

# Antimicrobial Activity of Beneficiated Kaolin Clay Supported With Silver and Zinc Oxide Nanoparticles for Removal of Total Coliforms, Faecal Coliforms, *Clostridium Perfringens* and *E. Coli* from Domestic Wastewater

OGUNDIPE F.O<sup>1</sup>, SAIDU M<sup>2</sup>., ABDULKAREEM, A.S.<sup>3</sup> and BUSARI, A.O<sup>2</sup>.

<sup>1</sup>Federal Ministry of Water Resources, Abuja, Nigeria

<sup>2</sup>Department of Civil Engineering, Federal University of Technology, Minna, Nigeria

<sup>3</sup>Department of Chemical Engineering, Federal University of Technology, Minna, Nigeria  
Corresponding Author: Ogundipe F.O

Date of Submission: 05-02-2023

Date of Acceptance: 20-02-2023

## ABSTRACT

This study describes antimicrobial effect of supported Beneficiated Kaolin Clay (BKC) with ZnO, AgO and Ag/ZnO nanoparticles for the removal of total coliforms, faecal coliforms, clostridium perfringens and E. Coliform from domestic wastewater using the nanocomposite adsorbents produced from kaolin clay, AgO and ZnO Nanoparticles formed in a single-step green synthesis process using Mangifera Indica plant extract. The crystal structure, morphology and formation of the nanocomposite kaolin clay adsorbents produced for the removal of these selected contaminants were confirmed using X-Ray Diffractometer (XRD), Dispersive X – Ray Fluorescence (XRF) Machine, High Resolution Transmission Electron Microscope (HRTEM) and Brunauer – Emmett – Teller (BET) Nitrogen Absorption Analyzer. Effect of temperature, contact time, and adsorbent dosage of the removal process were investigated. The order of removal efficiency for total coliforms, faecal coliforms, clostridium perfringens and E. Coli in the domestic wastewater is BKC/AgO/ZnO > BKC/AgO > BKC/ZnO > BKC. The nanocomposite adsorbents produced were potent against the test organisms. This

study showed clearly that the bactericidal effect of AgO nanoparticles was more effective than ZnO nanoparticles.

**Key words:** Green Synthesis, AgO, ZnO, Nanoparticles, Mangifera Indica, Kaolin, Kutigi

## I. INTRODUCTION

[5]. One of the major scientific challenges of this century is wastewater management and its potential impacts on freshwater quality. Globally, most of the wastewater from homes, cities, industry and agriculture flows back to nature without being properly treated or reused thereby polluting river water body. The wastewater, depending on its source, contains contaminants of high levels of microbes of serious concern. The economic and health impact arising from the effect of untreated wastewater discharging into river water body is quite enormous. [9]. Wastewater effluents particularly in developing countries like Nigeria are in most cases discharged into the adjoining environment with water bodies being mostly affected. Some of these wastewater effluents are untreated or inadequately treated before being discharged.

The water pollution caused by faecal contamination is a serious problem due to the potential for contracting diseases from pathogens. Coliforms such as *Escherichia coli* come from the same sources as pathogenic organisms. Total coliform counts give a general indication of the sanitary condition of water. They include bacteria that are found in water that has been influenced by surface water runoff and human or animal waste. These pathogens and other contaminants must be removed before the water could be declared safe for human consumption.

[4]. At present, the most extensively studied nanomaterials for water and wastewater treatment include carbon nanotubes (CNTs), nanocomposites, zero-valent metal nanoparticles and metal oxides nanoparticles. Among the metal oxide nanoparticles, metallic silver nanoparticles (AgO-NPs) have received considerable attention for their potential application as a biocide. [10]. The antibacterial activity of silver has been known for centuries and has led to the development of several Ag-based bactericidal products. [14]. Silver ions have been used as an antibacterial component in dental resin composites, in synthetic zeolites, and in coatings of medical devices. [1]. Antimicrobial activity of many nano-sized metal ions (nanoparticles) allows for design of nanocatalysts such as; AgCCA catalyst, N-doped TiO<sub>2</sub>, ZrO<sub>2</sub> and Zinc Oxide nanoparticles (ZnO – NPs).

[2][3][8][11][13][15][16]. As an adsorbent, ZnO was mostly applied to eliminate hydrogen sulphide (H<sub>2</sub>S) and recently, people have found that nanostructured ZnO could efficiently remove heavy metals, removal of Chromium VI, enhance adsorption of lead ion and recently use as antibacterial application. [6]. ZnO nanoparticles are synthesized by several different methods such as sol – gel techniques, wet chemical method, green chemistry and microwave method. The biosynthesis of ZnO nanoparticles of different size, ranging from 1 – 70nm, and shapes to include spherical, triangular and hexagonal has been conducted using bacteria, fungi and plant extracts. [12]. Synthesis of ZnO-NP involves the use of zinc chloride (ZnCl) as precursor. The reagent-grade ethanol is used for the cleaning of the nanoparticles.

This study showed the bactericidal effect of green synthesised silver and zinc oxide nanoparticles modified kaolin clay from Kutigi, Niger State, Nigeria. The choice of kaolin clay for this research work relates to its water treatment ability. Kaolinite is

widespread across Niger State, relatively free of charge, non-toxic and environmentally friendly. The choice of AgO and ZnO-NPs using *Mangifera Indica* leaves extracts as reducing agent is due to their antibacterial activities. [3]. ZnO nanoparticles synthesized using zinc chloride (ZnCl<sub>2</sub>) exhibited strong antibacterial prowess against both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction of metal oxides. [7]. The use of silver nanoparticles is effective against various microorganisms including plant pathogens.

## II. EXPERIMENTAL MATERIALS AND METHOD

The preparation of chemicals and materials involved are discussed in this chapter. Adsorption experiments were carried out at the National Water Quality Reference Laboratory, Minna, Niger State. The characterisation of Purified Kaolin Clay (BKC), BKC/ZnO, BKC/AgO and BKC/Ag/ZnO were done at the University of South Africa, Johannesburg in conjunction with Federal University of Technology Minna, Nigeria. The major materials used for this research work were kaolin clay collected from its deposit in Kutigi, Niger State, Nigeria and domestic wastewater collected from Shiroro hydro-power station's office building. Samples for microbial analysis were transferred aseptically into 2000 ml sterilized glass bottles and stored in sample cool boxes at 4 °C prior to analysis. The *Mangifera Indica* Plant extract was done at the National Water Quality Reference Laboratory, Minna, Niger State. The extract was used to synthesise ZnO and AgO Oxide nanoparticles. The BKC was modified with ZnO and AgO Oxide nanoparticles to produce AgO/BKC, ZnO/BKC and AgO/ZnO/BKC Nanocomposites.

**Beneficiated Kaolin Clay (BKC):** 200g of raw kaolin clay lumps were put in 4 Litres Beakers with 4 Litres of distilled water added. This represents 5 % w/w kaolin clay slurry in distilled water. The Beaker was stirred for 1 hour using Heildolph RGL500 High viscosity stirrer at control speed of 40 revolutions per minute for adequate dispersion of the kaolin clay particles in distilled water. The resulting mixture was allowed to swell in distilled water for 22hrs 57mins as calculated by Stoke's Law using equation 1.

$$u_s = \frac{g(\rho_p - \rho_w)d_p^2}{18\mu}$$

Where  $\rho_p$  = particle density, kg/m<sup>3</sup> (Kaolin clay particle = 1600kg/m<sup>3</sup>),  $\mu$  = liquid viscosity, kg/m.s (distilled water =  $8.90 \times 10^{-4}$  Pa.s),  $\rho_w$  = density of water, kg/m<sup>3</sup> (997kg/m<sup>3</sup>),  $u_s$  = Particle settling velocity, m/s,  $d_p$  = diameter of particle (m),  $g$  = acceleration due to gravity, m/s<sup>2</sup>(9.81m/s<sup>2</sup>),  $t$  = Settling Time,  $R$  = particle size (radius) of clay, assumed to be spherical ( $1\mu\text{m} = 1 \times 10^{-6}\text{m}$ ),  $h$  = Settling Height of Fluid (12cm = 0.12m), The resultant slurries were thereafter dried in a laboratory oven at a temperature of 105°C until the water is evaporated and the samples weight became constant. Acid activation was done by treating the beneficiated kaolin clay with 0.5M of HCl to remove carbonate and the resulting mixture was washed by 10% Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) to oxide organic matter (Bachiri et al, 2014). Filtration of the mixture was done using whatman filter paper. The residue from filtration was washed several times with distilled water and monitored until pH 7. The grinded beneficiated kaolin clay samples were stored in laboratory sterile 100 ml plastic bottle for characterisation and for further studies. The percentage yield (Y) of the beneficiated clay was calculated using equation 2.

$$Y = \frac{\text{Mass of Purified Kaolin Clay Produced}}{\text{Mass of Raw Kaolin Clay}} \times 100\%$$

Biomolecules present in plant extracts were used to reduce silver and zinc metal ions to nanoparticles (AgO and ZnO Nanoparticles) in a single-step green synthesis process using Mangifera Indica plant extract. The formation of nanoparticles was confirmed and a biological agent which include the various water-soluble plant metabolites such as alkaloids, phenolic compounds and tannin as a reducing agent were tested.

**Green Syntheses of Zinc Oxide:** 10g of Zinc Sulphate Heptahydrate (ZnSO<sub>4</sub>.7H<sub>2</sub>O) powder was measured into a 250 ml beaker and 100 ml of Deionised Distilled Water (DDW) was added. The solution was stirred using a magnetic stirrer for 30 min at 150 rpm. 50 mL of the prepared concentration was put in a 100 ml conical flask. The conical flask was put on the hot plate and temperature set to 80°C. Mangifera Indica leave extract was put in a burette, ready for titration. The solution was titrated, while continuously stirred at 80°C using magnetic stirrer

with the Mangifera Indica leave extract, drop by drop and very slowly until the light-yellow colour was formed. The titration was stopped immediately after the solution has changed to yellow, but the stirring was continued for 10 minutes until a colloid of the solution was obtained. The solution consumed 4.9 ml of the Mangifera Indica leave extract. The precipitate obtained was filtered by Whatman No. 1 filter paper and washed with DDW until the pH 7 was gotten. The precipitate was oven-dried at 105 °C for 6 hours and calcined in the furnace at 450 °C for 3 hours to obtain ZnO Nanoparticles (ZnO NP). The ZnO NP obtained was kept in a glass bottle for further characterisation and use.

**Green Synthesis of AgO Nanoparticles:** 5 g of Silver Nitrate (AgNO<sub>3</sub>) powder was measured into a 250 ml beaker and 100 ml of DDW was added. The solution was stirred using a magnetic stirrer for 30 min at 100 rpm. 50 mL of the prepared concentration was put in a 100 ml conical flask. The conical flask was covered with aluminium foil to avoid the photo degradation of silver. The conical flask was put on the hot plate and temperature set to 70°C. Leave extract was put in a burette, ready for titration. The Solution was titrated with the leave extract drop by drop and very slowly until the light-yellow colour formed. The titration was stopped immediately after the solution has changed to yellow. The solution consumed 2.8 ml of the Mangifera Indica leave extract. The precipitate obtained was filtered by Whatman No. 1 filter paper and washed with DDW until the pH 7 was gotten. The precipitate was oven-dried at 105 °C for 6 hours and calcined in the furnace at 450 °C for 3 hours to obtain AgO Nanoparticles (AgO NP). The dried AgO NP obtained was kept in a glass bottle for further characterisation and use.

**Synthesis of BKC/ZnO Nanocomposites:** 1 g of ZnSO<sub>4</sub>.7H<sub>2</sub>O powder was dissolved in 50 ml of DDW and stirred to get a precursor. The solution was stirred using a magnetic stirrer for 30 min at 150 rpm. 50 mL of the prepared concentration was put in a 100 ml conical flask. The conical flask was put on the hot plate and temperature set to 80°C. The solution was titrated, while continuously stirred at 80°C, with the Mangifera Indica leave extract until the light-yellow colour was formed. The solution consumed 2.6 ml of the Mangifera Indica leave extract. To the suspension formed, 10 g of the BKC was dispersed under vigorous stirring for 1 hour at 40

rpm. A homogeneous gel obtained was filtered by Whatman No. 1 filter paper and washed with DDW until the pH 7 was gotten. The gel was oven-dried at 105 °C for 6 hours and calcined in the furnace at 450 °C for 3 hours to obtain BKC/ZnO nanocomposite. The BKC/ZnO nanocomposite obtained was kept in a glass bottle for further characterisation and use.

**Synthesis of BKC/AgO Nanocomposite:** 0.5 g of AgNO<sub>3</sub> powder was dissolved in 50 ml of DDW and stirred to get a precursor. The solution was stirred using a magnetic stirrer for 30 min at 150 rpm. 50 mL of the prepared concentration was put in a 100 ml conical flask. The conical flask was put on the hot plate and temperature set to 70°C. The solution was titrated, while continuously stirred at 70°C, with the Mangifera Indica leave extract until the yellow colour was formed. The solution consumed 0.8 ml of the Mangifera Indica leave extract. 10 g of the BKC was dispersed into the suspension formed and vigorously stirred for 1 hour at 40 rpm. A homogeneous gel obtained was filtered by Whatman No. 1 filter paper and washed with DDW until the pH 7 was gotten. The gel was oven-dried at 105 °C for 6 hours and calcined in the furnace at 450 °C for 3 hours to obtain BKC/AgO nanocomposite. The BKC/AgO nanocomposite obtained was kept in a glass bottle for further characterisation and use.

**Synthesis of BKC/AgO/ZnO Nanocomposite:** 0.5 g of AgNO<sub>3</sub> and 1 g of ZnSO<sub>4</sub>.7H<sub>2</sub>O powder were

dissolved in two separate 50 ml of DDW and stirred to get precursors. The solutions were stirred using a magnetic stirrer for 30 min at 150 rpm. 50 mL each of the prepared concentrations of AgNO<sub>3</sub> and ZnSO<sub>4</sub>.7H<sub>2</sub>O were put in separate 100 ml conical flasks. The AgNO<sub>3</sub> and ZnSO<sub>4</sub>.7H<sub>2</sub>O solutions in the conical flasks were titrated with the Mangifera Indica leave extract under continuous stirring at 70°C and 80°C respectively until the light-yellow colour were formed. The AgNO<sub>3</sub> and ZnSO<sub>4</sub>.7H<sub>2</sub>O solutions consumed 0.7 ml and 1.5 ml of the Mangifera Indica leave extract accordingly. The two formed AgO and ZnO nanoparticles were mixed with 20 g of the BKC and vigorously stirred for 1 hour at 40 rpm. Three different mixing ratios of BKC/AgO/ZnO used were (20:0.5:1), (15:0.5:1) and (10:0.5:1) respectively. A homogeneous gel obtained was filtered by Whatman No. 1 filter paper and washed with DDW until the pH 7 was gotten. The gel was oven-dried at 105 °C for 6 hours and calcined in the furnace at 450 °C for 3 hours to obtain BKC/AgO/ZnO nanocomposite. The BKC/AgO/ZnO nanocomposite obtained was kept in a glass bottle for further characterisation and use.

**Characterisation:** The structural changes in Beneficiated Kaolin Clay (BKC), AgO Nanoparticles, ZnO Nanoparticles, AgO/BKC, ZnO/BKC and AgO/ZnO/BKC Nanocomposites were analysed using the stated equipment in Table 1.

**Table 1:** Characterisation of Beneficiated Kaolin Clay (BKC), AgO Nanoparticles, ZnO Nanoparticles, AgO/BKC, ZnO/BKC and AgO/ZnO/BKC Nanocomposites

S/N	Test Equipment	Uses	Location
1	X-Ray Diffractometer (XRD), Emma 0141, GCB SCIENTIFIC EQUIPMENT	Determination of mineral phases and compounds in materials. Study of crystal structure of the mineral phases and compounds in materials	University of South Africa (UNISA), Johannesburg, South Africa
2	Dispersive X – Ray Fluorescence (XRF) Machine, EDXRF-3600B, OXFORD INSTRUMENT	Chemical analyses of materials	University of South Africa (UNISA), Johannesburg, South Africa
3	High Resolution Transmission Electron Microscope (HRTEM), TECNAI G2, FEI Netherlands	Determination of Microstructure and particle size of materials	University of South Africa (UNISA), Johannesburg, South Africa

4	BET Nitrogen Absorption Analyser, TriStar II 3020, MICROMETRICS, USA	Surface area and pore size	University of South Africa (UNISA), Johannesburg, South Africa
5	UV – Spectrometer, UV – 1800 SHMADZU, Japan	Determination of purity and concentration of a solution	Centre for Genetic Engineering and Biotechnology, FUT Minna

**Domestic Wastewater Analysis:** The analyses were performed to determine the microbial loads of the wastewater. The analysed microbial parameters in the wastewater were total coliforms, faecal coliforms, clostridium perfringens and E. coli.

**Effect of Contact Time:** The antibacterial activities of BKC, BKC/ZnO, BKC/AgO and BKC/Ag/ZnO nanocomposites adsorbent against the total coliforms, faecal coliforms, clostridium perfringens and E. Coli in the domestic wastewater were observed at contact time of 10, 20, 30, 40, 50 and 60 minutes using an adsorbent dosage of 25g/100 ml of wastewater at a temperature of 29.5°C and pH of 6.9. The flasks were agitated at 40rpm at room temperature. The flask was removed from stirrer at 10mins interval for the contact time study. The samples were analysed after filtration by Whatman No. 42 filter paper. The treated wastewater was analysed for total coliforms, faecal coliforms, clostridium perfringens and E. Coli in accordance with APHA Methods (2017). The optimum contact time for the % removal efficiency for each parameter of interest was calculated using the formula 3.5.

**Effect of Adsorbent Dosage:** The effect of the adsorbent dosage on the removal of total coliforms, faecal coliforms, clostridium perfringens and E. Coli from wastewater using BKC, BKC/AgO, BKC/ZnO and BKC/AgO/ZnO were investigated with adsorbent dosage of 5 g, 10 g, 15 g, 20 g, 25 g and 30 g respectively. The experiments were performed by adding the measured weight of the adsorbent into 250 ml conical flasks containing 100 ml of the wastewater each. The study was done with the flasks stirred at 40rpm at room temperature. The supernatant was filtered and kept in bottle for analyses. The % removal efficiency for each parameter of interest was calculated using the formula 3.5.

$$\text{Percentage (\% Removal)} = \frac{C_i - C_e}{C_e} \times 100\%$$

Where;  $C_i$  = Initial Liquid Phase Concentration (mg/l),  $C_e$  = Equilibrium liquid phase concentration (mg/l),  $V$  = Volume of the solution (litre),  $M$  = Mass of the adsorbent (g).

**Effect of Temperature:** The effect of temperature on total coliforms, faecal coliforms, clostridium perfringens and E. Coli were investigated. The study was conducted at varying temperature of 40, 50, 60, 70 and 80°C. 25g of the adsorbent were contacted with 100 ml of wastewater in a corked 250 ml conical flask. The corked flasks were agitated in a water bath at the respective temperature. The supernatant was filtered and kept in bottle for microbial analysis. The % removal efficiency for each parameter of interest was calculated using the formula 3.5.

### III. RESULTS AND DISCUSSION

The XRD pattern showing various peaks produced in the characterisation of AgO and ZnO nanoparticles is presented in Figure 1 (A and B) respectively. The XRD spectra confirmed that the AgO and ZnO nanoparticles have polycrystalline hexagonal structure and crystallite size in the range of 30.6285nm and 32.038nm at Scherrer's constant of 0.94 and wavelength ( $\lambda$ ) of 1.5406 Å. According to XRF analysis, loss on ignition for the ZnO and AgO nanoparticles was found to be 52.13% and 51.1% respectively. The formation of AgO nanoparticles was also confirmed by the UV-Visible spectrometer analysis. The formation of absorbance peaks corresponded to the formation of AgO and ZnO nanoparticles spectrum at the characteristic absorption peak of 298.5 nm and 264.5 nm and absorbance of 0.444 and 0.977 respectively. The results of phytochemical screening of Mangifera Indica indicated a reasonable high phenol, flavonoids and tannin contents (Table 4.14) with biologically active compounds that possesses some disease preventive properties and was therefore a suitable phytochemical screening agent to produce AgO/ZnO nanoparticles. Phenol composition of the Mangifera Indica was found to be 5.348 mg/g, Flavonoid was 0.0183 mg/g and Tannin composition was 0.90 mg/g.

The crystal structure of the AgO and ZnO nanoparticle analysed by HRTEM are shown in Plate I (a – c) and II (a – c) respectively. The SAED pattern in Plate I (c) and II (c) suggested the AgO and ZnO nanoparticle were crystalline in nature.

The BET surface areas for ZnO and AgO nanoparticles produced were  $1.1045 \text{ m}^2\text{g}^{-1}$  and  $1.0545 \text{ m}^2\text{g}^{-1}$  respectively. The BET results showed that the adsorption-desorption isotherms for the ZnO and AgO nanoparticles possessed pore sizes that fell in

the mesopore widths and the quasi-overlapping adsorption-desorption curves in Figures 2 and 3 indicated clearly that the  $\text{N}_2$  adsorption-desorption isotherm curve for AgO and ZnO nanoparticle were classified as Type IV.

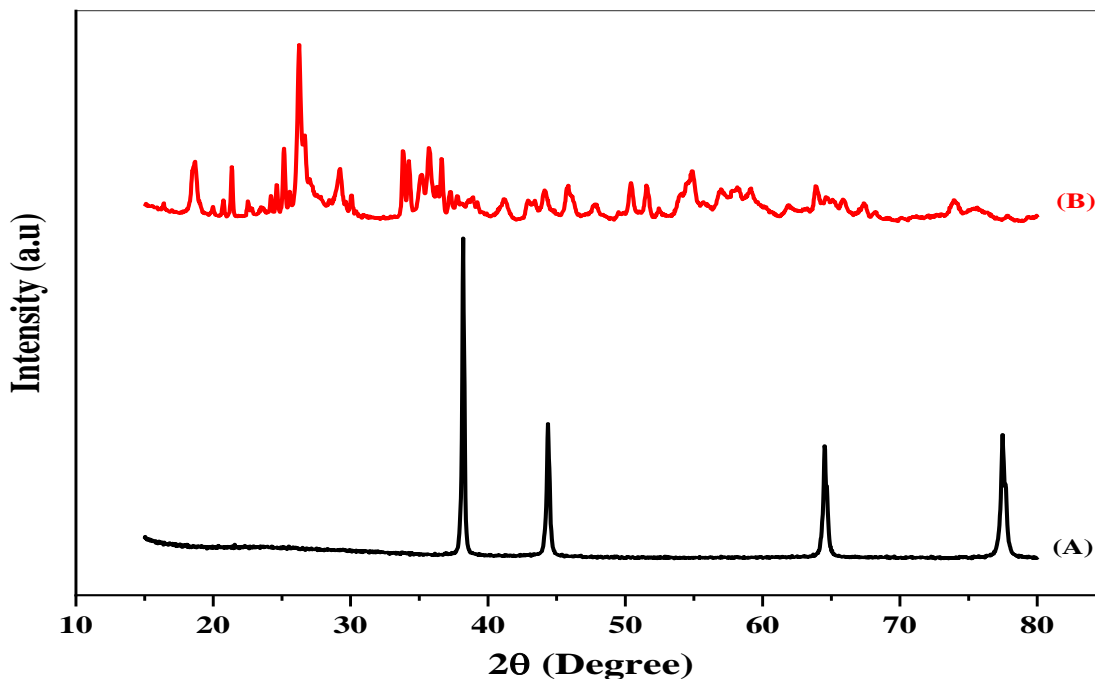


Figure 1: XRD patterns of (A) AgO and (B) ZnO

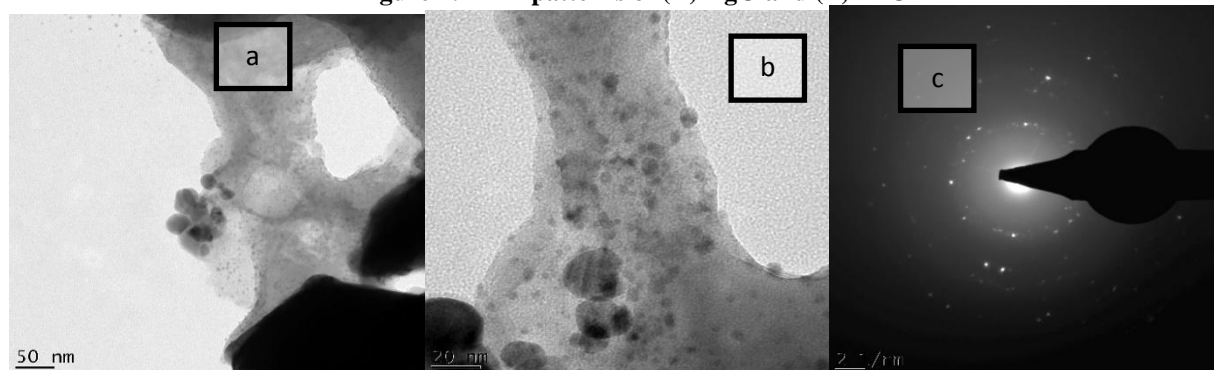
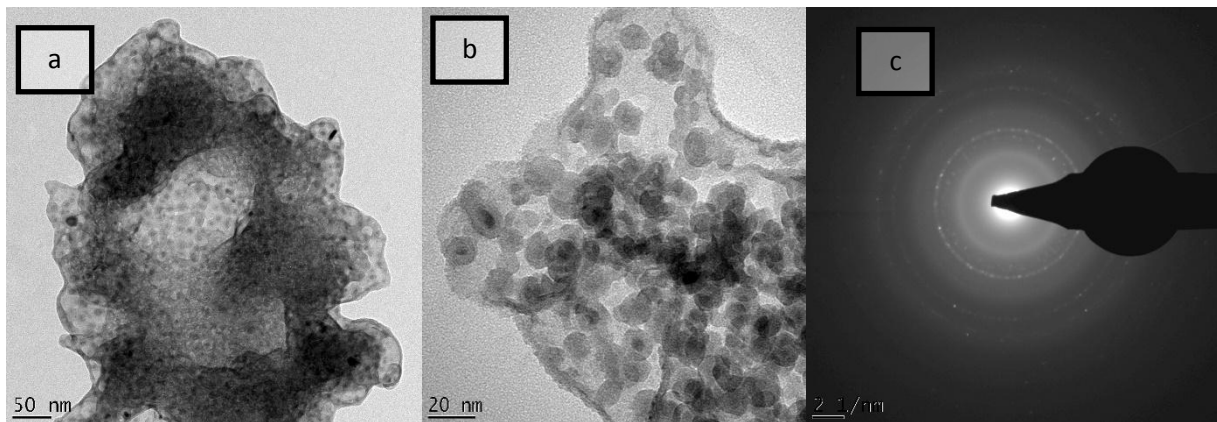
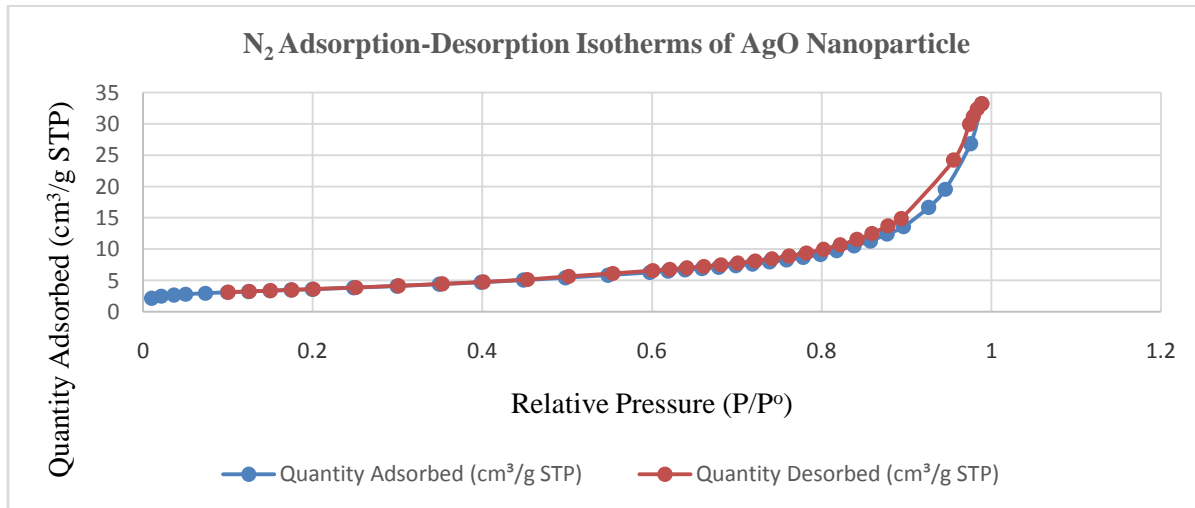


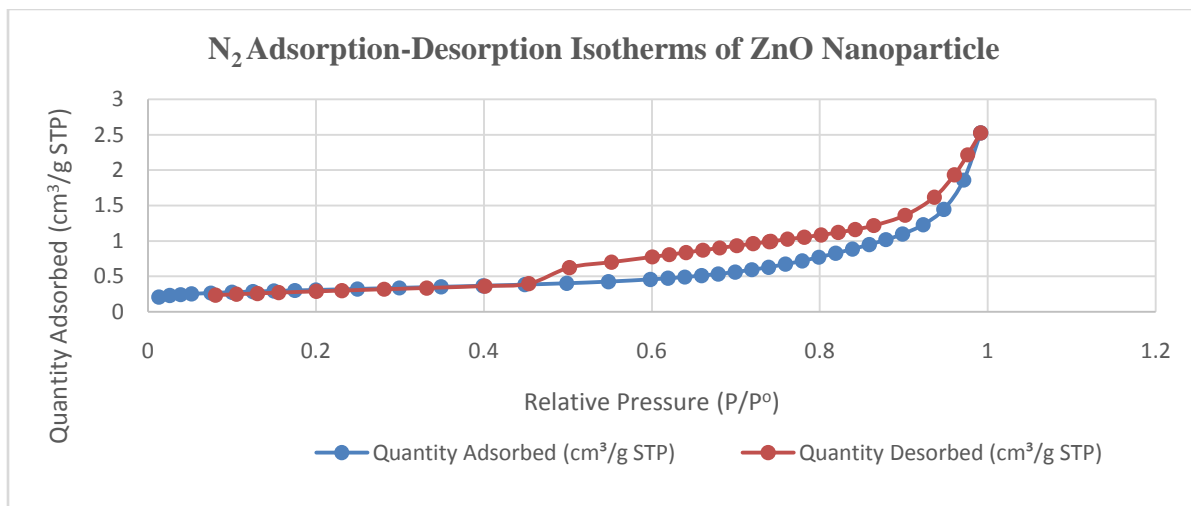
Plate I: HRTEM (a – c) and SAED (c) Images of AgO Nanoparticles



**Plate II:** HRTEM (a – b) and SAED (c) Images of ZnO Nanoparticles



**Figure 2:** N<sub>2</sub> Adsorption-Desorption Isotherms of AgO Nanoparticle



**Figure 3:** N<sub>2</sub> Adsorption-Desorption Isotherms of ZnO Nanoparticle

**Syntheses of Nanocomposite Adsorbents:** XRD profiles of the BKC impregnated with AgO and ZnO nanoparticles (BKC/AgO, BKC/ZnO and BKC/AgO/ZnO nanocomposite adsorbents) were found to contain different phases of kaolinite. The XRD analysis clearly showed the impregnation of Syn-Ag at 13.93 % on the BKC for BKC/AgO nano adsorbents, 7.90% of zinc sulphate hydrate on BKC for BKC/ZnO nano adsorbent and impregnation of silver – 3C and zinc sulphate hydrate on the BKC at 2.34 % and 3.80 % for BKC/AgO/ZnO respectively. The average Interplanar spacing (d-spacing) and

crystallite size for the Raw Kaolin Clay, BKC, BKC/AgO, BKC/ZnO and BKC/AgO/ZnO nanocomposite adsorbents were calculated to be 3.4994nm and 40.258nm, 3.532nm and 28.114nm, 3.818nm and 25.574nm, 4.712nm and 35.692nm, and 3.887nm and 26.934nm at Scherrer's constant of 0.94 and wavelength ( $\lambda$ ) of 1.5406 Å. The broad peaks formation of the XRD pattern showed that the raw kaolin clay, BKC, BKC/AgO, BKC/ZnO and BKC/AgO/ZnO nanocomposite adsorbents were polycrystalline in nature (Figures 4 and 5).

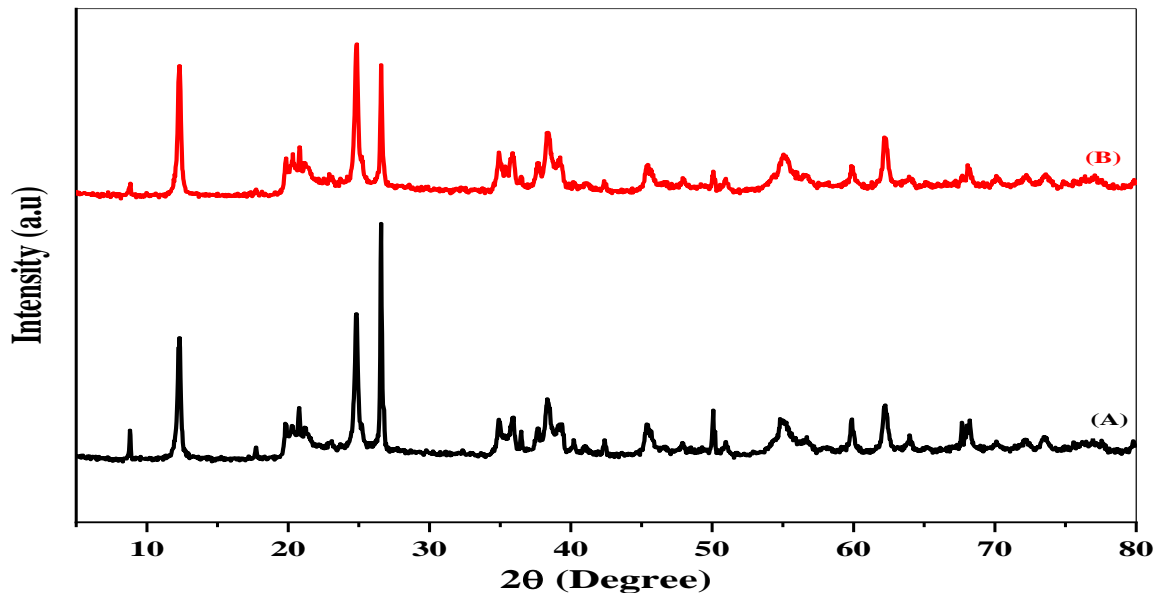
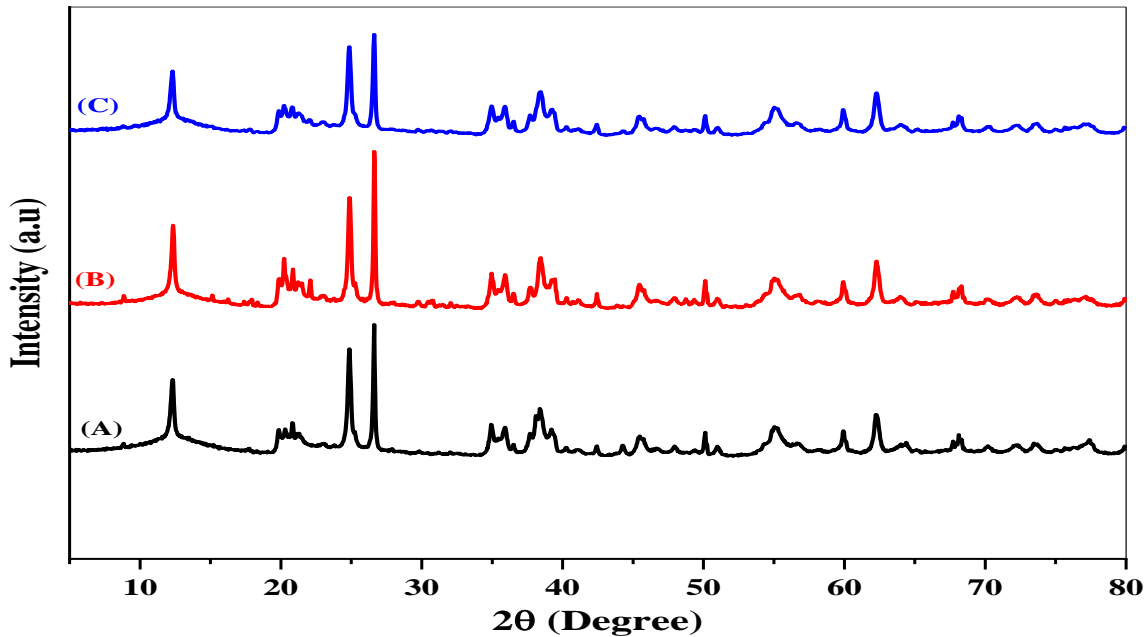


Figure 4: XRD patterns of (A) Raw kaolin and (B) beneficiated kaolin



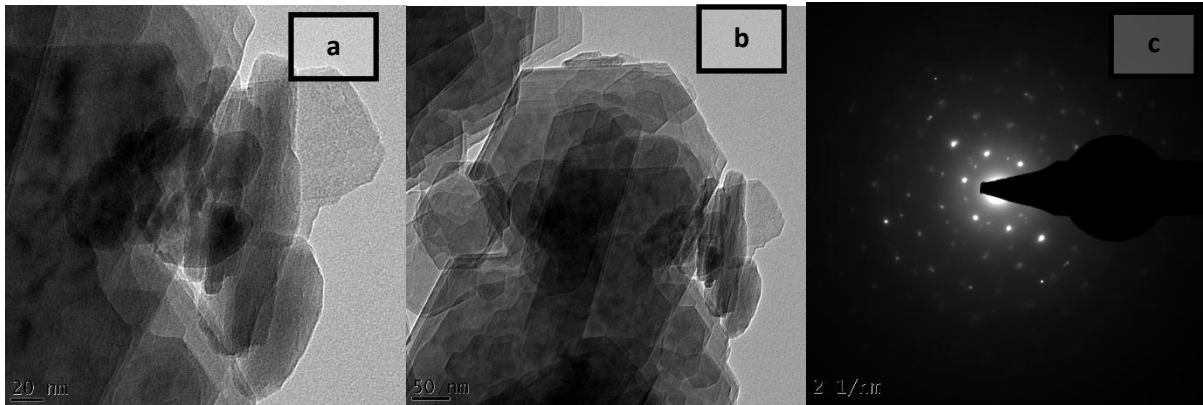


**Figure 5: XRD patterns of (A) AgO/Kaolin (B) ZnO/Kaolin and (C) AgO/ZnO/Kaolin**

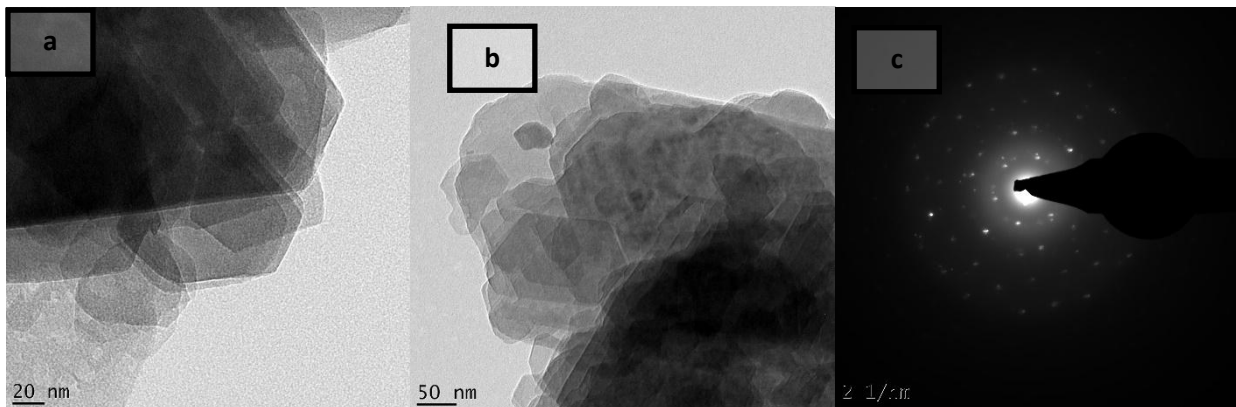
XRF results showed that the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio which is a function of the mineral phase present was found to be 1.540 in raw kaolin clay, 1.350 in BKC, 1.523 in BKC/AgO/ZnO, 1.517 in BKC/AgO and 1.4818 in BKC/ZnO nanocomposites adsorbents respectively. Clay minerals are classified by the ratio of their silica and alumina sheets. Alumina and silica in kaolin clay according to the literature are ratio **1:1**, one layer of silica for every layer of alumina. The  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio of BKC, BKC/AgO, BKC/ZnO and BKC/AgO/ZnO nanocomposite adsorbents as gotten in this study showed a purer kaolinite than the raw clay. Loss on ignition for the raw kaolin clay, BKC, BKC/AgO/ZnO, BKC/ZnO and BKC/AgO nanocomposites was found to be 8.90%, 11.62%, 12.37 %, 16.31% and 9.61 % respectively. The high LOI was attributed to the dehydroxylation reaction in the kaolin mineral. High LOI was also an indication

of potential normal porosity in the intended nano adsorbent kaolin clay

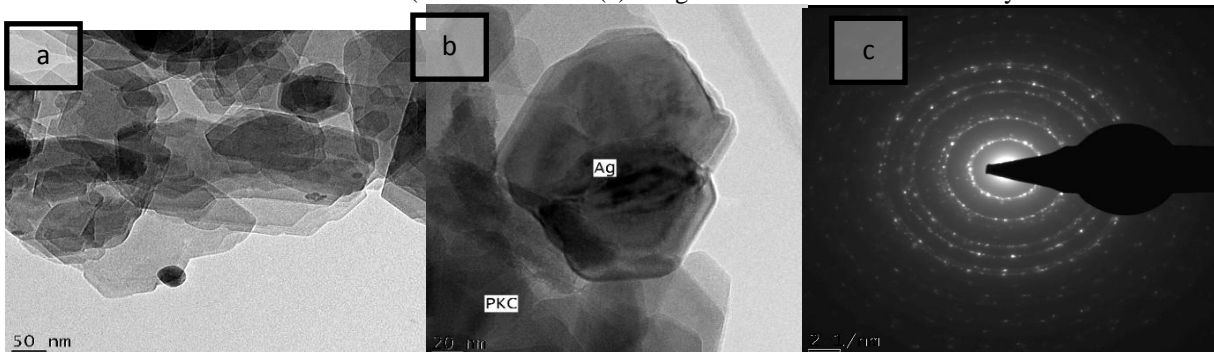
The HRTEM crystal structure of raw and beneficiated kaolin were presented in Plate III and IV. The bright spots and rings of the SAED pattern suggested that the raw kaolin clay and BKC are polycrystalline in nature and each ring depicted diffraction pattern of crystals of similar size with each bright spots reflecting individual peaks. The HRTEM crystal structure of BKC/AgO, BKC/ZnO and BKC/AgO/ZnO in Plate, V, VI and VII visibly showed the impregnation of AgO and ZnO nanoparticles on the beneficiated kaolin clay. The heavy dark colour images in Plate V, VI and VII indicated the presence of silver, zinc and Ag/Zn atoms while the light or grey colour indicated beneficiated kaolin crystal of different sizes.



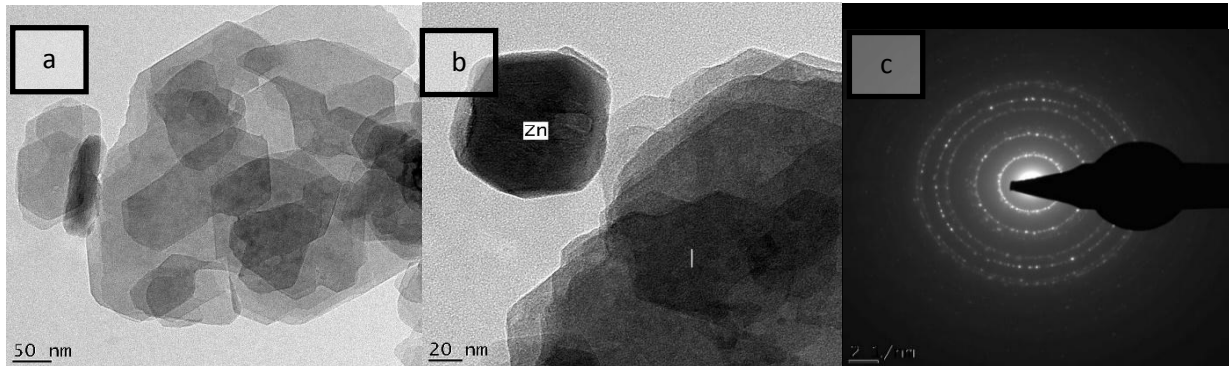
**Plate III:** HRTEM (a – b) and SAED (c) Images of Raw Kaolin Clay



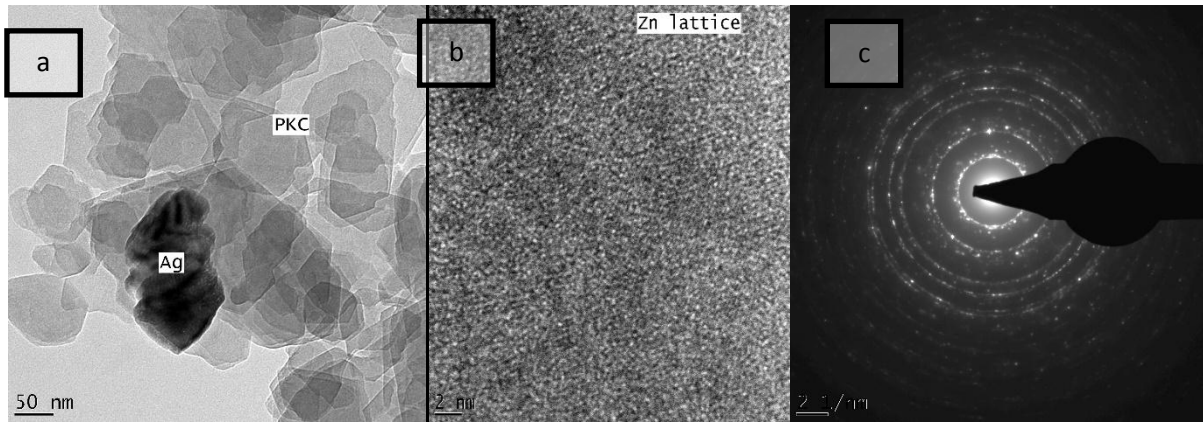
**Plate IV:** HRTEM (a – b) and SAED (c) Images of Beneficiated Kaolin Clay



**Plate V:** HRTEM (a – b) and SAED (c) Images of BKC/AgO Nanocomposite



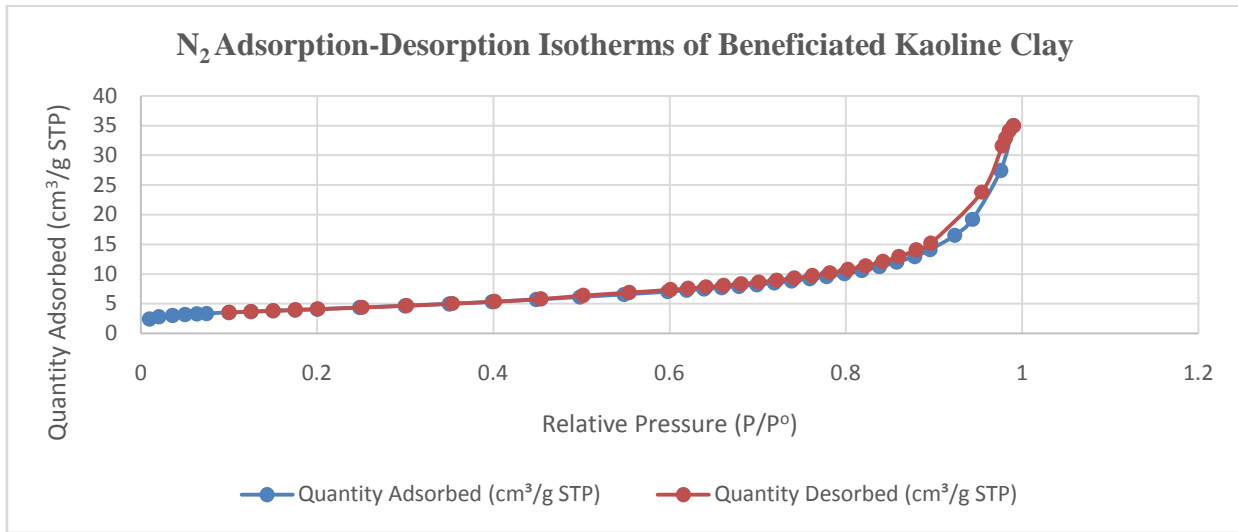
**Plate VI:** HRTEM (a – b) and SAED (c) Images of BKC/ZnO Nanocomposite



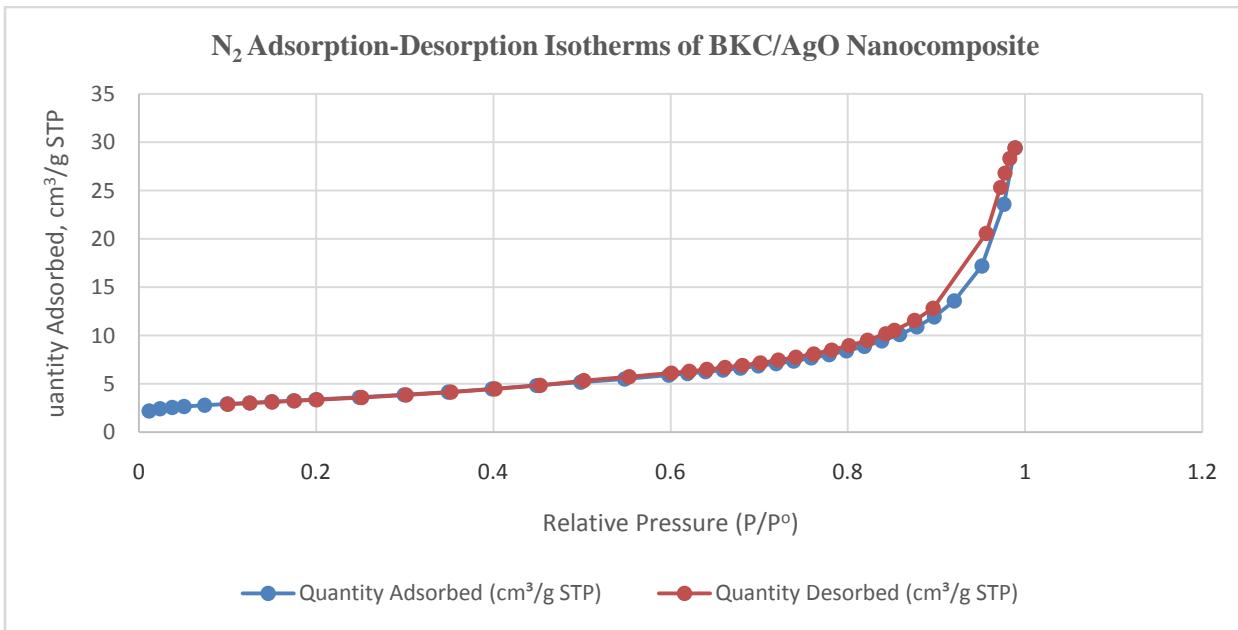
**Plate VII:** HRTEM (a – e) and SAED (f) Images of BKC/AgO/ZnO Nanocomposite

Nitrogen ( $N_2$ ) adsorption-desorption isotherm of the beneficiated kaolin clay, BKC/AgO, BKC/ZnO and BKC/Ag/ZnO nanocomposites adsorbents are displayed in Figures 6, 7, 8 and 9. The BET surface areas for BKC, BKC/ZnO, BKC/AgO and BKC/Ag/ZnO nanocomposites adsorbents are between  $11.9222 - 12.8278 \text{ m}^2\text{g}^{-1}$ , pore volume ranges from  $0.046930 - 0.063671\text{cm}^3/\text{g}$ , adsorption average diameter range from  $1.0422 - 1.1319 \text{ nm}$  and BJH adsorption average pore width range from  $13.8994 - 16.9233 \text{ nm}$ . It was also noticed that the

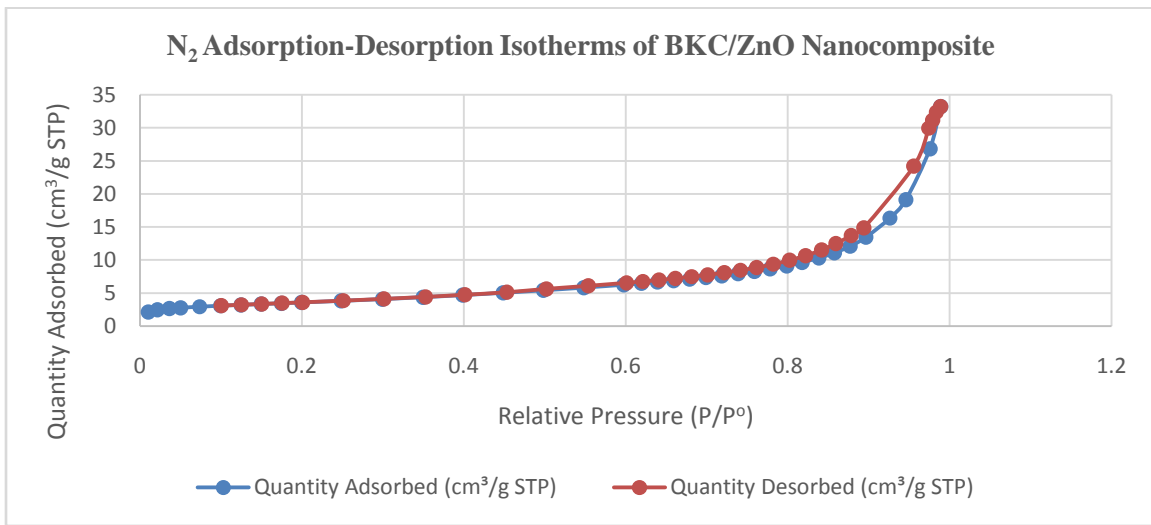
surface area, pore volume, pore diameter and pore width of the BKC/Ag/ZnO nanocomposite was appreciably higher than that of BKC/AgO and BKC/ZnO nanocomposites, the higher the surface area, the larger its adsorptive capacity. The BKC and three nanocomposite adsorbents produced belongs to type IV isotherm with a hysteresis loop, which resulted from capillary condensation in the mesopores (diameter,  $2 < d < 50 \text{ nm}$ ) at the relative pressure range of  $0.45 - 0.9, P/P_0$ .



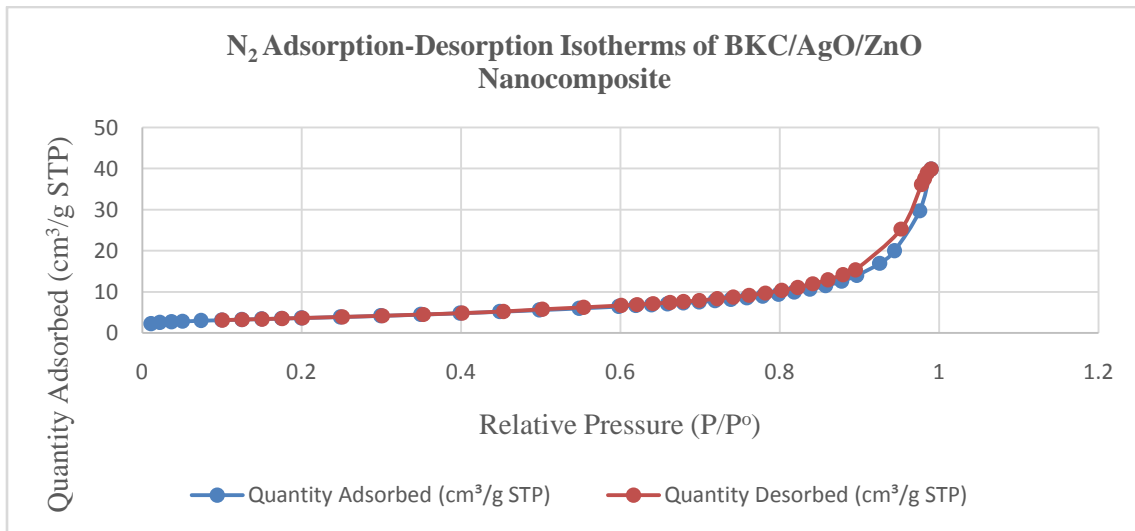
**Figure 6:** N<sub>2</sub> Adsorption-Desorption Isotherms of Beneficiated Kaolin



**Figure 7:** N<sub>2</sub> Adsorption-Desorption Isotherms of BKC/AgO Nanocomposite



**Figure 8:** N<sub>2</sub> Adsorption-Desorption Isotherms of BKC/ZnO Nanocomposite



**Figure 9:** N<sub>2</sub> Adsorption-Desorption Isotherms of BKC/AgO/ZnO Nanocomposite

**Domestic Wastewater Analyses:** The results of the microbial analyses of the domestic wastewater before treatment is presented in Table 2. The analysed parameters in the domestic wastewater were all above

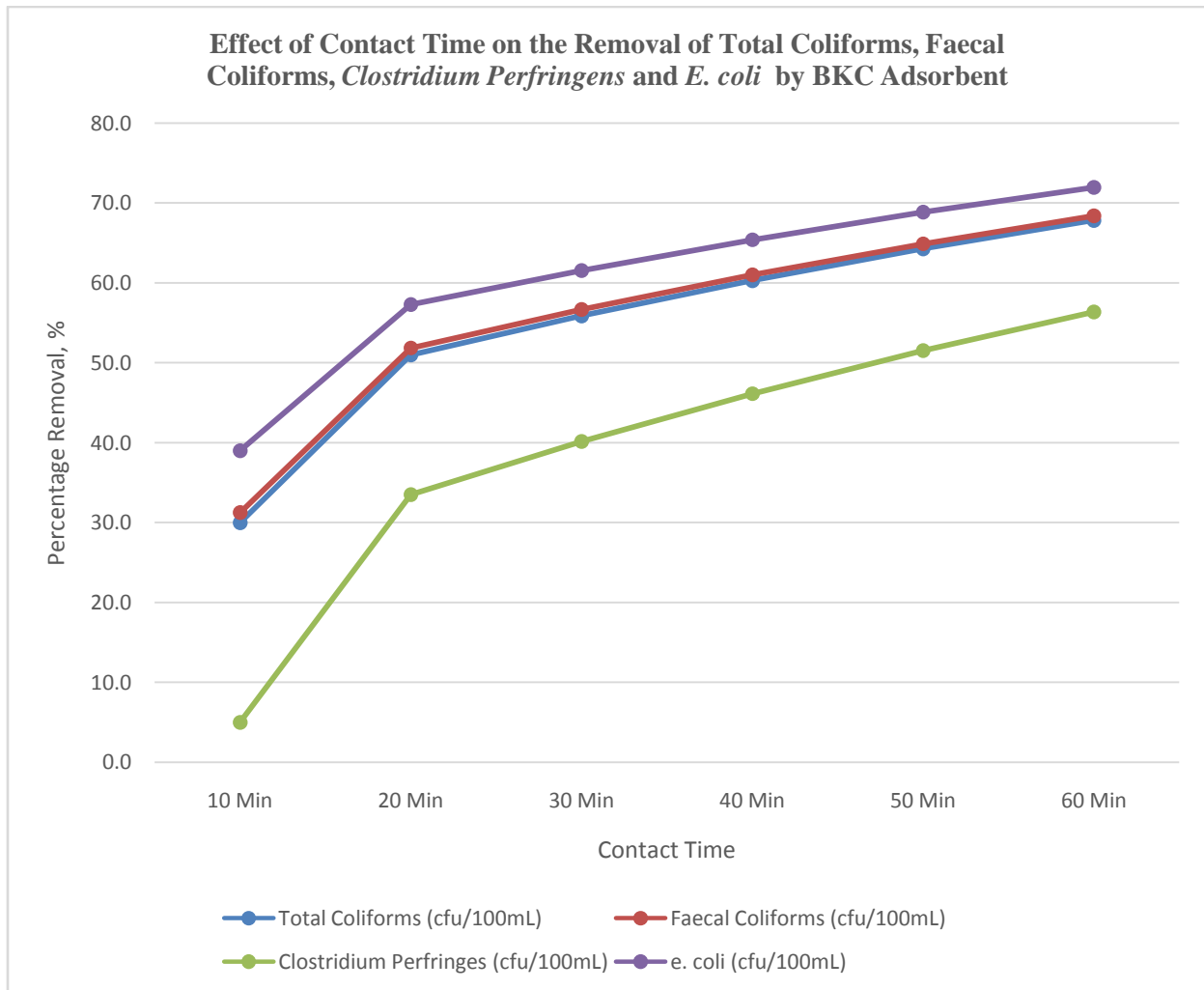
the specified maximum permissible limit set by National Environmental Standard, Regulation and Enforcement Agency (NESREA, 2011).

**Table 2:** Microbial Analyses of Wastewater before Treatment

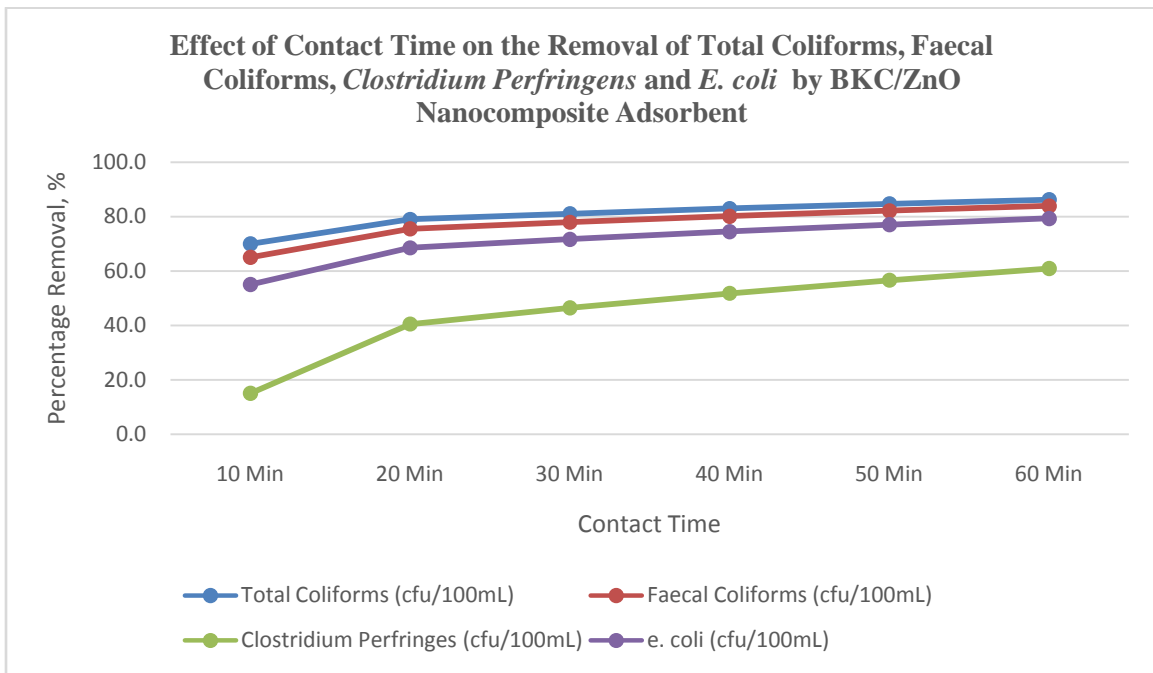
Parameters	Units	Wastewater	NESREA (2011)
Total Coliforms (TC)	cfu/100ml	6.45 x 10 <sup>3</sup>	5 x 10 <sup>3</sup>
E. coli	cfu/100ml	400	Absent
Faecal Coliforms	cfu/100ml	640	Absent
Clostridium Perfringens	cfu/100ml	48	Absent

**Effect of Contact Time on Microbial Parameters:** Figure 10 (a – d) describe the effect of contact time on the removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli onto BKC Adsorbent, BKC/AgO, BKC/ZnO and BKC/AgO/ZnO nanocomposite adsorbents. Rapid percentage removal was obtained between the contact times of 10 – 20 minutes for BKC adsorbent and BKC/ZnO nanocomposite adsorbent. The slight decrease in percentage removal of clostridium perfringens and E. Coli when treated with BKC adsorbent and BKC/ZnO nanocomposite adsorbents Figure 10 (a-b) with further increase in contact time

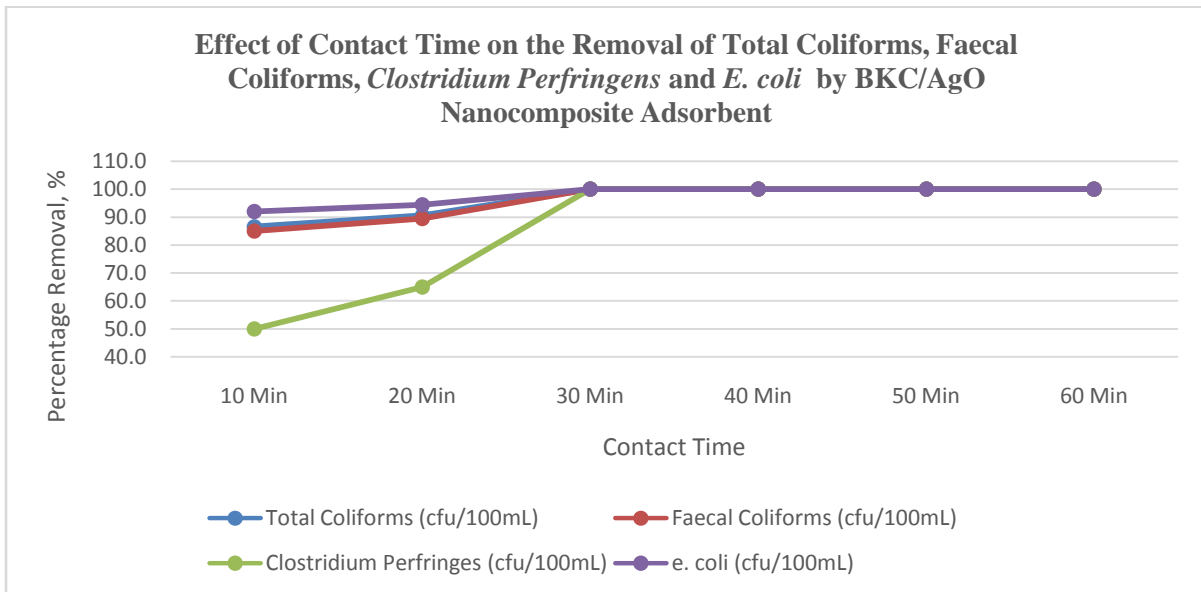
was due to strength of zinc nanoparticles against microorganisms. BKC/ZnO nanocomposite adsorbent also showed potential as an antimicrobial material in the removal of clostridium perfringens as shown in Figure 10 (b). The complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli at 20 minutes contact time for BKC/AgO and BKC/AgO/ZnO nanocomposite adsorbents was mainly due to the strong antibacterial activity of silver nanoparticles. For BKC/AgO and BKC/AgO/ZnO, all the microbial contaminants were removed within the first 20 minutes contact time.



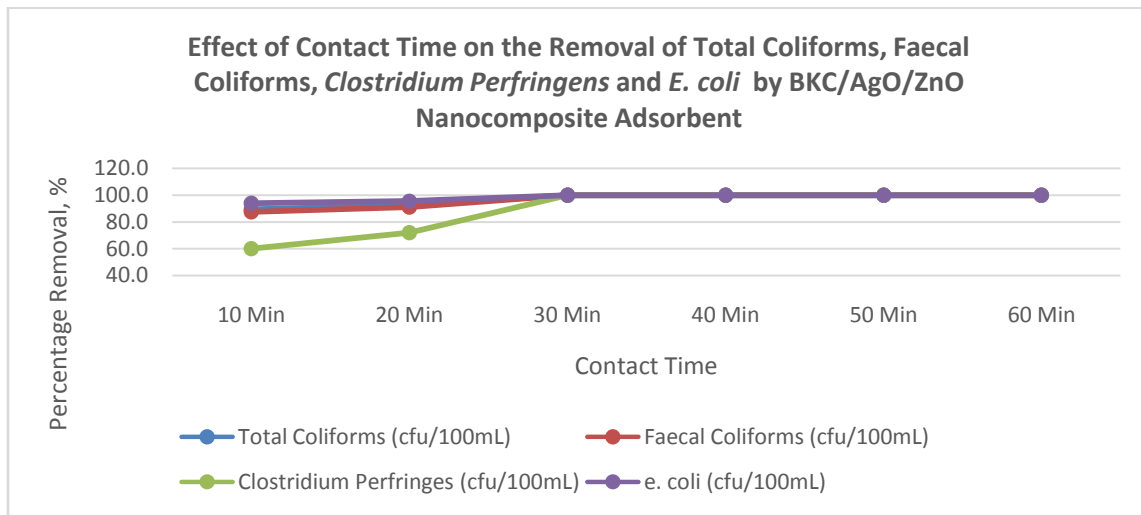
**Figure 10 (a): Effect of Contact Time on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC Adsorbent**



**Figure 10 (b): Effect of Contact Time on the Removal of Total Coliforms, Faecal Coliforms, *Clostridium Perfringens* and *E. coli* by BKC/ZnO Adsorbent**



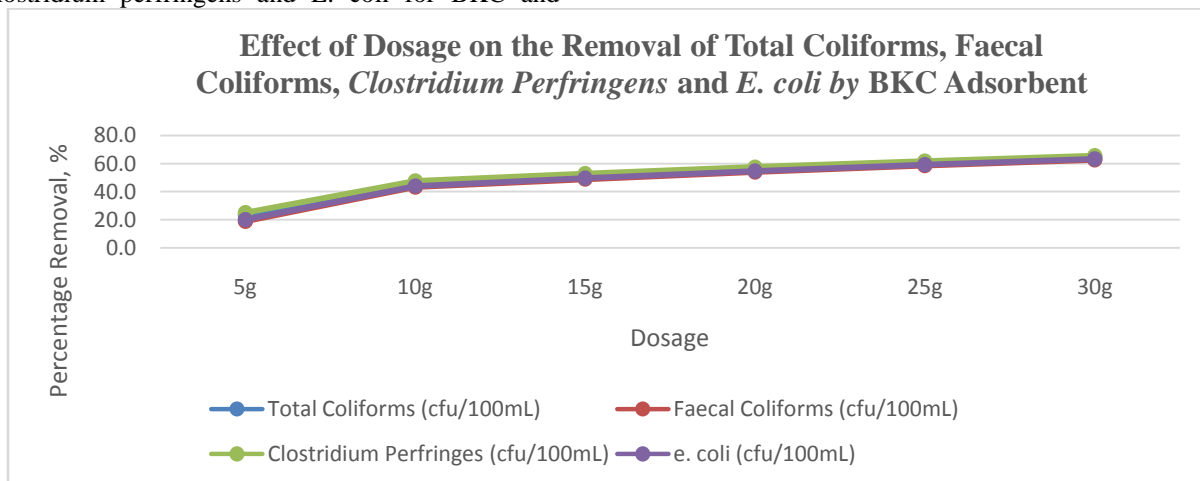
**Figure 10 (c): Effect of Contact Time on the Removal of Total Coliforms, Faecal Coliforms, *Clostridium Perfringens* and *E. coli* by BKC/AgO Adsorbent**



**Figure 10 (d): Effect of Contact Time on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC/AgO/ZnO Adsorbent**

**Effect of Dosage on Microbial Removal:** Figure 11 (a – d) describe the effect of dosage on the removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli onto BKC Adsorbent, BKC/AgO, BKC/ZnO and BKC/AgO/ZnO nanocomposite adsorbents. Rapid percentage removal was obtained between the adsorbent dosage of 5 – 10g for BKC adsorbent and BKC/ZnO nanocomposite adsorbent and continuous removal of microbes was noticed when BKC adsorbent and BKC/ZnO nanocomposite adsorbent was used to treat wastewater. The slight increase in percentage removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli for BKC and

BKC/ZnO nanocomposite adsorbents with further increase in dosages was due to low antimicrobial activity of the two adsorbents. The complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli at 15g dosage for BKC/AgO and BKC/AgO/ZnO nanocomposite adsorbents was mainly due to the antibacterial activity of silver nanoparticles. The complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli at 15 g dosages for BKC/AgO and BKC/AgO/ZnO nanocomposite adsorbents was strongly due to the antibacterial activity of silver nanoparticles as shown in Figures (c - d).



**Figure 4.11 (a): Effect of Dosage on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC Adsorbent**



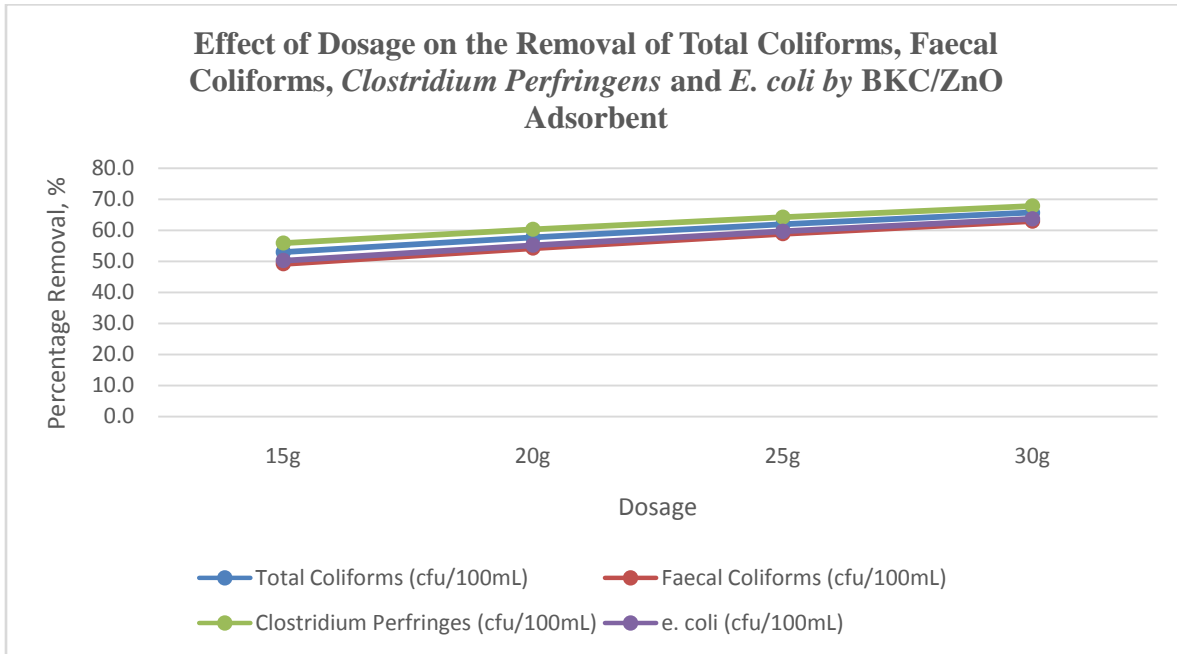


Figure 4.11 (b): Effect of Dosage on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC/ZnO Adsorbent

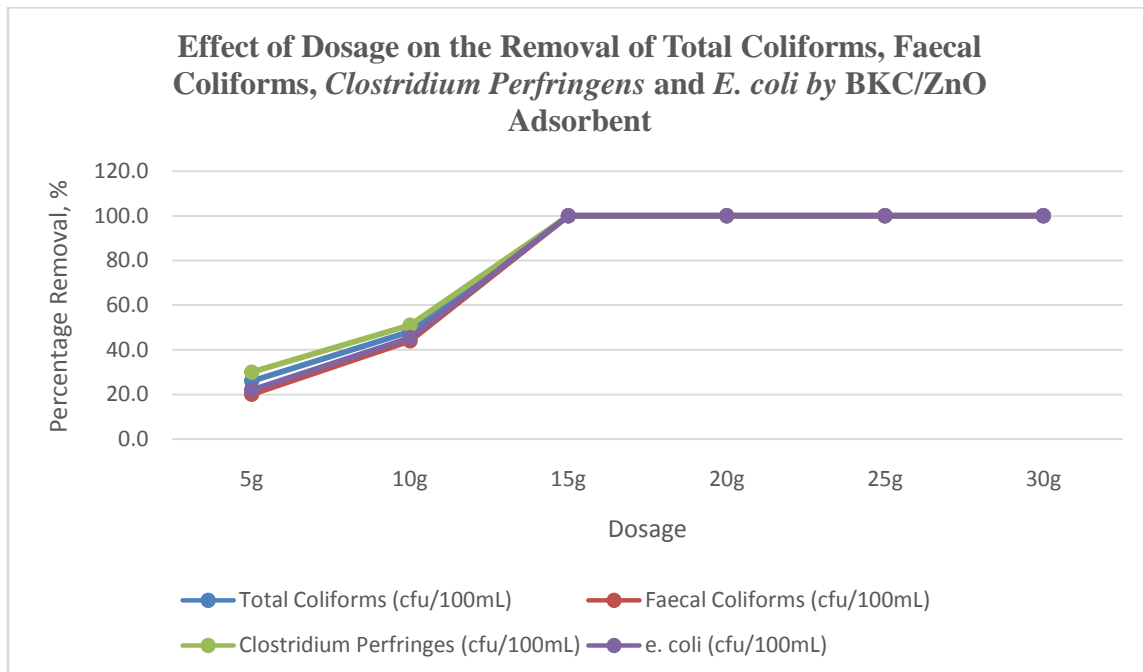
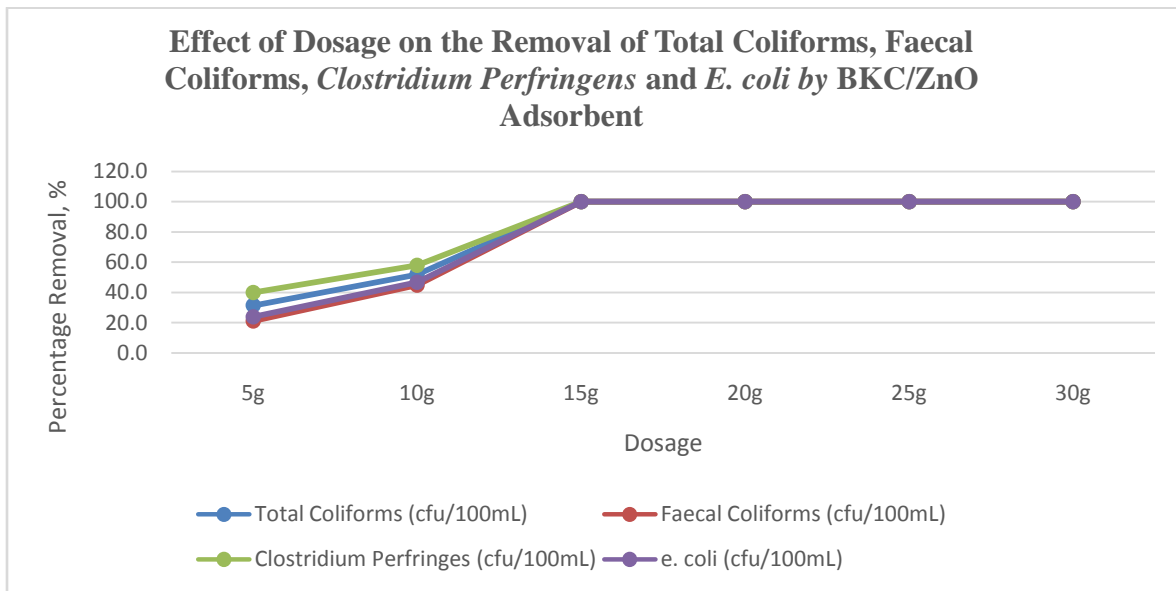


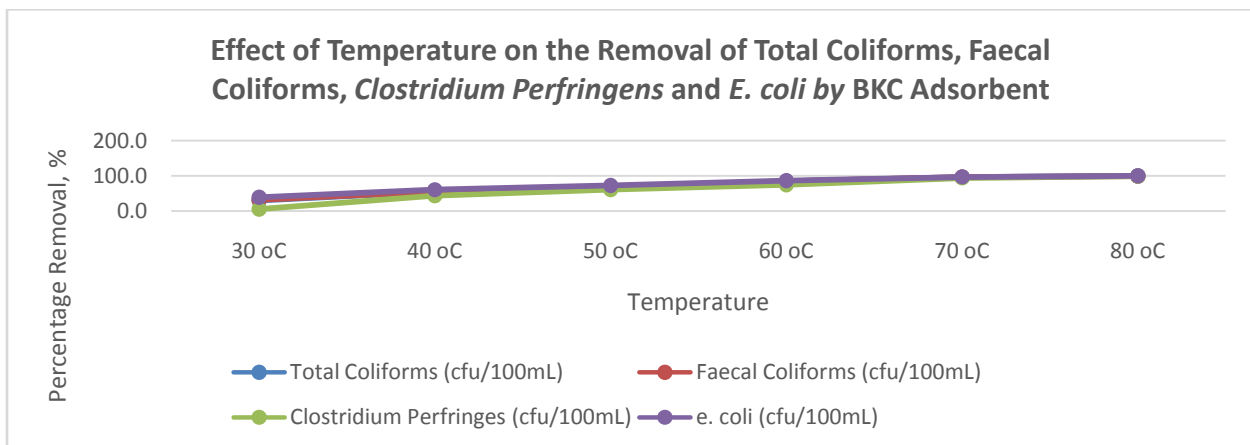
Figure 4.11 (c): Effect of Dosage on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC/AgO Adsorbent



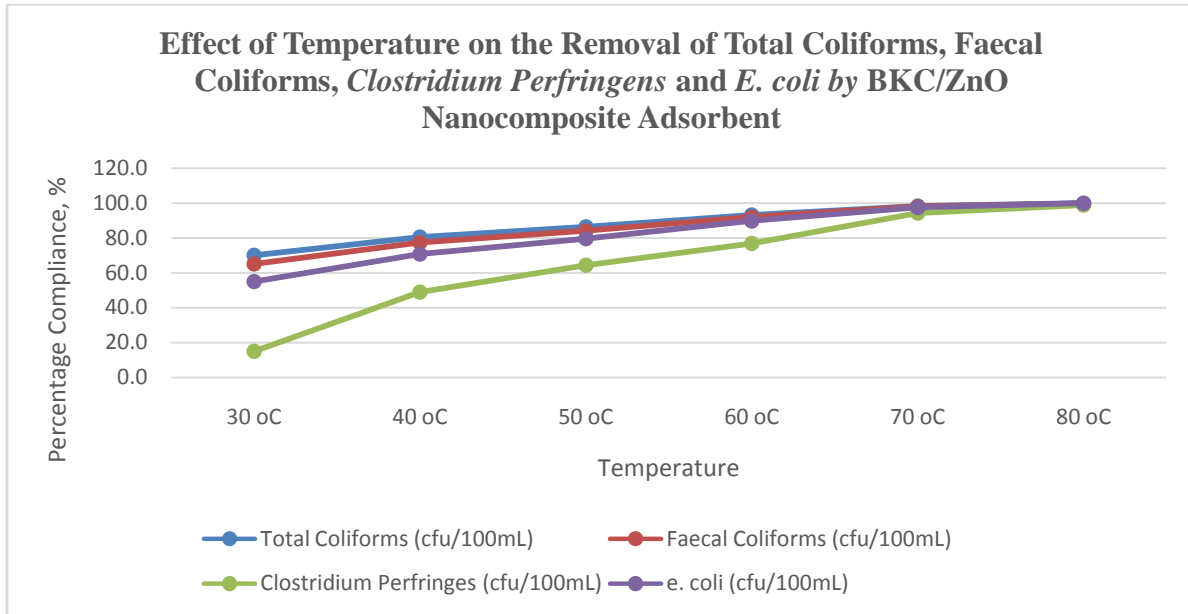
**Figure 4.11 (d): Effect of Dosage on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC/AgO/ZnO Adsorbent**

**Effect of Temperature:** The effect of temperature on the percentage removal efficiencies of total coliforms, faecal coliforms, clostridium perfringens and E. Coli by the BKC, BKC/ZnO, BKC/AgO and BKC/Ag/ZnO nanocomposites adsorbents are presented in Figures 4.12 (a – d). The observation revealed that an increase in the temperature to 50°C upward resulted in the complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli for BKC, BKC/ZnO, BKC/AgO and BKC/AgO/ZnO nanocomposite adsorbents. For BKC/AgO and BKC/AgO/ZnO, the temperature

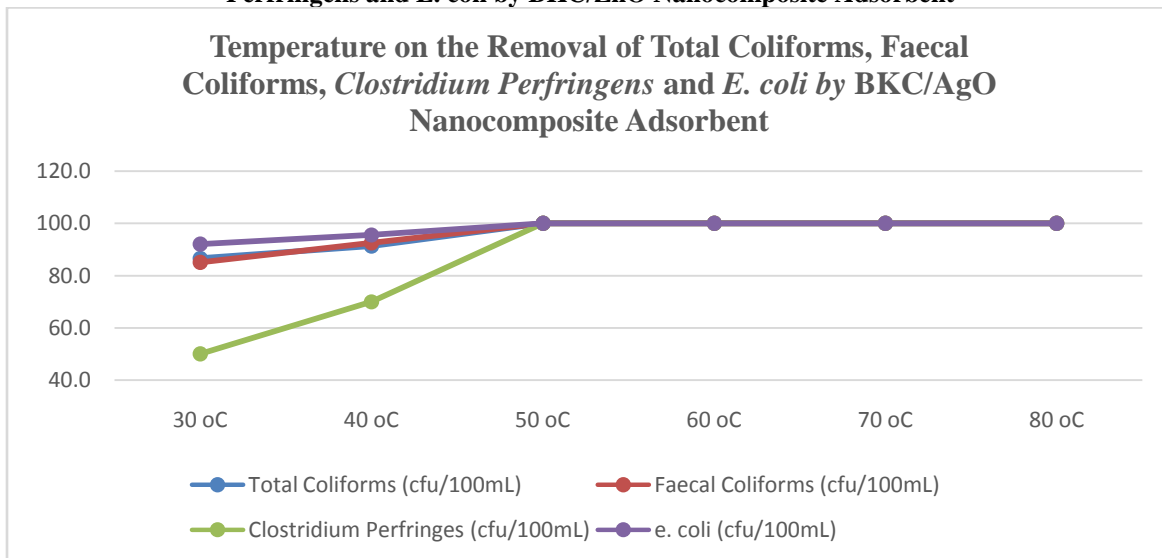
increment to 40°C resulted in the removal of all the microbial contaminants in the wastewater. The complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli at 40°C for BKC/AgO and BKC/AgO/ZnO nanocomposite adsorbents was strongly due to the antibacterial activity of silver nanoparticles as shown in Figures 12 (a - d), while the complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. Coli at 80°C for BKC and BKC/ZnO nanocomposite was mainly due to increase in temperature.



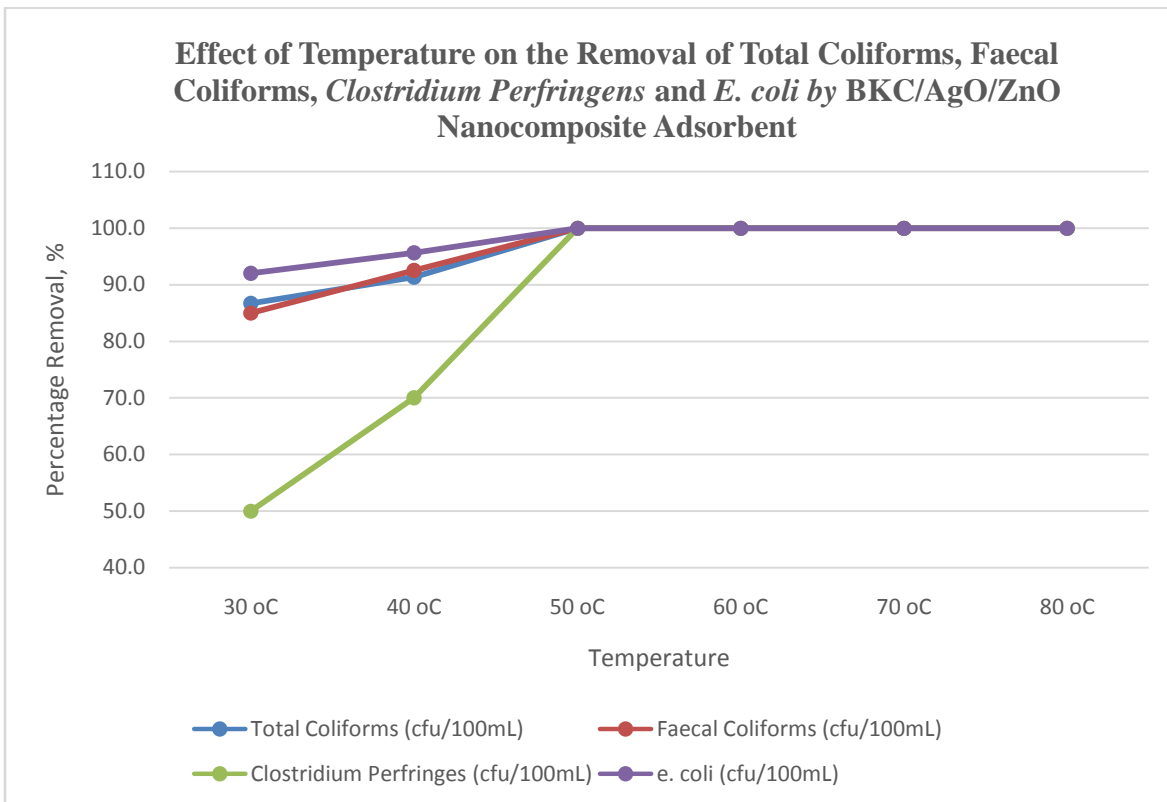
**Figure 4.12 (a): Effect of Temperature on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC Adsorbent**



**Figure 4.12 (b): Effect of Temperature on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC/ZnO Nanocomposite Adsorbent**



**Figure 4.12 (c): Effect of Temperature on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC/AgO Nanocomposite Adsorbent**



**Figure 4.12 (d): Effect of Temperature on the Removal of Total Coliforms, Faecal Coliforms, Clostridium Perfringens and E. coli by BKC/AgO/ZnO Nanocomposite Adsorbent**

**Antimicrobial Study Activities:** The antibacterial activities of BKC, BKC/ZnO, BKC/AgO and BKC/Ag/ZnO nanocomposites adsorbent against the total coliforms, faecal coliforms, clostridium perfringens and E. Coli in the domestic wastewater were observed at contact time of 10, 20, 30, 40, 50 and 60 minutes using an adsorbent dosage of 25g/100 ml of wastewater at a temperature of 29.5°C and pH of 6.9. It was observed that bacteria inactivation increased at the constant adsorbent dosage of 25 g with increase in contact time as presented in Table

3. There was a complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli at 30 minutes contact time for BKC/AgO and BKC/AgO/ZnO nanocomposite adsorbents as presented in Table 3. Anti-bactericidal effect of these two adsorbents could be attributed to the chemical properties of the nanoparticles in addition to the large surface area of kaolin. The antimicrobial effect of the AgO nanoparticles was more effective than ZnO nanoparticles

**Table 3:** Antimicrobial Study of BKC, BKC/ZnO, BKC/AgO and BKC/Ag/ZnO Nanocomposites Adsorbent

Microbial Parameters	Adsorbents	Wastewater Load	Anti-Microbial Effects at various contact time						Percentage Removal (%) at various contact time					
			10min	20min	30min	40min	50min	60min	10min	20min	30min	40min	50min	60min
Total Coliforms (cfu/100ml)	BKC	6000	4200	2940	2646	2381	2143	1929	30.0	51.0	55.9	60.3	64.3	67.9
	BKC/ZnO	6000	1800	1260	1134	1021	919	827	70.0	79.0	81.1	83.0	84.7	86.2
	BKC/AgO	6000	800	560	0	0	0	0	86.7	90.7	100.0	100.0	100.0	100.0
	BKC/AgO/ZnO	6450	680	476	0	0	0	0	89.5	92.6	100.0	100.0	100.0	100.0
Faecal Coliforms (cfu/100ml)	BKC	640	440	308	277	249	225	202	31.3	51.9	56.7	61.0	64.9	68.4
	BKC/ZnO	640	224	157	141	127	114	103	65.0	75.5	78.0	80.2	82.1	83.9
	BKC/AgO	640	96	67	0	0	0	0	85.0	89.5	100.0	100.0	100.0	100.0
	BKC/AgO/ZnO	640	80	56	0	0	0	0	87.5	91.3	100.0	100.0	100.0	100.0
Clostridium Perfringens (cfu/100ml)	BKC	40	38	27	24	22	19	17	5.0	33.5	40.2	46.1	51.5	56.4
	BKC/ZnO	40	34	24	21	19	17	16	15.0	40.5	46.5	51.8	56.6	61.0
	BKC/AgO	40	20	14	0	0	0	0	50.0	65.0	100.0	100.0	100.0	100.0
	BKC/AgO/ZnO	40	16	11	0	0	0	0	60.0	72.0	100.0	100.0	100.0	100.0
E. coli (cfu/100ml)	BKC	400	244	171	154	138	125	112	39.0	57.3	61.6	65.4	68.9	72.0
	BKC/ZnO	400	180	126	113	102	92	83	55.0	68.5	71.7	74.5	77.0	79.3
	BKC/AgO	400	32	22	0	0	0	0	92.0	94.4	100.0	100.0	100.0	100.0
	BKC/AgO/ZnO	400	24	17	0	0	0	0	94.0	95.8	100.0	100.0	100.0	100.0

**Comparative Analyses:** Table 4 showed the microbial results of the domestic wastewater treated with BKC/AgO and BKC/Ag/ZnO nanocomposite adsorbents were in accordance with NESREA standard.

**Table 4: Comparative Analyses**

Parameters	Wastewater	Nanocomposite Adsorbents				NESREA, 2011
		BKC	BKC/ZnO	BKC/AgO	BKC/AgO/ZnO	
Total Coliforms cfu/100ml	6.45 x 10 <sup>3</sup>	2348	2286	0	0	5 x 10 <sup>3</sup>
E. coli cfu/100ml	400	162	140	0	0	Absent
Faecal Coliforms cfu/100ml	640	260	200	0	0	Absent
Clostridium Perfringens cfu/100ml	48	15	10	0	0	Absent

#### IV. CONCLUSION

The nanocomposites adsorbent materials used in this study were efficient in the treatment of domestic wastewater against the test organisms. The bactericidal effect of AgO nanoparticles was more effective than ZnO nanoparticles. The results showed a complete removal of total coliforms, faecal coliforms, clostridium perfringens and E. coli at 30 minutes contact time and 40°C temperature for BKC/AgO and BKC/AgO/ZnO nanocomposite adsorbents. ZnO nanoparticle also showed potential as antimicrobial when the results obtained for BKC was compared with BKC/ZnO nanocomposite.

#### V. ACKNOWLEDGEMENT

**OriginLab Corporation, Northampton, USA:** Provision of Student version of OriginPro 2021b (trial) data and analysis software to calculate Full Width at Half Maximum (FWHM) of the selected peak. **Federal Ministry of Water Resources Abuja, Nigeria:** Free access to National Water Quality Reference Laboratory Minna. **Nanotech Research Group, Federal University of Technology, Minna:** Interfacing and liaising with the University of South Africa to characterize the kaolin clay and BKC samples.

**REFERENCE**

- [1]. Berekaa M.M. (2016). Nanotechnology in Wastewater treatment: influence of Nanomaterials on Microbial Systems. *International Journal of Current Microbiology and Applied Sciences*, Volume 5, Number 1, Pp 713 – 726.C
- [2]. Haritha Meruvu, Meena Vangalapati, Seema Chaitanya Chippada and Srinivasa Rao Bammidi (2011). Synthesis and Characterization of Zinc Oxide Nanoparticles and Its Antimicrobial Activity against *Bacillus Subtilis* and *Escherichia Coli*. *RasayanJ.Chem*, Vol.4, No.1 (2011), 217-222. ISSN: 0974-1496 CODEN: RJCABP
- [3]. Getie S, Belay A, Chandra Reddy AR and Belay Z (2017b). Synthesis and Characterizations of Zinc Oxide Nanoparticles for Antibacterial Applications. *J NanomedNanotechnol* 2017, S8. DOI: 10.4172/2157-7439.S8-004
- [4]. Haijiao Lu, Jingkang Wang, Marco Stoller, Ting Wang, Ying Bao, and Hongxun Hao. (2016). An Overview of Nanomaterials for Water and Wastewater Treatment. *Review Article.Hindawi Publishing CorporationAdvances in Materials Science and Engineering* Volume 2016, Article ID 4964828, 10 pages <http://dx.doi.org/10.1155/2016/4964828>
- [5]. Karishma K.C. and Mehali J.M. (2015). Application of Nanotechnology in Wastewater Treatment. *International Journal of Innovative and Engineering Research in Engineering*, Volume 2, Issue 1, Pp 21 – 25.
- [6]. Khushbu G. Patel, Rakshith R. Shettigar, and Nirendra M. Misra (2017). Recent Advance in Silica Production Technologies from Agricultural Waste Stream–Review. *Journal of Advanced Agricultural Technologies* Vol. 4, No. 3, September 2017. DOI: 10.18178/joaat.4.3.274-279
- [7]. Krishan K. Selwal, Vikas Sarsar, and Manjit K. Selwal (2013). Green synthesis of silver nanoparticles using leaf extract of *Mangifera indica* and evaluation of their antimicrobial activity. *Journal of Microbiology and Biotechnology Resesrach* 2013, 3 (5):27. ISSN: 2231 –3168, <http://scholarsresearchlibrary.com/archive.htm>
- [8]. Ming Hua, Shujuan Zhang, Bingcai Pan, Weiming Zhang, Lu Lv and Quanxing Zhang. (2012). Heavy metal removal from water/wastewater by nanosized metal oxides: A review. *Journal of Hazardous Materials* 211– 212 (2012) 317– 331,
- [9]. Okereke, J. N., Ogidi O. I., and Obasi K. O. (2016). Environmental and Health Impact of Industrial Wastewater Effluents in Nigeria - A Review, *Int. J. Adv. Res. Biol. Sci.* (2016). 3(6): 55-67 <http://s-o-i.org/1.15/ijarbs-2016-3-6-8>,
- [10]. Ralf Kaegi, Andreas Voegelin, Brian Sinnet, Steffen Zuleeg, Harald Haendorfer, Michael Burkhardt and Hansruedi Siegrist (2011). Behaviour of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant. ACS publications, [pubs.acs.org/est](https://pubs.acs.org/est). [dx.doi.org/10.1021/es1041892](https://doi.org/10.1021/es1041892) *Environ. Sci. Technol.* 2011, 45, 3902–3908.
- [11]. Rui Ma, Clément Levard, Jonathan D Judy, Jason M Unrine, Mark Durenkamp, Ben Martin, Bruce Jefferson, and Gregory Victor Lowry (2013). Fate of zinc oxide and silver nanoparticles in a pilot wastewater treatment plant and in processed biosolids. *Environ. Sci. Technol.* Downloaded from <http://pubs.acs.org>
- [12]. Sierra Maria J., Adriana P. Herrera and Karina A. Ojeda (2018). Synthesis of Zinc Oxide Nanoparticles from Mango and Soursop Leaf Extracts. *Contemporary Engineering Sciences*, Vol. 11, 2018, no. 8, 395 – 403, <https://doi.org/10.12988/ces.2018.8228>
- [13]. Stoyanova A., H. Hitkova, A. Bachvarova-Nedelcheva, R. Jordanova, N. Ivanova 1, and M. Sredkova. (2013). *Journal of Chemical Technology and Metallurgy*, 48, 2, 154-161
- [14]. Sukdeb Pal, Yu Kyung Tak, and Joon Myong Song (2007). Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*. *Applied and Environmental Microbiology*, Mar. 2007, p. 1712–1720 Vol. 73, No. 6 0099-2240/07/\$08.00\_0 doi:10.1128/AEM.02218-06
- [15]. Yang Zhang, Yongsheng Chen, Paul Westerhoff, Kiril Hristovski, and John C Crittenden (2008). Stability of commercial metal oxide nanoparticles in water. 42 (2008) 2204 – 2212, doi:10.1016/j.watres.2007.11.036



- [16]. Ying Chen, Hao Ding and Sijia Sun (2017).  
Preparation and Characterization of ZnO  
Nanoparticles Supported on Amorphous SiO<sub>2</sub>.  
Nanomaterials 7, 217;  
doi:10.3390/nano7080217  
[www.mdpi.com/journal/nanomaterials](http://www.mdpi.com/journal/nanomaterials)