-PAGE. The SDS-PAGE results displayed in Fig. 3 revealed two strong bands around 25 kDa and 37 kDa

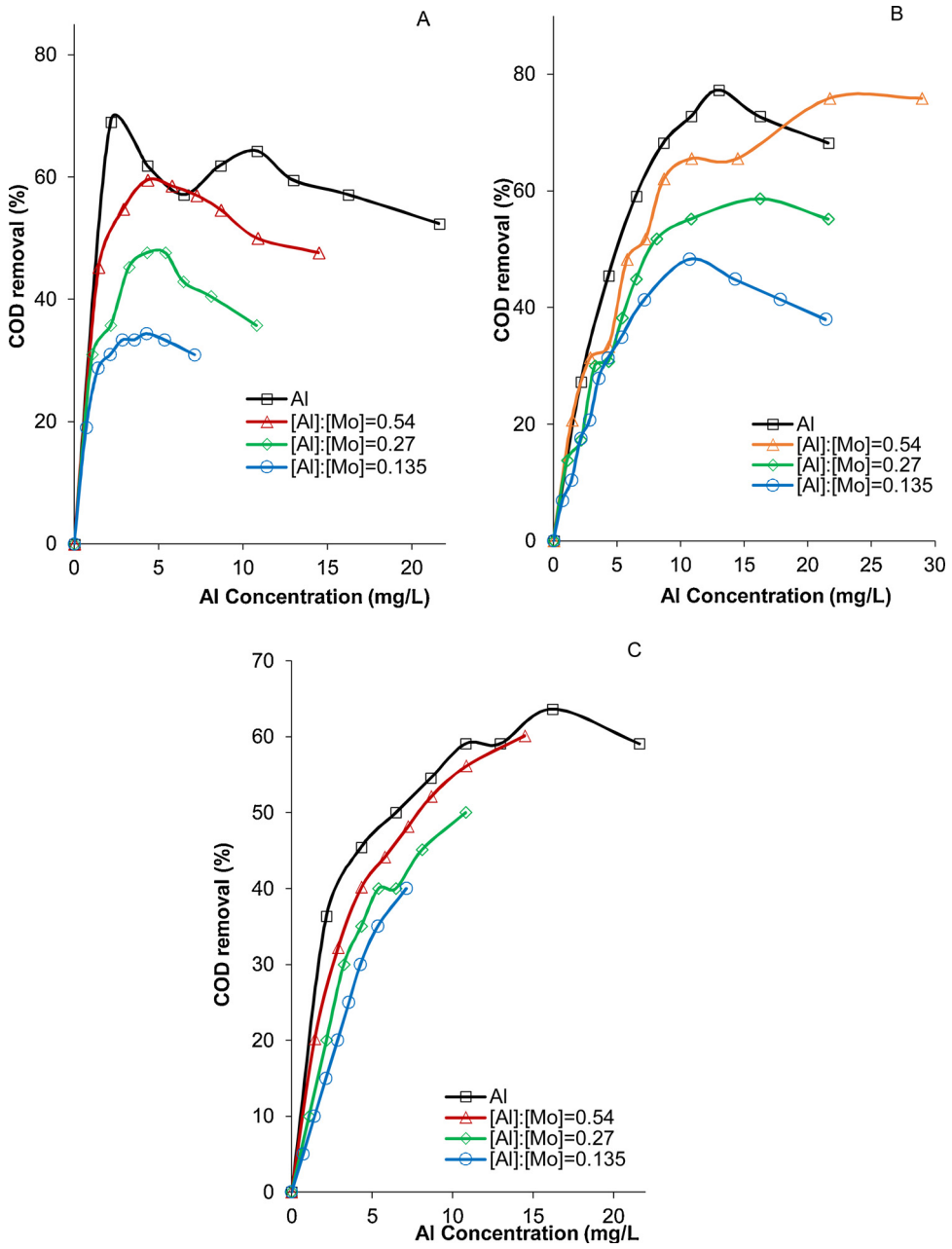

Fig. 6. COD removal: Synthetic water at pH = 6 (A); Synthetic water at pH = 8 (B); Hospital wastewater (C).

which suggesting that MOP was composed by dimeric protein with mean molecular weight of 31.5 kDa. The results were in the best agreement with the earlier reported on *M. oleifera*; the molecular weights of coagulating protein were 26.5 kDa and 30 kDa [31,33].

3.2. Quality of water samples

The characteristics of water samples used in this study are displayed in Table 1. The results show that the high value of HW samples temperature of 29.8 °C is mainly due to the ambient temperature of the study area, which could favor the microbial development. The pH of samples was slightly basic suggesting the development of algae [34]. The samples are slightly turbid and moderately mineralized. The small amount of dissolved oxygen 0.27 mg L^{-1} and the high levels of COD 238 mg L^{-1}, BOD_5 132.21 mg L^{-1} and UV_{254} 0.282 show that the samples were seriously polluted by pathogenic bacteria and also suggest that the bacteria used the dissolved oxygen for organic matters degradation. Bacteria such as *E. coli*, *P. aeruginosa*, *S aureus*, *Salmonella spp* and *V. cholerae* from HW samples had very higher values than the values defined in the Standard [35]. In addition, the bacteria used the dissolved oxygen for the nitrification of NO_3- ion to NO_2- ion, which justified the low concentration of NO_3- ion. Finally, based on the results, it clearly appeared that, the HW samples were polluted by pathogenic bacteria and must be treated before their disposal in environment. Thus, in this work, turbidity, COD, UV_{254}, *E. coli*, *P. aeruginosa* and *V. cholerae* were chosen as parameters to investigate the potential of extracted MOP and prepared MOP-PACl coagulant through the coagulation process.

3.3. Effects of Moringa oleifera seeds protein on turbidity, COD and UV_{254} removal efficiency

Jar-tests with MOP were conducted using synthetic water and hospital wastewater samples. The results obtained for the removal of

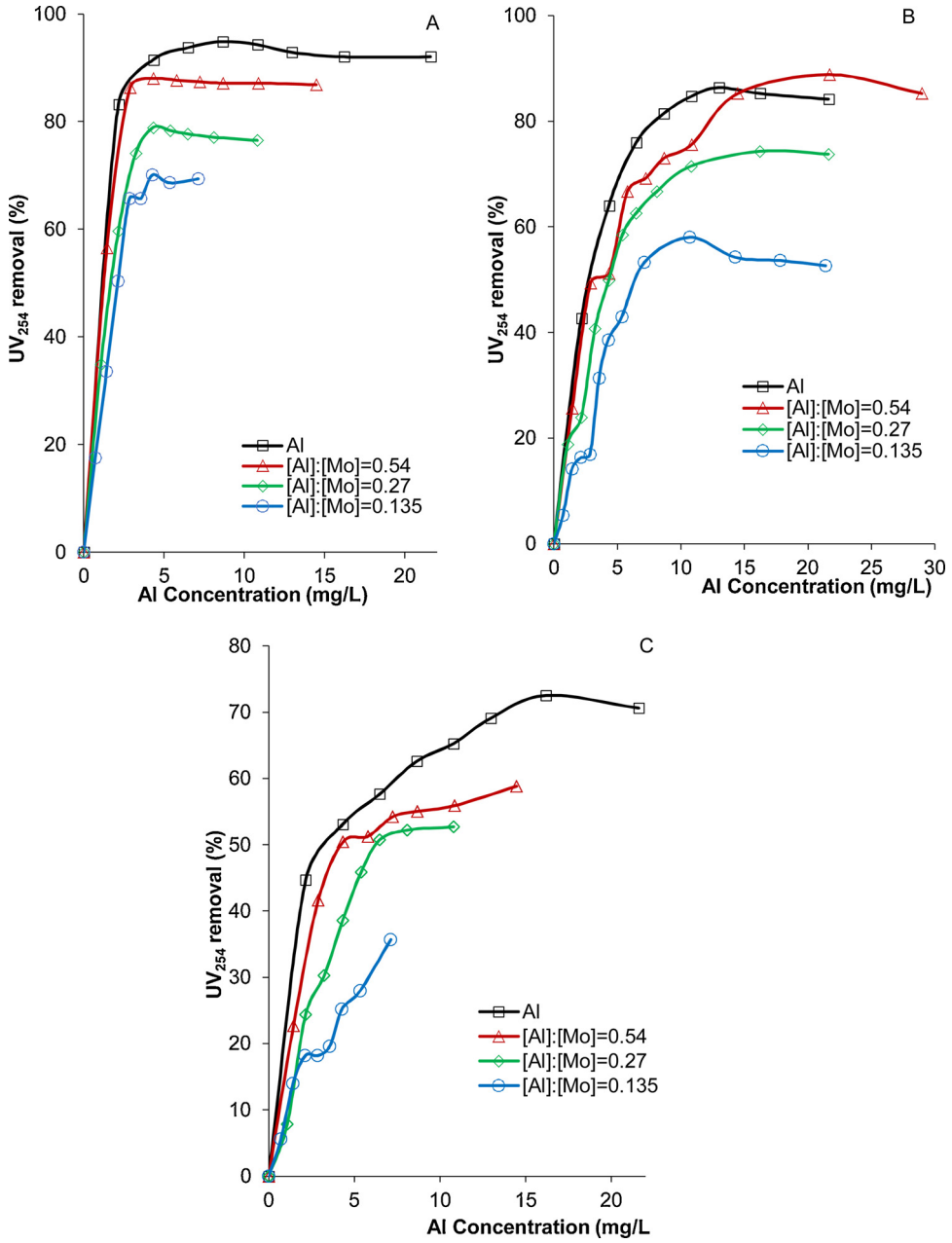

Fig. 7. UV_{254} removal: Synthetic water at pH = 6 (A); Synthetic water at pH = 8 (B); Hospital wastewater (C).

turbidity, COD and UV_{254} were showed in Fig. 4. It can be seen from Fig. 4A that the plots turbidity removal against coagulant dose have the same tend regardless the pH and the samples nature. The removal efficiency of turbidity from SW samples increased from 37.26 % to 82.28 % with increasing of the coagulant dosage from 50 mg L^{-1} to 400 mg L^{-1} at pH = 6 while at pH = 8, the turbidity uptake rises from 30.07 % to 48.14 % at the same MOP dose. The jar-test experiments performed with HW samples showed, an increase in turbidity removal rate from19.99 % to 64 % at natural pH of 7.37 when the MOP dose enhanced from 20 mg L^{-1} to 400 mg L^{-1}. The results also revealed that the turbidity removal rate at pH = 6 was higher than at pH = 7.37 and pH = 8. Furthermore, it has been reported that the isoelectric point of coagulating protein of *M. oleifera* seeds was between 10 and 11 [15]. So, at pH < 10, the nitrogen atom from dimeric protein of MOP would accept a proton from water samples and the MOP surface became positively charged. When MOP is introduced into the water samples, electrostatic attraction occurred between the negative load of pollutant particles from the samples and the positive load of dimeric protein from MOP surface, which leads to the aggregation of the pollutant molecules and their flocculation and sedimentation. Thereby, the removal of turbidity from water samples by MOP would take place by charge neutralization. However, given the pores observed on the MOP morphology, the possibly mechanisms whereby the pollutants from water samples have been removed by MOP are dominated by adsorption and charge neutralization [15,36]. From the results shown in Fig. 4B, the removal percentage of COD from SW samples increased from 18.18 % to 45.45 % at pH = 6, and from 18.18 % to 36.25 % at pH = 8 with rising of MOP dosage from 50 mg L^{-1} to 350 mg L^{-1}. When the experiments were carried out with HW samples, the COD removal efficiency enhanced from 11.53 % to 38.36 % with increasing in MOP dosage from 20 mg L^{-1} to 320 mg L^{-1}. Furthermore, the removal efficiency of UV_{254} increased from 16.91 % to 36.27 % at pH = 6, and from 11.94 % to 33.13 % at pH = 8 while it is seen a low uptake of UV_{254} from HW samples (Fig. 4C) which suggesting that HW samples contain

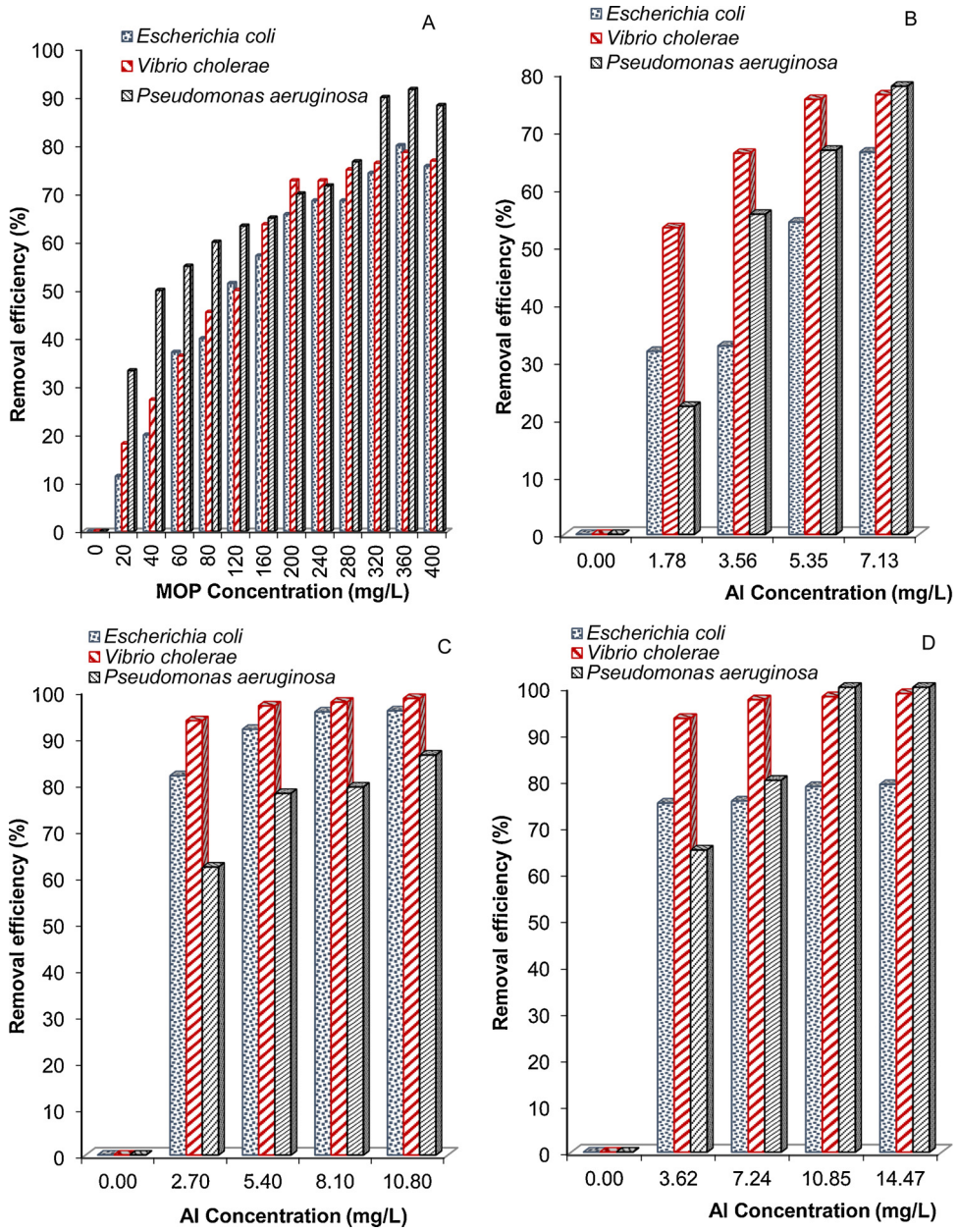

Fig. 8. Bacteria removed: MOP (A); 0.135 ratio (B); 0.27 ratio (C); 0.54 ratio (D).

the large amount of aromatic organic matter.

3.4. Effects of MOP-PACl on COD, turbidity and UV_{254} removal efficiency

The results of turbidity removal with MOP-PACl coagulant are shown in Fig. 5. The results highlighted that the turbidity removal efficiency increased with increasing in the coagulant dosage and the Al:MOP ratio. It is also showed that the turbidity uptake of 88.10 %, 95.05 % and 97.31 % from SW samples were reached at pH = 6 with Al dose in MOP-PACl coagulant of 4.27 mg L^{-1} and using Al:MOP ratios of 0.135, 0.27 and 0.54 respectively (Fig. 5A). However, when the assays were conducted using SW samples at pH = 8 (Fig. 5B) and HW samples (Fig. 5C), it was observed a slight decrease compared to the obtained results at 6 pH. The maximum of removed turbidity 90.45 % and 73.80 % were occurred at Al dosage of 21.6 mg L^{-1} using SW samples at pH = 8 and 10.80 mg L^{-1} with HW samples at Al:MOP ratio of 0.27. The results also revealed that, the MOP-PACl coagulant were really improved the turbidity removing with low Al:MOP ratio of 0.27.

From the results shown in Fig. 6, it can be seen that the rate of COD removed increased with increasing of the Al dosage. At pH = 6, the maximum COD removed 59.52 % from HS samples was achieved at Al dose of 4.32 mg L^{-1} in MOP-PACl coagulant with Al:MOP ratio of 0.54 (Fig. 6A). However, the COD removal efficiency of 58.62 % from SW samples at pH = 8 and 50 % from HW samples were reached using Al:MOP ratio of 0.27 with Al dosages of 21.70 mg L^{-1} and 10.80 mg L^{-1} respectively. It appeared from the results that the rate of COD removed using MOP-PACl coagulant was better than the level with MOP used as single coagulant.

The results displayed in Fig. 7 show a rising in the UV_{254} removal efficiency with increase in the Al:MOP ratios. Thus, at pH = 6, 70.07 % and 78.91 % of UV_{254} were removed using MOP-PACl coagulant with Al:MOP ratios of 0.135 and 0.27 at Al dosage of 4.32 mg L^{-1} (Fig. 7A). When the assays were carried out using SW samples at pH = 8 (Fig. 7B), the UV_{254} removal of 57.99 % with Al:MOP ratio of 0.135, and 74.30 % with 0.27 ratio were obtained using Al doses of 10.69 mg L^{-1} and 16.20 mg L^{-1} respectively, while 52.68 % of UV_{254}

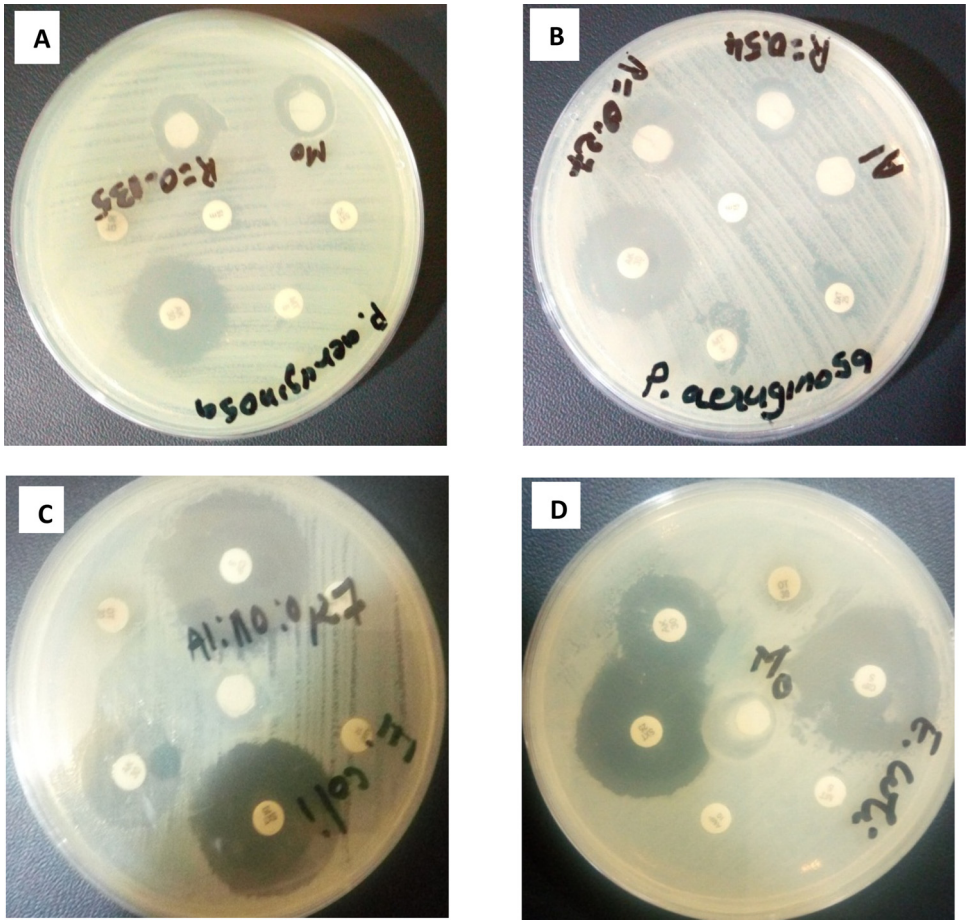

Fig. 9. Antibacterial effects against: P*seudomonas aeruginosa* (A, B); *Escherichia coli* (C, D).

Table 2
Inhibition zone diameter.

	Inhibition zone diameter (mm)	
	Escherichia coli	*Pseudomonas aeruginosa*
Al	12	18
MOP	15	16
0.135 ratio	16	17
0.27 ratio	16	20
0.54 ratio	17	16
OT	10	0
SXT	30	0
MET	0	0
AK	20	23
AMP	0	0
ERY	0	0
CIP	30	32

Oxytetracyclin: OT, Trimethoprim-sulfamethoxazole: SXT, Metronidazole: MET, Amikacin: AK, Ampicillin: AMP, Erythromycin: ERY, Ciprofloxacin: CIP.

was removed from HW samples using 0.27 ratio at Al dose of 10.80 mg L^{-1}. The results highlighted that the coating of PACl in MOP leads to improvement of effectiveness in the pollutants removing from contaminated water.

3.5. Antibacterial activity

The antibacterial efficiency of MOP and MOP-PACl coagulants were tested against bacteria such as *E. coli*, *V. cholerae,* and *P. aeruginosa* strains. The results shown in Fig. 8A revealed that the removal percentage of bacteria increase with increasing in the MOP dosage. So, it was also observed a rising in removal efficiency from 11.42 % to 75.71 % against *E. coli*, 18.91 % to 76.81 % against *V. cholerae,* and 33.33 % to 88.33 % against *P. aeruginosa* strains when increasing of the MOP dosage from 20 mg L^{-1} to 400 mg L^{-1}. The results obtained in this study were compared to the literature values for the removal efficiency in bacteria caused by *M. oleifera* seeds. Morgan et al. [37] reported 87 % against *E. coli* removed at 600 mg L^{-1} while 97.40 % against Salmonella and Shigella was observed by Vunain et al. [22]. The efficiency bacteria removed by MOP may be explained on the one hand by coagulation process which can remove the microorganisms associated with turbidity from water samples through the flocculation and sedimentation processes. On the other hand, the *M. oleifera* coagulating protein can destroy the inner and outer membranes of bacteria cells by the antioxidant compound 4 (α-L-Rhamnosyloxy) benzyl isothiocyanate molecules [38].

The results of assays performed with the MOP-PACl composite coagulant revealed that the antibacterial efficacy of composite coagulant increases with increasing of Al:MOP ratio and Al dosage from each composite coagulant. From the results shown in Fig. 8B, it is observed that the removal rate enhanced from 31.77 % to 66.35 % against *E. coli*, 53.22 % to 76.29 % against *V. cholerae* and 22.22 % to 77.78 % against *P. aeruginosa* strains with Al:MOP ratio of 0.135 at Al dosage in MOP-PACl rising from 1.78 mg L^{-1} to 7.128 mg L^{-1}. The Al:MOP ratios of 0.27 and 0.54 were effectively improved the rate of bacteria removed with 81.5 % to 95.75 % against *E. coli*, 93.6à % to 98.41 % against *V. cholerae* and 62.06 % to 86.21 % against *P. aeruginosa* strains with 0.27 ratio at Al dose rising from 2.70 mg L^{-1} to 10.8 mg L^{-1} (Fig. 8C). Moreover, 0.54 ratio caused removal rate of 75.11 % to 79.55 % against *E. coli*, 93.33 % to 98.66 % against *V. cholerae* and 65 % to 100 % against *P. aeruginosa* strains at Al dosage in MOP-PACl increased from

3.62 mg L^{-1} to 14.47 mg L^{-1} (Fig. 8D). The results also revealed that the Al:MOP ratio of 0.135 showed the same removal efficiency as MOP, but, the best antibacterial potential was occurred with Al:MOP ratio of 0.27.

Finally, the antibacterial activities of MOP and MOP-PACl were also compared against antibiotic-resistant bacteria strains such as *P. aeruginosa* and *E. coli* strains isolated from HS wastewater samples. The results displayed in Fig. 9 and Table 2 exhibited the antibacterial properties of tested coagulants marked by an inhibition zone with mean diameter of 16 mm against *P. aeruginosa* and 15 mm against *E. coli* strains with MOP, 17 mm against *P. aeruginosa* and 16 mm against *E. coli* strains with Al:MOP ratio of 0.135, 20 mm against *P. aeruginosa* and 16 mm against *E. coli* strains with Al:MOP ratio of 0.27, and, 16 mm against *P. aeruginosa* and 17 mm against *E. coli* strains with Al:MOP ratio of 0.54. These results also confirmed that Al:MOP ratio of 0.27 presents the best antibacterial effectiveness. However, the antibacterial potential of MOP was in best agreement with inhibition zone of 28.75 mm against *E. coli* strain and 29.75 mm against *P. aeruginosa* strain reported by Virk et al. [39] with extract concentration of 100 mg mL^{-1} which was higher than the extract concentration of 10 mg mL^{-1} used in the present study. Furthermore, the inhibition zone obtained with MOP-PACl was also in accordance with 15 mm reported by Katata-Seru et al. [24] for antibacterial effect of MOP-ferric chloride composite coagulant against *E. coli* strain. The results also highlighted that MOP and MOP-PACl coagulants used in this study presented the antibacterial property as Amikacin and Ciprofloxacin antibiotics against antibiotic-resistant bacteria strains.

4. Conclusions

Wastewater samples from the Departmental Hospital Center of Atakora in Benin were collected, characterized and then treated using *M. oleifera* seeds protein (MOP) as single coagulant and then as based composite coagulant at polyaluminum chloride (PACl). The results revealed a removal efficiency of 64 % turbidity, 16.54 % UV_{254}, 38.36 % COD, 75.71 % against *E. coli*, 76.82 % against *V. cholerae* and 88.83 % against *P. aeruginosa* strains using MOP. When MOP was used as coagulating aid to PACl, the removal rates of 73.80 % turbidity, 50 % COD, 52.68 % UV_{254}, 95.75 % against *E. coli*, 98.40 % against *V. cholerae* and 86.21 % against *P. aeruginosa* strains were obtained using Al:MOP ratio of 0.27. The antibacterial tests investigated revealed that 0.27 ratio had a best antibacterial potential with inhibition zone of 20 mm against *P. aeruginosa* and 16 mm against *E. coli* strains. Results from this study also shown that MOP-PACl composite coagulant well improved the treated water quality. In addition, 0.27 ratio appeared as the best suitable for the achievement in eliminating of the pollutants characterizing our wastewater samples due to its less aluminum and more MOP amount content. So, the resulting sludge from its use would present high biodegradability index than those with higher ratios.

Declaration of Competing Interest

We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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