



## Antibacterial and Dye Removal Potential of Modified Kaolinite for the Treatment of Drinking Water

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### Abstract

**Objective:** Poor treatment of drinking water before distribution leads to the consumption of contaminants causing water borne type of ailments due to the presence of the pathogens and cancer related illnesses as result of the presence of dyes. This research focused on the development of new organoclay based composite that serves as both antibacterial and dye removing agent for the treatment of microbial and dyes contaminated water from the source.

**Material and Methods:** The cation exchange capacity (CEC) of the kaolinite was improved through acid treatment. Chlorhexidine- loaded zinc- kaolinite was prepared via adsorption of chlorhexidine acetate (0.5 mmol/L) on zinc-kaolinite. The composites were characterized using Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray (EDX). The antibacterial assays of the composites were conducted against *Staphylococcus aureus* (*S. aureus*) and *Salmonella typhi* (*S. typhi*) using disc diffusion technique (DDT), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).

**Results:** The CEC value of the acid treated kaolinite (Kaot2) was improved from 9.26 + 0.82 to 13.43+1.61 meq/100g, the morphology of the composite remains intact and indicate the presence of Zinc (Zn) after formulation. The target composite (Chx-Zn-Kaot2) shows its effectiveness against *S. aureus* and *S. typhi* showing the inhibition zones of 26 mm and 1.5 mm respectively. Similarly, MIC, with 120 mg/mL inhibit both organisms while MBC revealed that the target composite, 60 mg/mL kills *S. aureus* and 120 mg/mL kills *S. typhi* respectively. The dye removal efficiency of the target composite is concentration dependent at the highest concentration can remove up to 83.79% and 91.75% of both CR and MB dyes respectively.

**Conclusion:** The formulated target composite is a good candidate for the treatment of drinking water contaminated with such microorganisms and can be able to remove substantial content of dyes.

## Introduction

Access to portable drinking water is of great importance to human survival. In most of the developing countries the source is mainly from the tap which is sourced often from the dam or rivers and partially treated and distributing for consumption. Due to the improper treatment which is usually percolation and sedimentation, in which the growth of microorganisms is supported and some might be pathogenic strains. This product is chosen because the

materials used for the synthesis have low cytotoxicity due to their concentrations.

The carrier is clay type known as kaolinite is available and affordable specifically in Nigeria. This type of clay is available in some part of the country but it must be purified. The microbial strains used are *S. aureus* (Gram positive) and *S. typhi* (Gram negative). This product which is in powder form can be included in treating water from the treatment plant(s)



before finally discharge for consumption. This will kill the microorganism present and prevent the incidence of cholera and other related outbreak due to the ingestion of these pathogens in the drinking water. The raw materials to be used for the synthesis are affordable and available in the north western Nigeria.

Clay minerals are widely considered as promising materials by virtue of their availability, low cost and easy to manipulate. Among the manipulations include mechanical, physical and chemical processes. Clay minerals are negatively charged in nature; as such it might be a suitable carrier for positively charged particles or metal ions due to their unique characteristics, as such, they can be coupled with positively charged metallic ion such as zinc ion ( $Zn^{2+}$ ) to counterbalance the charges forming Zn-clay complex. Kaolinite as one of the naturally occurring clay and is traditionally used as a nano filler and added in paper coating agent, paints, inks extenders, pharmaceutical ingredient as well as a pharmaceutical excipient [1, 2]. Despite the low cost and its abundance in nature, kaolinite is rarely used as a carrier due to its lower specific surface area and lower cation exchange capacity (CEC) value [3, 4]. Thus, due to this poor absorption capacity of kaolinite as compared to the other clay counterpart, there is a need to improve the absorption efficiency of this clay minerals. For instance, montmorillonite was described to have high cation exchange capacity as well as broad specific surface area compared to the other clay minerals [5]. These clay minerals including, montmorillonite, smectite, bentonite, sepiolite are widely used as adsorbents due to their low cost [6]. Others like kaolinite and zeolite acquire unique characteristics which make them too suitable carriers for cationic materials [7]. However, treatment of clay with acid was report to have effect on adsorption of metals such as Arsenic, Chromium, Cadmium, Copper, Cobalt, Iron, Manganese, Lead, Nickel, Zinc and Mercury [8, 9].

Therefore, improving the absorption properties of the kaolinite is necessary in order to make it more suitable for experimental and practical purposes [10]. In this study, the aimed was to come up with the method that can enhance the absorption capacity of the kaolinite due to its availability as raw material. For instance, the acid activation is one of the chemical treatment used to activate clay minerals [11], such as montmorillonite [12, 13] and hallosite [14]. This acid activation involves the reaction of the clay minerals with an

acid solution such as hydrochloric acid, sulphuric acid, nitric acid among others [15]. Which leads to the alteration of the clay minerals through the functionalization of leached groups and due to that, the surface can be coated with organic molecules changing it to more hydrophobic and this can be used as nanocomposite polymer materials [16, 11].

Humans interact with microorganisms in almost all walk of life being small and invisible to the naked eyes. Some of them are of immense benefit to both plants and animals, human inclusive; while others are pathogenic to the extent of being fatal. To combat this pathogenic threat, several antibacterial agents were produced and commercialized [17]. Yet, the epidemic of the diseases caused by various pathogenic bacteria is alarming and is attributed to the antibacterial resistance in which the microorganisms often developed, contributing to the treatment complication [18]. Thus, development of antimicrobial agent is needed to curtail this menace.

With this continues demand, the development of antimicrobial composite using clay and metals as reported by [19, 20] and other used the organic compounds for the formulation against *E. coli* and *S. aureus* [21, 22].

Chlorhexidine is one of the examples of an organic compound with antibacterial activity. Chlorhexidine acetate; was described as an organic salt belonging to bisbiguanide family and possesses a broad antibacterial activity which is effective against both Gram negative and Gram positive bacteria [4, 23]. It is said to be mild with no side effect so far to humans and widely used in relieving inflammation, as disinfectant and in washing wound surface [23]. Besides the antibacterial properties of Chlorhexidine acetate, it also has organophilic ability to adhere to the bacterial cell surface as they are naturally hydrophobic [24].

In a similar way, it was reported that some of the metals exhibit antibacterial activity such as zinc, silver and copper [25]. Among the metal ions listed, the silver ion is having the highest antimicrobial effect against over 650 pathogens with relatively low toxicity on animal cells [26]. Silver ion is being used for decades as a disinfectant against both Gram-positive and Gram-negative bacteria with a broad spectrum [27]. The use of these metal ions with the propensity of killing microorganisms are now included in the area of research for their modification to synthesize effective antimicrobial products.

Therefore, in this study, Chlorhexidine loaded Zinc-kaolinite as a future antimicrobial agent composite is feasible. Zinc ions and Chlorhexidine cation as positively charged forming an electrovalent bond with negatively charged surface of kaolinite. The cell wall of microorganism can be attracted by this positively charged complex which are expected to have antimicrobial activity and synergistic action by disrupting the cell wall and subsequently the cytoplasmic membrane through induction of oxidative stress and leads to the rupture of the cell wall and death.

## Materials and Method

### Acid Activation Procedure of Kaolinite

Raw kaolinite sample was added to 1.0 M sulphuric acid ( $H_2SO_4$ ) in the ratio of 1:20 (Kaolinite: acid) and was stirred overnight (18-24hrs), allow to settle. The treated kaolinite sample was rinsed and decanted five times until the excess acid removed and finally, the samples filtered and oven dried at  $90^\circ C$ . The sample was then subjected to Cation Exchange Capacity according to Isah *et al.*, [28]

### Determination of Cation Exchange Capacity (C.E.C) Value

Firstly, 0.2g of the activated kaolinite sample was weighed, added into 50ml capacity falcon tube. Then, 6.6ml of 1.0 M ammonium oxaloacetate ( $NH_4OAc$ ) solution was pipetted in to the tube containing the sample. The tube was capped and then placed on orbital shaker at 250 rpm for 10 minutes. After the mixing, the mixture was centrifuged at 2000 rpm for another 10 minutes. The supernatant was discarded and the procedure repeated up to three more times. After that, 6.6 ml of 2-propanol (Isopropanol) were dropped into the tube so as to remove excess ( $NH_4OAc$ ). Then, re-cap and shaken again at 250 rpm using mechanical shaker for 10 minutes. This mixture was centrifuged for 10 minutes at 2000 rpm and the supernatant discarded. This process was repeated two more times. Finally, in order to leach ammonium ions ( $NH_4^+$ ) from the sample, 6.6ml of 1.0 M of prepared sodium acetate ( $NaOAc$ ) was added into the tube containing the sample. Tightly cap the falcon tube, and then placed on shaker at 250 rpm for 10 minutes. The leachate was added to 50 ml container (volumetric flask). This step will be repeated three (3) times. The sodium acetate solution was added in to the combined leachate solution until it reach 50 ml mark. In order to determine ammonium concentration that leached

out during the CEC process, UV-VIS spectrophotometer was used with the aid of ammonium test kit. The assessment was done in triplicates for each sample. After that the cation exchange capacity (CEC) value was calculated based on the absorbance obtain from UV- vis spectrophotometer. The calculation used to achieve it is as follows with 5.0 mg/L as an example:

Step 1: Convert 5.0 mg/L to g/L =  $5.0 \times 10^{-3}$  g/L

Step 2:  $50 \text{ mL} = (0.05 \text{ L}) = 5.0 \times 10^{-3} \text{ g/L} \times 0.05 \text{ L} = 0.00025 \text{ g } NH_4$

Step 3: Molar mass of  $NH_4 = 18 \text{ g/mol}$

Step 4: No. of moles in 0.2g of sample =  $0.00025 \text{ g} \div 18 \text{ g/mol} = 1.39 \times 10^{-5}$

$\therefore 1 \text{ g of sample} = 0.0000694444 \text{ mol/g}$

Step 5:  $1 \text{ meq} = 6.02 \times 10^{-20} \text{ adsorption site} = 0.001 \text{ mol}$ .

$\therefore \text{C.E.C in meq/g} = 0.000091666 \text{ mol/g} \div 0.001 \text{ mol} \times 1 \text{ meq} = 0.069444 \text{ meq/g} = 6.9444 \text{ meq/100g}$  or  $6.9 \text{ meq/100g}$ .

### Preparation of the composite

The preparation of composite was done by obtaining 10 beakers containing 50mL of chlorhexidine solution (0.05M). Into each beaker, 2g of Zinc-kaolinite (Zn-Kao) was added and stirred overnight at room temperature. The sample was filtered next day using filter paper. Liquid part discarded, while the solid portion was washed with distilled water twice and then, oven dry at  $80^\circ C$  overnight. The dried solid was crushed using mortar and pestle and then sieve. The samples were kept in a glass bottle prior to use.

### Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray (EDX) procedure

The composite was analysed by CARL ZEISS 35 VP Supra FESEM. The sample in a powder form and placed on to the sample holder, coated with platinum (Pt) by using auto-fine coated model JFC-1600. The coating process was done in 10 minutes. The coated samples each was placed on FESEM sample holder and observed using the magnitude of 10,000 and 50,000. FESEM instrument was equipped with Energy Dispersive X-ray (EDX) (JED-2300 analysis station). It was conducted at CS Nano research centre Malaysia.

### Antibacterial assay

## Disc Diffusion Techniques (DDT)

One of the antibacterial activity of the composite was assayed using Disc Diffusion Techniques (DDT). The method employed was according to the National Committee for Clinical Laboratory Standards [29]. In which 0.2 g of each was pressed by using hydraulic pressure pump at pressure of 300 psi in to pellets and then kept for later use. Each bacterium (*S. aureus* and *S. typhi*) were cultured on NA at 37°C for 24 hours; five (5) colonies of each organism were diluted with sterile 0.9% saline solution and the suspension adjusted to 0.5 McFarland turbidity ( $1.5 \times 10^8$  CFU). A sterile cotton swab(s) was used for inoculating the surface of another freshly new NA plate via rotating the plate every 60° for uniformity of organism. The pellets prepared were positioned over the surface of the agar plate. The diameter of inhibition (in mm) of each was measured after 16 to 18 hours of incubation.

## Minimum Inhibition Concentration (MIC) and Minimum Bactericide Concentration (MBC)

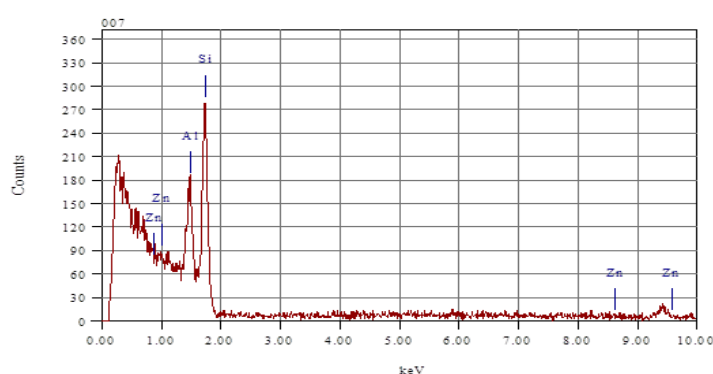
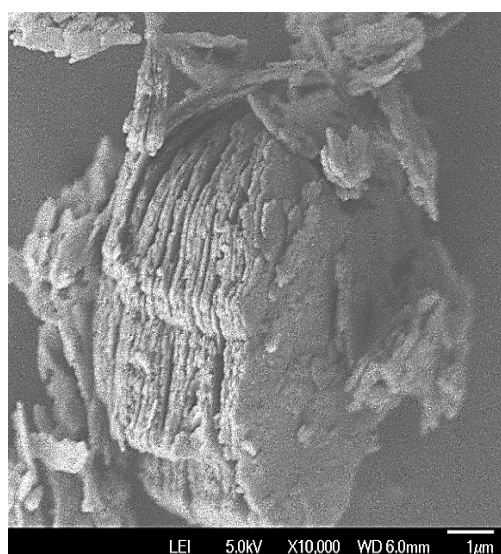
Determination of MIC, MBC and MFC of the composite against the test organisms were conducted in accordance with the Usman *et al.*, [30], with little modification. This was done by preparing concentrations using nutrient broth for bacterial studies. The concentrations were 120mg/mL, 60mg/mL, 30 mg/mL, 15 mg/mL and 7.5 mg/mL respectively. The concentration was aseptically inoculated with the test organism and incubated at 37°C for 24 hours. After which the broth was observed for the presence or absence of growth based on the turbidity. The MIC was taken as the lowest concentration that prevent the growth of the test organism. While in MBC, sample was taken from the broth with no visible growth in the MIC assay and sub-cultured on freshly prepared agar and incubated at 37°C for 24 hours. The MBC was taken as the concentration of in which the composite did not show any visible growth on new agar plate.

## Results

**Table 1:** Result of the CEC values of raw and acid treated Kaolinite which clearly shows there is an increase in the values after treatment of kaolinite with acid

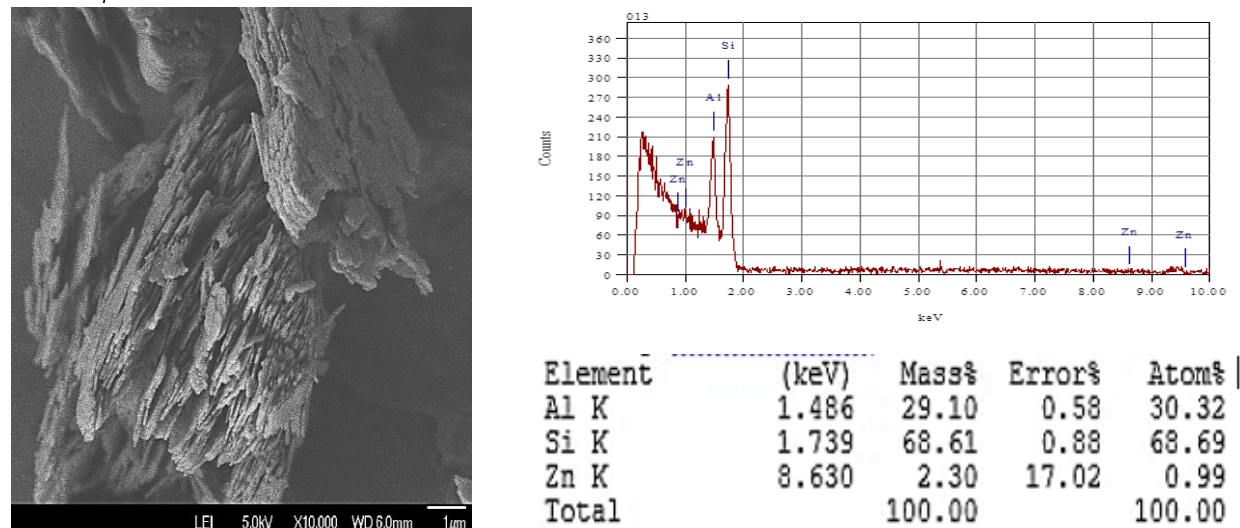
Samples	Description of acronyms	CEC Value (meq/100g)
Kao	Raw kaolinite	9.26 ± 0.82
Kao-t <sub>2</sub>	H <sub>2</sub> SO <sub>4</sub> -treated kaolinite	13.43 ± 1.61

Kao = raw kaolinite, Kao-t<sub>2</sub> = Acid treated kaolinite

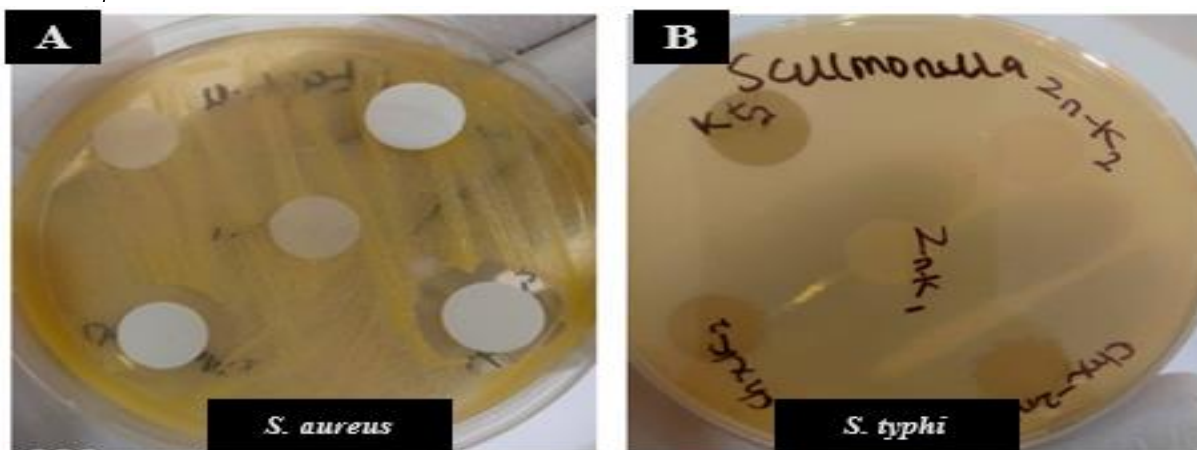


Element	(keV)	Mass%	Error%	Atom%
Al K	1.486	23.64	0.77	25.1
Si K	1.739	70.87	1.08	72.4
Zn K	8.630	5.49	21.24	2.4
Total		100.00		100.0

**Figure 1:** The FESEM-EDX of the Zn-Kaot<sub>2</sub> this shows the morphology of the acid treated kaolinite linked with zinc before the incorporation of the chlorhexidine acetate



**Figure 2:** The FESEM-EDX of the Zn-Kaot<sub>2</sub> this shows the morphology of the acid treated kaolinite linked with zinc after the incorporation of the chlorhexidine acetate



**Figure 3:** Result of disc diffusion technique showing the zones against (A) *S. aureus* and (B) *S. typhi* which is observed in the disc of Chx-Zn-Kaot<sub>2</sub>

**Table 2:** Result of Zone of inhibition of the composites using Disc Diffusion Technique (mm), this indicates that Chx-Zn-Kaot<sub>2</sub> shows wider zone of inhibition against both organisms

Organism	Kao-t2	Zn-Kao t2	Chx-Kaot2	Chx-Zn-Kao t2
<i>S. aureus</i>	NZ	NZ	20	26
<i>S. typhi</i>	NZ	NZ	1.5	1.5

NZ = No zone of inhibition

**Table 3:** Result of Minimum Inhibition Concentrations (MIC), the composite Chx-Zn-Kaot<sub>2</sub> which is the target, shows there is no turbidity observed at 30 mg/mL for *S. aureus* indicating it is the minimum concentration that inhibits the growth of this organism while *S. typhi* shows no turbidity at the concentration of 120 mg/mL indicating it is minimum inhibition concentration that halts its growth.

Sample	Organism	Concentrations (mg/mL)				
		120	60	30	15	Contro l
	<i>S. aureus</i>	+	+	+	-	+

Chx-Zn-Kaot <sub>2</sub>	<i>S. typhi</i>	+	-	-	-	+
	<i>S. aureus</i>	+	+	-	-	+
Chx-Kaot <sub>2</sub>	<i>S. typhi</i>	-	-	-	-	+
	<i>S. aureus</i>	-	-	-	-	+
Zn-Kaot <sub>2</sub>	<i>S. typhi</i>	-	-	-	-	+
	<i>S. aureus</i>	+	+	-	-	+
Kaot <sub>2</sub>	<i>S. typhi</i>	-	-	-	-	+

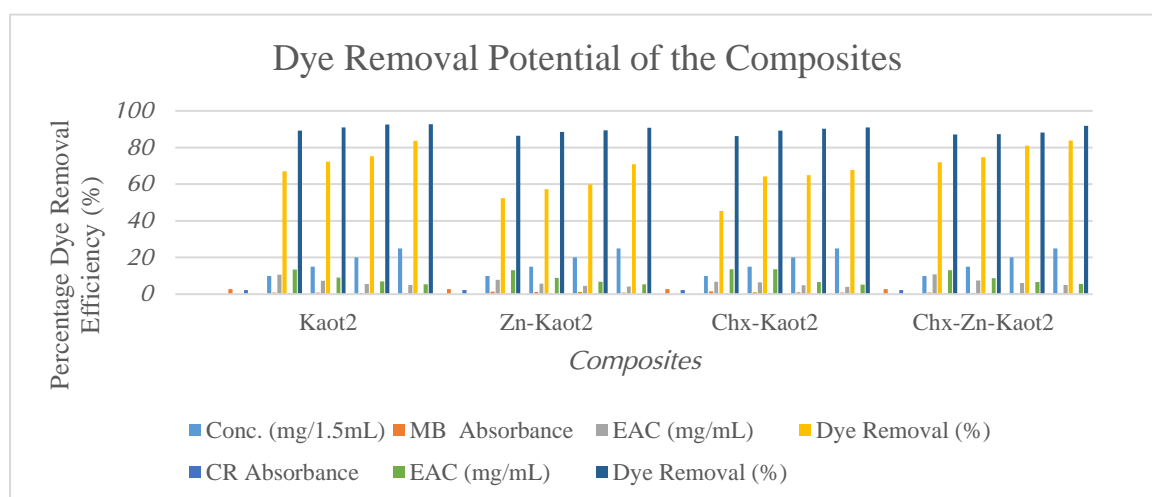
Positive represent = No turbidity, Negative represent = Turbidity present

**Table 4:** Result of Minimum Bactericidal Concentration (MBC), the target composite Chx-Zn-Kaot<sub>2</sub> kills *S. aureus* at 60 mg/mL while for the *S. typhi*, the concentration of 120 mg/mL kills it.

Organisms	Samples' Concentration (mg/ml)			
	Chx-Zn-Kaot <sub>2</sub>	Chx-Kaot <sub>2</sub>	Zn-Kaot <sub>2</sub>	Kaot <sub>2</sub>
<i>S. aureus</i>	60	120	>120	120
<i>S. typhi</i>	120	>120	>120	>120

**Table 5:** This table is showing the concentration of different composites and the percentage dyes they can remove. The percentage dye removal of both MB and CR are appreciably observed in the target composite Chx-Zn-Kaol<sub>2</sub> with 83.79% and 91.75% removal efficiency for CB and MB respectively

Composite	Conc. (mg/1.5mL)	MB Absorbance	EAC (mg/mL)	Dye Removal (%)	CR Absorbance	EAC (mg/mL)	Dye Removal (%)
Kaol <sub>2</sub>	0	2.794	0.00	0.00	2.194	0.00	0.00
	10	0.920	10.61	67.08	0.236	13.39	89.24
	15	0.775	7.23	72.26	0.200	9.09	90.88
	20	0.694	5.64	75.16	0.162	6.95	92.62
	25	0.460	5.01	83.54	0.159	5.37	92.75
Zn-Kaol <sub>2</sub>	0	2.794	0.00	0.00	2.194	0.00	0.00
	10	1.333	7.84	52.29	0.299	13.0	86.37
	15	1.196	5.72	57.19	0.252	8.85	88.51
	20	1.119	4.50	59.95	0.231	6.71	89.47
	25	0.813	4.24	70.90	0.202	5.45	90.79
Chx-Kaol <sub>2</sub>	0	2.794	0.00	0.00	2.194	0.00	0.00
	10	1.527	6.80	45.35	0.302	13.63	86.24
	15	1.000	6.42	64.21	0.238	13.55	89.15
	20	0.980	4.87	64.92	0.212	6.68	90.34
	25	0.920	4.02	67.71	0.200	5.17	90.88
Chx-Zn-Kaol <sub>2</sub>	0	2.794	0.00	0.00	2.194	0.00	0.00
	10	0.784	10.79	71.94	0.284	13.06	87.06
	15	0.704	7.48	74.80	0.279	8.70	87.28
	20	0.532	6.07	80.96	0.260	6.61	88.15
	25	0.453	5.03	83.79	0.181	5.51	91.75



**Figure 4:** Chart indicating the percentage potentiality of the prepared composite in removing methylene blue and Congo red dyes

$$\text{EAC (mg/mL)} = V(C_o - C_e)/m \dots (1) \text{ as in [41]}$$

$$\% \text{ Removal efficiency} = 100 - \frac{\text{Absorbance of the test}}{\text{Absorbance of the control}} \times 100 \dots (2)$$

EAC = Equivalent Adsorption Capacity (mg/ml), V = volume of the dye concentration used, Co = Initial concentration, Ce = Final concentration after 120 minutes [39]

## Discussion

Devising a good process of water treatment for the well-being of the community is paramount important. The poor treatment of the drinking water leads to many diseases associated with their contamination. In this study, the target composite was formed and it shows an excellent efficiency in killing the bacteria and dye removing effect.

The table 1, indicated the increase in cation exchange capacity of the kaolinite after being treated with 1.0 M of sulphuric acid, as such the composite can interact with cations substances whether organic or inorganic with the clay materials as reported by Wang *et al.*, [12] due to the increase in surface charges. This probes the report by Provis, [31], whose reported that treatment of clay with alkali improve the adsorption capacity of the natural clay. Also report revealed that acid causes leaching on the clay surface thereby increasing surface area [35]

FESEM linked with EDX as in figure 2; is the standard analytical method use to investigate elemental composition of a given material and provide more details in terms internal morphology which possess the magnification power of approximately 30,000 times [32]. Thus, this study, FESEM revealed the morphological features of the

composites before and after chlorhexidine was added. Figures 1 and 2, show the micrographic image together with the corresponding EDX spectra of these modified composites indicating the zinc ions remain linked to the treated clay. In all the images, acids treated and modified clay appeared to have the same morphology. Therefore, these results proved that structural framework of the composites remain intact prior to addition of chlorhexidine as well as after the incorporation and EDX was used in detecting the presence of metal ions in the samples [32]. Aluminum (Al) and silicate (SiO<sub>4</sub>) are the major components of the kaolinite. These spectra proved that the element zinc ion are effectively adsorbed on to the kaolinite studied and remain available after incorporating chlorhexidine. showing low intensity of the peak signifies the low zinc content used in this process; therefore, its loading on the kaolinite structural framework is expected to be low. A report was revealed that untreated kaolinite did not apparently adsorbed metal ion prior to acid treatment [32].

The above result of antibacterial effect in figure 3, indicate the effectiveness of this composite as

antibacterial agent against *S. aureus* and *S. typhi* that are often found in the contaminated water in which the later known to cause food and water borne related illnesses. Kaolinite itself has no charge naturally, but during the process to obtain the part of it, the charges generated due to the broken edges making it to have negatively charged edges and surfaces. The charges generated are not many but a lots of processes show that the charges can be enhance upon treatment with chemicals such as acid, alkali and other organic solvents. Many finding including this, proved that kaolinite has no antimicrobial effect but intercalating it with the chlorhexidine acetate and zinc in the composition proves the composition is a good antibacterial agent. In the composition, both zinc and chlorhexidine are positively charged inorganic and organic materials thus, making them attractable to the negatively charged kaolinite. During the action in an aquatic environment, there would be a dissociation to have a freely zinc ions and chlorhexidine which eventually attract the microbial cellular wall. Chlorhexidine is known to destruct the cell wall of the microorganisms and generation of oxygen reactive species leading to the puncture of the cytoplasmic membrane hence, the leaking of cytoplasmic content. The zinc also induces the generation of oxygen reactive species but in some bacterial species unlike the chlorhexidine, but their combination might provide the synergistic effect in

killing the microorganisms as shown in the figure 3 (b), in which the zone of inhibition was high compared to the one without zinc. However, the presence of chlorhexidine and zinc in a content when consume to cause any side effect have not been established. In the case of zinc, recently, Aliabadi *et al.*, [33]), reported that zinc complexes indicated a stronger antimicrobial effect against *S. aureus* and *S. epidermidis*. Similarly, microcapsules of encapsulated chlorhexidine acetate coated with silver particles exhibit a promising antibacterial effect for more than 30 days and this same method of modification can be applied to polymer film surface [34]. The synthesis of multifunctional antibacterial nanocoatings made from polydimethylsiloxane (PDMS) with sublimated chlorhexidine powder was effective against *Escherichia coli* K12, and confer no toxicity [16]. The report in one of the findings show that related composite containing chlorhexidine and zinc had no cytotoxic effect on hela cells [28].

The above data presented in table 5 and figure 4, show that this prepared composite (Chx-Zn-Kaot<sub>2</sub>) is an excellent candidate for removing the reference dyes used in this research. An appreciable percentage removal of dyes was an indication of being it as dye removal agent with an increase in the weight of the composite, the more the removing effect raised. Conversely, the equivalent adsorption capacity decreases upon increase in the composite

content. This is obvious due to the clumping nature of the natural clay, the more the content is increased, the lesser the access of the dye to the surface of the clay. This finding is consistent with the finding of Mukherjee *et al.*, [35], where a report emerged that treatment of natural clay with electrolytes enhance surface adsorption potential of dyes. Similarly, alkaline treatment of carbon from date press cake increases the surface area and enhances methylene blue adsorption [36]. Also activating carbon with calcium nitrate solution increases the adsorption capacity of the methylene up to tenth fold [37]. Recently, Tegin and Saka, [38], reported that chemical plus thermal activation of natural clay significantly improved the adsorption of methylene blue. Similarly, Unuabonah and Taubert, [39], used clay polymer for the successful water treatment.

of this study:

- The use of the formulated composite is said to safe as the concentration of the components used are not toxic.
- When the composite is used, there is need for subjecting it to filtration process to avoid suspended particles that cannot settle instantly.
- Similar work can be done for more prove.

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## Conclusion

The composite was successfully formed and characterized which found to have increased cation exchange capacity on the surface of kaolinite and nature of the composite was identified structurally and the composition showed the presence of each component used for its formation. The composite proven to perform double functions as antibacterial and dye adsorption effectiveness which make it to be a good candidate for treatment of contaminated water with microbes and dyes for proper hygienic water supply.

## Recommendations

The following are recommended based on the findings

## List of abbreviations

MHB = Müller-Hinton nutrient medium; MIC = Minimum inhibitory concentration; MBC = Minimum inhibitory concentration; DDT = Disk diffusion technique; CEC = Cation exchange Capacity; FESEM = Field emission Scanning electron microscopy; EDX = Element dispersive x-ray; MB = Methylene blue; CR = Congo red

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