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Anti-microbial and Cadmium Mitigating Effect of Magnesium Oxide-Carbonized *Hibiscus sabdariffa* (Zobo) Waste and Magnesium–Zinc Binary Oxide Nanocomposites in Contaminated Water

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Abstract

The need for active and inexpensive materials in combating menace of bacterial as well as heavy metals is very essential to the longevity of man and protection of his environment against deleterious substances such as bacterial and heavy metals. This research was designed to study the mitigative effect of MgO-carbonized zobo waste nanocomposite (MOCZWNC) and Mg-Zn binary oxide nanocomposite (MgZBONC) on *Salmonella typhi* – gram negative organism and *Staphylococcus aureus* – gram positive organism as well as eradication of cadmium ions in contaminated water. MgZBONCand MgOCZWNC was prepared, characterized and their adsorption ability was studied using adsorption isotherm evaluation. The mitigative effect of MgOCZWNC and MgZBONCon bacteria was evaluated using turbidimetric method. The maximum sorption capacity of MgOCZWNC and Mg-ZBONC for cadmium was 188.68 and 192.31 mg/g, respectively. The average crystallite size of MgOCZWNC and MgZBONC was 28.61 and 59.42 nm, respectively. The treated samples brought about a steady decrease in the population of *Staphylococcus* and *Salmonella* within 2 h, after about 2 h, the samples of staphylococcus and salmonella treated with MgOCZWNC continued to show continuity in its mitigative effect at decreasing the population of the bacteria with time whilethe staphylococcus and salmonella sample treated with MgZBONC experienced a slightly steady drop in their effectiveness after same nanocomposite period. The MgZBONC adsorbed cadmium ions more effectively and the MgOCZWNC was more efficient in severely reducing the population of staphylococcus and salmonella in contaminated water.

Keywords: Nanocomposites, antibacterial activity, mitigate, salmonella, staphylococcus

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Introduction

Water and food poisoning is an important health issues worldwide due to its fast contagiousness and lethality [1]. Staphylococcus, salmonella and cadmium are some notedmicrobes and heavy metal which are very harmful to human and its existence. There is need for potentially effective materials such as nanocomposites in mitigating the effect of these bacteria and heavy metals. A nanocomposite is a type of material composed of two or more phases or components, where at least one of these components has dimensions on the nanoscale (1–100 nm: 10^{-9}m) [2-3]. Metal oxide based nanocomposites are synthesized in order to modify their properties such as increased reactivity and efficiency [4-6]. Their ability to oxidize, reduce, precipitate and adsorb contaminants such as Pb^{2+}, Cd^{2+}, Ni^{2+} and microbes make them great adsorbents in water purification [7]. Nanomaterials are potential adsorbents and antimicrobial material because of their great chemical reactivity, large adsorption surface area, high porosity, enhanced structural properties, catalytic properties and chemical stability in solution, hence they can offer better selectivity, capacity and improved affinity towards heavy metals pollutants and also provide faster and efficient adsorption processes [3].

Pathogenic microbes such as *Staphylococcus aureus* (gram-positive bacteria), *Salmonella spp*. (gram-negative bacteria) are biological water pollutants that lead to various infectious diseases (typhoid, cholera, etc.) in humans [8]. Food and environmental contamination from both kinds of microbesmay occur directly from infected water and food-producing animals or may result from poor hygiene during production processes, or the retail and storage of food [8-9].

Heavy metal pollutants such as lead, cadmium, nickel are considered hazardous, toxic, carcinogenic and mutagenic because of their non-biodegradability [10].

In order to improve the water quality, protect human health as well as the ecological environment, it is therefore pertinent to use various nanocomposite to achieve this purpose. Antimicrobial agents have been extensively used to combat the presenceor proliferation of microorganisms; however they can be costly and require labor-intensive processing methods. Nanomaterials have been perceived to possess great antimicrobial properties and potentials [11]. Heavy metals can be removed from water by adsorption, chemical precipitation, ion exchange, etc. However, adsorption is considered the best treatment technique for the removal of heavy metals from water due to its high removal efficiency, cost-effectiveness, strong practicality, high applicability, good operability, low waste generation and low toxicity [12].

This investigation is envisioned to scrutinize the antimicrobial and cadmium mitigative effect of MgO-carbonized Zobo (*Hibiscus Sabdariffa*) waste nanocomposite (MgOCZWNC) and Mg-Zn binary oxide nanocomposite (MgZBONC) in reducing *Salmonella typhi*, *Staphylococcus aureus* and Cadmium (Cd^{2+}) in contaminated water.

Materials and Methods

Preparation of Mg-Zn binary oxide nanocomposite (Mg-ZBONC)

Mg-ZnBONC was synthesized by chemical co-precipitation method and thermal degradation method [13-14] with slight modifications. Solution A was made by liquefying 10.0 mmole of zinc sulphate ($ZnSO_4$) in 2%PVA solution in 100 ml distilled water in volumetric flask, and stirred to dissolve. Likewise, solution B was prepared by liquefying 10.0 mmole of magnesium sulphate ($MgSO_4$) in 2%PVA solution in 100 ml distilled water in volumetric flask, and stirred to dissolve. In preparing solution C, 20.0 mmole of sodium hydroxide (NaOH) was dissolved in 2%PVA solution in 100 ml distilled water in a volumetric flask and stirred vigorously. Solution C was added drop wisely from the burette to the mixture of solution A and B (which are in equal volume of 25 ml each) in a 500 ml flat bottom flask, with continuous stirring. The reaction mixture was continuously stirred for 2 h on a magnetic stirrer at a temperature of 90^{o}C. The solution was left to settlefor 6 h. The precipitate obtained was centrifuged and rinsedat the minimum five occasions with distilled water in recurring centrifugation to eliminate other unreacted reactants. The obtained precipitate was then dried in the oven at 100◦C and eventually, the Mg-Zn binary oxide nanocomposite was obtained by calcining the magnesium-zinc mixed hydroxide ($Mg(OH)_2$-$Zn(OH)_2$) at 950^{o}C for 6 h. The chemical equation of the reaction is revealed in equations 1 and 2:

$$MgSO_4\text{-}ZnSO_4 + 4NaOH \rightarrow Mg(OH)_2\text{-}Zn(OH)_2 + 2Na_2SO_4 \quad 1$$

$$Mg(OH)_2\text{-}Zn(OH)_2 \rightarrow \Delta MgO\text{-}ZnO \text{ (Mg-Zn Binary Oxide)} \quad 2$$

Preparation of MgO–Carbonized hibiscus flower (Zobo) waste Nanocomposite (MgOCZWNC)

Dried *Hibiscus sabdariffa* flower zobo flower was obtained from Egor market and further dried for one week to eliminate moisture. The zobo flower waste obtained after extraction of beverage (zobo drink) was dried in the oven at 85^{o}C for a week. The dried zobo waste obtained was carbonized in a muffle furnace (Carbolite, AAS 1110) at 300^{o}C for 30 min. The carbonized waste was ground using a British milling machine and sieved with mesh size 40 µm to get uniform particles.

MgO–Carbonized Zobo (*H. sabdariffa*) waste nanocomposite (MgOCZWNC) was prepared adopting [15] method with slight modification. Solution A was made by liquefying 10.0 mmole of magnesium sulphate ($MgSO_4$) in 2%PVA solution in 100 ml distilled water in volumetric flask and stirred vigorously, similarly, solution B was prepared by dissolving 20.0 mmole of sodium hydroxide (NaOH) in 2%PVA solution in 100 ml distilled water in a volumetric flask and stirred energetically. Solution B was put in drop wisely to solution Ain a 500 ml flat bottom flask from the burette, while stirring on a magnetic stirrer. The reaction mixture was stirred unceasingly for 1hour on a magnetic stirrer at a temperature of 90^{o}C after which 1 g of carbonized waste zobo (hibiscus flower) waste was added to the reaction mixture and allowed to stir for another 2 h. The reaction mixture was set asideto calm down for 24 h. The outcome was centrifuge and washed, at least 5 occasions. The outcome was calcinated in a muffle furnace at 290^{o}C for 90 min. After which the nanocomposite was taken out from the furnace and placed in a desiccator for 12 h. The chemical equations of the reaction are(equation 3, 4 and 5):

$$MgSO_4.7H_2O + 2NaOH \rightarrow Mg(OH)_2 + Na_2SO_4 + 7H_2O \quad 3$$

$$Mg(OH)_2 + C \rightarrow Mg(OH)_2\text{-}C \quad 4$$

$$Mg(OH)_2\text{-}C \rightarrow MgO\text{-}C \quad 5$$

Characterization of the nanocomposites

The nanocomposite prepared was characterized using Scanning electron microscopy (SEM; Phenom pro suite desktop scanning electron microscope), Fourier Transform-Infra Red (FT-IR System, spectrum BX, PerkinElmer, England) and x-ray diffraction (with X-Ray diffractometer, Schimadzu 6000 model).

Adsorbate preparation and adsorbent sorption analysis

Simulated contaminated water (adsorbates) of cadmium was prepared from its BDH analytical grade salts. The concentration of the cadmium ion in the prepared solution was affirmed with the use of atomic absorption spectrophotometer (AAS, Buck scientific model VGP-210). The adsorption efficiency of MgOCZWNC and MgZBONC for removing cadmium from contaminated water was studied through batch adsorption experiments. Batch adsorption parameter such as pH,time, adsorbent dosage and initial concentration was systematically varied and controlled to understand the effects of MgOCZWNC and MgZBONC on the removal of cadmium. The equilibrium amount of adsorbate adsorbed (adsorption capacities) and adsorption efficiency was calculated using the equation 6 and 7 [16-17]:

$$q_e = \frac{(C_o - C_e)}{M} \text{ x } V \qquad 6$$

$$\%E = \frac{(C_o - C_e)}{C_o} \text{ x } 100 \qquad 7$$

Where q_e is the amount of adsorbate absorbed in milligram per gram of the adsorbent; %E adsorption efficiency; C_o is the initial concentration of the metal ion before the adsorption process; C_e is the equilibrium concentration of the metal ion after adsorption process; M is the mass of the adsorbent and V is the volume of the solution in ml.

Application of adsorption isothermin adsorbent sorption analysis

The information about the nature of the interactions between the adsorbent and adsorbate molecules, as well as the affinity of the adsorbate for the adsorbent surface was obtained using a study of the graphical relationship between the amounts of adsorbate adsorbed onto the adsorbent's surface at equilibrium at constant temperature-adsorption isotherm [18]. Adsorption isotherm studies was carried out using Langmuir, Freundlich and Dubinin-Raduskevich adsorption isotherm models to evaluate the data obtained in order to predict whether the adsorption process occurred uniformly on the active site with same binding energies or the processes occurred on a multilayer sorption on a heterogeneous surfaces and active sites with different energies based on multilayer adsorption and equilibrium or to estimate the porosity of the adsorbent, the apparent energy of adsorption and the mechanism of adsorption whether physiosorption or chemosorption, respectively [19-20]. The linear equations of the models are shown in equations 8, 9 and 10:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{k_L q_m C_e} \quad \text{(Langmuir)} \qquad 8$$

$$q_e = \frac{1}{n}\log C_e + log K_f \quad \text{(Freundlich)} \qquad 9$$

$$\ln q_e = \ln q_m - \beta\varepsilon^2 \quad \text{(Dubinin-Raduskevich)} \qquad 10$$

Where: C_e is the equilibrium concentration of the adsorbate ($mg \cdot L^{-1}$),q_e is the equilibrium adsorption capacity of the adsorbent ($mg \cdot g^{-1}$), q_m is the maximum sorption capacity (mg/g),

k_L(L/mg) is the adsorption equilibrium constant (L/mg) at a given temperature related to the energy of sorption, it is a direct measure of the intensity of the adsorption process [18]. $K_f$andnare Freundlich constants depicting index of adsorption capacity and index of adsorption intensity or surface heterogeneity, respectively. $\frac{1}{n}$ ranges between 0 and 1(or n=1-10) is a measure of the favourability of the adsorption process, adsorption intensity or surface heterogeneity.As the value of $\frac{1}{n}$ gets closer to zero the surface becomes more heterogeneous. Whereas, a value below unity implies Chemisorptions process where 1/n above one is an indicative of cooperative adsorption [21]. β (mol^2/J^2) is activity coefficient constant which gives information on the average free energy of adsorption per adsorbate molecule when it is transferred to the surface of the adsorbent from infinity in the solution, $\varepsilon(J^2/mol^2)$ is related to the equilibrium concentration (Ce, mgL^{-1}) by this equation: [RT ln (1 + 1/C_e)]. The apparent energy (E, kJ/mol) of adsorption per adsorbate molecule (for removing a molecule from its location in the sorption space to the infinity) from Dubinin-Radushkevich isotherm model can be computed using equation 11:

$$E = (2\beta)^{-1/2} \qquad 11$$

Discerningthe nature of the sorption process could be forecasted based on the *E* value. If $E < 8$ kJ/mol, the sorption is of a physical nature, for $8 < E < 16$ kJ/mol, the sorption takes place by ion exchange, while for $E > 8$ kJ/mol, the sorption is of a chemical nature [18].

Study of the mitigative effect of MgOCZWNC and MgZBONC on *Salmonella typhi* and *Staphylococcus aureus*

Turbidimetric method of analysis was used to assess the mitigative effect of MgOCZWNC and MgZBONC on *Salmonella typhi* and *Staphylococcus aureus.* This process helps to determine the decimation of the bacteria with time. In this process, the spectrophotometer measures the quantity of light absorbed and transmitted through the sample. Through the degree of turbidity, the mitigative effect of the nanocomposite can be determined. When there is a high amount of bacteria, the turbidity will be high and less light is transmitted and vice versa. A control standard without the antibacterial agent (nanocomposite) was also made to confirm the effectiveness of the antibacterial agent. The readings in transmittance were plotted against time.

Prior to determining the antibacterial activity of the synthesized nanocomposites against *Salmonella typhi* and *Staphylococcus aureus*, the bacterial strains were

cultured under standard laboratory conditions by Enebeli Benneth Chukwudi, a Chief laboratory technologist and a specialist in the area of microbiology/plant pathology from the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Clinically isolated *Salmonella typhi* and *Staphylococcus aureus* on Petri dishes and prepared broth (nutrients) were acquired from University of Benin Teaching Hospital, Benin City. The nutrient broth were placed in two flasks with double distilled water, sterilized and activated in a pressure pot. Each surface of bacteria was swabbed by a sterile cotton swab (swab stick) and dissolved in a beaker containing 5ml of sterilized water. A syringe was used to measure 5 ml of the dissolved bacteria into each sample bottle appropriately labeled. Then 15 ml of the broth was measured into each labeled sample bottle containing each bacteria species. After which the nanocomposite material at the various concentrations were introduced into each sample bottle respectively at room temperature. Each sample bottle was incubated at 37ºC for 24 h. After incubation, each sample bottle was taken for absorbance transmittance measurement in the UV–Vis Spectrophotometer (JENWAY 6320D). The absorption spectrum of each sample is measured and noted at 0.5, 1, 1.5, 2 and 24 h, respectively.

Results and Discussion

Characterization of MgO-Carbonized *H. sabdariffa* (Zobo) waste (MgOCZWNC) and Mg-Zn binary oxide nanocomposites (MgZBONC)

The FT-IR spectra revealed the presence of O-H group at a peak - 3592-3000 cm^{-1} due to moisture and hydroxyl group on the MgO-carbonized *hibiscus sabdariffa* (zobo) waste, while the characteristic FT-IR spectra of Zn-Mg binaryoxide peak at 3905 -3324 cm^{-1} was assigned to O-H stretching vibration as a result of moisture. The peak at 2002 cm^{-1} indicated the existence of ketenimine (-C=C=N-) & isothiocyanate (-N=C=N-). The FT-IR spectrum also show the presence of N-H Stretch ascribed to the existenceof primary amide at a peak of 3502 cm^{-1}. The N-H stretch was also attributed to primary and secondary amine at a peak of 3398 and 3279 cm^{-1}. C-O stretch at 1156 cm^{-1} indicated the presence of acids, esters and anhydride. The peak at 721, 743 and 877 cm^{-1} predicted the presence of mono and meta-disubstituted aromatic compound. Mg-O stretching of MgO at 877 and 676 cm^{-1} (Fig. 1). Zn-Mg stretching of Zn-Mg binaryoxide at 99 and 917 cm^{-1} indicating the presence of Zn, Mg, and O within ZnO–MgO nanocomposites.

SEM micrographs was obtained using an accelerating voltage of 10 kV at x1,000 magnification. SEM micrographs clearly revealed that MgO-Carbonized zobo waste nanocomposite had a rough surface and variety of irregular pores. It has a crystalline texture in look. The SEM microgram of the MgO-ZnO binary oxide nanocomposite under the same SEM conditions revealed that its surface has smooth sheet-like flakes and crystalline look with a lesser amount of irregular pores (Fig. 2A and 2B).

X-ray diffraction pattern of the synthesised nanocomposites was obtained using the EMPYREAN-NGRL X-ray diffractometer with Cu K-Alpha radiation: 0.15406 nm (1.5406Å). In addition, the mean crystallite size was computedfrom the XRD data using the Debye-Scherrer formula equation:

$$D = k\lambda / \beta\cos\theta$$

Where D = Crystallite size; k = Scherrer constant (0.94); λ = X-ray wavelength (0.15406 nm); β = Full-width at half maximum of the peak (FWHM); θ = Peak position in XRD graph

The crystallite size (D) computed from XRD diffractogram data (Fig. 3) using the Debye-Scherrer formula equation, ranged from 11.71 - 48.70 nm with an average crystallite size of 28.61 nm for MgO-carbonized zobo (Hibiscus) waste nanocomposite (MgOCZWNC) and a range of 25.55 - 115.73 nm with an average crystallite size of 59.42nm was obtained for MgO-ZnO Binary Oxide nanocomposite (MgZBONC).

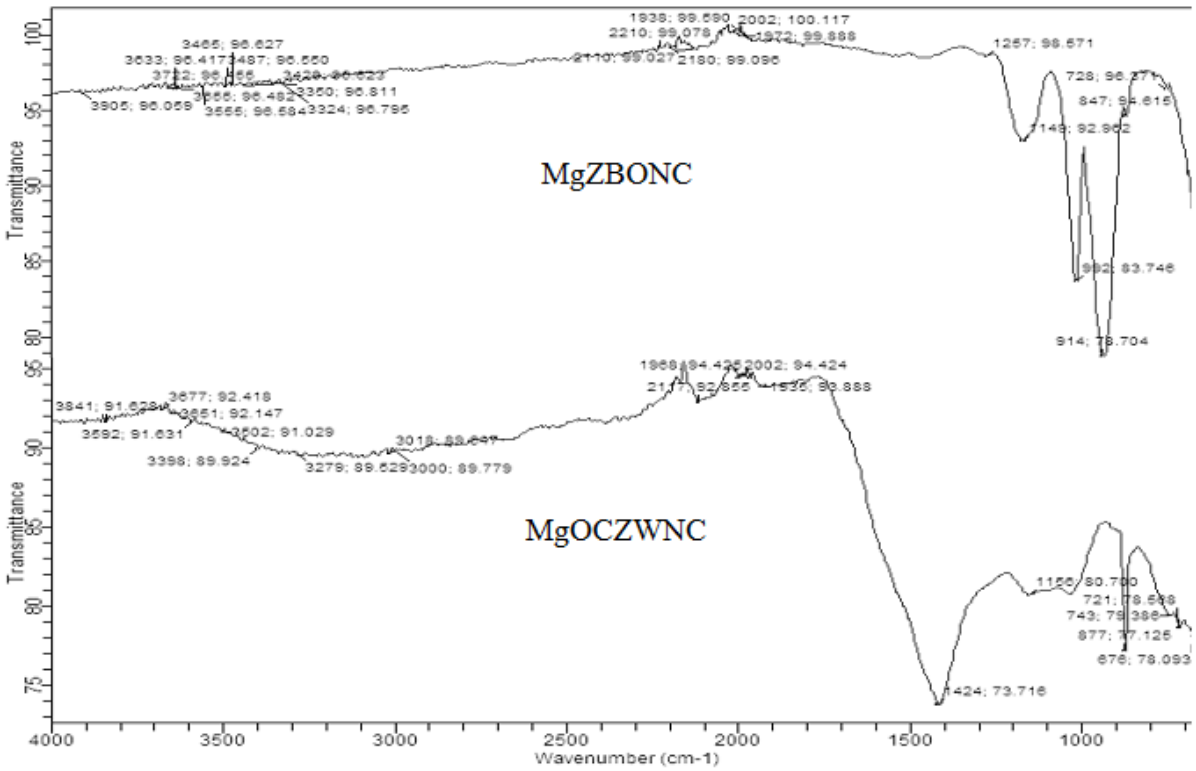

Figure 1: FT-IR spectra for MgZBONC and MgOCZWNC

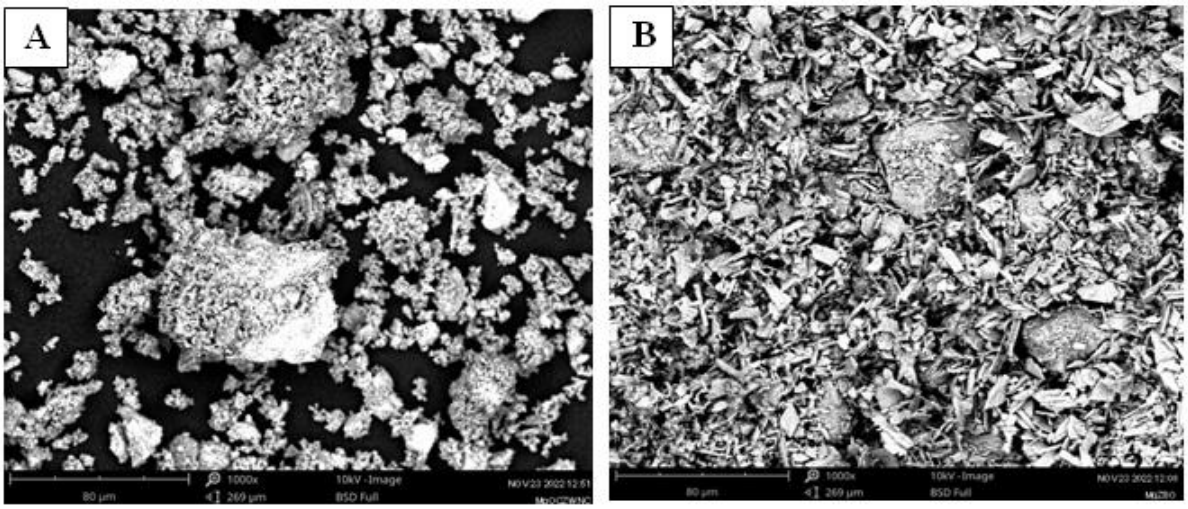

Figure 2: SEM image for MgO-Carbonized hibiscus waste nanocomposite (MgOCZWNC)-A and MgO-ZnO binary oxide nanocomposite (MgZBONC)-B

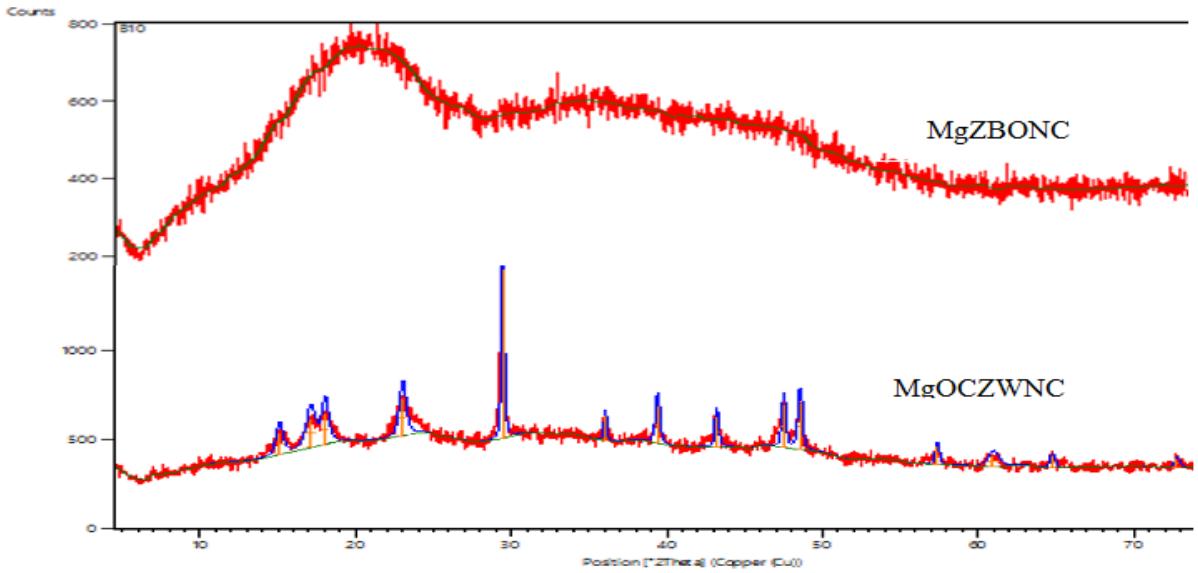

Figure 3: XRD diffractogram for MgZBONC and MgOCZWNC

Removal effect of MgOCZWNC and MgZBONC on cadmium from contaminated water

The coefficient of determination (R^2) obtained revealed that there was a goodness of fit of the models applied in interpreting the effects of MgOCZWNC and MgZBONC in removing cadmium from contaminated water. The R^2 values ranged from 0.83 to 0.95 (Table 1, Figs 4, 5 and 6). Langmuir adsorption isotherm study of MgOCZWNC and MgZBONC for cadmium ions revealed that the adsorbent had the highest sorption capacity of 188.68 and 192.31 mg/g, respectively. The Langmuir maximum sorption capacity is in agreement with the adsorption intensity of MgOCZWNC and MgZBONC for cadmium with a k_L value of 0.0823 and 0.224 respectively, this was corroborated the n_F of 0.84 and 2.66, respectively (Table 1). The $k_{F,}$ the index of adsorption capacity highest 15.09 and 42.24 for MgOCZWNC and MgZBONC, respectively, predicting that MgZBONC had a stronger adsorption capacity, hence a higher maximum adsorption of 192.31mg/g for cadmium ions. DRK adsorption isotherm study predicted that the adsorbent had the maximum sorption capacity of 94.72 and 147.14 mg/g (Table 1) respectively sequel to the higher amount of pores in the MgOCZWNC adsorbent.DRK adsorption isotherm study confirmed the superiority of MgZnBONC over MgOCZWNC in the removal of cadmium as a result of their apparent energy (E, kJ/mol) of adsorption per molecule or removal ofcadmium from its localein the sorption space to the infinity with a value of 707.13 J/mol for MgZnBONC and 34.45 J/mol for MgOCZWNC. The sorption pattern of the cadmium ion onto both adsorbent was by physiosorption.

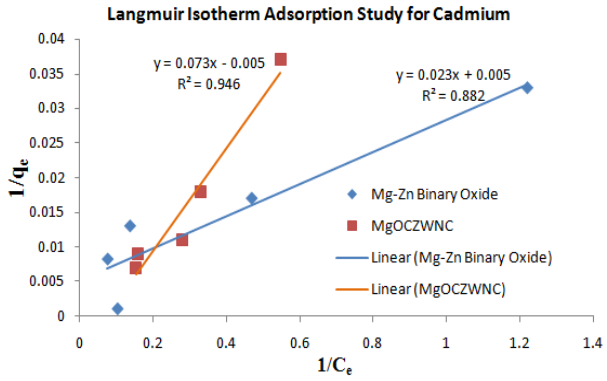

Figure 4: Langmuir adsorption isotherm study of MgOCZWNC and MgZBONC for cadmium

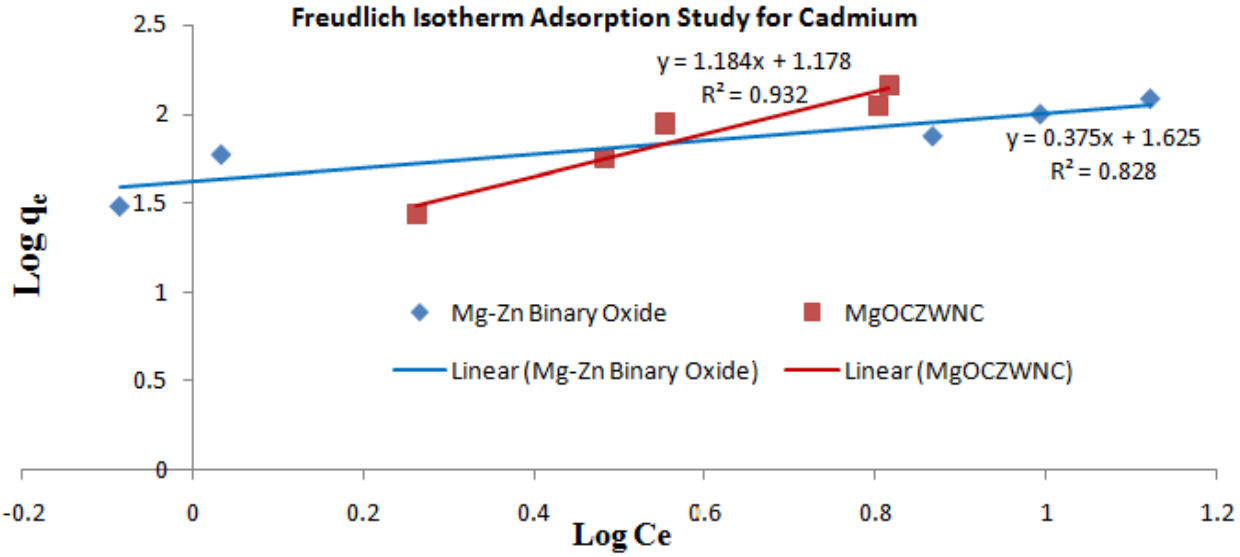

Figure 5: Freundlich adsorption isotherm study of MgOCZWNC and MgZBONC for cadmium

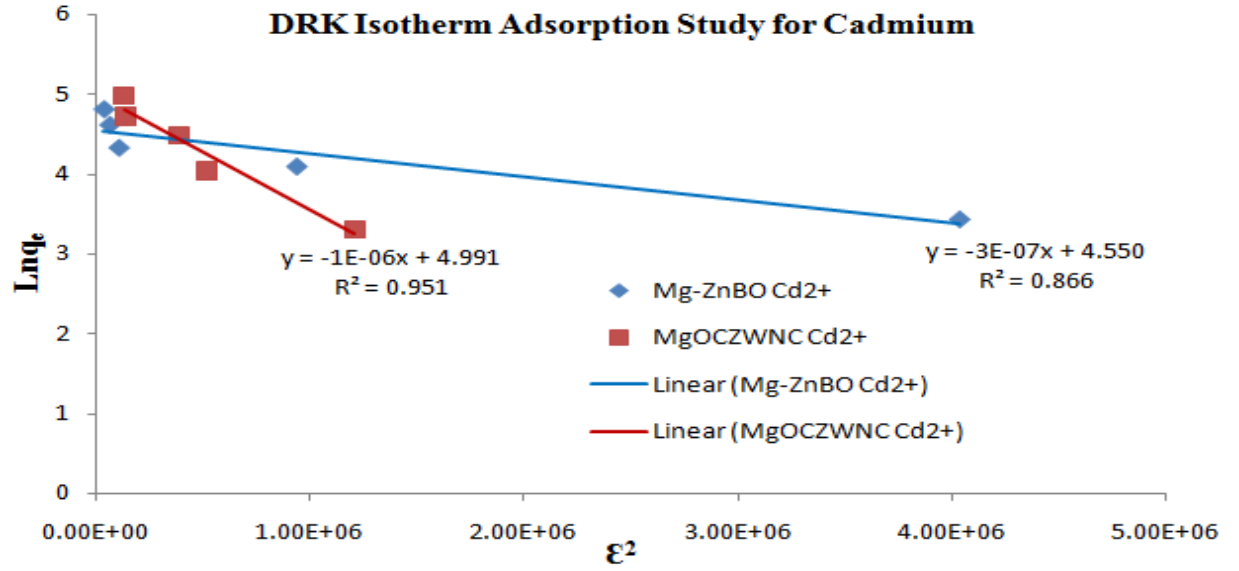

Figure 6: DRK adsorption isotherm study of MgOCZWNC and MgZBONC for cadmium

Table 1: Adsorption isotherm model parameters for cadmium adsorption

Adsorption Isotherm Model	Parameter	Unit	MgZBONC	MgOCZWNC
Langmuir	R^2	-	0.88	0.95
	q_m	mg/g	192.31	188.68
	K_L	-	0.22	0.08
Freundlich	R^2	-	0.83	0.93
	k_F	L/mg	42.24	15.09
	n_F	-	2.66	0.84
Dubinin Radushkevick (DRK)	R^2	-	0.87	0.95
	q_m	mg/g	94.72	147.14
	β	Mol^2/J^2	-3.0×10^{-7}	-1.0×10^{-6}
	E	kJ/mol	0.707.13	0.034.45

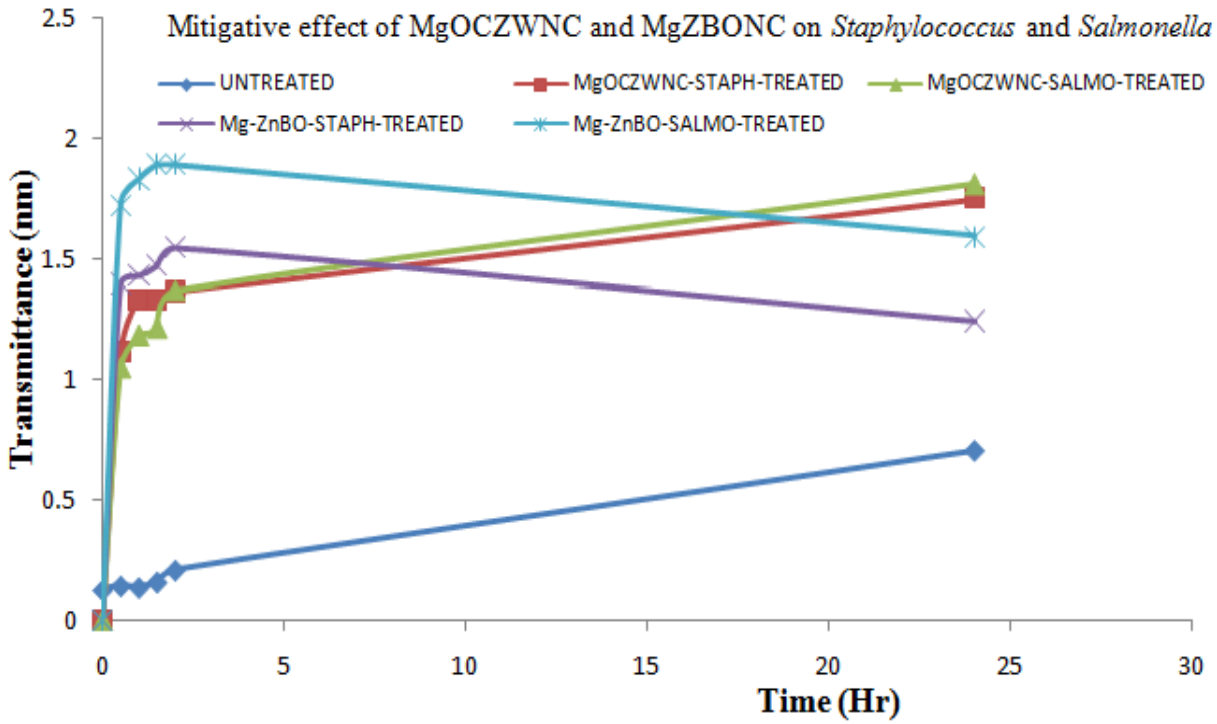

Figure 7: Mitigative effect of MgOCZWNC and MgZBONC on *Staphylococcus* and *Salmonella*

Mitigative effect of ZnOCZWNC and MgZBONC nanocomposites on *Salmonella typhi* and *Salmonella typhi* bacteria using turbidimetric method

The transmitted light from the UV–Vis Spectrophotometer (JENWAY 6320D) revealed there was a sharp rise in the transmitted light for all the nanocomposite within 2 h 30 min, however, the untreated sample displayed a very low transmittance compared to the treated samples of staphylococcus and salmonella.This observation revealed there was a steady growth and proliferation of the staphylococcus and salmonella bacteria without any interference hence the increase in turbidity whereas in the treated samples, at about 2 h 30 min, the growth of Staphylococcus and Salmonella was heavily mitigated as indicated by a higher light transmittance compared to the untreated (controlled) sample (Fig. 7). After about 2 h 30 min, the samples of staphylococcus and salmonella treated with MgOCZWNC continued to show a slightly steady rise in transmitted light indicating a continuity in the mitigation of this bacteria. The staphylococcus and salmonella sample treated with MgZBONC experienced a slightly steady decrease in transmitted light after about 2hrs 30min, indicating a drop in the effectiveness of the nanocomposite after that period. On the over all, the MgOCZWNC was more efficient in mitigating the growth of staphylococcus and salmonella in contaminated water.

Conclusion

The need for effective and low-cost materials for combating the menace of bacterial and heavy metals is very essential to the longevity of man and protection of his environment against deleterious substances such as bacterial and heavy metals which is the very basis of this research. The average crystallite size of MgOCZWNC and MgZBONC was 28.61nm and 59.42nm respectively. The MgZBONC adsorbed cadmium ions more effectively compared to MgOCZWNC. The adsorption pattern for cadmium onto MgZBONC and MgOCZWNC was by a physical process. The population of staphylococcus and salmonella in sample treated with MgOCZWNC and MgZBONC experienced a sharp decrease in the first 2 h compare to the untreated sample. After about 2 h, the samples of staphylococcus and salmonella treated with MgOCZWNC show continuity in its mitigative effect at decreasing the population of the bacteria with time while the staphylococcus and salmonella sample treated with MgZBONC experienced a slight drop in its effectiveness after 2 h. On the over all, the MgOCZWNC was more efficient in reducing the population of staphylococcus and salmonella in contaminated water.

Conflict of interest: The authors of this article declare no conflict of interest.

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