Original Research Article

DISINFECTION OF TANK WATER USING BIOGENIC SILVER NANOSILICA AS ANTI-MICROBIAL AGENTS IN JEDDAH CITY

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Abstract. The need for clean water is becoming urgent all over the world. The risk of microbial contamination of drinking water and its growing ability under poor nutritional conditions occur frequently and existing water management systems do not efficiently eliminate these micropollutants. The major goal of this research is to analyze and test the drinking water quality at random locations in Jeddah City and to investigate the integration of polyurethane foam coated nano-silica silver nanoparticles (NSAgNPs) into the water purification system.The study was conducted in Jeddah city at random intervals during August to December 2020. Twenty-five samples (n = 25) of drinking water (tank water) were collected from different locations in Jeddah. The water samples were found contaminated with *E. coli*, *Klebsiella spp.* and the most prevalent yeast species was Candida albicans. The multi-drug resistance isolates were positively identified by PCR amplification of 16S rDNA gene fragments. The antimicrobial activity of nano-silica silver nanoparticles (NSAgNP) was analyzed and their optimal concentrations that remove potentially harmful microbes were determined. The cytotoxicity of NSAgNPs by Sulforhodamine B colorimetric (SRB) assay against breast adenocarcinoma cell line (MCF-7) showed no cytotoxic effect at concentrations ≤100 µg/mL. The 50% growth inhibition concentration (IC 50) of NSAgNPs after 72 h was 35.8 µg/mL. The excellent performance of NSAgNPs can be attributed to the capping surface functional groups and smaller particle size 8.931 nm, as verified by TEM. In conclusion, the purified water obtained by using 100 mg/L NSAgNPs is free from microbial contaminants and completely safe to drink. We recommend the integration of biosynthesized foam coated silver nanoparticles with water purification system to achieve the desired final water quality.

Key words*: Drinking water, Nano-Silica Silver NanoParticles, Disinfection, E. coli, Klebsiella spp, Candida albicans, PCR, antibiotic resistance, Cytotoxicity.*

Introduction

The safety of drinking water is critical to public health. Disinfection is considered one of the most critical methods in water management because the disinfection process can destroy pathogens in drinking water. Nevertheless, traditional disinfection can have dangerous effects on human health because by-products of disinfection are produced, which are responsible for cancer and other diseases [1]. In addition, there are several difficulties and problems associated with the deterioration of water quality, such as microbes originating from surface

or groundwater sources, flooding that can cause the penetration of microorganisms into the drinking water pipes, leakage in the pipes through which microbial substances enter the drinking water network. The current research proposal suggests the application of nanotechnology approaches to water disinfection and provides a new attempt to develop clean and healthy water. About five million people every year suffer from inadequate sanitation, unsafe water, one of which is microbial disease (i.e. cholera) killing 50% of the global total [2]. Waterborne microbial infections had the greatest impact on children under the age of five (i.e., about 41% of global pathogenic infections). It is worth noting that drinking water must be safe for all residents, regardless of its source as well as free of chemicals that are hazardous to health, and must also be tasteless, odorless and colorless. Many waterborne diseases, be it bacteria, viruses, fungi, or protozoa, have made it interesting for government agencies and researchers to study water microbiology [3].

There are over 500 water- pathogens that pose a health danger in the water system of the United States (https://www.who.int/teams/environment-climate-change-and-health/water-sanitation-and-health/water-safety-and-quality/drinking-water-quality-guidelines) [4]. A wide range of fungal groups have been isolated from drinking water [5,6]. Some fungal species are considered highly allergic or can cause infections in immunocompromised individuals [7,8]. In addition, *Vibrio cholera*, *Salmonella enterica*, and *Shigella spp* are the most common waterborne enteric pathogens that have been eliminated by water disinfection and therefore are rarely a drinking water problem in developed regions [9]. However, Shiga toxin-producing *Shigella sonnei* and verotoxin-producing *E. coli* are still prevalent in the water of some developed countries and are transmitted from person to person and via contaminated food. Moreover, the prevalence of AMR *P. aeruginosa* and *C. difficile* may continue in sewage and river water and eventually enter drinking water [10,11]. Size controlled nanoparticles have come into focus because they offer solutions to technological and environmental problems. As a good antimicrobial agent, silver nanoparticles have been widely used to disinfect water [12]. Drinking water treatment with silver nanoparticles has recently attracted the attention of researchers because it efficiently kills wide range of bacteria, viruses, and many microbial pathogens, which is controlled by its small size which have large surface area with unique physicochemical and biochemical properties, besides its ability to be effectively immobilized on solid substances (e.g., silve/sand, silve/zeolite, and silver/fiber) [13,14]. The mechanism of antimicrobial action of AgNPs is not clearly known and continues to be debated. Silver nanoparticles (AgNPs) are extremely toxic to microorganisms and thus have significant antimicrobial effects on a variety of microorganisms including viruses [15,16], bacteria [17,18] and fungi [19, 20, 21]. In recent years, several theories reported that AgNPs are able to adhere to and subsequently changing the integrity of the bacterial cell wall, facilitating entry of nanoparticles in the cell membrane and thus increasing its permeability [22]. In addition, AgNPs can produce free radicals that can destroy the bacterial cell membrane, leading to cell death [23,24]. In addition, AgNPs can interact with the sulfur and phosphorus groups, resulting in DNA damage [25]. Moreover, during the degradation of AgNPs, Ag+ ions can inactivate several important enzymes due to interference with thiol groups that disrupt normal cellular function [26, 27, 28]. Therefore, metallic nanoparticles have entered our real life, and our exposure to these materials has increased our fears about their possible environmental health and potential risks. In an *in vitro* cell culture assay, AgNPs showed a possible cytotoxic effect on many human cell lines based on their dose, size and time, especially for

those with unique sizes ≤10 nm [29]. However, the biogenic AgNPs and their nanocomposites have both antimicrobial properties and no cytotoxic effects [30, 31]. This is because the biogenic nanoparticles are surrounded by capping surface functional chemical groups that maintain their biocompatibility and long-term stability and can interact with biological components through these active surface functional groups [31, 23]. In this work, we have prepared a new ecofriendly biogenic silver nanoparticles (NSAgNPs) for drinking water decontamination and explored their antimicrobial and cytotoxic activity.

Materials & Methods

Study area and water sampling

Drinking water samples from different locations across Jeddah city were collected as following: drinking water from tanks (25 samples), drinking water filtered with polypropylene filters (5 samples) and pure water from commercial bottles as control (5 samples). These locations were chosen because water is serving a great public need in the farming and manufacturing sectors. Drinking water can be contaminated with wastewater from underground. It is therefore necessary to study the microbiological quality of water at these sites. Water samples (storage tanks) were collected in sterile 50 ml falcon tubes at various sites during the period August to December 2020. Labelling samples was done correctly and the samples were sent to the laboratory for testing. Aliquots of the samples were cultivated for microbial screening using standard microbiological procedures [33].

Isolation and identification of microbial contaminants

One hundred fifty lilliliter (150 mL) of water samples, were filtered through 0.22 μm porous filters (Nitro-Cellulose Membrane Filters, Thomas Scientific) using a water pump. The aseptic membranes were applied to the plates, eliminating any air bubbles. The media used are: Cystine lactose electrolyte deficient (CLED) agar, Blood agar (CLED /BA), Nutrient agar for heterotrophic bacteria and Sabouraud Dextrose agar for fungi (bioMérieux SA, France). Each test was repeated three times. One millilitre of treated water was poured over the nutrient agar plates to differentiate heterotrophic bacteria. The plates were maintained at 37 ºC for incubation for 24 hours. Bacterial colonies were counted, characterized and recorded. Purified colonies were used for Gram staining, biochemical identification tests and PCR-based 16S rDNA and ITS rDNA identification.

Antimicrobial Susceptibility Testing

Sensitivity test of Kirby-Bauer disk diffusion method was carried out using antifungal and antibiotic discs at certain concentrations. The following antibiotic disks (Bioanalyse, LTD) were used at the indicated final concentrations: Amoxicillin (AMC-10 μg), Cefotaxime (CTX-25 µg), Ceftriaxone (CRO - 30 µg), Nalidixic acid (NA - 30 µg), Gentamicin (CN 20 µg), Chloramphenicol (C- 30 µg), Fluconazole10 µg, Miconazole 10 µg and Clotrimazole10 µg. Since there is a significant amount of resistance to these antibiotics, they were selected [34]. A bacterial suspension was prepared by transferring a single bacterial colony into 3 ml of sterile distilled water. 100 μl of each suspension was spread on the top surface of Mueller-Hinton agar plates. Antimicrobial disks were placed on the surface of the plates and maintained in the 37 ℃ incubator for 24 hs. The zone of inhibitions were measured and National Committee for Clinical Laboratory Standards (NCCLS) reference values were used

to establish the resistance categories for the various isolates according to the defined National Committee for Clinical Laboratory Standards [35].

Genomic DNA extraction

Following the instructions of the manufacturer, gDNA was extracted from the bacterial cultures with the QIAamp DNA Mini Kit. The yeild of DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific, USA) and the DNA quality was tested using a 1% agarose gel.

Detection of microbial contamination by DNA-PCR

16S and ITS rDNA-PCR fragments were amplified by using Qiagen®Taq PCR Master Mix Kit. Bacterial universal 16S rDNA primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1392R (5'-GGTTACCTTGTTACGACTT-3') and fungal ITS rDNA primers: ITS1 5'-TTGTGTTGCTACATCACCAAC-3' and ITS4 5'-TTTGCTGGCAACTTGATTACC-3' were used. Diluted DNAs of concentration 0.5 ng/µl, was added to 25 µl master mix containing 2 µl (25 pmol) 16S rDNA primer mix/ or ITS primer mix, 75 mM of the dNTPs, 15 mM MgCl2 and 15U Taq DNA polymerase. PCR negative control containing 2 µl 0.1 × TE (Tris EDTA) was used. DNA amplifications were done with a thermal cycler (3Prime Thermal Cycler, 3PRIMEX/02, Bibby Scientific Ltd). The PCR program consisted of a total of 35 cycles, the initial hard denaturation cycle at 95°C for 3 min, followed by 35 cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 2 min, followed by a final extension cycle at 72°C for 10 min and a 10°C soak. Ten microliters of each PCR product was run on a 1% agarose gel containing 0.5 µg/ml ethidium bromide and visualized using a UV transilluminator.

Preparation of biogenic nano-silica-silver nanoparticles (NSAgNPs).

Twenty milligram of Nano-silica was applied to 25 mL of a 1 mM $AgNO_3$ solution (pH 6.0) and incubated with rotation (120 rpm) for 2 hours at 30 °C. 5 mL of *A. niger* filtrate containg 6 mg/ mL protein mixture, kindly provided by Al-Zubaidi et al. College of Science, University of Jeddah **[36]** was added and incubated for further 48 h at 30 °C with shaking. The development of NSAgNPs was observed by the color shift of the nano-silica from colourless to a darken brown color. A control experiment was carried out without the use of the protein supplement under the same conditions. The synthesized NSAgNPs were precipitated by centrifugation (10.000 rpm, 15 min) and let to dry in a hot air oven at 50 °C for 5 h. The physicochemical properties of the dried NSAgNPs were analyzed by UV-vis spectroscopy, Transmission electron microscopy (TEM), Dynamic light scattering (DLS) and Zeta potential using (Malvern Instruments).

Silver nanoparticles characterization

UV–vis spectroscopy

The characterization of the biogenic NSAgNPs was carried out according to the method described by **Gurunathan et al. [37]**. Biosynthesized nanoparticles were studied using UV-Vis spectroscopy to measure their size and shape. The spectra were analyzed with a UV-visible spectrophotometer (UV-2450, Shimadzu, Tokyo, Japan). A rapid and marked change in the color of the solution is an early indication of the formation of NSAgNP. Three ml of

the suspension was transferred to UV spectrum at an absorbance of 300 to 700 nm (UV-spectroph; Shimadzu, Japan) evaluate the colour of the solution.

Transmission electron microscopy (TEM)

The shape and size of the biosynthesized silver nanoparticles (NSAgNP) were determined using Transmission Electron Microscope (TEM, JEM2100, Jeol, Japan). Few drops of the silver nanoparticle suspension (usually about 5 µl) were loaded on a carbon coated copper grid and then air dried on a filter paper for 5 -10 min. The grid can then be observed directly in a TEM once the medium has evaporated.

Coating of Polyurethane foam with silver nanoparticles

The experiment was divided into two separate parts. The first section of the experiment involved the use of 100 mg/L of silver nanoparticles (NSAgNPs) and mixed with water samples contaminated with microbes at different periods of time (15, 1h, 2h, and 24 h). The other part of the experiment was to coat a 20 cm polyurethane foam 1 mm thick with 100 mg/ L silver nanoparticles for full coverage and then air-dried. NSAgNPs coated foam is placed in water samples contaminated with microbes and allowed to bind with the microbial communities present in the water samples for various duration 1 h, 2 h, and 24 h. The purified water samples will then be tested for the presence or absence of microbial contamination.

Bacteriological analysis of water samples

Water samples were taken after treatment with nano-silver particles (NSAgNPs). The samples were analyzed bacteriologically for *E.coli*, *K. pneumoniae*, and *Candida spp.* using NSAgNPs coated foam membrane filtration following standard microbiological techniques.

Cell culture

MCF-7 breast adenocarcinoma cell lines were maintained in DMEM media supplemented with100 mg/mL streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetalbovine serum (FBS) in 5% CO_2 incubator at 37 °C in humidified conditions.

Cytotoxicity of NSAgNPs

Cell viability was evaluated by Sulforhodamine B colorimetric (SRB) assay. Aliquots of 100 µL cell suspension (5x10^3cells) were added to each well in 96-well plates and allowed to grow for 24 h in complete media. Hundred microliter of different concentrations of NSAgNPs (001,01,1,10 and 100 µg/ml) were added to the cell culture. After having been in a normal cell culture medium in an incubator for 72 hours, the media was replaced by150 µL of 10% TCA and cells were incubated for another 1 hour at 4°C. After incubation, the cells were washed 5 times with distilled water and 70 µL SRB solution (0.4 %w/v) were applied to the cells and the plates were kept in the dark at RT for 10 minutes. The plates were soaked in 1% acetic acid thrice then drained and dried off overnight. Then, 150 µL of Tris-HCl (10 mM) buffer was applied to facilitate the dissolving of protein-bound SRB stain. The O.D. was measured at 540 nm using a BMGLABTECH®-FLUO starOmega microplate reader (Ortenberg, Germany).

Statistical Analysis

Each experiment was repeated three times. The results were plotted as a mean with the range of measurement (i.e. mean ± standard deviation). One-way analysis of variation was used to investigate some differences in performance. Differences were done using SPSS software (SPSS Inc, Chicago, IL, USA).

Results

Characteristics of collected tank water samples

The locations and, the water samples ID collected from the points-of-use in north region of Jeddah city, Saudi Arabia is shown in **Fig.1, Table 1**. Tank water samples were transfered directly from the household tanks in sterile bottles to the laboratory under sterile conditions for physical and microbiological examination.

Fig. 1. Map of Saudi Arabia showing Jeddah city and the sites of collected tank water samples.

Table 1. Collected tank water samples and location coordinates

Sample ID	Coordinates
S1, S2, S5, S7, S19, S22	320° NW 21°28'49"N 39°13'53"E
	128° SE 21°28'37"N 39°13'50" E
	0° N 21°30'4" N 39°14'23" E
	1°N 21°34'34"N 39°10'32"E
S3, S4, S8, S10	359° N 21°30'24" N 39°18'5" E
	0° N 21°30'4" N 39°14'23" E
	144° SE 21°30'17" N 39°14'23" E

	39° NE 21°31'15" N 39°21'15" E
S6, S9	8° N 21°30'13" N 39°14'26"E
S11 to S18, S20, S21, S23, S24, S25	1°N 21°31'20" N 39°21'35" E
	79° E 21°46'17" N 39°5'37" E
	79° E 21°46'17" N 39°5'37" E
	123° SE 21°46'29" N 39°6'15" E

Physical parameters of tank water samples were tested in comparison with other filtered water to investigate the efficiency and the reliability of these filters in water treatment. Turbidity, Electrical conductivity (EC) and pH-value were measured to evaluate the quality of collected tank water samples as well as the efficiency of filtration system used in control samples (**Table 2**).

Turbidity is a crucial water quality metric, widely used to determine the quality of drinking water. The degree of water turbidity and microbiological contaminants is significantly independent. Water turbidity in the study area was found to be quite high as shown in **Table 2.** Turbidity avarege of water samples ranged between 18.5±0.03 to 26±0.29 NTU and the means and the standard deviations of the electric conductivity was 279.4±50.9 µS/cm.

The average of total microbial colonies/100 ml was 92.8±48.8 and 76% of tested water samples were found to be contaminated with mixture of coliforms (*E.coli*, *K. pneumoiae* and *Candida spp* **(Fig. 2, Table 2**). The presence of high percentages of coliform in potable water has the potential to cause microbial growth again and also the formation of biofilm. The existence of this coliform in potable water is not generally an indication of recent water pollution by fecal matter, the presence or absence of such bacteria is sometimes used to assess if the water disinfection process is working properly.

Quality parameters of tested water samples prior and post treatment with NSAgNP

Four controlled experiments were conducted (**Table 2**) to evaluate the capacities and limitations of the coated NSAgNPs polyurethane foam filter (current study) compared to standard filtration methods used in drinking water decontamination. Twenty five tank water samples before treatment with NSAgNPs (n=25), tap water/polypropylene home filter (n=5), pure water/commercial bottles (n=5) and tank water samples after treatment with NSAgNPs coated polyurethane foam filter (n=25) were analyzed for their quality in terms of physical and microbiological parameters. The aim of the experiment is to select the most satisfactory and economically optimal water filtration system suitable for domestic use. The results showed a sharp decrease in the value of turbidity, electrical conductivity, pH and total colonies in the case of polyurethane foam filter coated with NSAgNPs. The proposed nanosilver (NSAgNP) was very efficient in removing microbial colonies from contaminated water samples compared to other filter systems. All the water samples analyzed showed average turbidity to be quite high with average between 18.5±0.03 to 26±0.29 NTU and high EC with average 279.49±50.93 µS/cm in comparison with the control filtered water samples.

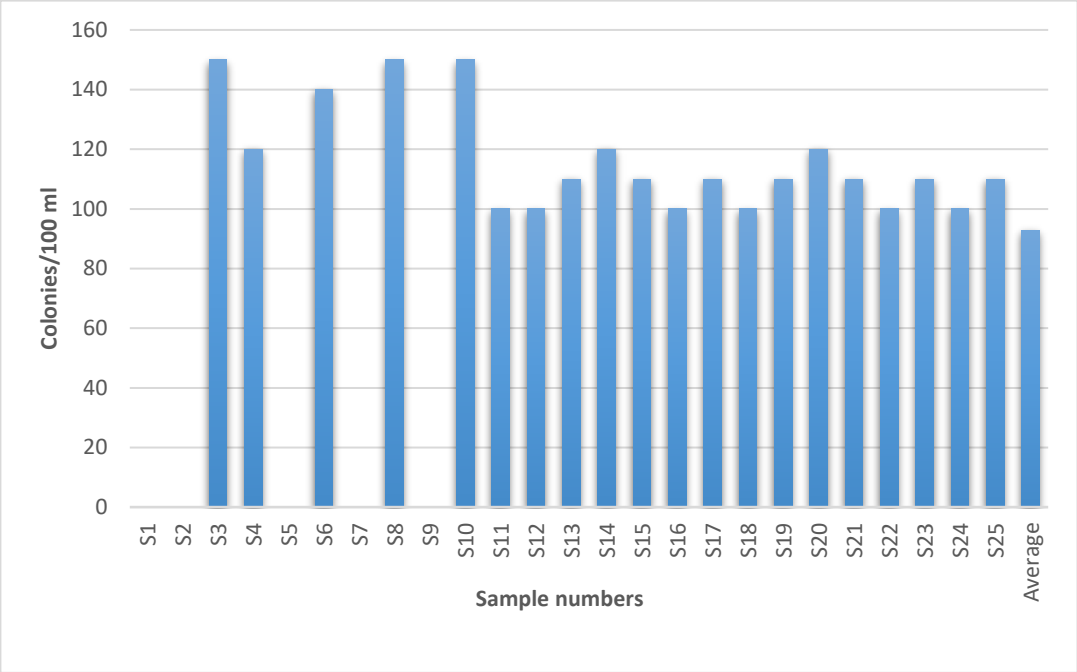

Fig. 2. Total microbial colonies in tested tank water samples (n=25)

Table 2. Quality parameters of tested water samples prior and post treatment with NSAgNPs

Parameters	Tank Water samples prior treatment with NSAgNPs (n=25)	Tap Water / polypropylene home filter (n=5)	Pure water/Commercial bottles (n=5)	Tank Water samples post treatment with NSAgNPs coated Polyurethane foam filter (n=25)	WHO limits
pH	7.89± 0.19 *p* <0.05	7.46±0.08 *p* <0.05	7.2±0.1 *p* <0.05	6.94±0.10 *p* <0.05	6.5-8.5
Turbidity (NTU/JTU)	26±0.293 *p* <0.05	18.5±0.03 *p* <0.05	0.5 ±0.04 *p* = 0.13	0.05±0.03 *p* =0.401	5.0
Total Colonies/100ml	92.8±48.8 *p* <0.05	Nill	Nill	Nill	-
Electric Conductivity (µS/cm)	279.49±50.9 *p* <0.05	95.5±19.5 *p* <0.05	< 0.05	1.419±0.14 *p* <0.05	-
Temp (°C)	25.0	25. 0	25.0	25.0	-

(NTU): nephelometric turbidity units, Turbidity less than (1 NTU) is a desirable water characteristic. *P* value > 0.05 means non significance (no difference between treatment groups), *p* values < 0.05 means significance.

Microbial identification

Microbial isolation and identification of water samples was performed by biochemical and molecular techniques. **Fig. 3** showed the microorganisms isolated from tested water samples using the filter membrane technique as menthioned under the materials and methods and identified on Cystine lactose electrolyte deficient (CLED) agar, Blood agar (CLED/BA) and Sabouraud Dextrose agar. (6/25, 24%) water samples (S1, S2, S5, S7, S19, S22) showed no

growth of any microorganisms on the tested media, while (4/25, 16%) (S3, S4, S5, S8) showed mixed growth of *Klebsiella pneumoniae* & *Candia spp.* Meanwhile, (2/25, 8%) (S6 and S9) water samples showed mixed contamination with *E coli* & *Candia spp.* However, 52% of water samples showed *Candida spp* growth.

The antibiotic sensitivity test were examined against isolated microbes and their efficacy was evaluated according to its qualitative and quantitative effects by the presence or absence of inhibition zones (**Table 3**). *K. pneumoniae* showed multidrug resistance to Ceftriaxone (CRO- 30 µg) and Chloramphenicol (C-30 µg). While *E. coli* showed multidrug resistance to Amoxicillin (AMC-10 μg), Gentamicin (CN 20 µg), and Chloramphenicol (C-30 µg). *Candida spp* showed resistance to Fluconazole (10 µg), Miconazole (10 μg), and Clotrimazole (10 μg). A significant difference in resistant and susceptibility pattern was found between isolated microbes. In all the cases p-value was < 0.05. Among *E.coli*, only two isolates showed resistant to Amoxicillin, Gentamicin and Chloramphenicol. Whereas, only three isolates of *K. pneumoniae* showed resistant to Ceftriaxone and Chloramphenicol. However, only five isolates of *Candida spp* showed resistance to Fluconazole, Miconazole, and Clotrimazole.

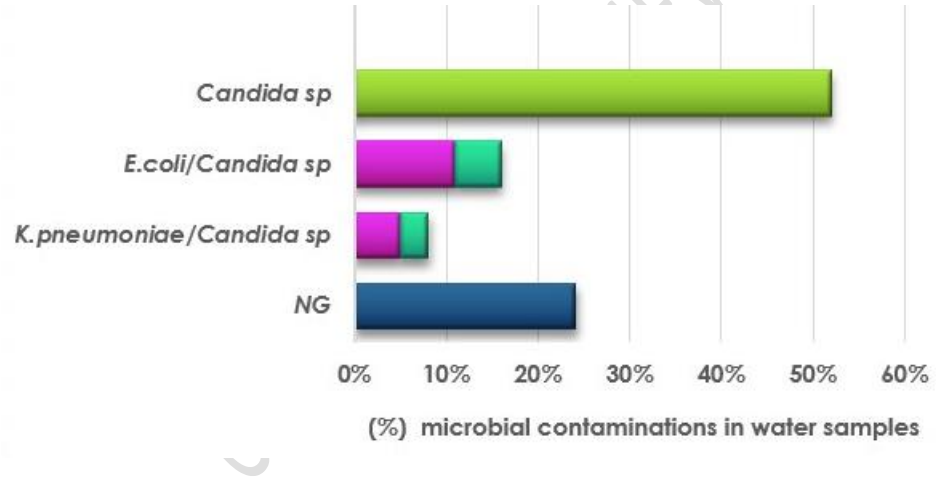

Fig. 3. Microbial contaminants isolated from tank water samples collected from different locations in Jeddah city. NG: 24% of water samples showed no growth of any of microorganisms. 8% of water samples showed mixed organisms (*K. pneumoniae* and *Candida sp*). 16% of water samples showed mixed contamination (*E. coli/Candida sp*.). 52% of water samples showed whitish colonies of *Candida sp.* on Sabouraud Dextrose Agar (SDA).

Table 3. Diameter of zone of inhibition generated against isolated water bacteria by antibiotic susceptibility testing.

No.	Antimicrobial Disk	Inhibition zone (mm)				
		Inhibition zone generated		Reference values		
		E. coli	*K. pneumoniae*	Resistant	Intermediate	Susceptible
1	Amoxicillin (AMC-10 μg)	13	25.0	≤16	17-19	≥20
2	Cefotaxim (CTX-25 µg)	29	27.8	≤23	24-29	≥30
3	Ceftriaxone (CRO- 30 µg)	28	19.9	≤23	24-27	≥28
4	Nalidixic acid (NA- 30 µg)	20	18.2	≤12	13-15	≥16
5	Gentamicin (CN 20 µg)	12	17.6	≤16	17-19	≥20
6	Chloramphenicol (C- 30 µg)	11	12.6	≤14	-	≥15
7	Fluconazole 10 μg	22	21.2	≤14	15-18	≥19

8	Miconazole	10 μg	19	17.0	≤ 9	10-14	≥15
9	Clotrimazole	10 μg	22	17.3	≤11	12-19	≥20

Molecular identification by PCR

Polymerase chain reaction was used to amplify the partial gene sequences of 16S rDNA amplified from both *K. pneumoniae* and *E. coli* as well as the ITS rDNA region from ninteen Candida spp. **Fig. 4** showed the size of the PCR products successfully amplified from *K. pneumoniae*, *E. coli* (~ 600 bp) and *Candida spp* (538 bp).

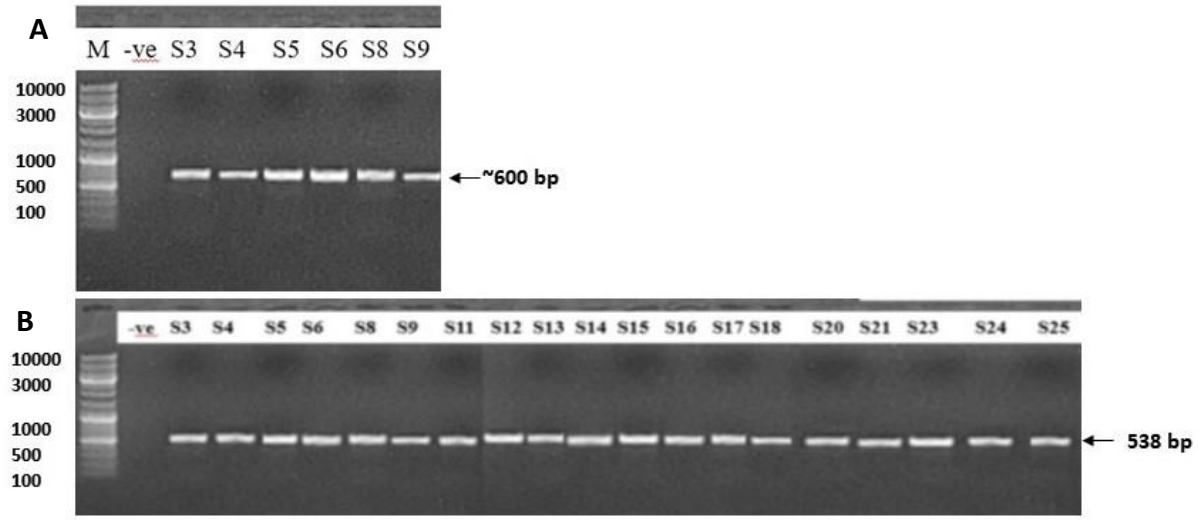

Fig. 4. 1% agarose gel electrophoresis showing the amplified PCR products of A) 16S rDNA from *K. pneumoniae* and *E. coli*. B) ITS region of rDNA from *Candida spp*. M: Thermo Scientific GeneRuler DNA Ladder Mix. The size of the PCR products is indicated by arrows.

Biosynthesis and characterization of silver nanoparticles (NSAgNPs)

The biosynthesized silver nanoparticles using protein extract of *A niger* (AgNPs) was immobilized on the solid support nanosilica was characterized using UV-vis spectroscopy, TEM, DLS and Zeta potential (**Fig. 5**). A gradual shifting in the colour of nano-silica from white (**Fig. 5A, left**) to light brown to dark brown (**Fig. 5A, right**) has developed as a consequence of the reductionof silver ions by the protein extracted from *A niger* filtrate. This suggested the formation of AgNPs on the surface of nano-silica, which was named as nano-silica silver nanoparticles (NSAgNPs). In this process, the negatively charged protein interacting with silver nanoparticles (AgNPs) as an oxidizing and reducing agent. NSAgNPs was then precipitated by centrifugation, dried at 50 °C and finally suspended in dH_2O. TEM photographs clearly demonstrate the presence of well scattered AgNPs around the surface of nano-silica particles (**Figs. 5 B & C**). Lattice space of the selected region scatter pattern (inset) of NSAgNPs of avergae size approximately 8.391 nm in diameter was shown in **Fig. 5D**. TEM multiple images demonstrated incredibly nanospheres of NSAgNPs (**Fig. 1E &F**) with average dimensions of 8.39 to 25 nm. The UV-vis spectra (**Fig. 5G**) of the suspended solution showed a strong peak at 436 nm due to the surface plasmon resonance curve (SPR) of the nanoparticles, zeta potentials range between -17.8 to - 4.88 mV (**Fig. 5H**).

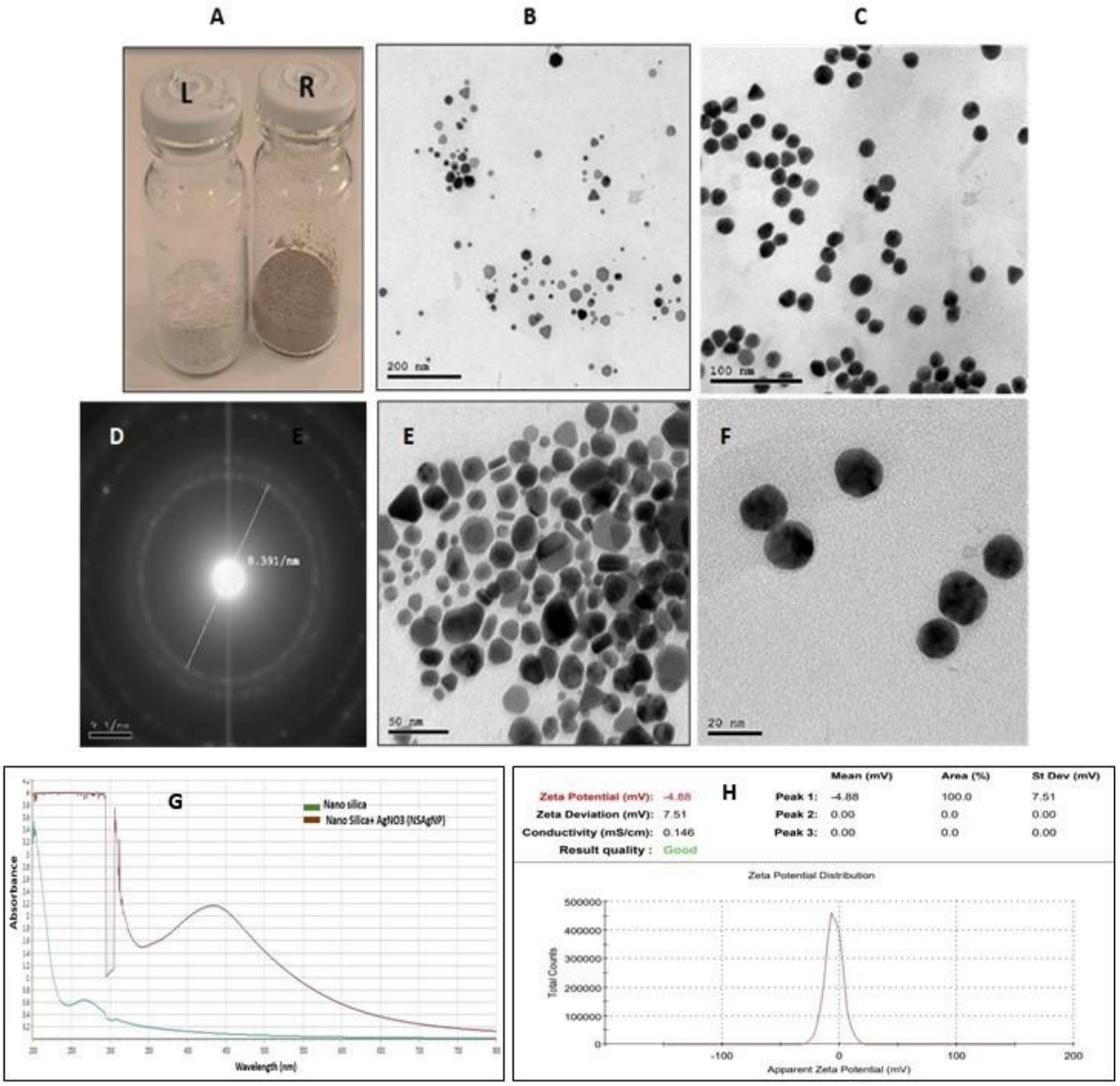

Fig. 5. Photos (A) of silica nanoparticle on the left (L), and NSAgNPs on the right (R); (B&C). TEM micrograph demonstrating formation of NSAgNPs of Avg Size (25 ± 5 nm); lattice space (D) and selected region scatter pattern (inset) of NSAgNPs of avergae size 8.391 nm diameter; (E&F) TEM image showing high resolution Spherical like-shape AgNPs; (G) UV-visible absorption spectrum of nano-silica prior and post reaction with $AgNO_3$ solution in presence of A. niger protein; (H) Zeta Potentials distribution spectrum of NSAgNP (-17.8 to - 4.88 mV mV).

Antimicrobial activity of NSAgNPs

The antimicrobial activity of (NSAgNPs) was analyzed by disk diffusion assay at various concentrations (10, 50, 100 µg/ml). The diameter of inhibition zones were recorded and tabulated in **Table 4.** The results in **Fig. 6** showed that, at concentration 100 µg/ml, NSAgNPs nanoparticles had strong antibacterial activity against *K. pneumoniae*, the maximum zone of inhibition was 28.17±0.68 mm, followed by *E. coli* (25.84 ± 0.32 mm) and *Cndida spp* (23.42 ± 0.46 mm). *P*-values were <0.05 which mean that there is a greater difference in the microbial response at concentrations (10, 50, 100 µg/ml) of NSAgNP.

Table 4. Inhibition zone of NSAgNPs against microbial pathogens using disc diffusion method

Microorganisms	Zone of inhibition (mm)			
	Concentration of NSAgNPs			*P -value*
	10 µg/ml	50 µg/ml	100 µg/ml	
Klebsiella pneumoniae	12.6 ± 0.42	19.05 ±0.49	28.17 ± 0.68	< 0.0001
E. coli	12.8 ± 0.44	17.55 ± 0.25	25.84 ± 0.32	< 0.0001
Candida albicans	13.1 ± 0.33	18.55 ± 0.25	23.42 ± 0.46	< 0.0001

p-values < 0.05 means statistically significant.

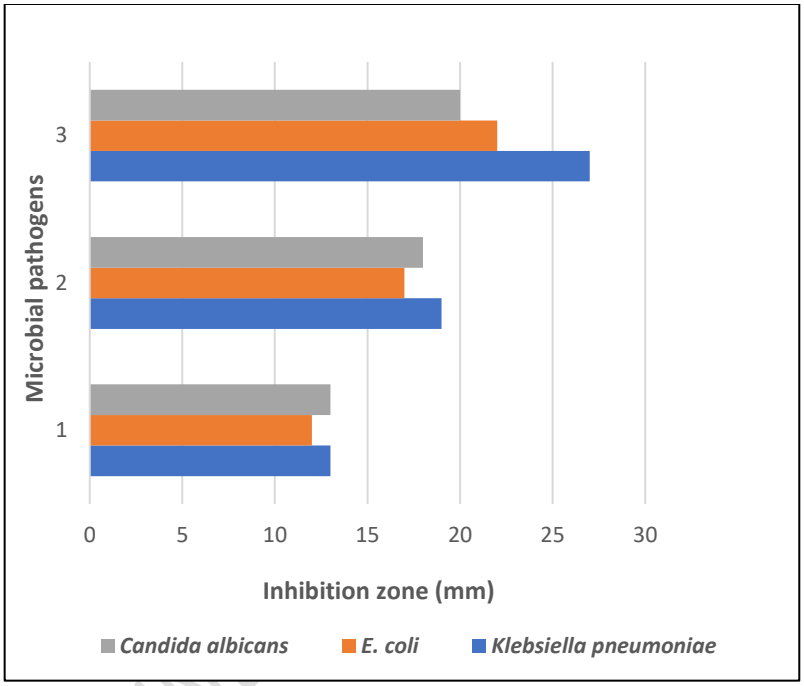

Fig. 6. Antimicrobial activity of NSAgNPs against isolated pathogens at concentrations (10, 50 and 100 µ/ml).

The results presented in **Table 5** revealed that the effect of biogenic silver nanoparticles (NSAgNPs) at concentration (100 µg/mL) was significantly reduced total microbes to 0.02 x 10^2 CFU/100 ml, 0.03 x10^2, and 0.02 x10^3 CFU/100 ml after 15 min. The greatest antibacterial activity against microbial contaminants with 100% elimination efficiency has been acheived by increasing the treatment time to 24 h (**Table 5**). It can be concluded that the treatment with 100 µg/mL NSAgNPs substantially decrease the average values of *K. pneumoniae*, *E. coli*, and *Candida spp* counts. The *P*- value was < 0.0001 which means that significantly tank water treated with NSAgNP has a great reduction of microbial pathogen after 15 min in comparison to control (water contaminated with 6x10^4 CFU/100ml).

Table 5. Effect of contact time on microbial elimination from water samples after treatment with 100 mg/L NSAgNP.

Concentration	Contact Time	*E. coli*	*K. pneumoniae*	*Candida spp*	*P*- value
	(CFU/100 ml)				
100 mg/ L	Control	6×10^4	6×10^4	6×10^4	
	15 min	0.02×10^2	0.03×10^2	0.02×10^3	< 0.0001
	1 h	0	0	0	
	2 h	0	0	0	
	24 h	0	0	0	

p- values < 0.05 means statistically significant

Cytotoxicity of NSAgNPs

The biogenic silver nanoparticles (NSAgNPs) were evaluated for cytotoxicity by SRB assay on MCF -7 breast adenocarcinoma cell lines. Cytotoxicity assay is one of the most commonly used methods to assess cell viability. The SRB assay depends on the ability of SRB to bind cellular components of proteins and determine total biomass. The cytotoxicity of biosynthesized NSAgNPs on MCF-7 at the concentrations (0.001, 0.01, 0.1,1.0, 10, 100, 1000 µg/ml) demonstrated an increase in the number of apoptotic cell lines with the increase in nanoparticle concentration (**Fig. 7**). The cell inhibition rate (IC50) was 35.8 µg/mL and the percent cell viability was maximum (98.1 to 995%) at very low concentrations of biosynthesized NSAgNPs as shown in **Fig. 7.** Effective cell inhibition was achieved at a maximum concentration of 1000 µg/mL of NSAgNPs. After 72 h of exposure, a dose-dependent effect on cell inhibition was observed. These results demonstrate that cytotoxicity increases with dose and time. An IC50 of 35.8 µg/mL suggests that the biogenic silver nanoparticles are effective as antimicrobial agents without harmfull effect on human health at the proposed concentrations.

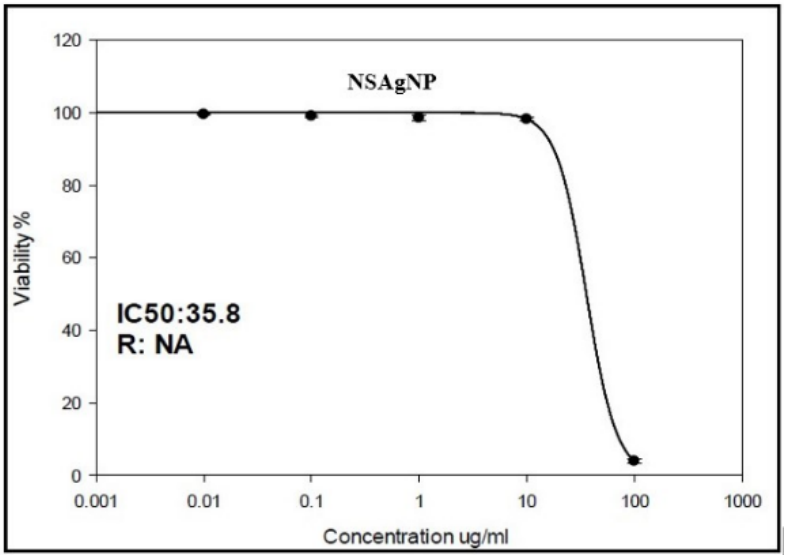

Fig. 7. MCF-7 Cell viability 72 h after treatment with the biogenic silver nanoparticles (NSAgNP)

Discussion

In this study, a preliminary survey in the north region of Jeddah city, at random intervals during the period August to December 2020 was conducted. Twenty-five tank water samples (n = 25) were collected to investigate the quality of drinking water regarding physical parameters and microbial contamination in addition to 5 samples of drinking water filtered with polypropylene filters (n=5) and 5 samples of pure water from commercial bottles as control (n=5) for comparison. All the water samples analysed showed average turbidity to be quite high with average between 18.5±0.03 to 26±0.29 NTU and high EC with average 279.49±50.93 µS/cm in comparison with the control filtered water samples. Turbidity is a crucial water quality metric, widely used to determine the quality of drinking water [38]. Turbidity can be used for the operational inspection of treatment systems and the efficiency of clarification substances used in domestic and small drinking water sources [39, 40, 41, 42, 43, 44]. Turbidity exceeding 1–2 NTU decreases the efficiency of chlorination by rising chlorine requirement and possibly shielding microorganisms from inactivation [45, 46]. Moreover, turbidity can reduce the efficiency of ultraviolet (UV) light disinfection by decreasing UV light transmission or by protecting microorganisms from inactivation [47, 48, 49, 50, 51, 52, 53].

The average of total microbial colonies/100 ml is 92.8±48.8 and 76% of tested water samples were found to be contaminated with mixture of coliforms (*E. coli, K. pneumoiae* and *Candida spp*). The presence of high percentages of coliform in potable water has the potential to cause microbial growth again and also the formation of biofilm. The existence of this coliform in potable water is not generally an indication of recent water pollution by faecal matter, the presence or absence of such bacteria is sometimes used to assess if the water disinfection process is working properly. These data corroborate the results of [54] who discovered that 28% of water samples come from tube wells tested positive for faecal coliforms and 10.5% tested positive for *E. coli*. However, 73.96% of the water samples from households were contaminated with faecal coliforms while 34.7% had *E. coli*.

Biochemical isolation and identification of microbial isolates contaminating water samples were performed using the filter membrane technique [55] and identified on Cystine lactose electrolyte deficient (CLED) agar, Blood agar (CLED/BA) and Sabouraud Dextrose agar. The results show that 6 (24%) out of 25 water samples showed no growth of any species on selected media. 16% (4/25) showed mixed growth of *Klebsiella pneumoniae* & *Candia spp.* 8.0% (2/25) showed mixed growth of *E. coli* & *Candia spp.* While, 52% (13/25) showed only the growth of *Candida spp*. All bacteriological techniques followed the basic fundamental protocols in clinical bacteriology as described by [56]. One of the most commonly used and effective techniques in the early detection and identification of pathogenic microbes, particularly *Candida albicans*, is polymerase chain reaction [57]. All in all, the PCR approach used in the current study was simple to conduct, fast to identify all the microorganisms, and highly accurate. PCR parameters for *Candida spp.* is similar to that of bacterial DNA amplification except the annealing temperature of the species-specific primers. The “presence or absence” of the DNA bands has simplified the process of agarose gel stained with ethidium under UV light. 16S rDNA gene fragments were successfully amplified from both *K. pneumoniae* and *E. coli* with PCR product size (~ 600 bp). 16S rDNA sequence is a widely used as bacterial gene marker in most scientific studies for a number of reasons. It can be used for reproducible and reliable bacterial identification, and also to define

the species and for useful distinction between the bacterial strains. The same results obtained by [58, 59]. The internal transcribed spacer (ITS) region of the rRNA gene was selected as a target sequence because it is the most reliable gene for identification and differentiation between *Candida species* [60]. Silver nanoparticles have been employed in the medical industry, especially for wound dressings, heart valves, medical face masks, bandages, and water filtration systems [61, 62, 63]. The biosynthesis of new nanostructured materials with high adsorption has been attracting significant interest lately [64, 65, 66]. In this study, we fabricated a biogenic NSAgNPs on a nanosilica support and simultaneously used to coat a 20 cm polyurethane foam 1 mm thick for full coverage and then air-dried 1.0 mM Silver nitrate solution was first mixed with nano-silica powder, in which Ag+ ions rapidly adsorbed on the electronegative surface of nano-silica [67]. These negative charges on the surface of nano-silica are due to the presence of vast amounts of hydroxyl groups [68].

UV spectroscopy is widely used to identify nanoparticles. Researchers have also used the UV spectroscopy technique to study the structure of nanoparticles after reduction, as well as to test their stability.

The presence of a strong absorption peak at 436 nm of NsAgNPs due to plasmon resonance (SPR) characteristic explores their shape and size [69]. According to the literature, a peak around 400 nm is characteristic of a spherical shape [70]. These outcomes were identical to those obtained in previous study [71, 72].

In order to better describe the size and shape of the particles, TEM analysis is recommended for nanoparticles. An image is generated as the electrons are passed through the specimen [73]. The results of the TEM images confirmed the small nanoparticles of average size 8.39 nm with a spherical shape with excellent uniformity and well-defined structure

The polydispersity index enabled classify homogeneous nanoparticle size. According to the manufacturer (Malvern Instruments), the typical polydispersity value is known to be between 0.08 and 0.7; the greatest polydispersity value is above 0.7. As a result, we conclude that the nanoparticles were uniform in size. When tested in the Zetasizer™ evaluation package, the nanoparticles showed Z-potency in the range of (-17.8 to - 4.88 mV mV), suggesting greater system stability [74].

Nano synthetic silver nanoparticles (NSAgNPs) were found to be relatively inexpensive, nontoxic, and environmentally friendly, and showed complete elimination of microbial organisms in contaminated water samples at a concentration of 100 mg/L with contact times ranging from 1 h to 24 h. The results showed a complete reduction in the number of bacteria to nil. The nano-silver coated Polyurethane foam also removed all the microbial species found in the tested water samples. Similar results were obtained by [75, 76, 71, 72, 77, 78].

Regarding the antimicrobial activity, NSAgNPs were evaluated against two Gram-negative bacteria (*K. pneumoniae* and *E. coli*) and one fungal isolate (*Candida spp.*) by disk diffusion assay at various concentrations 10, 50, 100 µg/ml. The inhibition zones were measured in millimeters in diameter and the minimum concentration of the NSAgNPs required to inhibit visible growth of the bacteria was determined after 24 hr at concentration 100 µg/ml. Therefore, NSAgNPs exhibit an efficient antibacterial activity against the tested pathogens in a shorter time at low concentration. Their small size may contribute to the efficient binding and uptake of bacteria resulting in their death [79, 80].

Cell Viability test is key toxicological measures to understand the cellular response to a toxicant. They offer information on cell death, survival, and other metabolic processes. We exploited the highly sensitive Sulforhodamine B colorimetric (SRB) to study the activity of NSAgNPs on MCF-7 breast adenocarcinoma cell lines. This assay depends on SRB's capacity to bind cellular components and quantify overall biomass. The results showed that at higher concentrations of biosynthesized NSAgNP, the apoptosis rate of MCF -7 cell lines was greatly increased. The IC50 of cell inhibition of silver nanoparticles was observed at 35.8 µg/mL. The viability of breast cancer cells was maximum (98.1 to 99.5 %) at concentrations (0.001, 0.01, 0.1, 1.0, and 10 µg/ml) of biosynthesized NSAgNP. Complete cell inhibition of MCF -7 was achieved at a maximum concentration of 100 µg/mL of NSAgNP. These data demonstrate dose- and time-dependent cytoxicity. Similar results were obtained by AshaRani et al. [81] whose results of nanoparticles showed no significant cytotoxicity in cancer cells (U251) and fibroblasts (IMR-90). Numerous studies have shown that nanoparticles possess toxic potential in relation to different tumors, Chen et al. [82] showed that hydroxyapatite nanoparticles inhibited the proliferation of K-562 cell growth. Shafagh et al. [83] determined the cytotoxicity of copper oxide nanoparticles with respect to K-562 cells and showed great potential reduction in cell viability (57%). Biogenic silver nanoparticles synthesized from the leaf extracts of Piper longum plant showed superior cytotoxic activity on HEp-2 cell lines [84]. Biosynthesized LdAgNPs using different aqueous extracts displayed anticancer effect against HepG2 and PC3 cancer cell lines [85].

One of the potential applications of antimicrobial biogenic silver nanoparticles produced in the current study is to integrate it with existing water disinfection technology. The use of membranes in purification of drinking water has been rising rapidly [86, 87]. Unfortunately, fouling the membrane material making it not efficient for water purification. Furthermore, the abundance and diversity of microbial contaminants in water usually requires multiple treatment steps. Incorporation of polyurethane foam coated biogenic nanoparticles (current study) enables its reactivity rather than a basic physical barrier and achieves multiple goals in one integrated system. The persistence of nanomaterials in drinking water is of concern not only because of the economic cost of water recovery but also because of the potential health consequences of removing them from drinking water [88, 89, 90, 91, 92]. The biosynthesized foam coated nano silica silver nanoparticles (NSAgNPs) developed in the current study, showed complete reduction of microorganisms in contaminated tank water samples at optimal concentration (100 mg/L) and it does not have any detrimental effect on the treated water which encourages the incorporation of these nanoparticles into drinking water purification systems. An analysis conducted at two German wastewater treatment plants showed that up to 96.4 percent of AgNPs were removed, with residual effluent concentrations varying from 0.7 to 11.1 ng/L. Despite the high removal efficiency, the authors calculated a cumulative release to society of 33 kg AgNPs per year for the whole world based on these data [93]. AgNPs have received considerable attention due to the potential environmental problems associated with their processing, use, disposal and application.

Conclusion

In the current study, the biosynthesized silver nanoparticles (NSAgNP) demonstrate strong antibacterial activity and are safe for use in drinking water treatment systems. Each

purification technology eliminates only one form of contaminant, so none can be counted upon to eliminate all forms of contamination to the maximum requirements. A well-integrated water purification system utilizes a combination of biosynthesized foam coated nanoparticles with purification technologies can achieve the desired final water quality. This combination of purification methods together with sufficient pre-treatment would have the highest quality water that is almost free of ionic, organic, and microbial contamination. We conclude that contamination, is mainly caused by low awareness and lack of personal and domestic hygiene practices, which should be evaluated. Then the necessary measures should be taken to improve the mentality of good health standards and the polluted domestic water should be controlled with the currently proposed nanotechnology system to ensure clean, healthy water.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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REFERENCES

[1] Li XF, Mitch WA. Drinking Water Disinfection By-products (DBPs) and Human Health Effects: Multidisciplinary Challenges and Opportunities. Environmental Science and Technology, 2017;52(4).

[2] WHO, (2020). https://www.who.int/news-room/fact-sheets/detail/drinking-water.

[3] Yates MV. Drinking Water Microbiology Encyclopedia of Microbiology, 2019;83–89.

[4] Water Sanitation and Health: Domestic water quantity, service level and health. WHO, 2020;1-76, Second edition, available: https://www.who.int/teams/environment-climate-change-and-health/water-sanitation-and-health/water-safety-and-quality/drinking-water-quality-guidelines.

[5] Al-Gabr HM, Zheng T, Yu X. Fungi contamination of drinking water. Rev Environ Contam Toxicol. 2014; 228:121-39.

[6] Ali EAM, Abdel-Rahman TMA, Sayed AE, Mohsen T, and Abd Al Khalek SH. Occurrence of Fungi in Drinking Water Sources and Their Treatment by Chlorination and UV-Irradiation. Egypt. J. Bot., 2017;57(3): 621-632.

[7] Hageskal G, Lima N, Skaar I. The study of fungi in drinking water. Mycological research. 2009 ;1;113(2):165-72.

[8] Bartemes KR, Kita H. Innate and adaptive immune responses to fungi in the airway. The Journal of allergy and clinical immunology, 2018;142(2):353–363.

[9] Koutsoumanis K, Allende A, Ordonez AA, Bover-Cid S, Chemaly M, Davies R, et al. Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. EFSA Journal.,2020;18(1):5967.

[10] Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, et al. Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environmental health perspectives, 2013;121(9):993-1001.

[11] Khalifa M, Bidaisee S. The importance of clean water. Sch J Appl Sci Res., 2018;1(7):17-20.

[12] Deshmukha SP, Patila SM, Mullania SB and Delekar SD. Silver nanoparticles as an effective disinfectant: A review. Materials Science & Engineering C, 2019;97:954–965.

[13] Agnihotri S, Mukherjiab S, Mukherji S. Immobilized silver nanoparticles enhance contact killing and show highest efficacy: elucidation of the mechanism of bactericidal action of silver. Nanoscale, 2013;5:7328

[14] Yusuf M. Silver Nanoparticles: Synthesis and Applications Handbook of Ecomaterials, 2018;2343–2356.

[15] Borrego B, Lorenzo G, Mota-Morales JD, Almanza-Reyes H, Mateos F, López-Gil E, et al. Potential application of silver nanoparticles to control the infectivity of Rift Valley fever virus in vitro and *in vivo*. Nanomedicine: Nanotechnology, Biology and Medicine. 2016;12(5):1185-92.

[16] Lysenko V, Lozovski V, Lokshyn M, Gomeniuk V, Dorovskih A, Rusinchuk N. Nanoparticles as antiviral agents against adenoviruses. Adv Nat Sci Nanosci Nanotechnol., 2018; 9(2):025021.

[17] Kalhapure RS, Sonawane SJ, Sikwal DR, Jadhav M, Rambharose S, Mocktar C, et al.. Solid lipid nanoparticles of clotrimazole silver complex: an efficient nano antibacterial against Staphylococcus aureus and MRSA. Colloids and Surfaces B: Biointerfaces. 2015;1; 136:651-8.

[18] Mohamed A, Fouda A, Elgamal SM, EL-Din Hassan S, Shaheen IT, Salem SS. Enhancing of cotton fabric antibacterial properties by silver nanoparticles synthesized by new Egyptian strain *Fusarium keratoplasticum* A1-3. Egyptian journal of chemistry, 60 (Conference Issue (The 8th International Conference of The Textile Research Division (ICTRD 2017), National Research Centre, Cairo 12622, Egypt. 63-71.

[19] Krishnaraj C, Ramachandran R, Mohan K, Kalaichelvan PT. Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012; 93:95-9.

[20] Ahmad T, Wani IA, Manzoor N, Ahmed J, Asiri AM. Biosynthesis, structural characterization and antimicrobial activity of gold and silver nanoparticles. Colloids and Surfaces B: Biointerfaces. 2013;107:227-34.

[21] Talapko J, Matijevic T, Juzbašic M, Antolovic-Požgain A and Škrlec I. Antibacterial Activity of Silver and Its Application in Dentistry, Cardiology and Dermatology. *Microorganisms,* 2020;8:1400.

[22] Joshi AS, Singh P, Mijakovic I. Interactions of Gold and Silver Nanoparticles with Bacterial Biofilms: Molecular Interactions behind Inhibition and Resistance. International Journal of Molecular Sciences, 2020; 21:7658.

[23] Lara HH, Garza-Treviño EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. J. Nanobiotechnol., 2011;9:30.

[24] Slavin YN, Asnis J, Häfeli UO, and Bach H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity Journal of nanobiotechnology, 2017;15(1):65.

[25] Dhanalekshmi KI, Meena KS. DNA intercalation studies and antimicrobial activity of Ag@ ZrO_2 core–shell nanoparticles *in vitro*. Materials Science and Engineering, C. 2016 ;1;59:1063-8.

[26] Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International nano letters 2. 2012; 32.

[27] Abdal Dayem A, Hossain MH, Lee SB, Kim K, Saha SK, Yang GM, Choi HY, Cho SG. The Role of Reactive Oxygen Species (ROS) in the Biological Activities of Metallic Nanoparticles, Int. J. Mol. Sci. 2016;18:120.

[28] Khan AA, Manzoor KN, Sultan A, Saeed M, Rafique M, Noushad S, Talib A, Rentschler S, Deigner HP. Pulling the Brakes on Fast and Furious Multiple Drug-Resistant (MDR) Bacteria. Int. J. Mol. Sci., 2021;22, 859

[29] Liao C, Li Y, Tjong SC. Bactericidal and Cytotoxic Properties of Silver Nanoparticles. Int. J. Mol. Sci., 2019;20(2): 449.

[30] Gopinath V, Priyadarshini S, Loke MF, Arunkumar J, Marsili E, MubarakAli D, et al. Biogenic synthesis, characterization of antibacterial silver nanoparticles and its cell cytotoxicity. Arabian Journal of Chemistry, 2015;10, (8): 1107-1117.

[31] Singh P, Garg A, Pandit S, Mokkapati V, Mijakovic, I. Antimicrobial Effects of Biogenic Nanoparticles. Nanomaterials (Basel, Switzerland), 2018;8(12):1009.

[32] Khandel, P, Yadaw, RK, Soni, DK, Kanwar L, Shahi SK. Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. J. Nanostruct. Chem., 2018;8:217–254.

[33] Andrew DE, Lenore SC. Standard Methods for Examination of Water and Wastewater, 21st Edition, Baltimore, Maryland: APHA, AWWA, WEF (2005)

[34] Kirby W, Bauer W, Sherris J, Turck M. Antibiotic susceptibility testing by a standardized single disk method. The American Journal of Clinical Pathology, 1966;45(4):493– 496.

[35] National Committee for Clinical Laboratory Standards, "Performance standards for antimicrobial disk and dilution susceptibility tests for bacterial isolates from animals,"

National Committee for Clinical Laboratory Standards, Wayne, Pa, USA, Approved standard, M31-A19, (11):1999.

[36] Al-Zubaidi S, Al-Ayafi A, Abdelkader HS. Biosynthesis, Characterization and Antifungal Activity of Silver Nanoparticles by Aspergillus Niger Isolate. Journal of Nanotechnology Research, 2019;2: 022-035.

[37] Gurunathan S, Han JW, Kim ES, Park JH, Kim JH. Reduction of graphene oxide by resveratrol:A novel and simple biological method for the synthesis of an effective anticancer nanotherapeutic molecule. Int. J. Nanomed. 2015; 10, 2951–2969.

[38] WHO (2017). Guidelines for drinking-water quality, fourth edition incorporating the first addendum. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/water-quality/guidelines/en/).

[39] Elliott MA, Stauber CE, Koksal F, DiGiano FA, Sobsey MD. Reductions of *E. coli*, echovirus type 12 and bacteriophages in an intermittently operated household scale slow sand filter. Water Res., 2008;42(10–11):2662–70.

[40] Preston K, Lantagne D, Kotlarz N, Jellison K. Turbidity and chlorine demand reduction using alum and moringa flocculation before household chlorination in developing countries. J. Water Health. 2010;8(1):60–70.

[41] Kotlarz N, Lantagne D, Preston K, Jellison K. Turbidity and chlorine demand reduction using locally available physical water clarification mechanisms before household chlorination in developing countries. J. Water Health., 2009;7(3):497–506.

[42] WHO, (2011) Evaluating household water treatment options Geneva: World Health Organization
(http://wwwwhoint/water_sanitation_health/publications/2011/household_water/en/, accessed 27 September 2016).

[43] Mwabi JK, Mamba BB, Momba MNB. Removal of Escherichia coli and faecal coliforms from surface water and groundwater by household water treatment devices/systems: a sustainable solution for improving water quality in rural communities of the Southern African development community region. *Int. J. Environ. Res. Publ. Health*, 2012;9(1):139–70.

[44] WHO/UNICEF (2012), A toolkit for monitoring and evaluating household water treatment and safe storage systems Geneva: World Health Organization, United Nations Children's Fund (UNICEF) (http://wwwwhoint/water_sanitation_health/publications/toolkit_monitoring_evaluating/en/, accessed 27 September 2016).

[45] Le Chevallier MW, Evans TM, Seidler RJ. Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. Appl. Environ. Microb., 1981;42(1):159–67.

[46] Śmiech KM, Kovács T, Wildschut RF, et al. Thermal disinfection of hospital wastewater in a pilot-scale continuous-flow system. Appl. Water Sci., 2020;10: 101.

[47] Christensen J, Linden KG. How particles affect UV light in the UV disinfection of unfiltered drinking water. J. Am. Water Works Ass., 2003;95(4):179–89.

[48] Batch LF, Schulz CR, Linden KG. Evaluating water quality effects on UV disinfection of MS2coliphage. J. Am. Water Works Ass., 2004;96(7):75–87.

[49] Passantino L, Malley J, Knudson M, Ward R, Kim J. Effect of low turbidity and algae on UV disinfection performance. J. Am. Water Works Ass., 2004;96(6):128–37.

[50] Amoah K, Craik S, Smith DW, Belosevic M. Inactivation of Cryptosporidium oocysts and Giardia cysts by ultraviolet light in the presence of natural particulate matter. J Water Supply Res, 2005;54(3):165–78.

[51] Templeton MR, Andrews RC, Hofmann R. Removal of particle-associated bacteriophages by dual-media filtration at different filter cycle stages and impacts on subsequent UV disinfection. Water Res., 2007;41(11):2393–406.

[52] Cantwell RE, Hofmann R, Templeton MR. Interactions between humic matter and bacteria when disinfecting water with UV light. J. Appl. Microbiol., 2008;105(1):25–35.

[53] Kollu K, Ormeci B. Effect of particles and bioflocculation on ultraviolet disinfection of Escherichia coli. Water Res., 2012;46(3):750–60.

[54] Mahmud ZH, Islam MS, Imran KM, Hakim SA, Worth M, Ahmed A, Hossan S, Haider M, Islam MR, Hossain F, Johnston D. Occurrence of Escherichia coli and faecal coliforms in drinking water at source and household point-of-use in Rohingya camps, Bangladesh. Gut pathogens. 2019 Dec;11(1):1-1.

[55] Mulamattathil SG, Bezuidenhout C, Mbewe M, and Ateba CN. Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng, South Africa, and Characterization Using Their Antibiotic Resistance Profiles. Journal of Pathogens, 2014; ID 371208, 11.

[56] WHO Library Cataloguing-in-Publication Data Basic laboratory procedures in clinical bacteriology / J. Vandepitte [et al.]. 2nd ed. 1. Bacteriological techniques—standards 2. Laboratory techniques and procedures standards 3. Manuals I.Vandepitte, J. ISBN 92 4 154545 3 (NLM classification: QY 100)

[57] White PL, Perry MD, Barnes RA. An update on the molecular diagnosis of invasive fungal disease. FEMS microbiology letters. 2009 Jul 1;296(1):1-0.

[58] Amoon RH, Abdallha AH, Sharif AO, Moglad EH, Altyb HN, Elzaki SG, Salih MA. Molecular characterization of *Pseudomonas aeruginosa* isolates from Sudanese patients: A cross-sectional study. F1000Research. 2018;25;7(1135):1135.

[59] Johnson JS, Spakowicz DJ, Hong BY. et al. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat. Commun.*, 2019;10:5029.

[60] Desnos-Ollivier M, Ragon M, Robert V, Raoux D, Gantier JC, Dromer F. Debaryomyces hansenii (Candida famata), a rare human fungal pathogen often misidentified as *Pichia guilliermondii* (*Candida guilliermondii*). Journal of clinical microbiology. 2008 Oct 1;46(10):3237-42.

[61] Theivasanthi T, Alagar M. Electrolytic synthesis and characterizations of silver nanopowder. arXiv preprint arXiv:1111.0260. 2011 Nov 1.

[62] Mousavi SM, Hashemi SA, Ghasemi Y, Atapour A, Amani AM, Savar Dashtaki A, et al. Green synthesis of silver nanoparticles toward bio and medical applications: review study. Artificial cells, nanomedicine, and biotechnology. 2018;12;46(sup3): S855-72.

[63] Bandyopadhyay A, Chowdhury SK, Dey S, et al. Silk: A Promising Biomaterial Opening New Vistas Towards Affordable Healthcare Solutions. J. Indian Inst. Sci., 2019;99, 445-487.

[64] Gunawan P, Guan C, Song X, Zhang Q, Leong S S J, Tang C, et al. Hollow fiber membrane decorated with Ag/MWNTs: toward effective water disinfection and biofouling control. ACS Nano., 2011; 5:10033–10040.

[65] Natalio F, Andre R, Hartog AF, Stoll B, Jochum KP, Wever R, Tremel W. Vanadium pentoxide nanoparticles mimic vanadium haloperoxidases and thwart biofilm formation. Nat. Nanotech., 2012;7:530–535.

[66] Suvorov O, Balandin G, Shaburova L, Podkopaev D, Volozhaninova S, Ermolaeva G. Impact of Silver Nanoparticle Suspensions on Mixtures of Fungal and Bacterial Microorganisms of Food Production. 13th International Conference on Nanosciences & Nanotechnologies (NN16), July (5-8) 2016, Thessaloniki, Greece"

[67] Das SK, Khan MM, Parandhaman T, Laffir F, Guha AK, Sekaran G, et al. Nano-silica fabricated with silver nanoparticles: antifouling adsorbent for efficient dye removal, effective water disinfection and biofouling control. Nanoscale. 2013;21;5(12):5549-60.

[68] Liou TH, Yang CC. Synthesis and surface characteristics of nanosilica produced from alkali-extracted rice husk ash. Materials Science and Engineering, 2011;176 (7):521-529.

[69] Elemike EE, Onwudiwe DC, Ekennia AC, Sonde CU, Ehiri RC. Green Synthesis of Ag/Ag_2O Nanoparticles Using Aqueous Leaf Extract of Eupatorium odoratum and Its Antimicrobial and Mosquito Larvicidal Activities. Molecules 2017; 22, 674.

[70] Neto EAB, Ribeiro C, Zucolotto V. Síntese de Nanopartículas de Prata para Aplicação na Sanitização de Embalagens. Comunicado Técnico, 2008;99:1-4.

[71] Abdelkader H, Alzahrani H, Al-Ayafi A, Almalah H, and Al-Zubaidi S. Green synthesis, Characterization and Antimicrobial activity of Biosynthesized Silver Nanoparticles using *Ziziphus spina - christi* leaf extracts. Adv Microb Res, 2019;3: 010.

[72] Almalah HI, Alzahrani HA and Abdelkader HS. Green Synthesis of Silver Nanoparticles using *Cinnamomum Zylinicum* and their Synergistic Effect against Multi-Drug Resistance Bacteria. Journal of Nanotechnology Research, 2019;1: 095-107.

[73] Alaqad K, Saleh TA. Gold and Silver Nanoparticles: Synthesis Methods, Characterization Routes and Applications towards Drugs. Journal of Environmental & Analytical Toxicology, 2016;6(4)

[74] Bhatnagar S, Kobori T, Ganesh D, Ogawa K, Aoyag H. Biosynthesis of Silver Nanoparticles Mediated by Extracellular Pigment from Talaromyces purpurogenus and Their Biomedical Applications. Nanomaterials, 2019; 9:1042.

[75] Zaki NH, Husain Z. Enhanced antibacterial and anti-biofilm activities of biosynthesized silver nanoparticles against pathogenic bacteria. Journal of Genetic and Environmental Resources Conservation. 2016;4(2):197-203.

[76] Dehghanizade S, Arasteh J, Mirzaie A. Green synthesis of silver nanoparticles using Anthemis atropatana extract: characterization and in vitro biological activities. Artificial cells, nanomedicine, and biotechnology. 2018;2;46(1):160-8.

[77] Habibipour R, Moradi-Haghgou L, Farmany A. Green synthesis of AgNPs@ PPE and its *Pseudomonas aeruginosa* biofilm formation activity compared to pomegranate peel extract. International journal of nanomedicine. 2019;14:6891.

[78] Mohamed DS, Abd El-Baky RM, Sandle T, Mandour S A and Ahmed EF. Antimicrobial Activity of Silver-Treated Bacteria against Other Multi-Drug Resistant Pathogens in Their Environment. Antibiotics, 2020;9,181

[79] Mmola M, Le Roes-Hill M, Durrell K, et al. Enhanced antimicrobial and anticancer activity of silver and gold nanoparticles synthesized using Sargassum in cisifolium aqueous extracts. Mol.2016;21:1633.

[80] Majoumouo MS, Sibuyi NRS, Tincho MB, Mbekou M, Boyom FF, Meyer M. Enhanced Anti-Bacterial Activity of Biogenic Silver Nanoparticles Synthesized From Terminalia mantaly Extracts. Int. J. Nanomedicine, 2019;14:9031-9046.

[81] AshaRani PV, Mun GLK, Hande MP, and Valiyaveettil S. Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. ACS Nano, 2009;3(2): 279-290.

[82] Chen P, Dai H, Han Y, Yin M, and Li S. Effect of hydroxyapatite nanoparticles on K562 cells in vitro Journal of Wuhan University of Technology-Mater. Sci. Ed. 2008;23(2):222-4.

[83] Shafagh M, Rahmani F, Delirezh N. CuO nanoparticles induce cytotoxicity and apoptosis in human K562 cancer cell line via mitochondrial pathway, through reactive oxygen species and P53. Iranian journal of basic medical sciences. 2015;18(10):993.

[84] Jacob SJP, and Narayanan JSFA. Synthesis of silver nanoparticles using Piper longum leaf extracts and its cytotoxic activity against Hep-2 cell line Colloids and Surfaces B: Biointerfaces, 2012;91(1):212-214.

[85] Prasannaraj G, and Venkatachalam P. Green engineering of biomolecule-coated metallic silver nanoparticles and their potential cytotoxic activity against cancer cell lines Advances in Natural Sciences: Nanoscience and Nanotechnology, 2017;8.

[86] Marcucci M, Ciabatti I, Matteucci A, Vernaglione G. Membrane technologies applied to textile wastewater treatment. Annals of the New York Academy of Sciences. 2003;984(1):53-64.

[87] Bodzek M, Konieczny K, Kwiecińska A. Application of membrane processes in drinking water treatment–state of art. Desalination and Water Treatment, 2011;35(1-3):164-184.

[88] Wiesner, M.R., Lowry, G.V., Alvarez, P., Dionysiou, D., and Biswas, P. Assessing the risks of manufactured nanomaterials, Environ Sci Technol. 2006; 40(14), 4336–4345.

[89] Powell MC, Kanarek MS. Nanomaterial health effects-part 2: uncertainties and recommendations for the future. Wisconsin Medical Journal. 2006;105(3):18-23.

[90] Moore MN. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environment international. 2006;32(8):967-76.

[91] Benn TM, Westerhoff P. Nanoparticle silver released into water from commercially available sock fabrics. Environmental science & technology, 2008;42(11):4133-9.

[92] Saleem H, Zaidi SJ. Developments in the Application of Nanomaterials for Water Treatment and Their Impact on the Environment. Nanomaterials, 2020;10(9):1764.

[93] Li L, Stoiber M, Wimmer A, Xu Z, Lindenblatt C, Helmreich B, Schuster M. To What Extent CanFull-Scale Wastewater Treatment Plant Effluent Influence the Occurrence of Silver-Based Nanoparticles in Surface Waters? Environ Sci Technol. 2016,50, 6327–6333