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A COMPARATIVE STUDY OF ZEOLITE LOADED COPPER OXIDE AND IRON OXIDE NANOPARTICLES AND ITS EFFECT ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY AGAINST SIX BACTERIAL AND THREE FUNGAL ISOLATES OF SECONDARY EFFLUENT

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ABSTRACT

Nanomaterials are well suited for water purification, disinfection and wastewater treatment applications. Antimicrobial nanoparticles can disinfect polluted water without formation of harmful disinfection by-products. Hence the present study is focused on the comparison of Zeolite loaded Copper oxide and Iron oxide nanoparticles and its effect on antibacterial and antifungal activity against bacteria and fungi isolated from secondary effluent. The chemically synthesized Copper oxide nanoparticles loaded zeolite (CuONZ) and Iron oxide nanoparticles loaded zeolite (Fe₃O₄NZ) were characterized by UV-Vis, SEM - EDAX, XRD and FTIR. The antibacterial activity was carried out by both qualitatively and quantitatively. The antibacterial activity of CuONZ and Fe₃O₄NZ was tested against *E.coli*, *Klebsiellapneumoniae*, *Salmonella typhi*, *Shigella boydii*, *Staphylococcus aureus* and *Bacillus subtilis* isolated from secondary effluent and it was tested with CuONZ and Fe₃O₄NZ at the concentration of 0.25g in sterile effluent and at the concentration of 0.25g and 2.5g of CuONZ and Fe₃O₄NZ in non sterile effluent. Excellent antibacterial activity was obtained with CuONZ. The antifungal activity of CuONZ and Fe₃O₄NZ was tested against *Candida albicans*, *Aspergillus niger* and *Rhizopus* spp. and the results showed that CuONZ played a significant antimicrobial activity. The metal oxide nanoparticles were stable during contact with microorganisms. Hence, natural Zeolite loaded nanoparticles could find the subsequent application in the disinfection of secondary effluent and removal of pathogenic microorganisms in the tertiary stage of wastewater treatment.

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INTRODUCTION

Nanotechnology manipulates matter at the nanoscale (1–100 nm) (Doolette *et al.*, 2013) producing nanoproducts and nanomaterials that can have novel and size-related physico-chemical properties differing significantly from those from larger particles. The novel properties of nanomaterials have been exploited widely for use in medicine, cosmetics, renewable energies, environmental remediation, and electronic devices (Fabrega, 2011 and Theron *et al.*, 2008). Nowadays the potentials of novel nanomaterials are employed for treatment of surface water (Hrevonic *et al.*, 2012; Tiwar *et al.*, 2008) groundwater and wastewater contaminated by toxic metal ions, organic and inorganic solutes, and microorganisms (Dhermendra *et al.*, 2008 and Sharma 2012). Nanofiltration techniques are now widely used to remove cations, natural

organic matter, biological contaminants, organic pollutants, nitrates and arsenic from groundwater and surface water (Bhattacharya *et al.*, 2013). Treatment of waste water contaminated with pathogenic bacteria of concern in water is *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Vibrio cholerae* and enteropathogenic *Escherichia coli* (Hrevonic *et al.*, 2012). Effluents before their discharge into water bodies is, crucial both in current environmental research and legislation (Velhal *et al.*, 2012) since water is a vital component of life on earth. The use of metal oxide nanoparticles exhibiting the antimicrobial activity offers the possibility of an efficient removal of pathogens from wastewater since they have much larger surface areas than bulk particles and can also be functionalized with various chemical groups to increase their affinity towards target compounds (Rajic *et al.*, 2011; Palanisamy *et al.*, 2013 and Tiwar *et al.*, 2008). Magnetic nanoparticles (MNPs) are a class of engineered particulate materials less than 100 nm that can be manipulated under the influence of an external magnetic

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field. Magnetic nanoparticles display the phenomenon of super paramagnetism, not keeping magnetized after the action of magnetic field, offering advantage of reducing risk of particle aggregation. Each particle behaves like a paramagnetic atom, but with a giant magnetic moment, as there is still a well defined magnetic order in each nanoparticle (Faraji *et al.*, 2010). Cuprous oxide (Cu_2O), a well-established stoichiometric form of copper oxide, is a p-type metal oxide semiconductor having a cubic crystal structure and direct band gap of 2.2 e (Zahmakiran and Ozkar, 2009). Zeolites are microporous, aluminosilicate compounds which are natural minerals or synthesized. They were widely used in industry for water and waste water treatment, waste gas treatment, as catalysts, as molecular sieve, in the production of laundry detergents, nuclear processing, medicine and in agriculture purposes for the preparation of advanced materials and recently to produce the nanocomposites (Jahangirian *et al.*, 2013). Although nanomaterials are small size, despite using as the best stabilizers it appears that the generation of nanoparticles in systems with confined void spaces such as inside mesoporous and microporous solids is an efficient way of preventing aggregation. In this regard, Zeolite-Y has been considered as a suitable host providing highly ordered large cavities with a diameter of 1.3 nm (Zahmakiran and Ozkar, 2009). The present study is aimed to compare the effect of antibacterial and antifungal activity of Copper oxide and Iron oxide nanoparticles loaded zeolite against six bacterial and three fungal isolates of Secondary effluent and they were characterized by UV- Visible spectroscopy (UV-Vis), Scanning electron microscopy (SEM) and Energy- dispersive X-ray (EDX) spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy.

MATERIALS AND METHODS

All the chemicals used in the present study were analytical grade and used as such without further purification. Standard laboratory glass wares made of borosil were used. Effluent from the secondary stage of the biological wastewater treatment plant at Sewage Treatment Plant, Nesapakkam, Chennai, India was collected and the bacteria from the effluent were isolated by serial dilution technique using Nutrient agar (Peptone-2.5g; Beef extract 0.75g; Yeast extract-0.8g; NaCl-0.25; Distilled water-500 mL; Agar-7.5g; pH-7.0) by incubating at 37°C for 24h. The well-isolated typical bacterial colonies were pure cultured and maintained on Nutrient agar slants at 4°C. The isolates were identified based on their morphological, cultural characteristics and biochemical reactions using Bergey's Manual of Systematic Bacteriology (1984). The fungi were isolated by serial dilution technique was followed by the isolation on Potato Dextrose agar (PDA - Potato Starch from infusion-2.0; Dextrose-10.0g; Distilled water - 500ml; Agar-7.5; pH-3.0) by incubating for 7 days RT. The well-isolated typical colonies were pure cultured and maintained on PDA slants at 4°C. The fungal isolates were identified based on their macroscopic, microscopic features and biochemical reactions.

Chemical synthesis of Copper oxide nanoparticles loaded Zeolite

A 20.0 g of Zeolite was added to a solution of 7.2g Copper-Cu (II) acetate reagent in 400 mL distilled water and stirred for

4hrs at 80°C. After filtration, the Cu (II)-exchanged Zeolite was washed with distilled water thrice and dried at 60°C in a hot air oven. The dried Cu (II)-exchanged Zeolite was mixed with 400mL distilled water and sodium hydroxide 0.1M was added to solution Cu (II)-exchanged Zeolite, until pH reached to 8.0. After 2h reflux, precipitate was collected by filtration and washed with distilled water and dried in oven at 60°C. The product was calcinated at 350°C in hot air oven for 2h and stored for further use (Reza *et al.*, 2012).

Chemical synthesis of Iron oxide nanoparticles loaded Zeolite

A 4.0 g of zeolite was added to 40 mL of distilled water and slowly stirred for 10 min until homogenous suspension was obtained. Then FeCl_3 and FeCl_2 solutions with the molar ratio of 2:1 was added into the suspension and stirred for 30 min. While the mixture was being stirred vigorously, about 20 mL of 2.5 M NaOH solution was slowly added and vigorous stirring was continued for another 30 min. The synthesized zeolite / Fe_3O_4 NPs product was then filtered washed with distilled water and finally air dried at RT. All the experiments were performed at ambient temperature and pressure (Jahangirian *et al.*, 2013). The metal-loaded zeolites nanoparticles resulted in the formation of CuO, Fe_3O_4 supported on the Zeolite were named as CuONZ, $\text{Fe}_3\text{O}_4\text{NZ}$, respectively.

Characterization of Copper oxide and Iron oxide nanoparticles loaded zeolite

The chemically synthesized copper oxide and iron oxide nanoparticles loaded zeolite were analyzed by UV-Vis, SEM - EDAX, XRD and FTIR spectroscopy for its composition, structure, and various properties like physical, magnetic, etc.

UV- Visible Spectroscopy (UV-Vis) analysis

UV- Visible spectroscopy analysis of nanoparticles (0.1mg/ml in distilled water) with a resolution of 2.0 nm between 200 to 800 nm was carried out on an ELCO SL159 UV-Visible absorption Spectrophotometer at Alagappa Chettiar building, Anna university, Guindy, Chennai, India.

Scanning Electron Microscopy (SEM) analysis

Scanning Electron Microscopy for the analysis of surface morphology with a magnification of 12X to greater than 10,000X was carried out on a FEI Quanta FEG200, Netherland at Sophisticated Analytical Instrumentation Facility (SAIF) IIT, Chennai, India.

Energy- Dispersive X-ray (EDX) spectroscopy analysis

Energy-dispersive X-ray (EDX) Spectroscopy analysis for the determination of elemental composition was carried out in EDAX at SAIF, IIT, Chennai, India.

Transmission Electron Microscopy (TEM) analysis

TEM study was done to determine the size and shape of synthesized nanoparticles with a lattice resolution of 0.14 nm and a point to point resolution of 0.12 nm which have a

standard probe and a variable temperature probe (100 to 500 K) and was carried out in JEOL 3010 instrument with a UHR pole piece to find out exact particle size at IIT, Chennai, India.

Fourier Transform Infrared (FT-IR) Spectroscopy analysis

The synthesized nanoparticles were mixed with potassium bromide and converted to a pellet and analyzed with a scan range of MIR 50-4000 cm^{-1} with the resolution of 2.0cm^{-1} was carried out in BRUKER RFS system at SAIF, IIT, Chennai, India.

X-ray Diffraction (XRD) analysis

The X-ray diffraction pattern of synthesized nanoparticles were observed with a resolution of 120 and the intensity of 2 θ and carried out on ISO Debyelex (2002) INELCPS at Nuclear Physics Department, Guindy, Chennai, India.

Evaluation of antimicrobial activity of copper and iron oxide nanoparticles loaded zeolite

The antibacterial activity was carried out by both qualitatively and quantitatively (Hrevonic *et al.*, 2012).

Qualitative method

The antibacterial activity of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ was tested against four Gram-negative and two Gram-positive bacteria pre-grown on Nutrient agar for 24 h at 37°C . The bacterial biomass was suspended in the sterile 0.05 M NaCl solution. 1 mL of the suspended each bacterial biomass was inoculated into 25 mL of sterile effluent water. To each of the bottles, 0.25 g of the sterile CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ were added. The control bottles were left without addition of the nanoparticles. The bottles were sealed and incubated aerobically in a dark for 24 h in a water bath at 37°C under shaking condition at 70 rpm. The experiments were performed in the same way for fresh non-sterile effluent water with 0.25g and 2.5g of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ per 25mL of non-sterile effluent water. The viable cells were determined at the beginning, after short-term exposure for 1 h and 4h (corresponding to the lag phase of bacterial growth) and long-term exposure of 24h (corresponding to the stationary phase of bacterial growth). OD values were checked to estimate the range of high or low bacterial numbers in the bottles and 0.1 mL were aseptically inoculated onto the Nutrient agar plates by spread plate method and incubated at 37°C for 24 h. After the incubation period, the bacterial colonies were counted reported as CFU mL^{-1} .

Quantitative method

The quantitative antibacterial activity of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ was studied by Standard Disc Diffusion Method. Muller Hinton Agar (MHA-Beef infusion form-150.0g; Casein hydrolysate-8.7g; Starch-7.5g; Agar - 8.5 g; Distilled water - 500 ml; pH-7.3) plates were prepared and stored at 4°C . The bacterial isolates were inoculated in test tubes containing 2 mL of Nutrient broth separately and the turbidity was compared to 0.5 Mac Farland standards. The tubes were incubated at 37°C for 24 h. The isolates were inoculated on MHA plates by

streaking the swab over the entire surface of the plates and dried for 5 minutes. Sterile discs of 5mm in dia were loaded with 30 μL of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ at different concentrations like 10 mg, 20 mg, 30 mg, 40 mg, 50 mg of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ prepared in 1ml of the solvent and placed on to the respective plates. The positive controls of Tetracycline, Ciprofloxacin and Ampicillin and negative control of sterile disc were placed in a separate plate. All plates were incubated at 37°C for 24 h. The evaluation of antibacterial activity of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ was determined based on the size of the inhibition zone around the disc (Behera *et al.*, 2013).

Antifungal activity of copper and iron oxide nanoparticles loaded zeolite by disc diffusion method

Antifungal activity of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ was studied by Disc Diffusion method. PDA plates were prepared and stored at 4°C . The fungal isolates were inoculated onto the test tubes containing 2 mL of Potato Dextrose broth separately and incubated at 28°C for 48 h (Savithamma *et al.*, 2012). The isolates were inoculated on PDA plate by swabbing over the entire surface of the plates and dried for 5 min. Sterile discs of 5mm in dia were loaded with 30 μL of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ at different concentrations such as 100mg, 200mg, 300mg, 400mg, 500mg prepared in 1mL of solvent and placed on to the respective plates. The positive controls of Amphotericin B, Ketoconazole and Flucanazole and negative control of sterile disc were placed in a separate plate. All plates were incubated at RT for 24 h to 48 h. The evaluation of antifungal activity of CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ was determined based on the size of the inhibition zone around the disc.

RESULTS AND DISCUSSION

The secondary effluent sample was blackish grey in colour from which six bacteria such as *E.coli*, *Klebsiellapneumoniae*, *Salmonella typhi*, *Shigellaboydii*, *Staphylococcus aureus* and *Bacillus subtilis* and three fungi such as *Candida albicans*, *Aspergillus niger*, *Rhizopus* spp. were isolated (Mohamed 2011; Lokeshwari *et al.*, 2013).

Characterization of Copper oxide nanoparticles loaded zeolite and Iron oxide nanoparticles loaded zeolite

UV-Vis analysis

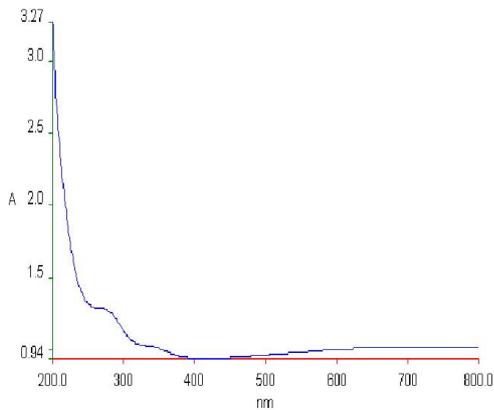
The chemically synthesized copper oxide nanoparticles loaded zeolite was light bluish in colour, powdery and soluble in distilled water and iron oxide nanoparticles loaded zeolite was brown in colour, powdery and also soluble in water (Figure. 1 & 2). Zeolite and both CuONZ and $\text{Fe}_3\text{O}_4\text{NZ}$ were analyzed by UV- Visible spectroscopy and obtained with peaks confirming the synthesis (Figure. 3), in which the zeolite exhibited an absorption peak at 270nm, CuONZ exhibited an absorption peak at 370 nm and $\text{Fe}_3\text{O}_4\text{NZ}$ exhibited an absorption peak at 260nm. The notable bands were observed at 260, 290, 340 and 440 nm and a broad absorption feature beyond 600 nm in the spectra of zeolite framework stabilized copper (I) oxide nanoparticles (Zahmakiran, 2009). The absorption peaks of iron oxide nanoparticles loaded zeolite at



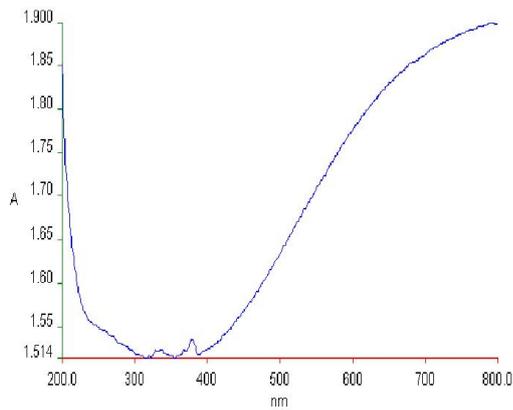
Figure 1. Copper oxide nanoparticles loaded Zeolite



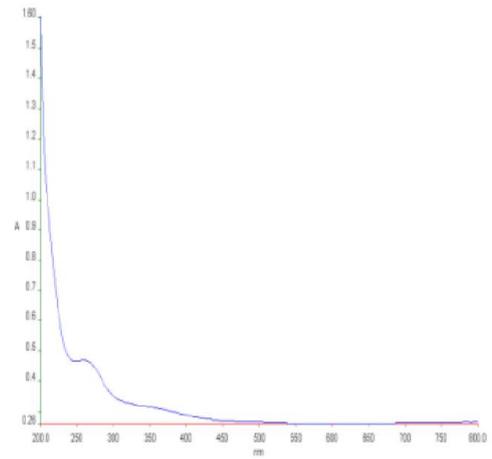
Figure 2. Iron oxide nanoparticles loaded Zeolite



a) zeolite



b) Copper oxide nanoparticles loaded Zeolite



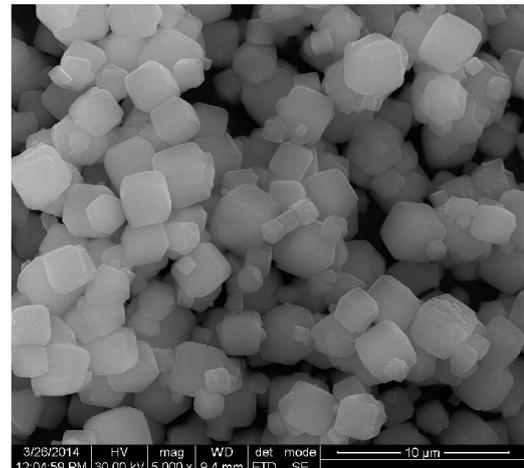
c) Iron oxide nanoparticles loaded zeolite

Figure 3. UV- Visible spectroscopy analysis of Zeolite, Copper oxide and Iron oxide nanoparticles loaded Zeolite

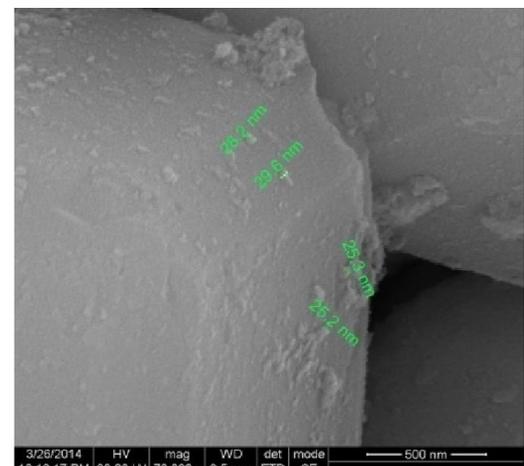
around 340 nm and 230 nm respectively observed (Meera, 2009). These peaks were due to the charge transfer spectra and characteristic peaks of Iron oxides.

SEM analysis

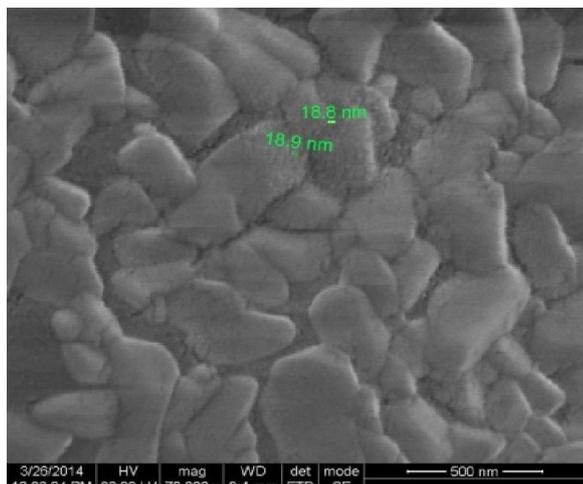
The SEM micrographs of Zeolite, CuONZ and Fe₃O₄NZ are shown in Figure. 4.



a) Zeolite



b) Copper oxide nanoparticles loaded Zeolite

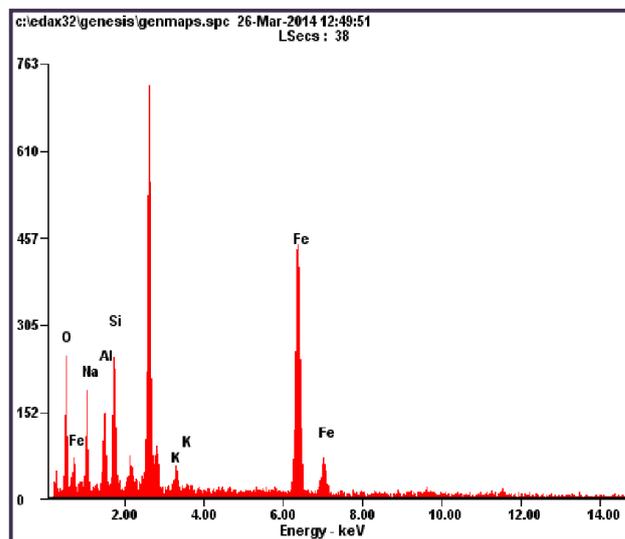


c) Iron oxide nanoparticles loaded zeolite

Figure 4. Scanning Electron Microscopy analysis of Zeolite, Copper oxide and Iron oxide nanoparticles loaded Zeolite

Element	Wt%	At%
OK	21.91	37.35
NaK	17.50	20.77
AlK	10.22	10.33
SiK	13.79	13.39
KK	01.42	00.99
FeK	35.16	17.17
Matrix	Correction	ZAF

b. Copper oxide nanoparticles loaded zeolite

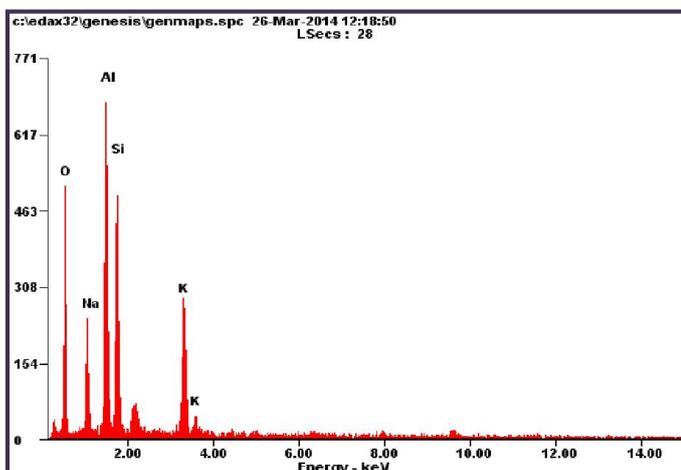


Element	Wt%	At%
OK	21.91	37.35
NaK	17.50	20.77
AlK	10.22	10.33
SiK	13.79	13.39
KK	01.42	00.99
FeK	35.16	17.17
Matrix	Correction	ZAF

c) Iron oxide nanoparticles loaded zeolite

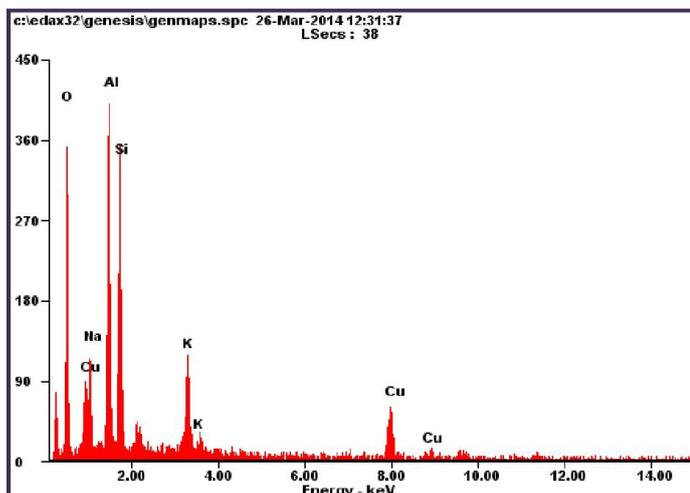
Figure 5. Energy Dispersive Spectroscopy (EDX) analysis of Zeolite, Copper oxide and Iron oxide nanoparticles loaded Zeolite

In SEM analysis the average size of CuONZ was measured as 27nm and Fe₃O₄NZ was measured as 18.8 nm. Both the nanoparticles were attached evenly on the surface of zeolite. Annamaria *et al.*, (2006) studied the influence of iron oxides on the structural properties of zeolite, the hysteresis loop of iron oxide confirmed its mesopores structure, typical for observation with aggregates. The crystallite size of both samples of iron oxide were calculated according to the Scherrer's formula, and the values 17.2 nm for the iron oxide 20°C and 19.1nm for the iron oxide 85°C were found. It could be assumed that the particles of iron oxide precipitated on the surface of zeolite, forming aggregates. In SEM micrograph the zeolite particles were agglomerated (Reza *et al.*, (2012). The EDAX results of zeolite, CuONZ and Fe₃O₄NZ are shown in Figure. 5 and it confirmed the presence of elements O, Na, Al, and Si by the indication of sharp signals with the zeolite weight percentage of 43.74%, 9.74%, 18.91% and 19.21 % respectively and CuO-NPs weight percentage of O, Na, Al, Si and Cu such as 42.71%, 8.71%, 18.41%, 17.99 % and 7.59% respectively and Fe₃O₄-NPs with the weight percentage of O, Na, Al, Si and Fe such as 21.9%, 17.50%, 10.22%, 13.79 % and 35.76% respectively.



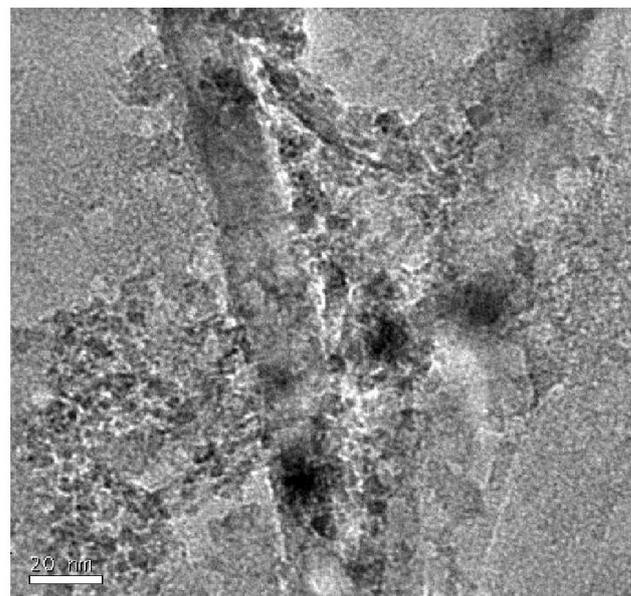
Element	Wt%	At%
OK	43.74	57.47
NaK	09.74	08.91
AlK	18.91	14.73
SiK	19.21	14.38
KK	08.39	04.51
Matrix	Correction	ZAF

a. Zeolite



TEM analysis

The TEM micrographs of Zeolite, CuONZ and Fe₃O₄NZ are shown in Figure. 6. In TEM analysis of CuONZ and Fe₃O₄NZ revealed the irregular and spherical structure of nanoparticles with size ranging from 10-20 nm. HR-TEM images revealed that nanocrystalline CuO particles were highly dispersed and no aggregation or cluster formation occurred. This might be due to the interaction between the zeolites active sites without damaging the zeolite crystal structure and the CuO surface, which have high surface energy due to its smaller size (Sakthivel *et al.*, 2008). The TEM image and histogram of particle sizes distribution of zeolite/Fe₃O₄ -NCs showed Fe₃O₄-NPs appeared as spherical morphology with mean diameters of 3.55 ± 1.03 nm which was obtained from counting of 169 particles in the image. The result was better compared that reported by Jahangirian *et al.*, (2013) who produced Fe₃O₄-NPs with average diameter of 7.5 nm when using FeCl₃ and FeSO₄ as iron sources and NH₄OH as precipitating agent.

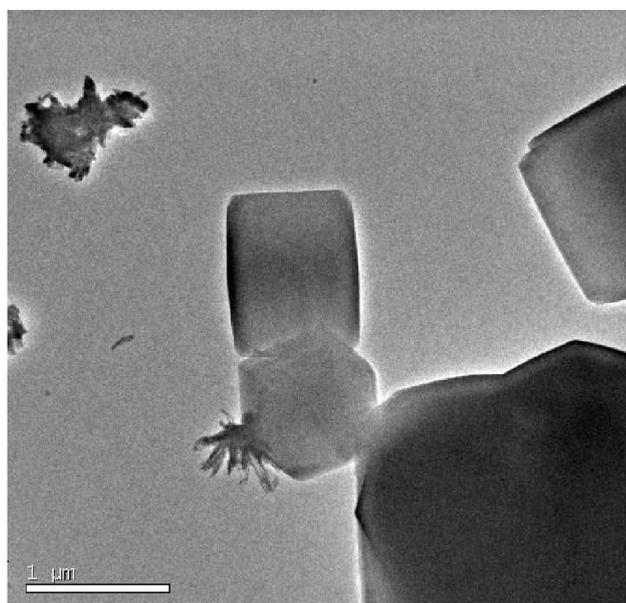


c) Iron oxide nanoparticles loaded Zeolite

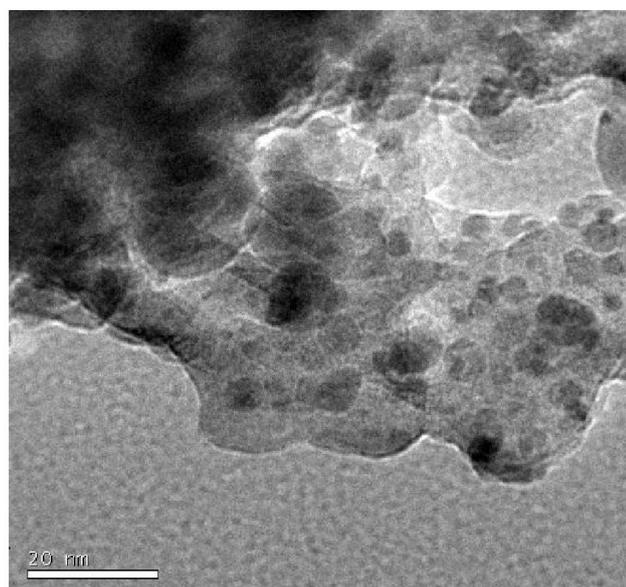
Figure 6. Transmission Electron microscopy analysis of Copper oxide and Iron oxide nanoparticles loaded Zeolite

FT-IR analysis

FT-IR spectra of Zeolite, CuONZ and Fe₃O₄NZ are shown in Figure. 7. FTIR analysis was carried out for further confirmation of Zeolite, CuONZ and Fe₃O₄NZ were observed in the range of 4000-400 cm⁻¹. FT-IR spectra of clean Zeolite have a strong absorption bands. The wide range of peaks appeared between 3000- 3700cm⁻¹ due to hydroxyl groups.



a) Zeolite



b) Copper oxide nanoparticles loaded Zeolite

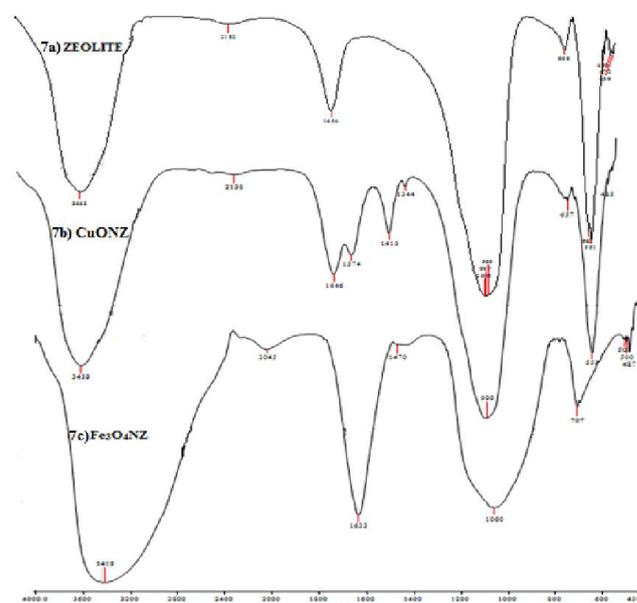


Figure 7. FT-IR Spectroscopy analysis of Copper oxide and Iron oxide nanoparticles loaded Zeolite

Vibrations within the network peak 450-1200cm⁻¹ showed no shift or broad disruption in the main absorption peak zeolite, due to presence of metal complexes or metal oxide nanoparticles. This phenomenon caused by low concentrations of metal in the zeolite was loaded. Most of the zeolite peaks were broad and strong. This range included 1100-1600cm⁻¹.

Table 1. Antibacterial activity of CuONZ and Fe₃O₄NZ on isolated bacteria with sterile effluent of secondary effluent

Bacteria	CuONZ 0.25g with sterile effluent (CFU mL ⁻¹)			Fe ₃ O ₄ 0.25g with sterile effluent (CFU mL ⁻¹)		
	1hr	4hr	24hr	1hr	4hr	24hr
<i>Escherichia coli</i>	NG	NG	NG	NG	NG	NG
<i>Klebsiella pneumoniae</i>	NG	NG	NG	NG	NG	NG
<i>Salmonella typhi</i>	10 ⁶ -10 ⁷	10 ⁵	6000	10 ⁷ -10 ⁸	10 ⁵ -10 ⁶	8000
<i>Shigellaboydii</i>	10 ⁶ -10 ⁷	10 ⁵	8000	10 ⁸ -10 ⁹	10 ⁶ -10 ⁷	10 ⁶ -10 ⁷
<i>Staphylococcus aureus</i>	10 ⁷ -10 ⁸	10 ⁵ -10 ⁶	NG	NG	NG	NG
<i>Bacillus subtilis</i>	2x10 ⁴	8000	4000	1x10 ⁴	5000	NG

NG = No Growth

Table 2. Antibacterial activity of CuONZ and Fe₃O₄NZ on isolated bacteria with non-sterile effluent of secondary effluent

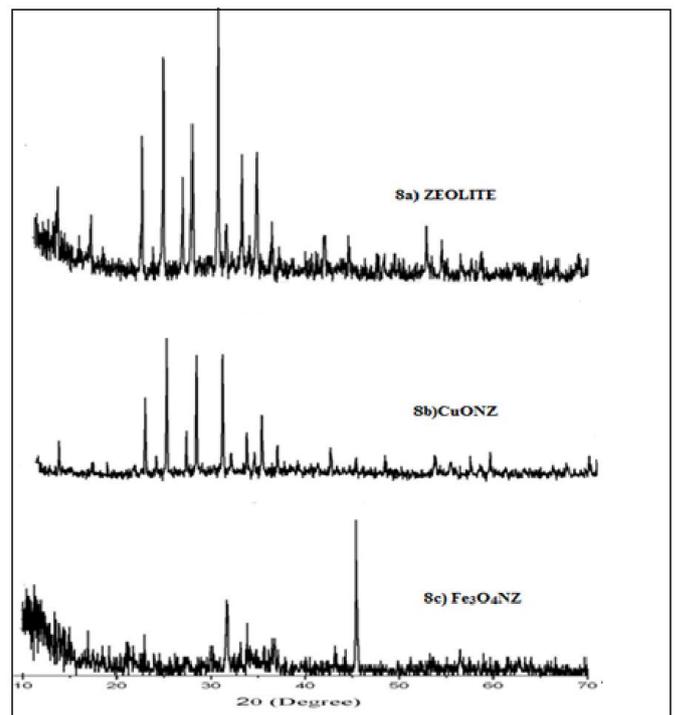
BACTERIA	CuONZ 0.25g with non-sterile effluent (CFU mL ⁻¹)			CuONZ 2.5g with non-sterile effluent (CFU mL ⁻¹)			Fe ₃ O ₄ 0.25g with non-sterile effluent (CFU mL ⁻¹)			Fe ₃ O ₄ 2.5g with non-sterile effluent (CFU mL ⁻¹)		
	1hr	4hr	24hr	1hr	4hr	24hr	1hr	4hr	24hr	1hr	4hr	24hr
<i>Escherichia coli</i>	500	600	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
<i>Klebsiellapneumoniae</i>	500	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
<i>Salmonella typhi</i>	10 ⁷ -10 ⁸	10 ⁶	1x10 ⁴	1600	800	NG	10 ⁴ -10 ⁵	6000	6000	10 ⁶ -10 ⁷	10 ⁴ -10 ⁵	1000
<i>Shigellaboydii</i>	10 ⁶ -10 ⁷	10 ⁵ -10 ⁶	5x10 ⁴	10 ⁷ -10 ⁸	10 ⁶ -10 ⁷	25000	10 ⁷ -10 ⁸	10 ⁶ -10 ⁷	10 ⁶ -10 ⁷	10 ⁷ -10 ⁸	10 ⁶ -10 ⁷	10 ⁶ -10 ⁷
<i>Staphylococcus aureus</i>	10 ⁵ -10 ⁶	1000	NG	5000	NG	NG	10 ⁴ -10 ⁵	6000	6000	8000	NG	NG
<i>Bacillus subtilis</i>	10 ⁶ -10 ⁷	10 ⁴ -10 ⁵	1x10 ⁴	10 ⁵ -10 ⁶	7000	1500	10 ⁶ -10 ⁵	8000	4000	5000	3000	NG

NG = No Growth

Based on FTIR spectrum of samples containing copper nanoparticles and compared with the pure zeolite spectrum, it was realized that the original zeolite spectrum (1005cm⁻¹) appeared in lower region and this peak had shifted 2cm⁻¹ and appeared in 999 cm⁻¹, which could be due by the interaction of zeolite network and copper oxide nanoparticles (Reza *et al.*, 2012). In FTIR analysis of Zeolite and Fe₃O₄NZ, the FTIR spectrum was related to the raw Zeolite and nanoscale samples Fe₃O₄ at Zeolite. The wide range peaks at 3000- 3800cm⁻¹ were due to hydroxyl groups. Vibrations within the network peak 450-900cm⁻¹ showed no shift or broad disruption in the main absorption peak zeolite, due to presence of metal complexes or metal oxide nanoparticles. This phenomenon caused by low concentrations of metal in the zeolite is loaded. Most of the zeolite peaks were broad and strong. Therefore, it was expected that the matrix within the zeolite peak special specific absorption peak is observed to be associated with the specimens with the range 1000-1600cm⁻¹. The FTIR spectrum of samples containing iron nanoparticles compared with pure zeolite spectrum can be realized that the original zeolite spectrum (1060cm⁻¹) appeared in lower region. This peak has shifted 2cm⁻¹ and appeared in 1470 cm⁻¹, which could be caused by the interaction of zeolite network and iron oxide nanoparticles (Jahangirian *et al.*, 2013).

XRD analysis

The XRD patterns of Zeolite, CuONZ and Fe₃O₄NZ are shown in Figure. 8. The XRD pattern of the zeolite and CuONZ showed peaks in the whole spectrum of 2θ values ranging from 10 -70. The XRD peaks indicated that the material consist of particles in nanoscale range. The diffraction peaks of zeolite located at 2θ = 12.4, 16, 21.6, 23.9, 26, 27, 29.8, 30.7, 32.4, 34.11, 41.4, 52.4 and the diffraction peaks of copper oxide nanoparticles loaded zeolite located at 2θ = 12.4, 16.1, 21.7, 22.8, 24, 26.1, 27.1, 29.9, 30.9, 32.5, 33.4, 34.2, 35.8, 40.1, 52.6, 56.5, 58.6, 69.1 and the diffraction peaks of iron oxide nanoparticles loaded Zeolite located at 2θ = 12.7,

**Figure 8. X-Ray diffraction analysis of Copper oxide and iron oxide nanoparticles loaded Zeolite nanoparticles**

13.4, 31.6, 45.4. The XRD patterns of Zeolite, CuONZ and Fe₃O₄NZ were corresponding to the Joint Committee on Powder Diffraction Standards (JCPDS) card No.77-0380. These results confirmed that there were significant amount of Fe₃O₄-NPs precipitated on the surface of zeolite as evidenced by the attraction of the powder particles of zeolite/Fe₃O₄- NPs (Jahangirian *et al.*, 2013). Sakthivel *et al.* (2008) given the powder XRD pattern of Zeolite and zeolite encapsulated nanocrystal line CuO. The presence of peaks corresponding to CuO (at 2θ= 34 and 2θ= 38) and confirmed the presence CuO in the zeolite. Pure C -Y zeolite also had small peaks

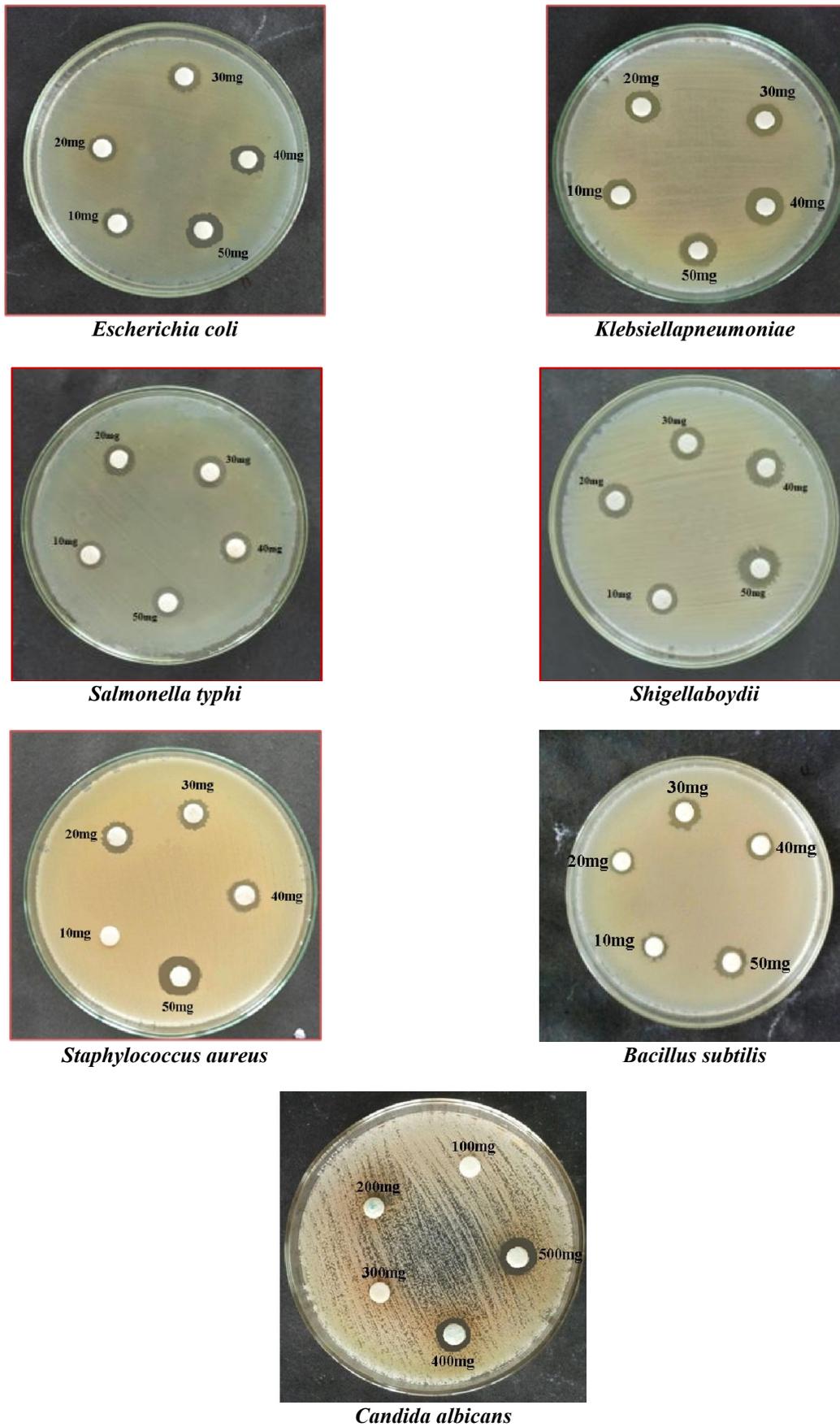
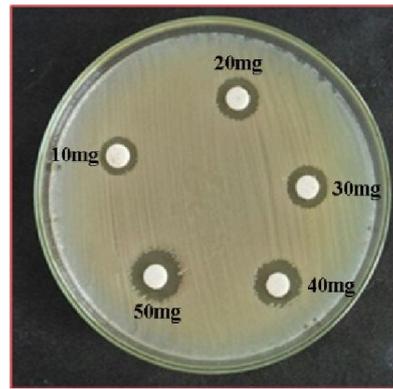
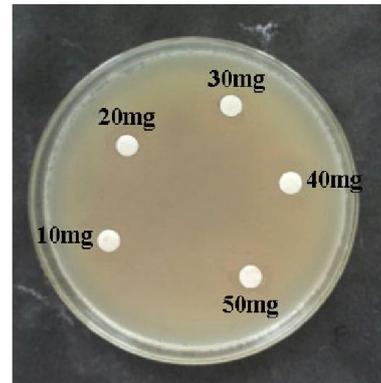
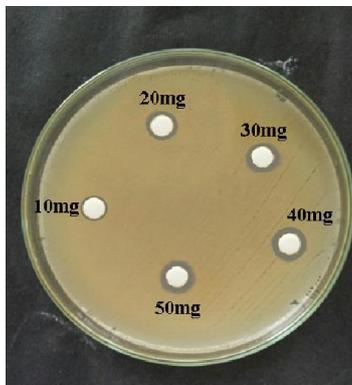
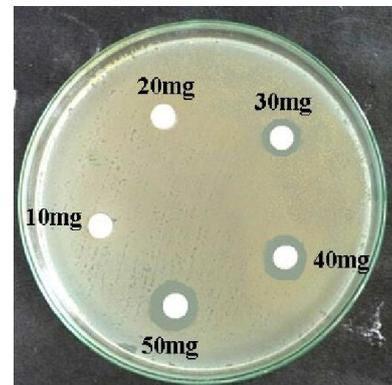


Figure 9. Antibacterial activity of Copper oxide nanoparticles loaded Zeolite (CuONZ) by Disc Diffusion method

*Escherichia coli**Klebsiella pneumoniae**Salmonella typhi**Shigella boydii**Staphylococcus aureus**Bacillus subtilis**Candida albicans***Figure 10. Antibacterial activity of Iron oxide nanoparticles loaded Zeolite (Fe_3O_4) by Disc Diffusion method**

corresponding to CuO and this might be due to the formation of trace amount of CuO. There have been no other changes in the other diffraction peaks of the zeolite and the crystallinity of the zeolite was maintained even after the base treatment of the zeolites. The XRD pattern of the zeolite and Fe₃O₄NZ showed peaks in the whole spectrum of 2θ values ranging from 10 -70. The XRD peaks indicated that the material consist of particles in nanoscale range.

Antibacterial activity of CuONZ and Fe₃O₄NZ by qualitative and quantitative method

In the present study the antimicrobial activity of copper oxide and iron oxide nanoparticles supported onto natural zeolite was investigated in the secondary effluent. In the qualitative antibacterial activity the numbers of bacterial viable cells were determined at the beginning of experiment and long-term exposure of 24 h (corresponding to the stationary phase of bacterial growth) shown in Table 1& 2. After 24 h of contact, the CuONZ showed consistently 100% of antibacterial activity against *Escherichia coli*, *Klebsiellapneumoniae* and *Staphylococcus aureus* and Fe₃O₄NZ showed consistently 100% of antibacterial activity against *Escherichia coli*, *Klebsiellapneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis* in both sterile and non-sterile effluent. Whereas, Fe₃O₄NZ didn't show any activity against *Shigellaboydii* when tested with both Qualitative and Quantitative method (Figure.9&10). When compared to Fe₃O₄NZ, the CuONZ showed the less number of viable bacterial cells of *Salmonella typhi* and *Shigellaboydii*. Excellent antibacterial activity was obtained for CuONZ and the antibacterial activity of Fe₃O₄NZ nanoparticles was less efficient. The antimicrobial activity of Cu₂O, ZnO and NiO nanoparticles supported onto natural Zeolite in the secondary effluent under dark conditions. After 24 h of contact the Cu₂O and ZnO nanoparticles reduced the numbers of viable bacterial cells of *Escherichia coli* and *Staphylococcus aureus* in pure culture for four to six orders of magnitude and showed consistent 100% of antibacterial activity against native *E. coli* after 1 h of contact during 48h exposures. The antibacterial activity of NiO nanoparticles was less efficient (Hrevonic et al., 2012).

Antifungal activity of CuONZ and Fe₃O₄NZ by disc diffusion method

Antifungal activity of CuONZ and Fe₃O₄NZ against *Candida albicans*, *Aspergillus niger* and *Rhizopus* spp. was studied by Disc Diffusion method. CuONZ showed susceptibility against *C. albicans* at the concentration of 400mg and 500mg. However, no inhibition area was identified for *Aspergillus niger* and *Rhizopus* spp. When compared to Fe₃O₄NZ, the CuONZ revealed a better antifungal activity against *C.albicans*. The results showed that CuONZ played a significant antibacterial activity when compared to Fe₃O₄NZ are shown in shown in Figure. 9. The silver nanoparticles were powerful antibacterial and antifungal agent against various pathogens. The antifungal activities of silver nanoparticles were tested against *Candida albicans* and no inhibition identified with *Aspergillus niger*. The minimum fungicidal concentration of *Candida* spp. was between 6.63 -18.75 µg ml⁻¹. Susceptibility of different pathogenic cultures against AgNPs results showed that AgNPs have great promise as antimicrobial agent against *C. albicans* (Chan, 2013).

Conclusion

In the area of water purification, nanotechnology offers the possibility of an efficient removal of pollutants and germs. It has made great improvements for handling water contamination problems and will clearly make further advancements in future. Nanotechnology based treatment has offered very effective, efficient, durable, less time and energy consuming with very less waste generations and eco-friendly approaches. The present study results proved the applicability of Copper oxide and Iron oxide nanoparticles loaded zeolite as a novel wastewater disinfection technique. The natural Zeolite loaded nanoparticles could find the subsequent application in the disinfection of secondary effluent water and removal of pathogenic microorganisms in the tertiary stage of wastewater treatment.

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