



## Microbiological Assessment and Treatment of Drinking Water Using Zinc Oxide Nanostructure in Khartoum, Sudan

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### KEYWORD

Drinking water; E.coli.  
Total coliform; Zinc  
oxide; antibacterial  
activity

### ABSTRACT

In this study, biological characteristics of drinking water were evaluated by purification method using ZnO nano structure as photo catalyst. Collection of 196 samples of water was carried out during January 2019-December 2019, covering three different seasons i.e. Autumn, Winter and Summer. Study area consisted of four lines of the Khartoum network according to (GPS) map. Samples were examined microbiologically to determine Total Coliform Count (TCC) by Most Probable Number (MPN) technique and fecal coliform (*E. coli*). ZnO semiconductor nanostructure was prepared and characterized using Scanning Electron Microscopy (SEM), Ultra Violet (UV) and X-Ray Diffraction (XRD) techniques. Microbiological analysis indicated that 66% and 37% of the samples tested were contaminated by Total and fecal coliforms, respectively. The highest Total Coliform contamination level (85.7%) was recorded in August during rainy season. While, during others seasons, the contamination levels were in the range of 39 - 82%. As for the water samples collected from different lines, highest level of contamination (100%) by total coliforms was recorded for line one, two and three in Winter. However, the lowest contamination level (28%) was recorded for line four in Winter. Fecal contamination was more frequently detected during rainy season in line one (100%), followed by line two and four (85%), while it was 57% in line three. In remaining seasons, the contamination levels were in the range of 14-71%, respectively. Almost 100% removal of *E. coli* was achieved by using only 0.01gm /100ml of ZnO nanostructure within 90 minutes in sunlight and the minimum bacterial contact time for removal was found to be 20minutes at 0.05gm/100 ml and 0.01gm/100 ml within 90 minutes in the dark. This study concluded that Khartoum drinking water was highly contaminated with total coliform and *E. coli*, and the contamination in rainy season was the highest. It is further concluded that the higher degradation percent of *E.coli* was achieved by ZnO catalyst in sun light.

### INTRODUCTION

In Sudan, there are different sources for drinking water supply, surface water from rivers and streams (mainly the River Nile and its main tributaries- the Blue Nile,

White Nile) rain water and ground water(Ahmed et al., 2002,Shanan et al., 2015).

The River Nile and its tributaries face many problems first: the discharge of untreated wastewater and sewage



from some industries in the River Nile, second: wastewater from refinery stations (containing polymers Most of the water treatment stations in the Khartoum State are old and designed to receive about 8000 ppm of suspended matter particles. In the 1980's, especially during and after the drought and desertification period, the amount of mud and suspended solids increased during flood period, amounting to about 21,000 ppm and the turbidity level reached about 6850 NTU (Taha and Ibrahim, 2002).

The drinking water distribution network of Khartoum was established in 1900 (Elhassan, et al., 1984). Water was fed from only two wells in Buri area to serve the army barracks and the government quarters at that time. During the years of 1925, 1927, 1954 and 1994 additional water sources were added: about 70 underground wells were dug to feed four stations (Ahmed, 2005).

Bacterial contaminated drinking water and poor sanitation has been incriminated among factors responsible for over a million deaths per year (Akporo, et al., 2011). Long storage of good-quality drinking water is a main factor of fecal coliforms contamination through hands or utensils (Jensen, P. 2002). It has been claimed that cold water storage tanks distributed in Khartoum area were contaminated; people play a major role in polluting storage tanks water (Bashir, E et al., 2005). Contamination by microorganisms can occur through improperly installed or through undetected leaks in the water pipe system (Gleeson, C et al., 1997). Studying the quality of water in Khartoum network, Musnad et al. (2010) showed that the bad physical conditions of the pipe's lines led to biological contamination. Ministry of Health reported in 2011 the microbiological contamination levels were found to be 10.3 % in Mogran water as to Buri 21.6% while in 2014, 15.3% contamination in Mogran and 44.7% in Buri.

El-Tingari (2010) also reported 25% of the samples of Khartoum network were found to be contaminated with coliform bacteria.

The presence of an epidemic water-related disease has been reported in many areas in Khartoum (WHO 2002) *E. coli* is an important pathogen, which could cause diarrhea in children (Guo et al.; 2005 and Yu et al., 2011), some kinds of *E. coli* can cause urinary tract

and other chemicals), third: agricultural chemicals waste (Taha and Ibrahim, 2002, Abu Sabah et al., 2018). infections, respiratory illness and pneumonia and other illnesses.

Several studies have been conducted regarding the bacteriological quality of drinking water in the different localities in Sudan such as in Nyala South Darfur, Sudan. (Amira A.A and Yassir M.E 2010), Sudan University of Science and Technology, Eltayib H A et al 2013), Khartoum State (Amira M E et al 2010 and Sana O and Rawda Y 2009), Dairy farm in Khartoum State Atif E A 2014), Shendi Town, River Nile State (Karbassi et al., 2008, Rychlik, 2011, Ahmed Adam Belal et al., 2021) and Abeer M. Abasset al 2022), results of all these studies were tested positive for fecal coliforms and Total coliform.

The most used water disinfection conventional methods are chlorination, ultraviolet treatment and ozonation. However, these methods have their limitations, chlorine and ozone has a tendency to form carcinogenic disinfection by-products (DBP) when added to water, moreover some of water-borne bacteria have increased their resistance to available disinfectants, this leads to higher required disinfectant doses, and hence the formation of higher amounts of DBP (Sara et al., 2015; Karsener et al., 2006; Cheng et al., 2010; Remucal & Manley, 2016). Hence, new treatment method needs to be considered to enhance effectiveness of disinfection. (Baruah et al., 2012) have extensively reviewed the prospects of photocatalysis as a promising method for water disinfection. Nanotechnology has a potential to advance water treatment by improving treatment efficiency, the use of metal oxide semiconductors in photocatalysis for environmental applications has been extensively investigated (ujishima A et al., 2007). Well known that the semiconductor material becomes more and more chemically active as the size of the particles get down to nanometer scale (Svrček et al. 2006).

Zinc oxide (ZnO) is a (II-VI) is stable under harsh processing conditions, which makes it suitable for antimicrobial applications. It is used in cosmetics (Sonia S et al., 2017), cellulose fiber (Varaprasad K. et al 2017), also incorporated as antimicrobial into textiles (Cloutier Met al., 2017). Moreover, for photonic applications, it is a bio-safe, biocompatible and low-cost production (Yu J. et al., 2008).



ZnO is increasingly recognized as a suitable alternative due to its bandgap energy of ZnO (3.37 eV), results in the transition of electrons from the valence band (VB) to the conduction band (CB). The result of this process is region of positive charge termed a hole (h<sup>+</sup>) in the VB, and a free electron (e<sup>-</sup>) in the CB (Alhamed and Abdulllah 2010). At the ZnO particle surface, the holes react with surface hydroxyl groups (OH) and adsorbed H<sub>2</sub>O molecules to form OH• radicals. Hydroxyl radicals have the power to oxidize the organic compounds adsorbed onto the semiconductor surface and inactivate microorganisms (Rincnet. *al.*, 2001).

Photocatalytic reactions, damage the cytoplasmic cell wall which cause the leakage of cell contents, and eventually cell death (Vijayaraghavan., 2012). ZnO is also reported to be strongly antibacterial on a variety of targets like Escherichia coli (E. coli), Staphylococcus

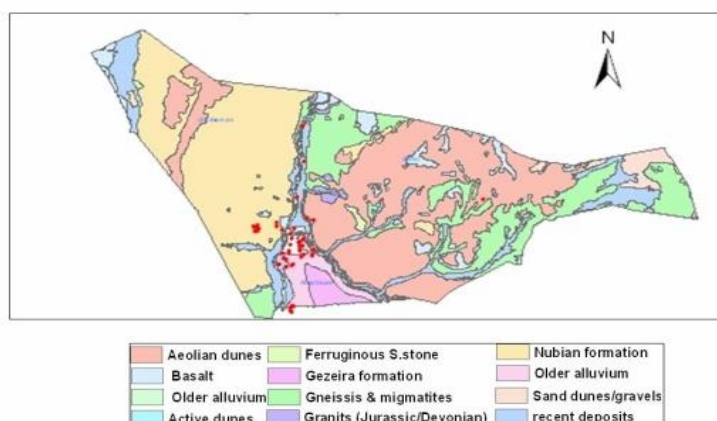
aureus (S. aureus), Bacillus subtilis (B. subtilis), (Z. Huanget. *al.*, 2008, Phys., 2009 and Zhang L., 2011).

This study aimed to assess the microbiological content in drinking water in Khartoum State, Sudan and to purify this water using ZnO nanostructure. The inactivation rates of gram-negative Escherichia coli exposed to ZnO in both Sunlight and dark conditions were also investigated. Moreover, the minimum inhibitory concentration (MIC) and the minimum bacterial contact time were estimated.

## Materials and Methods

### Samples collection

Water samples were collected from Khartoum State (032° 32' 00" E, 15° 38' 00" N). Fig (1) shows the geological map of Khartoum State and ground water boreholes location.



**Fig (1) Geological map of Khartoum State and ground water bore holes location**

The study area was divided into four lines of the Khartoum locality network according to (GPS) map as shown in Fig (2). Seven samples were collected from each of the four lines based on distance (distance between each sample points taken is one kilometer for each line).

The collection of 196 samples was carried in seven phases during the January 2019-December 2019, covering the three different seasons; rainy season, winter and summer.

The seven phases were:

- 1- Phase one: from 1st of January to 1st of February.
- 2- Phase two: from 17th of February to 1st of March.
- 3- Phase three: from 1st of April to the end of May.
- 4- Phase four: from 20th of June to the end of July.
- 5- Phase five: from 1st August to the end of September.
- 6- Phase six: from end of September to the end of October.
- 7- Phase seven: from end of October to the end of December.



**Fig (2) GPS Map of sampling location**

For the bacteriological tests, the outside nozzle of the tap was cleaned, turned on full and the water allowed to run for 1-2 minutes, after that the tap was sterilized by the flame. Then it was allowed to cool by running the water to waste for two minutes then the sample bottle was filled from a gentle flow of water and the cap of the bottle closed. Glassware to be used in the analysis, was washed thoroughly with de ionized water and left to dry, and then sterilized in a hot oven.

The water samples were collected in 200 ml sterile glass bottles, stored in an ice box, transported to the laboratory and immediately analyzed.

### Sample preparation

#### Chemical analysis of water samples:

Laboratory chemical analysis was carried out according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1971, 1985 and 1998, Richards 1954) and (Csuros et al., 2018).

#### Sterilization of glassware:

Before sterilization at 160°C for at least three hours (Harrigan and McCane, 1976), instruments such as loops, needles, forceps, spoons, and knives were sterilized by flaming directly after dipping in spirit.

#### Determination of coliform bacteria:

Determination of coliform bacteria was done by using the most probable number (MPN) technique according to (Sun et al., 2006).

#### Presumptive coliform test:

The medium used in this test was MacConkey broth double strength medium with neutral red indicator. The presumptive coliform test was carried out by inoculating the tubes of sterile MacConkey broth containing inverted Durham's tubes as follows (Harrigan, 1998, Li et al.2014, Remucal & Manley 2016).

1. 50 ml of the water sample were added to one bottle containing 50 ml of double strength MacConkey broth using a sterile measuring pipette.
2. 10 ml of each of the water sample was added to each of five universal tubes containing 10 ml of double-strength MacConkey broth.
3. 1 ml of each of the water sample was added to each of five universal tubes containing 5ml of double-strength of MacConkey broth.
4. The inoculated tubes were incubated at 37°C for 48 hours.



### Confirmed coliform test:

Each gas positive presumptive tube was inoculated into a tube containing 5 ml brilliant green bile broth with inverted Durham's tube. All tubes were incubated at 44 - 45°C for 24 hours to detect faecal coliforms. Negative tubes were discarded and results were recorded. Positive tubes were shown by gas production and turbidity appearance.

MPN of bacteria were calculated from the combination of confirmed positive, negative and presumptive results. The values were looked upon from probability formula in standard methods Maccrady's table and recorded as MPN/100 ml samples. (Sagar Aryal, 2018).

### Isolation of E. coli (completed test):

A loop immersed in brilliant green positive tubes was inoculated into 5 ml of peptone water and incubated at 44-45°C for 24 hours, a drop of Kovac's reagent (0.2 – 0.3 ml) was added. (Adzitey et al., 2015).

The dark red colour on the surface of the culture indicated a positive test for indole

### Preparation of zinc oxide (ZnO) nanostructure

Zinc Nitrate hexahydrate was prepared, then, 250 ml of it were mixed with 250 ml of the prepared Hexamethylenetetramine (HMTA) in a beaker (1000ml)

The mixture was heated to 78 °C in the oven for five hours. The white fine nanostructure ZnO was filtered, cleaned with deionized water and ethanol successively, then dried in air atmosphere at about 60° C (Chen *et al.*, 2000)

### Zinc oxide nanostructure characterization

#### XRD characterizations

ZnO X-ray diffraction (XRD) patterns were measured at Physics, Chemistry, and Biology (IFM) Department at Linköping University, Sweden using a Phillips PW 1729 powder diffractometer (Philips, PAN analytical, The Netherlands) equipped with Cu K  $\alpha$  radiation ( $\lambda = 1.5418 \text{ \AA}$ ), a generator voltage of 40 kV and a current of 40 mA.

Particle size was calculated from XRD diffraction pattern measured for ZnO particles using Scherrer equation.

### SEM Characterization

Field emission scanning electron microscopy was measured on a Jeol microscope, Model JSM-6700F, using the energy dispersive spectroscopic FE-SEM/EDS technique. SEM shows the surface morphology and an estimated size of the prepared ZnO particles.

### Results

#### Total Coliform Count (TCC):

Table 1. Shows the number of total coliforms in 100 ml of water samples from the seven phases. The contamination level with total coliform for the 28 samples from the four lines (CL) in the different points during the period (January 2019- December 2020), the highest (85.7%) contamination level was recorded in August (rainy season) In the remaining months, the contamination levels were in the range 39%- 82%. As for the four lines, the highest levels (100%) were recorded for line two and three in phase two (winter), line one and three in phase six (winter) and line one in phase seven, while the lowest (28%) was recorded for line four in phase six and seven.

**Table 1: Number of total coliforms in 100 ml of water sample**

Lines Phases	Line 1	Line2	Line3	Line4	CL%
Phase 1	50	5	0	13	76%
	17	5	0	0	
	3	35	35	0	
	35	35	25	8	
	0	20	35	0	
	0	1	8	5	
P C%	71%	85.7%	71%	42%	
	0	8	12	0	



Phase2	1	1	8	0	82%
	25	13	18	1	
	2	35	13	17	
	1	35	90	0	
	0	90	3	3	
	1	25	8	5	
P C%	71%	100%	100%	42%	
Phase 3	5	0	0	0	64%
	0	8	25	0	
	25	90	13	1	
	0	0	18	9	
	1	35	12	0	
	1	90	3	5	
	0	25	0	9	
P C%	57%	71%	71%	57%	
Phase 4	9	0	16	0	57%
	9	2	0	0	
	0	0	18	0	
	9	6	18	9	
	0	3	18	6	
	0	16	0	9	
	6	0	18	9	
P C%	57%	57%	71%	71%	
Phase 5	9	0	9	0	85.7 %
	9	2	3	6	
	16	0	16	0	
	9	9	9	18	
	16	18	9	9	
	9	6	3	16	
	6	9	4	6	
P C%	100%	71%	100%	71%	
Phase 6	0	0	0	0	39%
	18	0	0	0	
	0	16	9	1	
	16	16	6	2	
	0	18	16	0	
	0	0	2	0	
	0	0	0	0	
P C%	28%	42%	57%	28%	
Phase 7	16	16	0	0	64%
	9	0	0	0	
	6	1	6	16	
	3	6	18	18	
	18	18	18	0	
	1	0	6	0	
	9	2	0	0	
P C%	100%	71%	57%	28%	
MC %	100%	71%	57%	28%	
Average	-	-	-	-	66.8 %
SD	-	-	-	-	16.1

\*MC Monthly Contamination, \*\*PC Phase Contamination, \*\* \*CL Contamination of lines *E. Coli*

Based on the bacterial cultures results obtained, all samples studied in the seven phases (n= 196), the level of *E.coli* contamination was 37%. *E.coli* was present in

most of the samples taken from different points for each of the four lines.



As shown in (Table 2) *E. Coli* was more frequently detected in phase five (rainy season) (100%) line one, followed by line two, line four (85%) and line three (57%).

In the remaining months, the contamination levels were in the range 71% - 14%. As for lines, the highest levels (85.7%) were recorded for line three in phase two (winter) and phase three summer, while the record of

uncontaminated (0.00%) samples were found in all the four lines in phase one, line one, phase two and line four in phase three.

*E.coli* was observed to exceed the standard zero limits in most of the points in the four lines and during most of the phases.

**Table 2: *E.coli* contamination level in water samples on the four lines during the seven phases.**

Lines Phases	Line 1	Line 2	Line 3	Line 4
Phases 1	0.00%	0.00%	0.00%	0.00%
Phases 2	0.00%	14%	85.7%	57%
Phases 3	28.5%	14%	85.7%	0.00%
Phases 4	28.5%	28.5%	57%	42%
Phases 5	100%	85.7%	57%	85%
Phases 6	28.5%	71%	28.5%	14%
Phases 7	14%	28.5%	14%	14%
Average	28.5%	34.5%	46.8%	30.2%
SD	31.5	31.7	33.7	32.1

#### **Effect of time and amount of ZnO nanostructure on *E.coli* inactivation**

*E. coli* degradation with time was studied in both sunlight and dark conditions, at 10, 20, 30, 60, and 90 minutes, to verify the optimal contact time.

Initial concentrations of *E. coli*  $5 \times 10^5$  cfu/ml were added to serial amounts of ZnO nanostructure (0.01 gm, 0.02 gm, 0.03 gm, 0.04 gm, and 0.05 gm /100 ml, respectively to determine the minimal inhibitory concentration.

#### ***Escherichia coli* bacteria culture isolated from clinical specimens and Zinc oxide nanostructure (ZnO):**

*Escherichia coli* bacteria (*E. coli*), were isolated from clinical specimens, obtained from National Health Laboratories, and were examined using ZnO nanostructure. The experiment was conducted as follows:

- 1- Two sets of six bottles that contained 100 ml distilled water each was sterilized in autoclave at 121 C° for 15 minutes.
- 2- Serial concentrations of zinc oxide nanostructure (ZnO) were measured, (0.01, 0.02, 0.03, 0.04 and

0.05 gm) and added subsequently into five bottles that contained 100 ml distilled water of the two sets.

- 3- 0.1 ml of the standard bacterial suspension *E.coli*  $\sim 5 \times 10^5$  cfu/ml taken from McFarland standard solution was added to each of the twelve bottles using sterile pipette.
  - 4- One bottle from each set was used as control of culture with 0.1ml bacteria suspension *E.coli* without adding ZnO.
  - 5- Six bottles of set one were exposed to the sunlight and the other six bottles (set two) were covered with aluminum foil to protect samples from the light.
  - 6- The temperature range 32-37C° for the two sets and the pH was 8.3.
- 0.1 ml from each of the twelve bottles were withdrawn using a micro pipette after (0, 10, 20, 30, 60 and 90 minutes) and cultured in nutrient agar media spread on the plates using sterile spreaders then all the plates were incubated in 37 C° for 24 hours.
- 7- The *E.coli* bacteria counts were taken using colony counter. The disinfection efficiency, *E*, is calculated as follows:

$$E = \frac{C_i - C_f}{C_i} \times 100$$



Where:

Ci and Cf are the initial and final most probable number (MPN) /100.

Experiment was repeated two times to take the values the consistent results were reported, and the odd ones were ignored.

### Inactivation of *E. coli* in the dark

To study the efficiency of ZnO nanostructure in the absence of the sunlight, the experiment was conducted in the dark using the same amounts of ZnO used in the sunlight experiment, same concentration of *E. coli*  $5 \times 10^5$  cfu/ml and same temperature.

As shown in (Table 3), after elapse of 10 minutes, the number of *E. coli* colonies in all of ZnO nanostructure amounts were higher than the control sample and the

*E. coli* degradation was observed as the amount of ZnO nanostructure increased.

After the elapse of 20 minutes no inhibition was observed, at 0.01gm, degradation of *E. coli* was found (39 - 47 17% ) at 0.02gm, (29 - 47 49% ) at 0.03gm, (16 - 47 66%) at 0.05gm.

After the elapse of 30 minutes, the degradation of *E. coli* was found (38 - 64 68%) at 0.01gm, (24 - 64 62%) at 0.02gm, (38 to 64 59%) at 0.03 gm, 33 to 64 49%) at 0.04gm and (26- to 264 48%) in 0.05 gm.

After the elapse of 60 minutes, no trend of *E. coli* degradation was observed, and after 90 minutes almost a complete degradation was observed in all amounts of ZnO nanostructure added.

**Table 3: Number of *E. coli* per 100 ml distilled water using serial concentrations of zinc oxide nano structure in dark at different times.**

Concentration of ZnO mg (100ml) <sup>-1</sup> Time	Control	0.01	0.02	0.03	0.04	0.05
10 minutes	33	56	38	37	57	uncountable
20 minutes	47	46	39	24	32	16
30 minutes	64	38	24	26	33	26
60 minutes	37	43	67	uncountable	17	63
90 minutes	53	0	6	2	0	0

### Photo degradation of *E. coli* in the sunlight

To study the effect of ZnO photo catalytic efficiency, the samples were exposed to sunlight. As shown in (Table 4), After 20 minutes, 0.01gm of ZnO showed a higher number of colonies than the control and the number of colonies decreased to half in 0.02gm ZnO, while 0.03gm, 0.04gm and 0.05gm, showed a complete inhibition (100%) of the bacterial growth.

After 30 minutes contact time, 0.01gm of ZnO showed a higher number of colonies than the control and no

inhibition was found in 0.02gm, while a complete inhibition (100%) of the bacterial growth was observed in 0.03gm, 0.04gm and 0.05gm of ZnO.

At 60 and 90 minutes contact time a complete inhibition (100%) of the *E. coli* was observed in all concentrations of ZnO nanostructure, it was observed that as time increased the inactivation of *E. coli* increased.





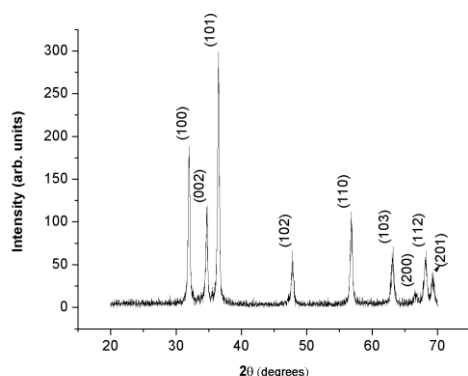
**Table 4: Number of *E. coli* per 100 ml distilled water using serial concentrations of zinc oxide nano structure in sunlight at different times.**

Concentration of ZnO mg.(100ml) <sup>-1</sup> Time	Control	0.01	0.02	0.03	0.04	0.05
10 minutes	24	68	53	44	56	20
20 minutes	33	43	15	13	14	0
30 minutes	17	43	16	0	0	0
60 minutes	12	0	0	0	0	0
90 minutes	20	0	0	0	0	0

From the above results the minimum inhibitory concentration of ZnO in sunlight was found to be 0.01gm/ 100 ml within 60 minutes and the minimum bacterial contact time for removal was found to be 20 minutes at 0.05gm/100 ml. This indicates the role of time of incubation and amount of ZnO nanostructure added.

#### XRD Result:

The X-ray diffraction pattern of the nanostructures ZnO is shown in Fig 3. All the peaks belonging to ZnO, indicate formation of single-phase ZnO with wurtzite structure. The average particle size of the sample was found to be ~13.21 nm which is derived from the FWHM of more intense peak corresponding to 101 planes located at 36.33° using Scherrer's formula.

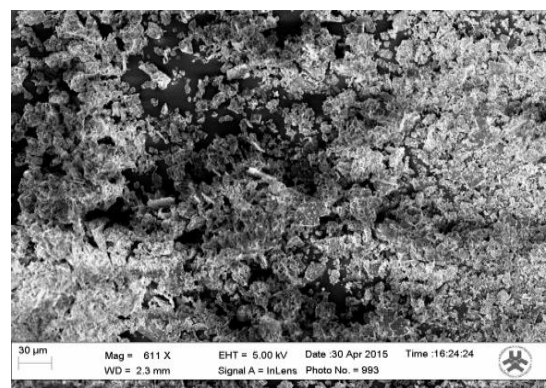


**Fig 3: X-ray diffraction patterns of nanostructure zinc oxide (ZnO) particles.**

#### SEM Result:

The SEM image demonstrates clearly the formation of ZnO nanostructures. Figure 4 and, show SEM images magnification for ZnO nanostructures. All the

nanostructures were having a diameter of around 50 nm.



**Fig 4: X-ray diffraction patterns of nanostructure zinc oxide (ZnO) particles**

#### Discussion

The(TCC) in Khartoum State network has been studied by(Yagoub& Ahmed, 2009)who have tested 120 samples at winter season, 73.3% of them showed positive bacterial growth. Ibrahim, (1999) tested 101 samples at rainy season; his results showed that Khartoumcenter, with its extensively treated water, found to contain the highest coliform bacterial count. WhileMohammed, (2013) tested 384 samples and results showed that the highest (100%) recorded in January, October and December, while during the remaining months, the contamination levels were in the range of 71.99 - 96.99%.

El-Tingari (2010)also reported 80% of the samples studied have total viable count, 25% of the samples of Khartoum network were found to be contaminated with



coliform bacteria and the highest value of total coliform was reported in Burri.

A study conducted by Musnad et al., (2010), showed that 25% of the samples (treated surface water) and 45% of the samples (well water) were contaminated with coliform bacteria.

The presence of bacteria in water pipes could be attributed to cross-contamination between the municipal water supply and sewer, due to leaky pipes and lack of water pressure (Semenza et al. 1998). According to WHO report, re-growth of thermo tolerant coliforms in the distribution system are unlikely unless sufficient bacterial nutrients are present or the water temperature is above 15°C, and there is no residual free chlorine (World Health, 1996).

The microbiological results in this study indicate that the total viable count of water samples showed that the water in the network distribution system is, generally, highly polluted by bacteria including coliform as an indicator of faecal contamination in all seven phases.

This indicates that the current treatment by chlorination is not enough to prevent microbial contamination with bacteria or the process undertaken for chlorination is not adequate.

It was found that water distribution systems that contain large quantities of unlined cast-iron and unlined ductile-iron pipes frequently experience problems with coliform violations and taste and odor complaints ((Lechevallier et al., 1996); Van der Wende and Characklis 1990; Van der Kooij and Oorhuizen 1997)

Corrosion products also can accumulate nutrients for the growth of microorganisms (Van der Wende and Characklis 1990;(Sellar& Watt, 1996);(Hamishehkar et al., 2016)(Lechevallier et al., 1996).

(Edam & Abdelgalil, 2022) tested the seasonal quality of water sources in El Obied, North Kordofan State, Sudan. Their results illustrated that all samples collected showed positive results of E. coli. Moreover, Hanan B. (Hanan B. Alkhiry, 2020), Investigated the microbial contents in drinking water in Omdurman, Khartoum State, Sudan. Their results show that most of the samples were contaminated by bacteria, including Escherichia coli, and the contamination percentage

washigh in the rainy season and summer, while the least level was found in winter season. These results agree with what found by Mohamed (2013) who carried out an E. coli examination on 384 samples from Khartoum state network. The reported results showed that the highest level of E.coli was found in October (96%) and November (68%); but less in September (25%).

The World Health Organization (WHO) as well as Sudanese Standard and Meteorology Organization guideline for drinking water quality does not allow any detection of coliforms or E. coli in 100 ml of drinking water. In the present study, however, most water samples were likely contaminated with bacteria pathogens as coliforms were detected in most of the samples (WHO,2011). A high count of fecal coliforms in drinking water can be evidence for fecal contamination and the occurrence of waterborne diseases in the community. High count of total coliforms may not directly show pathogenic bacteria contamination; this at least indicates chlorination has not been done properly, which in turn implies pathogenic bacteria contamination of the drinking water.

These indicate that water-quality problems are rampant in water-delivery systems of the country.

This contamination indicates an ineffective treatment or contamination during transportation in the distribution system (Chan et al.,2007). Such contamination may be due to leakage from pipelines as some of these pipes are old and cracked.

High level of the turbidity values in the network system, might cause the bacterial re-growth. This fact indicates that the treatment by chlorination is not enough to prevent microbial contamination.

From the ZnO nanostructure investigation results it was concluded that, the minimum inhibitory concentration of ZnO nanostructure in the dark was found to be (0.01gm/100 ml) within 90 minutes from the obtained results, the inactivation efficiencies for E.coli which were isolated from clinical specimens (Stack Labs.) and under sunlight were found to be higher than in the dark conditions. This might be attributed to that Zn+2 concentration was observed under both conditions, while cell membrane damage and DNA degradation were observed only under sunlight conditions.



Inactivation under dark conditions was hence attributed to the bactericidal effect of Zn<sup>2+</sup> ions, while inactivation under sun light conditions was due to the combined effects of Zn<sup>2+</sup> ions and photo catalytic. (Sabkota et al., 2011). It is also believed that the antimicrobial activity of ZnO may be attributed to photocatalytic activity. By absorbing UV light which activates its interaction with bacteria, ZnO nanoparticle suspensions can produce ROS such as H<sub>2</sub>O<sub>2</sub> which has phototoxic effect on bacteria (Sirelkhatim et al., 2015). Moreover, ZnO nanostructure was found to dissolve slowly in aqueous media releasing Zn<sup>2+</sup> ions (Han et al. 2010).

It has been suggested that Zn<sup>2+</sup> ion could accumulate in the cell membrane and (Baruah et al., 2012). instigated the disruption of the membrane in the experiment by (Li et al., 2008 and Atamaca et al., 1998). While, cell membrane damage and DNA degradation were observed only under sun light conditions.

(Abdelhadi 2012) in his study also showed that, sample that with ZnO nanostructure in the dark have not showed degradation products, which indicates that the cell death is not due to cell components degradation as it was found when the reaction was conducted under sunlight.

A major advantage of using ZnO nanostructure as antimicrobial in water purification is the possibility of point-of-use treatment systems (Q. Li et al., et. 2008). Moreover, ZnO photocatalyst material can be engineered to absorb visible light thereby enhancing solar photocatalysis. S. Baruah et al., 2008.; 2005 S. Baruah (2009).

## Conclusion

The microbiological analysis indicated that Khartoum drinking water samples were highly contaminated with total coliform and E. coli, and the contamination in rainy season was the highest. It is highly recommended to carry out bacteriological and chemical examination frequently and regularly for the water entering the distribution system and the water in the distribution system for the control of the hygienic quality of the water supply. Water circulating in the distribution system whether treated or not, should not contain any organisms which may be of fecal origin, if coliform

organisms are found, further investigation is required to determine their source. Frequent examinations are essential for hygienic control. Moreover, results of this study indicate that zinc oxide nanostructure had strong antibacterial activity against E. coli and sensitive to the sun light. Higher degradation percent of E. coli was achieved by the ZnO catalyst in sun light, as compared to the inactivation of ZnO NPs in the dark.

## References

1. Abdelhadi A. O. (2012). Water Disinfection by Photo-Degradation of Microorganisms Using Natural Dye-Sensitized ZnO Catalyst. M.Sc. Thesis. Faculty of Graduate Studies, Najah National University, Nablus, Palestine. 74-76.
2. Abeer M. Abass, E. A. Muna, Y. A. Sabiel, Hind E. Ahmed, A. M. Abeer, O. A. Alia, Sana A. Abdelaziz and Ghada H. Abdelnab (2022), Assessment of Bacterial Contamination in Drinking Water Sources in Khartoum/ Sudan, Journal of Advances in Microbiology(1): 50-56.
3. Akpor, O. B. and M. Muchie (2011), Challenges in meeting the MDGs: The Nigerian drinking water supply and distribution sector. J. Environ. Sci. Technol., 4: 480-489.
4. Alhamed M. and Abdullah W. (2010). Structural and optical properties of ZnO:Al films prepared by the sol-gel method. JED, 7 246-252.
5. Atmaca S, Gül K. and Cicek R. (1998). The Effect of Zinc on Microbial Growth. J. of Medical Sciences Tr, 28 (6): 595-597.
6. Baquero F., Coque MT., Cruz F. (2011). Ecology and evolution as targets: 10254 Afr. J. Biotechnol. The need for novel Eco-Evo drugs and strategies to fight antibiotic resistance. Anti microb. Agents Ch., 55:3649-3660.
7. Baruah, S., Jaisai, M., Imani, R., Nazhad, M. and Dutta, J. (2010). Baruah, S., Pal, K. S., and Dutta, J. (2012). Nanostructured Zinc Oxide for Water Treatment. Nanoscience and Nanotechnology-Asia 2 (2): 90-102.
8. Bashir, E. A (2005), Assessment of the Quality of Drinking water in Khartoum State M.Sc. Thesis, Faculty of Science, University of Khartoum, Sudan, May 2005.
9. Chen C W, Chen K H, Shen CH, Ganguly A, Chen L C, Wu J J, Wen H I and Pong WF (2006)



- Anomalous blue shift in emission spectra of ZnO nanorods with sizes beyond quantum confinement regime. *Appl. Phys. Lett.*, 88: 241905.
10. Cheng Z, Ting K, Tao Y, Goh A and Yin Xi (2010). Studies On Water Treatment Using Nano-Semiconductors as Photocatalysts Sustain. *Environ. Res.*, **20**: (5) 281-286.
  11. Cloutier M, Mantovani D, Rosei F. (2015), Antibacterial coatings: challenges, perspectives, and 591 opportunities. *Trends Biotechnol.*; 33:637–52.
  12. Gleeson, C. and N.F. Gray, (1997), *The Coliform Index and Waterborne Disease: Problems of Microbial Drinking Water Assessment*. Spon Press, London.
  13. Guo T., Ma YL., Guo P., Xu ZR. (2005). Antibacterial effects of the Cu (II)-exchanged montmorillonite on *Escherichia coli* K88 and *Salmonella choleraesuis*. *Vet. Microbiol.*, **105**:113-122.
  14. Han J., Qiu W. and Gao W. (2010). Potential dissolution and photo dissolution of ZnO thin films. *J. Hazards Mater***15**: 22-115.
  15. Huang Z, Maness P, Blake D M and Wolfrum E J (2000) Bactericidal mode of titanium dioxide photocatalysis. *J. Photochem. Photobiol. A*, **130** 163.
  16. Jensen, P.K., J.H. Ensink, G. Jayasinghe, W. van der Hoek, S. Cairncross and A. Dalsgaard, (2002), Domestic transmission routes of pathogens: The problem of in-house contamination of drinking water during storage in developing countries. *Trop. Med. Int. Health*, 7: 604-609.
  17. Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, 2008), Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. *Water Res*; 42:4591–4602.
  18. Rincn A. G., Pulgarin C, Adler N. and Peringer P. (2001). Interaction between *E. coli* inactivation and DBP-precursors dihydroxybenzene isomers in the photocatalytic process of drinking-water disinfection.
  19. Sabkota A., Anceno A. A., Baruah S., Shipin V. O. and Dutta J. (2011). Zinc oxide nanorod mediated visible light photoinactivation.
  20. Sara C Mosheka, , Suprakas Sinha Ray aMaurice S. Onyango, Maggie N B Moba (2015), Preparation and antibacterial activity of chitosan-based nanocomposites containing bentonite-supported silver and zinc oxide nanoparticles for water disinfection , *Applied Clay Science* 114, 330-339
  21. Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM. (2015), Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Lett* ;7:219–42.
  22. Sonia S, Kumari HLJ, Ruckmani K, Sivakumar M.(2017), Antimicrobial and antioxidant potentials of biosynthesized colloidal zinc oxide nanoparticles for a fortified cold cream formulation: apotent nano cosmeceutical application. *Mat Sci Eng C-Mater*597;79:581–9.
  23. Taha MM and Ibrahim A A (2002) The Standards and the Health: A paper presented in Conference of Drinking Water: Risks & Treatments, UNESCO CHWR. Khartoum. Sudan, October 2002, pp 116- 132.
  24. Ujishima A, Zhang X and Tryk D A (2007), *Int. J. Hydrogen Energy* 32 2664–72.
  25. Varaprasad K, Raghavendra GM, Jayaramudu T, Seo J. (2016), Nano zinc oxide-sodium alginate antibacterial cellulose fibres. *Carbohydr Polym*;135:349–55.
  26. Vijayaraghavan R. (2012), Zinc oxide based inorganic antimicrobial agents. *Int J Sci Res*;1:35–46.
  27. Zhang LL, Chen B, Xie LL, Li ZF. (2011). Study on the antimicrobial properties of ZnO suspension against Gram-positive and Gram-negative bacteria strains. *Adv Mat Res*;393–5:1488–91.