Assessment of carbon nanotubes and silver nanoparticles loaded clays as adsorbents for removal of bacterial contaminants from water sources

M. E. M. Hassouna, M. A. ElBably, Asmaa N. Mohammed and M. A. G. Nasser

ABSTRACT

This work evaluated the antimicrobial efficacy of kaolin clay and its loaded forms with carbon nanotubes (CNTs) and silver nanoparticles (AgNPs) against bacterial isolates from different water supplies (tap, underground and surface water) in addition to wastewater. A total of 160 water samples were collected from different water sources in the investigated districts. Samples were cultured for isolation and serological identification of pathogenic bacteria. AgNPs were synthesized by a typical one-step synthesis protocol, where CNTs were carried out in a reactor employing the double bias-assisted hot filament chemical vapor deposition method. Both were characterized using transmission electron microscopy, infrared and X-ray fluorescence (XRF) spectroscopy. The antimicrobial efficacy of each of natural kaolin clay, AgNPs- and CNTs-loaded clays were evaluated by their application in four concentrations (0.01, 0.03, 0.05 and 0.1 ppm) at different contact times (5 min, 15 min, 30 min and 2 h). AgNPs-loaded clays at concentrations of 0.05 and 0.1 mg/l for 2 h contact time exhibited a higher bactericidal efficacy on *Escherichia coli* and *Salmonella* spp. (70, 70, 80 and 90%, respectively) compared to CNTs-loaded clay. Concluding, the application of AgNPs-loaded clay for removal of water bacterial contaminants at a concentration of 0.1 ppm for 2 h contact times resulted in highly effective removals.

Key words | bacterial contaminants, drinking water, kaolin clay, nano-adsorbents

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INTRODUCTION

There is no doubt that drinking water sources receive heavy loads of microorganisms through several means such as industrial, agricultural, and domestic wastes (Annual Drinking Water Quality Report 2005). A wide spectrum of pathogenic agents can be found in water and monitoring of their presence on a routine basis is impractical. Traditionally, microbial safety of drinking water has been confirmed by monitoring for the absence of microorganisms of fecal origin (Le-Chevallier & Au 2004).

To reduce the incidence of waterborne diseases and to make water suitable for drinking, removal of pathogenic organisms, fecal matters, suspended solids, algae, organic matters, and harmful chemicals from polluted water is mandatory (Gupta & Chaudhuri 1995). The removal capacity of a multiple-barrier water disinfection device for protozoa, bacteria, and viruses was studied and the efficiency of the multiple-barrier device in removing *Escherichia coli* was close to 100%, and more than 87% of *Cryptosporidium* oocysts and more than 98% of *Giardia* cysts were removed. Close to 100% of coliphages were removed and 99.6% of the adenovirus was removed (Espinosa-García *et al.* 2014). Moreover, for wastewater, stabilization ponds and

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constructed wetlands are efficient for the reduction of *Cryptosporidium* in wastewater, especially when the retention time is longer than 20 days with suitable sunlight and temperature while high rate filtration and chlorine disinfection are inefficient for the reduction of *Cryptosporidium* in effluents (Nasser 2016).

This study focused on nanotechnology and its applications which is considered as one of the rapidly developing sciences. As demand for fresh drinking water is increasing, nanotechnology can contribute notable development and improvement to water treatment processes. The disinfection process is the last and most important step in water and wastewater treatment processes. Some nanomaterials can be used as disinfectants due to their antimicrobial properties (Hossain *et al.* 2014). Carbon-based nanomaterials such as carbon nanotubes (CNTs), activated carbons, fullerene and graphene are widely used as the currently most promising functional materials due to their high adsorption capacities. Therefore, graphene nanosheets will challenge the current existing adsorbents, including other types of carbon-based nanomaterials (Yu *et al.* 2015).

Nano-sorbent materials such as CNTs and poly materials are applied to remove heavy metals, organics and biological impurities (Savage & Diallo 2005). CNTs have been gradually applied to the removal of organic contaminants (e.g., dyes, pesticides, and pharmaceuticals/drugs) from wastewater through adsorption processes (Yu *et al.* 2014), besides this, CNTs as adsorbent media have been proved to be able to remove a wide range of contaminants including bacteria (Akasaka & Watari 2009). CNTs with different diameters would have different specific surfaces, which would affect their adsorptive properties. However, the effects of different outer diameters of CNTs on adsorption of organic contaminates has not been investigated up to now (Yao *et al.* 2014).

Meanwhile, application of silver nanoparticles (AgNPs) has been extensively studied in the food industry for drinking water treatment (Konopka *et al.* 2009; Kumar & Raza 2009; Zhao *et al.* 2010). The antimicrobial activity of silver can be mainly attributed to interactions of silver ions with thiol groups of cellular proteins, leading to their inactivation. Processes such as cell respiration and ion transport across membranes, are also employed (Marambio-Jones & Van Hoek 2010). However, the mechanisms of toxic action for AgNPs are still not very well defined (Fabrega *et al.* 2011). The use of AgNPs in drinking water treatment has extensively become of interest (Feng *et al.* 2000; Jain & Pradeep 2005). However, addition of silver to water in such concentration that shows bactericidal activity does not impair the taste, color, odor, and other physicochemical characteristics of water (Klueh *et al.* 2000).

The present study aimed to evaluate the antimicrobial efficacy of natural kaolin clay and nano-adsorbent materials (CNTs and silver nitrate-loaded clay) against isolated bacteria from different water supplies (tap, underground and surface water) commonly used for human and animal drinking in addition to wastewater.

MATERIALS AND METHODS

Study area and period

A cross-sectional study was carried out in nine different rural areas in Beni-Suef Province (coordinates: 29°04'N 31°05'E), Egypt during the period from September 2014 to July 2015. These areas are suffering from health problems related to water quality on both human and animal levels.

Water sample collection

A total of 160 water samples were collected from main water sources intended for animal and human drinking. Samples represented three different water supplies (tap, underground [hand pumps], surface water [water channel] source) beside wastewater ($n = 40$ of each) in investigated districts. The tap and hand pump water samples were collected in 250 ml sterilized Schott Duran Bottles. For this purpose, the outlets of the taps and hand pumps were thoroughly disinfected by ethyl alcohol 70%, water was allowed to flow and then water samples were taken. Surface water and wastewater samples were collected 20 cm below the surface of the water using sterile plastic syringes (50 ml capacity) according to the method described previously (Azam *et al.* 2012). Sampling bottles were tightly capped, properly labeled and identified according to their source and site and immediately sent to the laboratory in an ice box for further microbiological examination according to standard guidelines (American Public Health Association (APHA) 2012).

Isolation and identification of bacterial pathogens

Isolation of bacterial pathogens

E. coli was isolated on MacConkey agar (Oxoid; CM 0115) and Eosin Methylene Blue agar (EMB; Oxoid; CM 69) plates meanwhile, *Salmonella* and *Shigella* species were isolated by pre-enrichment of water samples on buffer peptone water, then enrichment of samples on Selenite F broth, followed by isolation of the typical organism on selective medium, Xylose Lysine Deoxycholate agar (XLD) (Collee *et al.* 1996).

Biochemical and serological identification of bacterial pathogens

All colonies with different characteristics on EMB and XLD were sub-cultured onto nutrient agar for purification. Enteric bacteria isolated on respective selective or differential media were identified on the basis of their colony and morphological characteristics and then using API 20E (Biomerieux, Crappone France); final verification based on serological identification was carried out at the Ministry of Health, Cairo, Egypt, using techniques adopted by Sojka (1965). Serotyping was carried out by the slide agglutination technique using diagnostic polyvalent and monovalent *E. coli* O & K antisera.

Antimicrobial efficacy of natural kaolin and nano-adsorbent materials against isolated bacterial pathogens

The antimicrobial effects of natural kaolin clay and its loaded forms were evaluated against a total of 50 isolated bacterial pathogens from three different water supplies in addition to wastewater in the investigated districts.

Preparation of natural kaolin clay and its loaded forms

Natural clay pretreatment. Kaolin clay was collected from kilometre 18 of the Eastern Minia/Beni-Suef road. The pretreatment of kaolin was carried out by milling it to the sub-micro size. It was then washed with distilled water several times and after that dried in an oven at 100°C. Milling of the clay to the sub-micro size was then repeated, due to the agglomeration of clay particles which resulted from repeated washing and drying, and after that it was ready for use. Sub-micro sized clay agglomerated together to form micro granules after 10 h millings (1 μm in diameter) is shown in Figure 1.

CNTs-clay preparation. The composite of CNTs and clay was prepared by using kaolin as a catalyst during the synthesis of CNTs using the chemical vapor deposition technique (Pastorkova *et al.* 2012). The results indicated that CNTs clay composite was successfully fabricated and the clay and CNTs were not exfoliated by each other. The morphology of multi-walled carbon nanotubes (MWCNTs) kaolin clay composite was characterized by transmission electron microscope (TEM). Figure 2 shows the tubular structure of MWCNTs. Furthermore, it is clear that the outer walls of MWCNTs were deformed and amounts of amorphous carbon were deposited. From the high resolution TEM (HR-TEM) image of CNTs, the internal diameter of MWCNTs is approximately (7.06 nm) and the external diameter is nearly (15.66 nm).

Kaolin clay appears separated or linked with CNTs in Figure 3 which indicates that small amounts of clay are present inside CNTs due to their small nano-size (0.21 nm) and a large amount of clay is dispersed on the outer wall surface. These results revealed that the MWCNTs/kaolin composite are not exfoliated by each other (Pastorkova *et al.* 2012).

AgNPs-clay preparation. The effectiveness of AgNPs as an antimicrobial was determined. Whereas silver nitrate was used to investigate the effect of silver ions, stable AgNPs of <100 nm in size were synthesized through a one-step protocol according to the method described by Vigneshwaran *et al.* (2006) with some modifications. Five grams of natural clay were added to 100 ml of deionized water and heated to complete digestion, then 5 ml of 0.1 N solution of silver nitrate ($AgNO_3$) (FW 169, 87 Gamma laboratory chemicals, Assay: Min 99.0%) were added and stirred well. This mixture was put into a dark glass bottle and autoclaved. After preparation of AgNPs-loaded clay, it was kept in a dark glass bottle away from direct sunlight at room temperature (25°C), and the size of AgNPs-loaded clay particles was

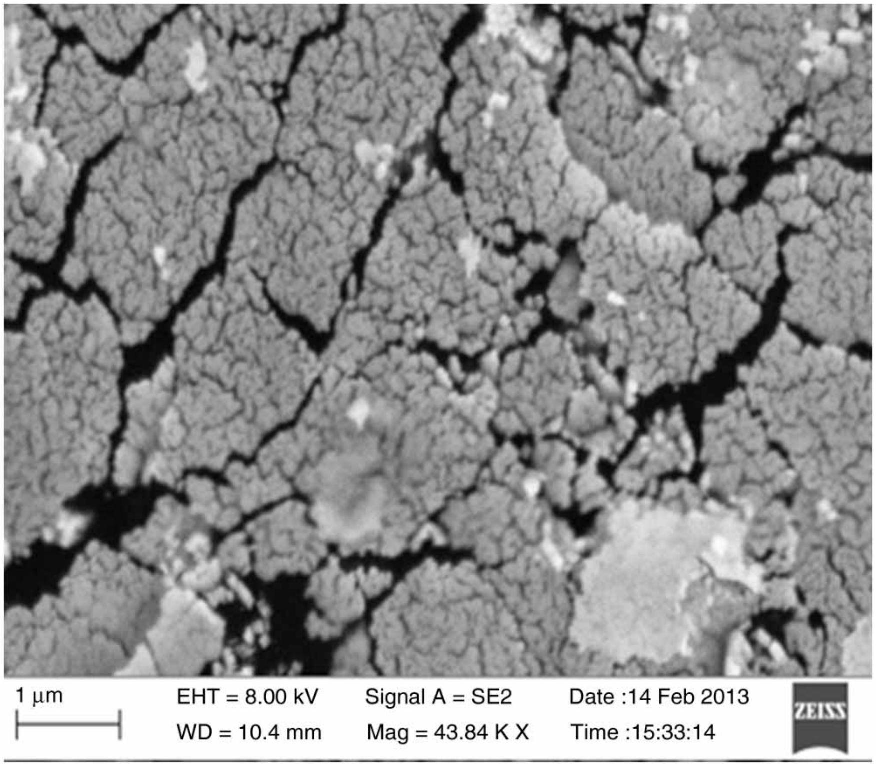

Figure 1 | Scanning electron microscope image of natural kaolin clay after 10 h milling.

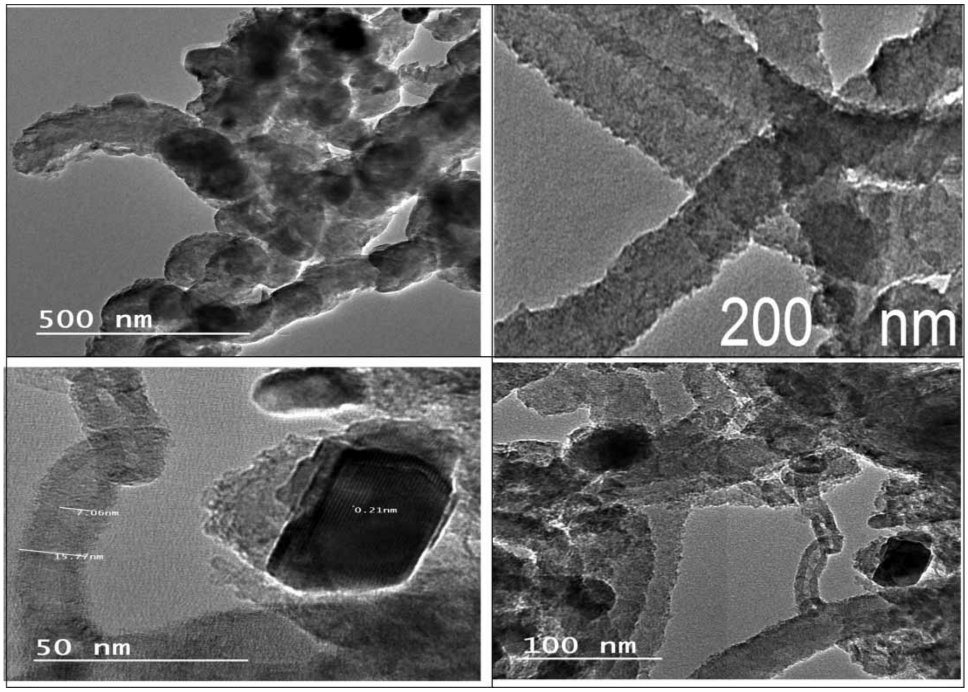

Figure 2 | TEM images for CNTs-clay composite at different magnifications.

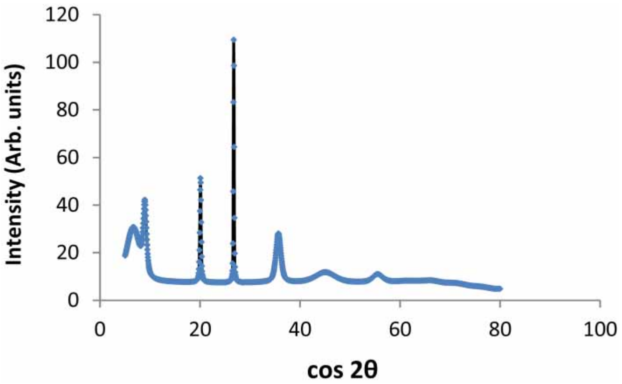

Figure 3 | The X-ray diffraction pattern of CNTs-clay composite.

measured by TEM (JEOL-JEM- 100CX II) and their size ranged from 5–40 μm (Figure 4). The elemental analysis by wavelength dispersive X-ray fluorescence (XRF) spectrometry revealed that the initial wt % of Ag_2O (12.64%) has been added to the kaolin/$AgNO_3$ loaded sample (Table 1), which was completely absent in the natural clay. This assures the successful loading of the clay with $AgNO_3$. These data were confirmed through the observation of two peaks centered at energies very close to their tabulated line energies in the range 2.86–3.51 (Figure 5); one lies at 3.12 which is narrow and strong (1^{ry} diffraction) with an intensity of 2.34 K and the other is less intense, lying at 3.25 with an intensity (2^{ry} diffraction) of 1.04 K. Compared with the X-ray diffraction (XRD) pattern for the natural clay (Hassouna *et al.* 2014) such peaks are replaced by Si, Al, Ca and Mg ones that are normally found in clays. The total concentration of AgNPs stock was measured by graphite furnace atomic absorption (Model 210VGP) at the National Research Center, Cairo, Egypt.

Evaluation methods of natural kaolin and its loaded form

The antibacterial efficiency of kaolin clay and nano-adsorbent materials was determined using the broth macro dilution technique according to Li *et al.* (2008) with some modifications. Five sterile test tubes for each tested material (each test tube containing 1.2 ml homogeneous bacterial suspensions) were prepared in Brain Heart Infusion (BHI), then 0.8 ml of the tested material suspension were aseptically added to four test tubes to obtain final concentrations of 0.01, 0.03, 0.05 and 0.1 mg/l. Each of the natural kaolin, CNTs-loaded clay and AgNPs-loaded clay treatments were thoroughly mixed separately and allowed to interact with bacteria at different contact times (5, 15, 30 min and 2 h). The remaining test tube (the fifth one) was used as the negative control (1.2 ml bacterial suspension without any adsorbent material). Then, 1 ml of inocula was transferred to BHI-containing tubes, for incubation at 37 °C for 24 h. A sample was considered positive if it was exhibiting medium turbidity and the formation of a thin surface skin or of a precipitate in the bottom of the

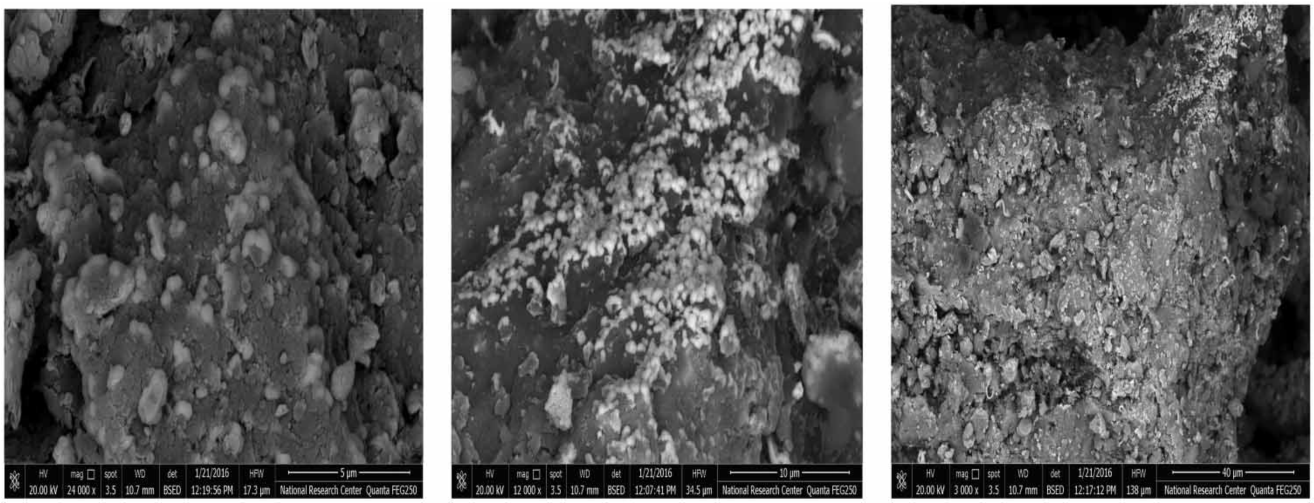

Figure 4 | TEM images for AgNPs-clay composite at different magnifications.

Table 1 | Elemental analysis of the kaolin/$AgNO_3$ loaded sample by wavelength dispersive XRF spectrometry

Main constituents (wt %)	Sample no. (1)
SiO_2	39.01
TiO_2	1.09
Al_2O_3	19.21
$Fe_2O_3^{tot.}$	8.74
MnO	0.02
MgO	1.48
CaO	2.76
Na_2O	0.39
K_2O	0.93
Ag_2O	12.64
P_2O_5	0.09
SO_3	0.04
Cl	2.00
L.O.I	11.39
Cr_2O_3	0.028
NiO	0.010
CuO	0.008
ZnO	0.026
PbO	0.034
Rb_2O	0.007
Ga_2O_3	0.005
As_2O_3	0.019
Y_2O_3	0.010
SrO	0.024
Nb_2O_5	0.013
ZrO_2	0.034

tube. After incubation, the suspension was spiked in solid medium (trypticase soya agar) to confirm the presence or absence of microorganisms tested against various tested materials and exposure times. The absence of bacterial growth in plates indicates the effectiveness of the adsorbent.

Statistical analysis

The data collected and recorded on specifically designed forms were entered in the Microsoft Excel spreadsheet then prepared for analysis. The frequent distribution of bacterial isolates from different water sources was calculated by using percentage values and analyzed using a Chi-square test (SPSS, version, 22).

RESULTS

The frequent distribution of pathogenic bacteria isolated from different water sources in (Table 2) clarified that there were in total 121 bacterial isolates out of 160 examined samples. The highest percentages of bacterial contaminants were isolated from waste and surface water sources (100% of each) at $X^2 = 9.52$ ($P < 0.05$), while the lowest percentages were presented in underground and tap water (20% and 5% respectively). Moreover, the predominant bacterial isolates were *E. coli* and *Shigella flexneri* followed by *Salmonella* spp., *Klebsiella aerogenes* and *Klebsiella pneumoniae* (34.7, 24.7, 16.5, 14.0 and 9.9%, respectively).

Concerning serological identification of *E. coli* isolated from different water sources (Table 3), it has been found that the predominant *E. coli* serotypes in different water sources were O128:k67, O157:k-, O111:k58 and O55:k59. The highest serotype percentages in wastewater were O157:k- and O111:k58 (33.3% and 25.9%, respectively) followed by O128:k67 and O55:k59 (18.55% of each). However, in surface water, the highest percentage of *E. coli* serotype was O157:k- followed by O128:k67, O55:k59 and O111:k58 (30.7, 23.07, 23.07 and 15.3%, respectively) while in underground water, O128:k67 and O111:k58 accounted for 50% each.

A comparison of the antimicrobial efficacy of natural kaolin clay with nano-adsorbent materials against bacterial isolates from different water sources is presented in Table 4. It has been revealed that AgNPs-loaded clay at a concentration of 0.1 mg/l had the highest antimicrobial activity against *Salmonella* spp. (90%) followed by *E. coli*, *Klebsiella pneumoniae* and *Shigella flexneri* (80% each) and *Klebsiella aerogenes* (70%) after 2 h of exposure time. The antimicrobial activity of CNTs-loaded clay at the same concentration was 70% for each of *Salmonella* spp. and *Klebsiella pneumoniae*, while it was 60% for *E. coli* isolates.

The results in Table 5 show the antimicrobial efficacy of AgNPs-loaded clay at different exposure times. It was

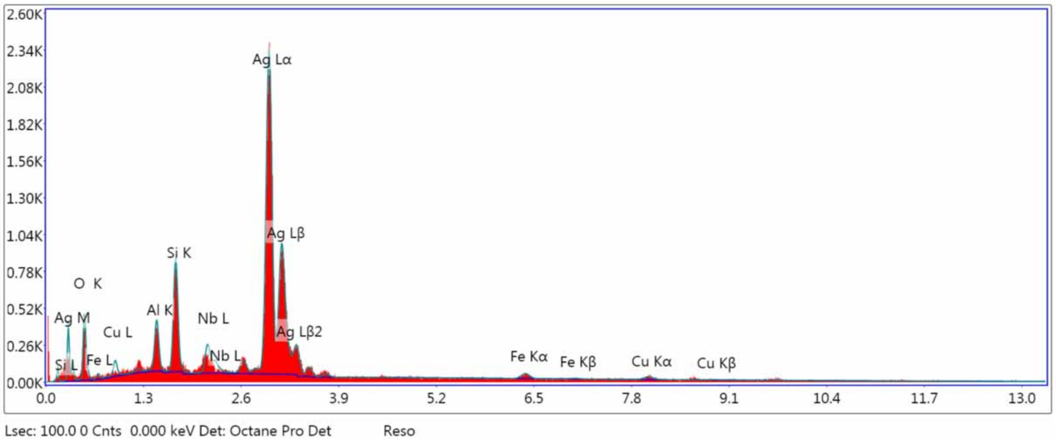

Figure 5 | The XRD pattern of AgNPs-clay composite.

Table 2 | The frequency distribution of pathogenic bacteria isolated from different water sources

Bacteriological finding	**Total**		**Bacterial isolates (%)**					
						Klebsiella		
Water samples	**Examined (No.)**	**Positive No. (%)**	**Total (No.)**	***E. coli***	***Salmonella* spp.**	***pneumoniae***	***aerogenes***	**Shigella *flexneri***
Tap	40	5.0	5	ND	ND	20	60	20
Underground	40	20.0	12	16.6	16.6	ND	33.3	33.3
Surface	40	100	46	28.2	15.2	17.3	10.8	28.2
Waste	40	100	58	46.5	18.9	5.1	8.6	20.6
Total	160		121	34.7	16.5	9.9	14.0	24.7

ND: not detected, $X^2 = 9.52$, $P < 0.05$.

Table 3 | Serological identification of *E. coli* isolated from different water sources

Isolated bacteria		***E. coli* serotypes (%)**			
Water samples	**Total isolated (No.)**	**O128:k67**	**O157:k-**	**O111:k58**	**O55:k59**
Tap	0	ND	ND	ND	ND
Underground	2	1 (50)	ND	1 (50)	ND
Surface	13	3 (23.07)	4 (30.7)	2 (15.3)	3 (23.07)
Waste	27	5 (18.5)	9 (33.3)	7 (25.9)	5 (18.5)
Total	42	11 (26.19)	13(30.9)	10 (23.8)	8 (19.04)

ND: not detected.

found that the highest antimicrobial activity was achieved at the concentration dose of 0.1 mg/l and 2 h exposure time for all tested bacterial isolates (*Salmonella* spp. was 90%, followed by *E. coli*, *Klebsiella pneumoniae* and *Shigella flexneri* at 80% each). Meanwhile all bacterial isolates showed a highly resistant profile to other concentrations (0.01, 0.03 and 0.05 mg/l) at different exposure times (5 min, 15 min, and 30 min, respectively).

Table 4 | Antimicrobial efficacy of natural clay and nano-adsorbent compounds against isolated bacteria of different water sources after two-hour exposure time

Bacterial isolates (spp.)	***E. coli***			***Salmonella* spp.**			***Klebsiella***						***Shigella flexneri***		
							pneumoniae			***aerogenes***					
Tested compounds dose(ppm)	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Natural clay (2.0)	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
CNTs-clay (0.1)	60	10	30	70	10	20	70	20	10	60	10	30	40	40	20
AgNPs-clay (0.1)	80	10	10	90	10	0.0	80	20	0.0	70	20	10	80	10	10

S: Sensitive (absence of bacterial growth), I: Intermediate (moderate bacterial growth), R: Resistant (bacterial growth).

Table 5 | Antimicrobial efficacy of AgNPs-loaded clay against isolated bacteria from different water sources at different exposure times

		Antimicrobial activity (%) of AgNPs-clay at different exposure times											
		5 min			**15 min**			**30 min**			**2 h**		
Bacterial isolates (spp.)	**Doses (mg/l)**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
E. coli	0.01	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	20	80
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	40	60	0.0	40	60
	0.05	0.0	0.0	100	0.0	20	80	20	50	30	70	10	20
	0.1	0.0	20	80	0.0	40	60	80	20	20	80	10	10
Salmonella spp.	0.01	0.0	0.0	100	30	10	60	30	20.0	50	40	30	30
	0.03	0.0	0.0	100	40	20	40	30	40	30	60	30	10
	0.05	0.0	0.0	100	60	20	20	60	30	10	70	20	10
	0.1	0.0	20	80	70	0.0	30	70	20	10	90	10	0.0
Shigella flexneri	0.01	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	30	70
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	50	50	0.0	50	50
	0.05	0.0	0.0	100	0.0	40	60	50	40	10	70	20	10
	0.1	0.0	20	80	0.0	50	50	70	20	10	80	10	10
K. pneumoniae	0.01	0.0	0.0	100	0.0	0.0	100	0.0	30	70	0.0	40	60
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	40	60	0.0	50	50
	0.05	0.0	0.0	100	0.0	60	40	60	20	20	60	30	10
	0.1	0.0	40	60	0.0	50	50	60	30	10	80	20	0.0
K. aerogenes	0.01	0.0	0.0	100	0.0	0.0	100	0.0	20	80	0.0	20	80
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	40	60	0.0	40	60
	0.05	0.0	0.0	100	0.0	50	50	40	20	40	50	20	30
	0.1	0.0	30	70	0.0	40	60	30	50	20	70	20	10

S: Sensitive (absence of bacterial growth), I: Intermediate (moderate bacterial growth), R: Resistant (bacterial growth).

The presented data in Table 6 clarify that the antimicrobial efficacy of CNTs-clay was higher against *Salmonella* spp. and *Klebsiella pneumoniae* (70% for each) compared to *E. coli*, *Klebsiella aerogenes* and *Shigella flexneri* (60, 60 and 40%, respectively). Moreover, the same bacterial isolates revealed that highly resistant patterns were present at low tested concentrations of CNTs at different exposure times.

DISCUSSION

Drinking water has the potentiality to transport microbial pathogens to a great number of individuals, causing subsequent illness, which is well documented in countries at all levels of economic development (Payment 1997). In this study, results revealed that the highest percentages of bacterial isolates (*E. coli* and *Salmonella* spp.) were observed

Table 6 | Antimicrobial efficacy of CNTS-loaded clay against isolated bacteria from different water sources at different exposure times

		Antimicrobial activity (%) of CNTS-clay at different exposure times											
		5 min			**15 min**			**30 min**			**2 h**		
Bacterial isolates (spp.)	**Doses (mg/l)**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
E. coli	0.01	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.05	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.1	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	60	10	30
Salmonella spp.	0.01	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.05	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.1	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	70	10	20
Shigella flexneri	0.01	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.05	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	30	10	50
	0.1	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	40	40	20
K. pneumoniae	0.01	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.05	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	40	50	10
	0.1	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	70	20	10
K. aerogenes	0.01	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.03	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
	0.05	0.0	0.00	100	0.0	0.0	100	0.0	0.0	100	40	20	40
	0.1	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	60	10	30

S: Sensitive (absence of bacterial growth), I: Intermediate (moderate bacterial growth), R: Resistant (bacterial growth).

in wastewater followed by surface water supply as compared to other water sources in the investigated districts. These results could be attributed to the impact of unsupervised anthropogenic activities including excessive usage of fertilizers and pesticide, waste disposal and industrial waste, seepage from septic tanks and construction of water pipes. The surface water presented significant acute toxicity, and could cause diseases if used in livestock farms without being adequately treated. This represents a dangerous public health risk, which needs future evaluation and control. This was in agreement with Melegy *et al.* (2014) who showed that pathogenic bacteria can occur in surface water in large numbers, either being excreted in feces or occurring naturally in the environment. Moreover, Mohammed (2016) revealed that in surface water, *E. coli* was isolated in the highest percentage (17/56; 56.7%) followed by *Staph. aureus* (11/56; 36.7%), *Salmonella* spp. (8/56; 26.7%), *Strept. faecalis* (7/39; 23.3%), *Shigella flexneri* (5/56; 16.7%), *Proteus* spp. (5/56; 16.7%) and *Klebsiella pneumoniae* (3/56; 10.0%) at $X^2 = 9$, $P \leq 0.01$. Sources of fecal contamination include humans, livestock and wild animals; in general, human fecal waste gives rise to the highest risk of waterborne disease (Craun 1996). The most sporadic cases of waterborne intestinal illness will not be detected, or if detected may not be recognized as water-related, while it is estimated that water, sanitation, and hygiene are responsible for 40% of all deaths and 5.7% of the total disease burden occurring worldwide (Prüss *et al.* 2002).

E. coli is a widely used bio-indicator of fecal contamination in water sources. External contact and subsequent ingestion of bacteria from fecal contamination can cause detrimental health effects (Money *et al.* 2009). In this study, it has been found that the predominant *E. coli* serotypes in different water sources were O128:k67, O157:k-, O111:k58 and O55:k59. Environmental Protection Agency and epidemiological investigations, suggested that cultural fecal indicator counts are valid predictors of disease risk (Sinton *et al.* 1994) and sewage can serve as a vehicle for bacteria to enter into human and non-human hosts either by direct contact or through contamination of drinking water supplies (Boczek *et al.* 2007).

The current increased attention is directed to health problems associated with drinking water quality at both human and animal level. In particular, communities who use surface water supplies are of special concern, since their drinking water is commonly not treated or monitored for contamination on a routine basis. The present study evaluated the efficiency of natural kaolin and nano-adsorbent materials for the removal of water contaminants, and subsequently risk hazards to human and animal health. The present study revealed that different types of microorganisms vary in their response to the tested material of kaolin clay, CNTs-loaded clay, and AgNPs-loaded clay. Natural kaolin clay was the least effective against all bacterial pathogens. The results also suggested that the antimicrobial effects of kaolin and nano-adsorbent materials are not only dependent on the types of material but also on their concentrations and exposure times. A previous study showed that AgNPs supported on granular activated carbon gave good results against *Escherichia coli* (Acevedo *et al.* 2014), instead, in the present work we used kaolin as a support material which is highly available and cost effective. The efficiency of AgNPs-clay was highly effective against bacterial pathogens at concentrations of 0.05 and 0.1 mg/l. These results are in agreement with Sondi & Salopek-Sondi (2004) who suggested that the antimicrobial effects of the AgNPs on bacteria were dependent on the concentration of Ag in the nanoparticles (NPs); where increasing the concentration of NPs delayed the growth of bacteria. The treated bacterial cells were significantly changed and showed major damage, which was characterized by the formation of 'pits' in their cell walls. Previous studies have shown that antimicrobial formulations in the form of NPs could be used as effective bactericidal materials due to their enhanced reactivity, resulting from their high surface/volume ratio (Pal *et al.* 2007; Choi *et al.* 2008; Park *et al.* 2009). Particularly, silver in the form of NP (AgNPs) is known to exhibit strong biocidal effects on different bacterial species (Sondi & Salopek-Sondi 2004; Rai *et al.* 2009). Moreover, silver ions and silver-based compounds are highly toxic to Gram-negative and Gram-positive microorganisms (Bae *et al.* 2010; Fayaz *et al.* 2010; You *et al.* 2011; Maillard & Hartemann 2013).

CNTs are graphene sheets rolled into a tube and possibly capped by half a fullerene. They can be either single-walled (SWNTs), a single pipe with a diameter from 1 to 5 nm, or multi-walled (MWNTs), with several nested tubes, at lengths varying from 100 nm up to several tens of micrometers (Li *et al.* 2008). In the present study, the antimicrobial activity of CNTs against bacterial isolates from different water sources revealed that the sensitivities (%) of almost all bacterial pathogens to CNTs were 70% after 2 h exposure time.

However, the bacterial isolates which showed resistant to CNTs were *E. coli*, *Klebsiella aerogenes* (30% each), *Salmonella* spp., *Shigella flexneri* (20% each) and *Klebsiella pneumoniae* (10%) after 2 h exposure time. The results could be interpreted to suggest that the main factors which controlled their sensitivity were nanomaterial (CNTs) concentration and contact times. These results are supported by Kang *et al.* (2007) who clarified that antimicrobial activity of CNTs requires direct contact between CNTs and target microorganisms. Suspension of non-functionalized CNTs in water is extremely difficult and does not provide enough CNT-microbe contact for disinfection. Accordingly, the antibacterial activity of CNTs could be exploited by coating CNTs on a reactor surface in contact with the pathogen-laden water, also it has been found that using immobilized SWNTs on a membrane filter surface led to 87% killing of *E. coli* in a 2 h contact time.

CONCLUSION

Based on the present results, it can be concluded that the application of AgNPs-loaded clay for removal of water bacterial contaminants resulted in highly effective removals at a concentration of 0.1 ppm and 2 h contact time, and that CNTs-loaded clay was also effective, but to a lesser extent, at the same concentration and exposure time.

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