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Efficiency of silver nanoparticles against bacterial contaminants isolated from surface and ground water in Egypt

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ABSTRACT

The bactericidal efficiency of silver nanoparticles (AgNP) was evaluated against bacteria isolated from surface and ground water samples in Egypt. The AgNP were synthesized by typical one-step synthesis protocol, and were characterized using transmission electron microscopy and atomic absorption spectrophotometer. The bactericidal efficiency of AgNP was evaluated by its application in three concentrations *i.e.*, 0.1, 0.05 and 0.01 ppm to water sample, and allowed to interact with bacteria for different duration *e.g.*, 5 min 15 min, 30 min, 1 h and 2 h. Then, the bactericidal efficiency of AgNPs was determined by comparing the counted bacteria before and after the treatments. Higher mean values of total bacterial count (TBC), total coliform count (TCC), and total streptococcal count (TFS) were detected in surface water than in ground water. Also, the results showed that TBC, TCC and TFS exceeded permissible limits. Application of AgNP at different concentration, the number of bacteria in TBC was significantly reduced in all AgNP-exposed samples as compared to the control group (p<0.05). The highest concentration of AgNP exhibited highest bactericidal efficiency in TBC, where, after two hours, 0.1, 0.05 and 0.01 mg/L AgNP was found to be sufficient to inhibit 91.85, 89.14 and 74.92%, and 92.33, 85.23 and 53.17% in TBC of surface and ground water, respectively. Moreover, the inhibition efficiency of the highest concentration (0.1 ppm) against TCC reached to 98.10 and 99.88% in surface water and 95.54 and 99.20% in ground water after 1 h and 2 h, respectively. Similar results were found against TFS count. The AgNPs were found to be effective against bacteria of water origin.

Keywords

AgNP, Bacterial contaminant, Efficiency, Ground water, Surface water

ARTICLE HISTORY

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INTRODUCTION

Water-borne disease is considered as the leading cause of death in many countries especially in developing countries (Yehia and Sabae, 2011). In the world, over one billion people lack access to fresh water, and this condition is responsible for death of two million people per anum (WHO, 2004; UNESCO, 2006; Halem et al., 2009; WHO/UNICEF, 2012).

Drinking water must meet specific criteria and standards ensuring safety; the water should be free from pathogenic microorganisms and hazardous compounds (WHO, 1993). Unfortunately, drinking water sources receive heavy loads of microorganisms through several ways like industrial, agricultural, and domestic wastes (Annual Drinking Water Quality Report, 2005).

Detection of organisms that are normally present in feces of human and other warm-blooded animals is used as the indicator of fecal contamination. Indicator bacteria such as total coliform bacteria, fecal coliform, and fecal streptococci are widely used for the assessment of fecal contamination and possible water quality deterioration in fresh water sources (APHA, 1993, 2005).

To reduce the incidence of water-borne diseases and to make the water suitable for drinking, removal of pathogenic organisms, fecal matters, suspended solids, algae, organic matters, and harmful chemicals from pollued water is mandatory (Gupta and Chaudhuri, 1995). Nanotechnology has opened an alternate way for water purification. Application of silver nanoparticles (AgNPs) have been extensively studied in food industry for drinking water treatment (Silvestry-Rodriguez et al., 2008; Konopka et al., 2009; Kumar and Raza, 2009; Zhao et al., 2010).

The use of AgNP in drinking water treatment is relatively new and has recently become of interest (Jain and Pradeep, 2005; Feng et al., 2000). However, addition of silver in water in such concentration that shows bactericidal activity does not impare the taste, color, odor, and other physicochemical characteristics of water (Klueh et al., 2000), and has no apparent detrimental effects on mammalian cells (Yahya et al., 1992).

The present study was conducted to evaluate the bactericidal efficiency of AgNPs against bacteria isolated from surface water and underground water in Egypt.

MATERIALS AND METHODS

Sample collection: A total of twenty seven surface water samples were collected from different regions of Nile River and Al-Ibrahimeya Canal in Assiut Governorate, Egypt (**Figure 1**). The water samples were collected in accordance with the standard methods for the examination of water and wastewater (APHA, 2005).

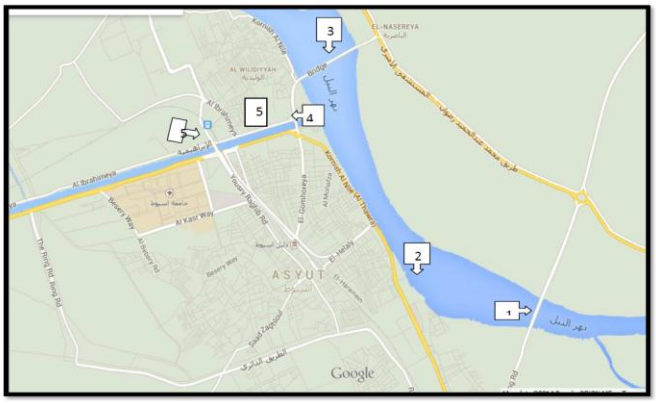

Figure 1: Google map illustrating different regions of water sample collection. 1- Al-Wasta Bridges , 2- The area facing to Al-Helaly Street, 3- Al-Khazan Bridge, 4- Al-Baladya Club Bridge, 5- 25th January Bridge, 6- Al-Azhar Bridge.

In addition to surface water samples, ground water samples (n=23) were also collected from 11 different hand-pumps wells located at Assiut Governorates, Egypt. All the hand-pump wells were located at localities and were using for human and animal drinking.

Preparation of silver nanoparticles (AgNPs)

Stable AgNPs of <100 nm in size were synthesized through one-step protocol as per the method described by Vigneshwaran et al. (2006). In brief, 1 g of soluble starch was added to 100 mL of deionized water and heated till complete dissolution, and 1 mL of 100 mM aquous solution of silver nitrate ($AgNO_3$) crystal (FW 169, 87 Gamma laboratory chemicals, Assay: Min 99.0%) was added and stirred well. This mixture was put into dark glass bottle and autoclaved. The resulting solution was clear yellow in color indicating the formation of AgNPs.

After preparation of AgNPs, the stock solution was kept in dark glass bottles away from direct sunlight at room temperature (25^0C), and the size of AgNP was measured by transmission electron microscopy (TEM) (JEOL-JEM- 100CX II) at Electron Microscopy Unit, Assiut University, Egypt. Total concentration of AgNP stock was measured by Graphite Furnace Atomic Absorption (Model 210VGP) at the Faculty of Science, Assiut University, Egypt.

Antibacterial experiments

Application of silver nanoparticles: For each water sample, bactericidal assay was carried out in four sterile conical flasks of 500 mL capacity, each flask contained 250 mL of water sample, and AgNP suspension was aseptically added to three flasks to obtain a final treatment of 0.01, 0.05 and 0.1 mg/L. Each AgNP treatment was thoroughly mixed and allowed to interact with bacterial communities present in the collected water samples for different durations like 5, 15, 30, 60 and 120 min. The remaining water sample in the 4^{th} flask was used as the negative control (water sample without any AgNPs).

Viability of bacteria before and after the test: Viability of bacteria was examined using different bacteriological tests after 5 min, 15 min, 30 min, 1 h, and 2 h of interaction. After the end of each contact time, sufficient amount of mixture of water sample and AgNP was transferred aseptically into sterile bottle, and AgNP was quenched by adding 5 g/L sodium

thiosulfate ($Na_2S_2O_3$) to stop the antimicrobial reaction between AgNPs and bacteria, as described in the European Quality Standards (NEN, 1997).

The same amount of $Na_2S_2O_3$ was also added to each control negative flask, to exclude any factor that could affect the viability of bacteria except AgNP, then it was cultured on the same media to represent the counting before treatments.

A tube without water sample but containing MacConkey Broth/Azide Dextrose Broth plus autoclaved distilled water was used as negative growth control for Most Probable Number (MPN). Also a petri-dish contained plate count agar only, and another contained agar plus autoclaved distilled water used as negative growth control for total viable bacterial count (TVBC). Enumeration of total viable bacteria by Pour Plate Method (PPM), Total Coliforms (TC) and Fecal Streptococci (FS) was done according to the method described by APHA (2005).

Evaluation of antibacterial efficacy of AgNP: Antibacterial efficiency of AgNPs was obtained by comparing the number of bacteria before and after the treatment for each case. Percent of antibacterial efficacy was calculated using the following equation.

$$\text{Disinfection efficacy \%} = \frac{(C0 - C) \times 100}{C0}$$

Where, C0 is the initial bacterial count in raw water (control negative), C is count of bacteria after a certain contact time of the treated water (Li et al., 2006).

Statistical analyses: Analysis of variance of data was computed using the General Linear Models (GLM) Procedure of SAS software version 9 (SAS, 2009). Furthermore, data were subjected to analyze the variance using ANOVA. The results were presented as mean and standard error for each variable. Differences among treatment mean were tested by using Duncan's new multiple range test (Duncan, 1955). Pearson Correlation was made to measure the correlation between the estimated variables. *p*-value was considered statistically significant when $p<0.05$.

RESULTS AND DISCUSION

The synthesized AgNP was examined by Transmission Electron Microscopy (TEM) images showing spherical nanoparticles of 8.26-31.1 nm in size (**Figure 1**).

Data presented in **Table 1** showed the bacteriological quality of water samples collected from surface and ground. The mean values of TBC were 812.5±63.8 and 498.5±81.7/mL for surface and ground water, respectively. The mean values of TCC were 436.8±47.6 and 291.8±70.4 for 100 mL of surface water and ground water, respectively, while the mean values of TFS were 135.3±20.6 and 37.1±7.1 for 100 mL of surface and ground water, respectively. From these results, it was observed that the higher mean values of TBC, TCC and TFS were detected in surface water as compared to ground water.

In our study, finding of TBC was higher than the safe limits approved by the European Economic Community (EEC, 1998), Water Environment Federation (WEF, 1998), WHO (2006) and Egypt Standards (2007). Moreover, the results of TCC was higher than the safe limits approved by Cabelli (1978), EEC (1998), WEF (1998), US EPA (2001), WHO (2006), and Egypt Standards (2007). The results of TFS was higher than the safe limits approved by Egypt Standards (2007).

Our results showed that TBC, TCC and TFS exceeded permissible limits; these results were in agreement with the findings of Yousseff and Sabah (2007), Shash et al. (2010), Saad et al. (2012) and Othman et al. (2012). The presence of high counts of TBC, TC and FS indicated that the water received large quantities of domestic, industrial and agricultural wastes (WHO, 1993; Ali et al., 2000).

The antimicrobial effects of the AgNPs on bacteria was dependent on the concentration of Ag in the nanoparticles; where increasing the concentration of nanoparticles delayed the growth of bacteria. The treated bacterial cells were significantly changed and

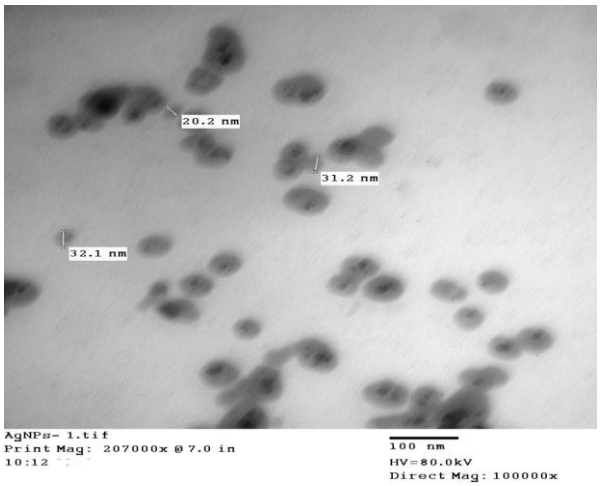

Figure 1. Transmission Electron Microscopy (TEM) images of AgNP showed spherical shapes of nanoparticles and their sizes ranged between 8.26- 31.1.

Table 1. Bacteriological quality of different water source.

Bacterial count	Mean±SE	
	Surface water	Ground water
Total Bacterial count (TBC)/mL	812.5±63.8	498.5±81.7
Total Coliform count (TCC)/100 mL	436.8±47.6	291.8±70.4
Total Fecal Streptococcal count (TFS)/100 mL	135.3± 20.6	37.1±7.1

Table 2. Mean values of Total Bacterial Count (TBC), Total Coliform Count (TCC) and Total Fecal Streptococcal Count (TFC)of Surface water after Application of Ag Nps.

Treatment	Contact time	Total Bacterial Count (Mean±S.E/mL)	Inhibition %	Total Coliform Count (Mean±S.E/100ml)	Inhibition %	Total Fecal streptococcal Count (Mean±S.E/100ml)	Inhibition %
0.1 ppm	Control	530.1±144.0 ^{ab}		309.4±117.5 ^{a}		25.3±5.8 ^{b}	
	5 Min	409.0±166.2 ^{abc}	22.85	145.1±62.2 ^{abc}	53.10	4.4 ±0.5 ^{c}	82.70
	15 Min	204.4±94.0 ^{cd}	61.44	50.5±18.3 ^{bc}	83.69	3.1 ±0.7 ^{c}	87.64
	30 Min	106.2±37.6 ^{d}	79.96	18.6 ±9.6 ^{bc}	93.99	3.8 ±1.8 ^{c}	84.84
	1 h	57.0±13.3 ^{d}	89.25	13.8 ±6.4 ^{c}	95.54	2.7 ±0.5 c	89.29
	2 H	40.6±13.7 ^{d}	92.33	2.5 ±0.9 ^{c}	99.20	0.5 ±0.3 ^{c}	98.02
0.05 ppm	Control	412.0±74.1 ^{abc}		255.4±117.6 ^{ab}		15.9 ±2.4 ^{b}	
	5 Min	192.2±49.8 ^{cd}	53.36	193.3±114.1 ^{abc}	24.34	6.7±2.6 ^{c}	58.12
	15 Min	142.7±40.4 ^{d}	65.36	115.8±63.1 ^{abc}	54.67	3 ±0.8 ^{c}	81.15
	30 Min	103.8±34.6 ^{d}	74.80	39.3 ±16.7 ^{bc}	84.60	2.5±0.9 ^{c}	84.55
	1 h	91.3±28.5 ^{d}	77.85	37.6 ±16.4 ^{bc}	85.27	1.6 ±0.9 ^{c}	90.05
	2 H	60.9±17.6 ^{d}	85.23	40.8±26.4 ^{bc}	84.04	1.3 ±0.4 ^{c}	91.62
0.01 ppm	Control	553.4±187.3 ^{a}		310.6±134.7 ^{a}		70.0±18.8 ^{a}	
	5 Min	297.4±87.8 ^{bcd}	46.27	218.3±113.7 ^{abc}	29.74	6.08 ±1.67 ^{c}	91.30
	15 Min	236.7±75.9 ^{cd}	57.23	194.2±102.2 ^{abc}	37.48	12.4 ±4.1 ^{bc}	82.25
	30 Min	257.8±72.3 ^{cd}	53.42	129.9±62.8 ^{abc}	58.18	3.5±0.9 ^{c}	95.06
	1 h	269.4±76.1 ^{cd}	51.33	118.6±63.2 ^{abc}	61.82	15.6 ±8.5 ^{bc}	77.72
	2 H	259.2±95.4 ^{cd}	53.17	109.3±62.8 ^{abc}	64.82	9.7 ±2.6 ^{bc}	86.12

^{a,b,c,d,e,f,g} Values within columns with no common superscript differ significantly (p<0.05). $^{b-e=bcde,}$ $^{d-g=d,e,f,g}$

Table3. Mean values of Total Bacterial Count, Total Coliform count and Total Fecal Streptococcal count of ground water samples after treatment by AgNPs.

Treatment	Contact time	Total Bacterial Count (Mean±S.E/mL)	Inhibition %	Total Coliform Count (Mean± S.E/100ml)	Inhibition %	Total Fecal streptococcal Count (Mean±S.E/100ml)	Inhibition %
0.1 ppm	Control	530.1±144.0 ^{ab}		309.4±117.5 ^{a}		25.3±5.8^{b}	
	5 Min	409.0±166.2 ^{abc}	22.85	145.1±62.2 ^{abc}	53.10	4.4 ± 0.5 ^{c}	82.70
	15 Min	204.4±94.0 ^{cd}	61.44	50.5±18.3^{bc}	83.69	3.1 ± 0.7 ^{c}	87.64
	30 Min	106.2±37.6 ^{d}	79.96	18.6 ± 9.6 ^{bc}	93.99	3.8 ± 1.8 ^{c}	84.84
	1 h	57.0±13.$_{3}$ ^{d}	89.25	13.8 ± 6.4^{c}	95.54	2.7 ± 0.5 ^{c}	89.29
	2 H	40.6±13.7 ^{d}	92.33	2.5 ± 0.9 ^{c}	99.20	0.5 ± 0.$_{3}$ ^{c}	98.02
0.05 ppm	Control	412.0±74.1 ^{abc}		255.4±117.6 ^{ab}		15.9 ± 2.4 ^{b}	
	5 Min	192.2±49.8 ^{cd}	53.36	193.3±114.1 ^{abc}	24.34	6.7±2.6 ^{c}	58.12
	15 Min	142.7±40.4 ^{d}	65.36	115.8±63.1 ^{abc}	54.67	3 ± 0.8 ^{c}	81.15
	30 Min	103.8±34.6 ^{d}	74.80	39.$_{3}$ ± 16.7 ^{bc}	84.60	2.5± 0.9 ^{c}	84.55
	1 h	91.3±28.5 ^{d}	77.85	37.6 ± 16.4 ^{bc}	85.27	1.6 ± 0.9 c	90.05
	2 H	60.9±17.6 ^{d}	85.23	40.8±26.4 ^{bc}	84.04	1.3 ± 0.4 c	91.62
0.01 ppm	Control	553.4±187.$_{3}$ ^{a}		310.6±134.7 ^{a}		70.0±18.8 ^{a}	
	5 Min	297.4±87.8 ^{bcd}	46.27	218.3±113.7 ^{abc}	29.74	6.08 ± 1.67 ^{c}	91.30
	15 Min	236.7±75.9 ^{cd}	57.23	194.2±102.2 ^{abc}	37.48	12.4 ± 4.1 ^{bc}	82.25
	30 Min	257.8±72.$_{3}$ ^{cd}	53.42	129.9±62.8 ^{abc}	58.18	3.5±0.9 ^{c}	95.06
	1 h	269.4±76.1 ^{cd}	51.33	118.6±63.2 ^{abc}	61.82	15.6 ± 8.5 ^{bc}	77.72
	2 H	259.2±95.4 ^{cd}	53.17	109.3±62.8 ^{abc}	64.82	9.7 ± 2.6 ^{bc}	86.12

^{a,b,c,d} Values within columns with no common superscript differ significantly (p<0.05).

Table 4. Effect of different concentrations of AgNP on mean of microbial contamination of surface water and ground water samples.

Treatment	Mean of TBC/mL		Mean of TCC/100 mL		Mean of TFS/100 mL	
	Surface water	Ground water	Surface water	Ground water	Surface water	Ground water
Control	812.5 ±63.8[a]	498.5 ± 81.7[a]	436.8±47.6[a]	291.8 ± 70.4[a]	135.3± 20.6[a]	37.1 ±7.1[a]
0.1 ppm	192.3±19.3[c]	163.4 ± 40.4[c]	60.4 ± 15.2[b]	46.1 ±18.7 [b]	36.0±7.3[b]	2.9±0.4[b]
0.05ppm	192.0 ±13.9[c]	118.2±17.0[c]	90.6 ± 22.2[b]	85.4 ±27.2[b]	26.0 ±6.3[b]	3.0 ±0.6[b]
0.01 ppm	303.5 ± 25.7[b]	264.1 ± 36.1[b]	89.7 ± 17.0[b]	154.1 ± 37.1[b]	29.0 ±5.6[b]	9.5 ±2.0[b]

[a,b,c,d] Values within columns with no common superscript differ significantly (p<0.05).

Table5. Effect of different contact times of AgNP on microbial contamination isolated from surface water and ground water samples.

Treatment	TBC/mL				T C C/100 ml				TFS/100 ml			
	Surface water		Ground water		Surface water		Ground water		Surface water		Ground water	
	Mean ±S.E /mL	Inhibition %	Mean± S.E /mL	Inhibition %	Mean±S.E /mL	Inhibition %	Mean± S.E /mL	Inhibition %	Mean± S.E /mL	Inhibition %	Mean ±S.E /mL	Inhibition %
Control	812.± 63.8 [a]		498.5 ± 81.7 [a]		436.8 ±47.6 [a]		291.8 ± 70.4 [a]		135.3 ± 20.6 [a]			
5 Min	362.9± 31.6 [b]	55.33	299.5± 64.8[b]	39.92	178.1 ± 35.4 [b]	59.23	185.5 ± 56.9 [ab]	36.42	40.40 ± 8.4 [b]	70.13	37.1 ± 7.1 [a]	84.60
15 Min	271.9 ± 28.3 [c]	66.54	194.6 ± 42.1 [bc]	60.96	108.8 ± 25.1 [bc]	75.10	120.2 ± 40.5 [bc]	58.83	34.5± 9.9 [b]	74.52	5.7 ± 1.0 [b]	83.32
30 Min	225.4± 25.2 [cd]	72.26	155.9 ± 30.3 [c]	68.72	74.8 ± 23.5 [cd]	82.88	62.6 ± 34.4 [bc]	78.54	37.7± 9.2 [b]	72.12	6.2 ± 1.5 [b]	91.22
1 h	169.8± 19.3 [de]	79.10	135.3 ± 30.3 [c]	72.85	29.8 ± 10.1 [d]	93.19	56.7 ± 22.2 [c]	80.58	22.2 ± 7.5 [b]	83.59	3.3 ± 0.7 [b]	82.12
2 h	116.4± 17.6 [e]	85.67	120.2 ± 34.3 [c]	75.89	9.7 ± 6.5 [d]	97.77	50.8 ± 223.0 [c]	82.58	17.0 ± 5.8 [b]	87.42	6.6± 2.9[b]	89.62

[a,b,c,d,e] Values within columns with no common superscript differ significantly (p<0.05).

showed major damage, which was characterized by the formation of "pits" in their cell walls (Sondi and Salopek-Sondi, 2004).

Effects of AgNP on bacteria of surface water

Antibacterial effects of AgNP in TBC: The statistical analysis of **Table 2** showed that at the concentrations of 0.1, 0.05 and 0.01 ppm, the TBC was significantly reduced in all AgNP-exposed samples as compared to the control group (p<0.05). Moreover, at the initial concentration (0.1 ppm), there were significant difference of activity among 1 h, 2 h and 5 min (p<0.05), while in 0.05 ppm, there were significant differences between 2 h and 5 min (p<0.05). At the 3rd concentration used (0.01 ppm), there were significant differences among 1 h, 2 h and 5 min (p<0.05) (**Table 2**). Also, the results showed that the highest antibacterial effect was found with the highest concentration of AgNP (0.1 ppm) at maximum time used (2 h), followed by 0.05 ppm, while the 0.01 ppm showed the least reduced from 917.7±114.8 to 74.8±16.7, followed by 0.05 ppm after 2 h (81.5±15.6), 0.1 ppm after 1 h (105.2±16.2), and then 0.05 after 1 h (147.8±20.6) and their inhibitions were 91.85, 89.14, 88.54 and 80.30%, respectively (**Table 2**).

Antibacterial effect of AgNP in TCC: The statistical analyses of **Table 2** revealed that in all concentraions of AgNPs used, the TCC was significantly reduced in as compared to the control group (p<0.05), Moreover, in 0.1 ppm, there were significant difference of activity among the time periods of 1 h, 2 h and 5 min (p<0.05), while in 0.05 and 0.01 ppm, there were significant differences between 2 h and 5 min (p<0.05). Our results showed that the highest mean of TC was found in the control group of the three concentrations (502.4±89.4, 426.8±83.9 and 381.1±75.1 CFU/100 mL), while the lowest mean was found at 0.1 ppm of AgNP after 2 h (0.63±0.36) followed by 0.05 ppm after 2 h (4.9±3.9); 0.1 ppm after 1 h (9.5±4.9), and 0.05 ppm after 1 h (13.3±6.4) (**Table 2**).

Antibacterial efffect of AgNP in TFS: In all the three different treatment groups of AgNP (0.1 ppm, 0.05 ppm and 0.01 ppm), the mean values of TFS count were significantly reduced as compared to the control group (p<0.05). However, the analysis of variance showed no significant differences among the five different contact times (5 min, 15 min, 30 min, 1 h and 2 h) (**Table 2**). From the results of **Table 2**, it was revealed that the highest mean of FS was found in the control groups of the three treatments (201.8±48.8, 108.8±30.8 and

95.3±17.2 CFU/ 100 mL for 0.1, 0.05 and 0.01 ppm of AgNP, respectively), while the lowest means were 0.1 ppm (16.8±10.4), 0.05 ppm (17.1±10.4) and 0.01 ppm (17.1±9.9) after 2 h. The results of **Table 2** revealed that the highest concentration (0.1 ppm) resulted in 91.68 and 86.14% inhibition in TFS after 1 h and 2 h, respectively, in addition to 84.25 and 84.44% inhibition in TFS at 0.05 ppm, and 0.01 ppm caused 82.02 and 77.21% inhibition after 2 h and 1 h, respectively.

Effect of AgNP on bacteria of ground water

Antibacterial effect of AgNP in TBC: The lowest mean of TBC was found at 0.1 ppm of AgNP after 2 h (40.6±13.7) followed by 0.1 ppm after 1 h (57.0±13.3), 0.05 ppm after 2 h (60.9±17.6), and 0.1 after 30 min (106.2±37.6) (**Table 3**). The data analysis shown in **Table 3** illustrates that at the 1st (0.1 ppm) and the 2nd (0.05 ppm) concentrations, the TBC was significantly reduced in all AgNP-exposed samples as compared to the control group except between 5 min and the control group. However, at the 0.1 ppm concentration, there were significant differences between 2 h, 1 h, 30 min and 5 min (p<0.05), while at the 2nd concentration (0.05 ppm), the analysis of variance showed no significant differences among the five contact times (5 min, 15 min, 30 min, 1 h and 2 h). The statistical analysis showed that at the 0.01 ppm, the TBC was significantly reduced in all AgNP-exposed samples as compared to the control group (p<0.05). However, the analysis of variance showed no significant differences among the five contact times (5 min, 15 min, 30 min, 1 h and 2 h) (**Table 3**).

The results of **Table 3** showed that there were variations between the efficiency of AgNPs at different concentrations, where the highest concentration exhibited highest antibacterial activity in TBC of ground water samples, and the efficiency reached to 92.33 and 89.25% after 1 h and 2 h, respectively, and the percentage of inhibition was greater than the other two concentrations at the same times (85.23% for 0.05 ppm and 53.17 % for 0.01 ppm at 2 h).

Antibacterial effects of AgNP in TCC: Findings of this study revealed that the highest mean of TCC was found in the control groups of the three concentrations (309.4±117.5, 255.4±117.6 and 310.6±134.7 CFU/100 mL), while the lowest mean was found with 0.1 ppm of AgNP used after 1 h and 2 h (2.8±0.9 and 13.8±6.4, respectively) (**Table 3**). The statistical analysis of the data presented in **Table 3** showed that at the 1st used concentration, the TCC was significantly reduced in all AgNP-exposed samples as compared to the control group (p<0.05) except between 5 min and the control group, however the analysis of variance showed no significant differences among five contact times (5 min, 15 min, 30 min, 1 h and 2 h). While for the 2nd (0.05 ppm) and the 3rd (0.01 ppm) concentrations used, the analysis of variance showed no significant differences among the five contact times (5 min, 15 min, 30 min, 1 h and 2 h).

The results presented in **Table 3** showed that there were variations between the efficiency of AgNPs at different concentrations, where the highest concentration (0.1 ppm) showed in the highest antibacterial activity in TCC with the bacteria of ground water samples, and its efficiency reached to 95.54 and 99.20% after 1 h and 2 h, respectively. Furthermore, the significant differences were found only between the dcontact times and the control group of 0.1 ppm of AgNP and the inhibition percentage of TCC was greater than the other two concentrations at the same times (84.04% for 0.05 ppm and 64.82% for 0.01 ppm at 2 h).

Antibacterial effect of AgNP in TFS: Our results showed that the highest mean of TFS was the control group of the three concentrations (25.3±5.8, 15.9±2.4 and 70.0±19.0 CFU/100 mL for 0.1, 0.05 and 0.01 ppm of AgNP, respectively). While the lowest mean was at 0.1 ppm of AgNP after 2 h contact time (0.5±0.3) followed by 0.05 ppm after 2 h contact time (1.3±0.4), as shown in **Table 3**. Moreover, the statistical analysis of the data presented in **Table 3** showed that at the 1st, 2nd and 3rd concentrations, the FSC was significantly reduced in all AgNP-exposed samples as compared to the control group (p<0.05). However, the analysis of variance showed no significant differences between the different five contact times (5 min, 15 min, 30 min, 1 h and 2 h) and each other of the three concentrations.

Table 3 revealed that there were variations among the efficiency of AgNPs at different concentrations, where the highest concentration (0.1 ppm) showed the highest antibacterial activity against FS of ground water samples and its efficiency reached to 98.02% followed by 0.05 ppm, which resulted in 91.62% inhibition of TFS count.

From **Table 2** and **Table 3**, it was observed that the bactericidal efficiency of AgNP was higher in surface water than ground water in addition to the survival rate in TBC decreased with the increase in the concentration of AgNP, moreover, the bactericidal efficiency of AgNP increased with increasing times

with bacteria in all concentrations of the two water sources. These findings were in agreement with findings of Shrivastava et al. (2007), Choi and Hu (2008), and Pranab et al. (2011); however, our finding disagreed with the results of Bradford et al. (2009).

Effect of different concentrations of AgNP (0.1, 0.05 and 0.01 ppm) on the overall mean of microbial contamination of surface and ground water samples.

Different concentrations of AgNP on TBC: Data presented in **Table 4** showed the effect of different concentrations of AgNP (0.1, 0.05 and 0.01 ppm) on the overall mean of TBC of surface water and ground water samples. The mean values at 0.1, 0.05, 0.01 ppm and the control group were (192.3 ±19.3, 192.0±13.9, 303.5±25.7 and 812.5±63.8 CFU/mL) and (163.4±40.4, 118.2±17.0, 264.1±36.1 and 498.5±81.7 CFU/mL) in surface water and ground water samples, respectively. The statistical analysis of the data presented in **Table 4** showed that, in all water sources, the TBCs were significantly reduced in all AgNP-exposed samples as compared to the control group (p<0.05), moreover, in surface water and ground water samples, the 1st (0.1 ppm) and the 2nd (0.05 ppm) concentrations were significantly differed from the 3rd (0.01 ppm) concentration (p<0.05), however, there was no significant differences between the 1st and the 2nd concentrations.

Different concentrations of AgNP on TCC: Data presented in **Table 4** showed the effect of different concentrations of AgNP (0.1, 0.05 and 0.01 ppm) on the overall mean of TCC of surface water and ground water samples. The mean values at 0.1, 0.05, 0.01 ppm and the control samples were (60.4±15.1, 90.6±22.2, 89.7±17.0 and 436.8±47.6 CFU/100 mL) and (46.1±18.7, 85.4±27.2, 154.1±37.1 and 291.8±70.4 CFU/100 mL) in surface water and ground water, respectively.

The statistical analysis of the data presented in **Table 4** showed that in all water sources, the TCCs were significantly reduced in all AgNP-exposed samples as compared to the control group (p<0.05), however, the analysis of variances showed no significant differences between the three concentrations of AgNP of the two water sources.

Different concentrations of AgNP on TFS: Data presented in **Table 4** showed the effect of different concentrations of AgNP (0.1, 0.05 and 0.01 ppm) on the overall mean of TFS of water samples collected from surface water and ground water samples. The mean values at 0.1, 0.05, 0.01 ppm of AgNP and the control group were (36.0±7.3, 26.0±6.3, 29.0±5.6 and 135.3±20.6 CFU/100 mL) and (2.9±0.4, 3.0±0.6, 9.5±2.0 and 37.1±7.1 CFU/100 mL) in surface water and ground water samples, respectively.

The statistical analysis of **Table 4** showed that in all water sources, there were significant differences between the three used concentrations and the control groups (p<0.05), however the analysis of variances showed no significant differences between the three concentrations of AgNP of the two water sources.

From **Table 2** and **Table 3**, it could be summarized that the AgNPs exhibited highest bactericidal efficiency against TFS in ground water followed by surface water. Moreover, the bactericidal efficiency of AgNPs increased with the increase of its concentrations and contact times with bacteria.

Effect of different contact times of AgNP on the overall mean of Microbial contamination of surface water and ground water samples.

Different contact times of AgNP on TBC: The statistical analysis of the data of **Table 5** showed that there were significant differences between all contact times and the control groups at all water sources (p<0.05). Moreover, in TBC of surface water, there were significant differences between the mean at 2 h and the mean values at 5 min, 15 min, 30 min, 1 h and 15 min, 5 min; 30 min and 5 min and between the mean of 15 min and 5 min (p<0.05), however, there were no significant differences between 2 h and 1 h; 1 h and 30 min and between 30 min and 15 min Furthermore, in TBC of ground water there were significant differences between the mean at 2 h, 1 h, 30 min and the mean at 5 min (p<0.05), however there were no significant differences in between 2 h, 1 h, 30 min, 15 min

Different contact times of AgNP on TCC: The statistical analysis of the data of **Table 5** showed that there were significant differences between all contact times and the control groups at all water sources (p<0.05). Moreover, in TCC of surface water, there were significant differences between the mean at 2 h, 1 h and the mean at 15 min, 5 min and between the mean at 30 min and 5 min (p<0.05), while in TCC of ground water, there were significant differences between the mean at 2 h, 1 h and the mean at 5 min (p<0.05).

Different contact times of AgNP on TFS: The statistical analysis of **Table 5** showed that there were significant

differences between all contact times and the control groups at all water sources (p<0.05), however the analysis of variance of TFS showed no significant differences between all contact times and each other at the two water sources.

From the above data, it was clear that the bactericidal efficiency of AgNPs increased with the increase in its concentration and contact time with the bacteria. These findings might be attributed to the treated bacterial cells, and showed major damage, which was characterized by the formation of "pits" in their cell walls, which exhibited a significant increase in permeability, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane and, finally, causing cell death. The concentration of the nanoparticles gradually decreases, allowing resumed growth of bacterial cells. This process is governed by the interaction of these particles with intracellular substances of the destroyed cells, causing their coagulation and removal from the liquid system (Sondi and Salopek-Sondi, 2004). Our finding agreed with the results of Cowan et al. (2003), Sondi and Salopek-Sondi (2004), Yoon et al. (2007), Tiwari et al. (2008), Kim et al. (2011), Tuana et al. (2011), and Nawaz et al. (2012) who proved that there were a positive correlation between the elevated concentration of AgNPs and the inhibition of *Escherichia coli*.

CONCLUSION

The results describes the possibilities of the use of AgNP as an alternate in water treatment facilited by bactericidal acitivity of AgNP. The AgNPs are proved to be efficient against fecal bacterial indicators and TBC. We recommend that immediate action is needed to prevent the bacterial contaminants leaching into drinking water source as well as the hand-pump wells should be dug deeper and far away from the sewage tank or any sources of water contaminations. Studies of long-term toxicological effects of AgNP on different microorganisms (such as viruses, algae and protozoa) that contribute to water-borne diseases are suggested. Moreover, further researches are needed to fully understand the behavior of AgNPs in natural water condition such as dissolution, cluster formation and aggregations, and the factors that control their occurrence.

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