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RESEARCH ARTICLE

BIOCONVERSION OF SILVER AND NICKEL IONS INTO NANOPARTICLES BY *ACHROMOBACTER SP*. STRAIN MMT AND THEIR APPLICATION IN WASTEWATER DISINFECTION

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ABSTRACT

Biological synthesis of metal nanoparticles was one of the most brilliant green methods for nanoparticles (NPs) synthesis. For this reason, the nitrate reductase-producing bacterium *Achromobacter* sp. MMT was employed to produce both silver (AgNPs) and nickel (NiNPs) nanoparticles (NPs). Subsequently, produced NPs were characterized by UV-vis, EDX, XRD, ξ potential and TEM. The surface plasmon resonance of AgNPs and NiNPs was exhibited at 420 and 395 nm, respectively. The EDX revealed strong signal with atomic percentages 71.4 % and 5.8% of silver and nickel, respectively. XRD indicated an ultra-fine nature and small crystallite size of studied NPs. Zeta potential (ξ) recorded -52.2 mV and -31.8 mV for AgNPs and NiNPs, respectively. In the same order, TEM analysis revealed that AgNPs and NiNPs have a teeny, uniform, spherical NPs ranging from 0.72 to 1.4 nm and from 31.0 to 31.7 nm. Both studied NPs exhibited antimicrobial activity against aerobic, anaerobic, Gram negative, Gram positive, mold, yeast, biofilm and algae. NPs were applied in various wastewater samples proved their efficiency in disinfection process.

INTRODUCTION

Bio-nanotechnology has emerged as integration among biotechnology and nanotechnology for developing biological synthesis and environmental-benign technology. Where, it combines biological principles with physical and chemical approaches to produce nano-sized particles with specific functions. It also represents an economic substitute for chemical and physical methods of nanoparticles formation Metallic nanoparticles have studied extensively because of their exceptional physicochemical characteristics including catalytic, optical properties, electron properties etc. Silver, aluminum, gold, zinc, carbon, titanium, palladium, iron, nickel, fullerenes, copper etc. have been rottenly used for the synthesis of nanoparticles (Fatemeh *et al*., 2010; Zaki *et al*., 2011).

Green synthesis provides alternatives for chemical and physical methods as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and harsh toxic chemicals and does not cause any harm to human and domestic animals health (Tiwari *et al*., *2008;* Salvadori, *et al*., 2015; Pattanayak and Nayak, 2013)

Wide application had been achieved by incorporation of nanoparticles. Depending on physicochemical, optical, electrical, mechanical, optoelectronics properties of NPs, make them an interesting candidate for industrial, medical and environmental fields (Prathna *et al*., 2010; Sahayaraj and Rajesh, 2011; Rai and Bai, 2011; Pattanayak and Nayak, 2013; Salvadori, *et al*., 2015).

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Traditionally, chlorination is the most used approach to disinfect both water and wastewater. However, chlorine is suffering from the disadvantage of chlorine residual even at small concentration was toxic to aquatic life (Victoria , 2002; Tree *et al.*, 2003).This led to test and examines other alternative. Recently, NPs exhibits good biocide effect against wide range of microorganisms, under different aeration conditions and with different sources of effluents with different chemical characters (Zaki *et al.*, 2011; Zaki *et al.*, 2014).

In this regard, recently in our lab *Achromobacter* sp. strain MMT was isolated as an efficient nitrate reductase producer (Eltrahony *et al.*, 2015). As known that nitrate reductase-producing organisms are nanoparticles producers, in the present study, synthesis of nanosilver and nanonickel particles using strain MMT was investigated. Produced nanoparticles were characterized using TEM, XRD, EDX and other techniques. NPs were examined as antagonistic agent against bacteria, yeast, fungi and algae.

MATERIALS AND METHODS

Chemicals, strain, cultural conditions and biosynthesis of NPs

Silver (3 mM $AgNO_3$) and Nickel (1.5 mM of Ni $(NO_3)_2$ nitrates required for synthesis of NPs were obtained from Sigma-Aldrich. Other required chemicals were purchased from Merck. *Achromobacter sp*. MMT used in this study was previously identified as a nitrate reductase producer by Eltrahony *et al.* (2015). Culturing, media, and production of NPs were performed as described elsewhere (Eltrahony *et al.* 2015). The initial pH of all media was adjusted to 7.2 to 7.5 with NaOH (1M) and HCl (0.5M). All media were prepared with distilled water and sterilized at 121◦C for 20 min. After incubation period for 120 h, the MMT cells containing NPs were collected by centrifugation at 10000 rpm for 20 min and were disrupted by ice cold TSE buffer (pH 8); incubated for 30 min at 30^{O}C with vortex every 10 min as described by Va´zquez-Laslop *et al*. (2001).

Characterization of produced NPs

As described previously by Zaki *et al.* (2011), the reduction of $AgNO_3$ and Ni $(NO_3)_2$ ions to NPs could be optically examined by color changes of the bacterial cells and slightly surrounding media. Produced NPs were characterized by UV-Vis spectrophotometer (Labomed. model UV–Vis Double beam spectrophotometer) in the wavelengths ranging from 200-800 nm. The phase purity was determined by X-ray Diffractometer (XRD Schimadzu-7000, USA). The chemical composition of the NPs was examined using EDAX (JEOL JSM 6360LA, Japan -Faculty of Science- Alexandria University). The size, shape, and morphologies of the formed NPs and its producing cells were determined by applying TEM (JEOL JEM-1230, Japan- Faculty of Science- Alexandria University).

The electrostatic potential, hydrodynamic diameter and particle size distribution of NPs were performed through dynamic light scattering (DLS) technique using Zetasizer nano (ZS, Malvern Instruments, Worcestershire, UK; Faculty of pharmacy-Alexandria University). The NPs were equilibrated at 25°C for 120 sec in a zeta cell then placed in the analyzer chamber equipped with a HeNe laser operating at 632.8 nm and a scattering detector at 173 degree. The data were analyzed by Zetasizer software 6. The mean ± standard deviation (SD) of at least two independent measurements expressed the results (Zetasizer Nano Series User Manual, 2004).

The antagonistic effect of NPs against various organisms

Quantitative assay_(MIC) for planktonic bacteria, yeast and molds

The lowest concentration inhibits microbial colony formation by serial two folds dilution of NPs (Ag and Ni), metal ions ($AgNO_3$ and Ni $(NO_3)_2$), antibiotics (rifamycin, streptomycin and tetracycline) and chlorine were determined with concentrations (0.075, 0.15, 0.3, 0.6, 1.2 µg/ml), (0.075, 0.15, 0.3, 0.6, 1.2 µg/ml), (0.016, 0.032, 0.064, 0.128, 0.256 µg/ml) and (0.075, 0.15, 0.3, 0.6, 1.2 µg/ml), respectively. About 10^6 CFU/ml of examined microbes listed in Table (2) were inoculated on Mueller Hinton Agar**.** Media were supplemented with the above mentioned concentrations of each tested antimicrobial agent, separately. The bacterial inoculated plates were incubated at 35°C ± 2°C for 24h, while fungal plates were incubated at 25°C ± 2°C for 2-3 days. The lowest concentration that locked or prevented the microbial growth was determined (Kora and Arunachalam, 2010; Mazumder *et al.*, 2013).

Anti-biofilm effect by tissue culture plate method

The Gram negative *P. aeruginosa* and Gram positive *S. aureus* bacteria were selected for biofilm formation (Kora and Arunachalam, 2010). The effect of two concentrations (0.15 and 0.3 mg/ml) of NPs on biofilm formation was examined on sterile 96-well flat bottom polystyrene microtitre plate wells inoculated with 100 µl of Tryptone Soy Broth (TSB) containing 10^8 CFU/ml and loaded with NPs in comparable to metals ions, antibiotics and chlorine solutions.

These plates were incubated in static condition at 37^{O}C for 24 h. Positive controls wells were maintained with medium containing bacterial suspension, while negative control wells contain sterile TSB only. Then, the wells contents were removed by tapping the plates (Gupta *et al.*, 2013) and washed three times with sterile phosphate buffer saline to remove loosely attached bacteria (planktonic).

Wells were stained with 150 µl of 0.25% crystal violet and incubated for 30 min. Further these wells were washed, air dried, bound stain was solubilized in 150 µl of 95% ethanol and the absorbance at 595 nm was recorded using the plate reader (Tecan Infinite M200, Switzerland). These optical densities (OD) values were considered as an index of bacteria adhering to the surface and forming a biofilm. However, the lower absorbance value revealed the more intensive anti-biofilm effect. The percent inhibition of the biofilm activity was calculated as described in the following equation:

% Inhibition of adhesion = [(A- A_0/ A) × 100]

Where**,** A represents the absorbance of the positive control wells and A_0 reflects the absorbance of the treated wells with antimicrobial agent (Ibrahem *et al*., 2014).

Effect of NPs on algae Chlorella vulgaris

The algae were cultured in 250 mL Erlenmeyer flasks containing 60 mL of sterilized BBM medium (Vishnu and Sumathi , 2014) which were capped with loose cotton, and these flasks were placed on incubator at 25^{O}C under illumination with daily cycles of 12h light and 12h night for 7 days. To determine the effect of NPs, about 0.15 mg/ml was added to the algal culture during the inoculation step. The cell density of the culture was monitored by counting with a hemocytometer under light microscope (Olympus BH-2, Japan) as reported by (Barhoumi and Dewez, 2013). The inhibitory rate was calculated as described in the above section.

Application of NPs in real water and wastewater samples

Different water and wastewater samples were collected to study the effect of biomimetic NPs on their microbial content in comparable to chlorine solution. These samples were collected in January 2015 from water and wastewater (for sampling sites see Table 5). Chemical analysis of the collected water samples for pH, Na^-, K^-, Cl^+, salinity, TDS, EC, NH_4^+, No_2^-, NO_3^-, PO_4^- and SO_4^- were performed. Subsequently, water and wastewater samples were treated with 0.1 and 0.3 mg/ml of NPs for 30 min, 60 min and 2h as a contact time.

While, the standard concentrations 5 and 10 mg/l of chlorine applied usually to disinfect water and wastewater were used. Pour plate method was used for determination of bacterial count in each concentration and contact time for NPs and chlorine.

The bacterial load within each sample without treatment was performed as positive control plates. The inoculated NB plates were incubated at 30°C on inverted position and the number of colonies on the petri plates was counted after 24h. The colony forming units (CFUs) were calculated by multiplying the number of colonies by the dilution factor (Sanpo *et al.,* 2013). The inhibitory effect percentage of each concentration and contact time for NPs and chlorine was calculated according to the following formula **(**Robin *et al*., 2013).

$$\text{Inhibitory effect (\%)} = \frac{\text{No. of colonies in control- No. of colonies in treatment}}{\text{Number of colonies in control}} \times 100$$

RESULTS AND DISCUSSION

As shown in Figure 1, the reduction of $AgNO_3$ and of Ni $(NO_3)_2$ to AgNPs and NiNPs could be visually approved by color changes of the bacterial cells and slightly surrounding media from yellow to dark brown/black**.** The variation in the color may be due to the difference in the nature, size and shape of the metal particles by relative activity of bacterial cells.

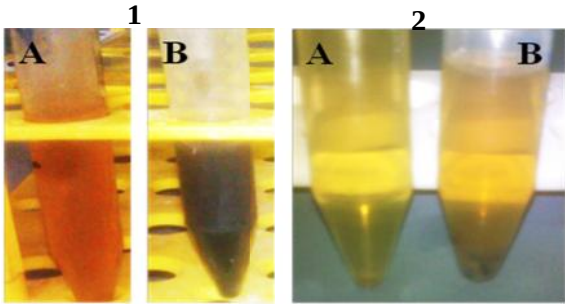

Figure 1 Visual inspection of NPs synthesized by strain MMT; 1) AgNPs, 2) NiNPs, A) media containing metal ions before inoculation, and B) biosynthesized NPs in cells and in surrounding media.

The UV-vis spectroscopy revealed absorbency peaks of both AgNPs and NiNPs in the range from 370 to 450 nm (Figure 2). The maximum peak of Ag was at 420 nm, while Ni gave its maximum peak at 395 nm. This may be due to the presence of a dipole plasmon resonance which may be attributed to the formation of small seeds of silver oxide and nickel oxide, respectively (Sathyavathi *et al*., 2014; Ahmad *et al*., 2015). All UV absorption peaks characterized with the absence of long tailing on large wavelength suggested absence of aggregation (Minaeian *et al*., 2008).

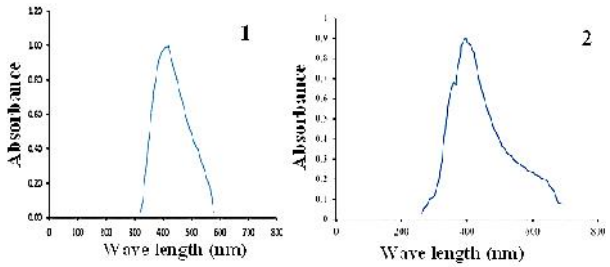

Figure 2 UV-Vis absorption spectrum of biosynthesized NPs; 1) AgNPs, and 2) NiNPs.

As illustrated in Table (1), the atomic percentage of the NPs revealed strong signals of Ag (71.4 %), and Ni (5.8%) confirming their nanoparticles formation. Presence of other elements conjugated with biosynthesized NPs could be observed in high percentage specially sulfur and phosphorus. Such point could be explained by the presence of bacterial biomolecules that contain polar phosphorus backbone (phospholipids, ATP, DNA and RNA) and proteinogenic amino acids as cysteine and/or methionine (Park *et al.,* 2009; Shakoori *et al*., 2012; Sugiyama *et al*., 2013).

Table 1 EDX of biosynthesized NPs; a) AgNPs, and b) NiNPs

a

Elements	%
Na	4.3
S	22.6
Ag	71.4
Fe	1.7

b

Elements	%
Na	3.2
S	32.7
Ni	5.8
P	39.4
Cl	8.4
K	3.5
Ca	7.0

As shown in Figure (3), XRD peaks of NPs appeared sharp, clearly distinguishable and broad, which indicates the ultra-fine nature and small crystallite size. However, it contains no other phase indicates the purity of the samples (Reddy *et al*., 2014; Mohameed *et al*., 2015). The XRD spectrum of AgNPs shows characteristic intense peaks at 27.94, 32.27, 38.3 46.34 and 67.48 which corresponds to hkl of (110, 111, 200, 211and 222) planes of face centered cubic silver. These peaks corroborate with the standard Ag_2O (JCPDS 76-1393) (Dhoondia and Chakraborty, 2012; Nwanya *et al*., 2013). For NiNPs the characteristic diffraction peaks are weak due to low concentration of NiNPs. The peaks appeared at 2Θ = 37.18º, 43.2º and 62.8º are indexed as (111), (200), and (220), respectively and represents face-centered cubic (FCC) crystalline structure of NPs. These diffraction peaks matched with the standard spectrum (JCPDS, No. 04-0835) and (JCPDS card no. 47-1049) (Hada *et al*., 2013; Khalaji, 2015).

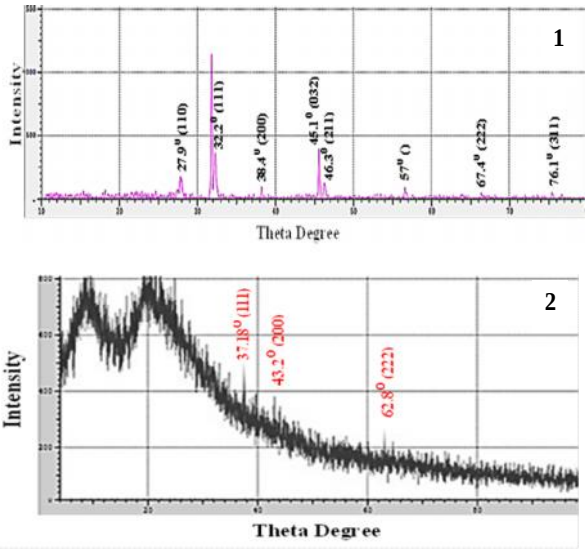

Figure 3 XRD pattern of NPs biosynthesized by strain MMT; 1) AgNPs, and 2) NiNPs.

The electrostatic potential (The Zeta potential "ξ") that exists at the shear plane of a particle, which is related to both surface charge and the local environment of the particle was -52.2 mV and -31.8 mV for AgNPs and NiNPs, respectively as a mean of 3 measurements (Figure 4). The zeta potential is a measurement tool of charge stability and controls all particle-particle interactions within a suspension. The magnitude of the zeta potential is predictive of the colloidal stability. However, a dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or -30 mV, which means that particles with zeta potentials more positive than +30 mV are normally considered stable, as well as the particles with zeta potentials more negative than -30 mV (Saeb *et al*., 2014).

Out of the results, zeta potential was found to be advantageous as recorded a higher value which is due to greater electro-static repulsion between the particles that results in "Brownian motion". Such motion keep them in a state of animated suspension for a much longer time and by such way minimizing aggregation/flocculation and exhibiting monodispersion and stability (Gil *et al*., 2013; Li, 2004). The negative singe of zeta potential is due to bacterial proteins which NPs were suspended in. Such bacterial biomolecules as proteins (carrying negative charge suggested being due to negatively charged amino acid as aspartate and glutamate) and sugar-phosophate backbone (nucleic acid residues including both DNA and RNA) (Filiz and Koç, 2014). Such biomolecules at which NPs were imbedded consider being as capping, stabilizing and functionalizing agent in which preventing aggregation and agglomeration (Zaki *et al*., 2014).

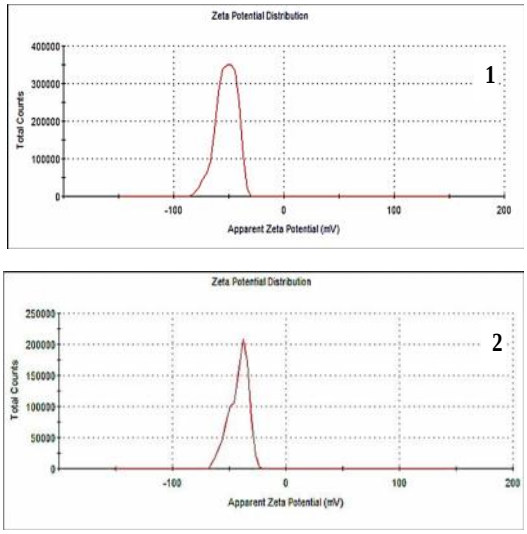

Figure 4 Zeta potential analysis of NPs; 1) AgNPs, and 2) NiNPs

As reported by Madhavi *et al*. (2013) and Sun *et al*. (2006) the range for the PDI is from 0 to 1 (values close to zero indicate a homogeneous dispersion and those greater than 0.5 indicate high heterogeneity). DLS analysis revealed that the size (hydrodynamic diameter) of AgNPs was 717, 42 and 6.7 nm with intensity 5.2%, 1.8% and 93%, while its PDI was 0.234. However, the DLS size of NiNPs were 483 and 87 nm with intensity 96.8% and 3.2%, respectively, while it's PDI was 0.434. DLS analysis suggested that AgNPs and NiNPs were homogenous dispersity. Larger PDI of NiNPs was observed parallel with high percentage of large protein particles (96.8%) that coagulates low percentage of NiNPs in aggregates.

The size, shape, and morphologies of the formed NPs and its biofactory were investigated by TEM. Figure (5-1) showed numerous electron opaque nanoparticles appear to be teeny, uniform, spherical and monodispersed without distinct aggregation ranging in size from 0.72 to 1.4 nm of AgNPs. The AgNPs scattered as seeds like in the periplasmic space of the bacterial cells, which is between the outer cell wall and inner plasma membranes. Such results were in agreement with (Zaki *et al*., 2014). However, Figure (5-2) illustrates bioconversion of nickel salt to nanoparticles. It appears that viable cell with the cytoplasmic compartment migrates slightly from the cell wall as small distance exists between the cell wall and cytoplasmic membrane. In addition, numerous large (31.7 nm) and spherical nanoparticles entrapped in aggregation at the cytoplasmic compartment of the cell. Nevertheless, in general TEM, EDX and DLS were concordant in low concentration of biosynthesized NiNPs.

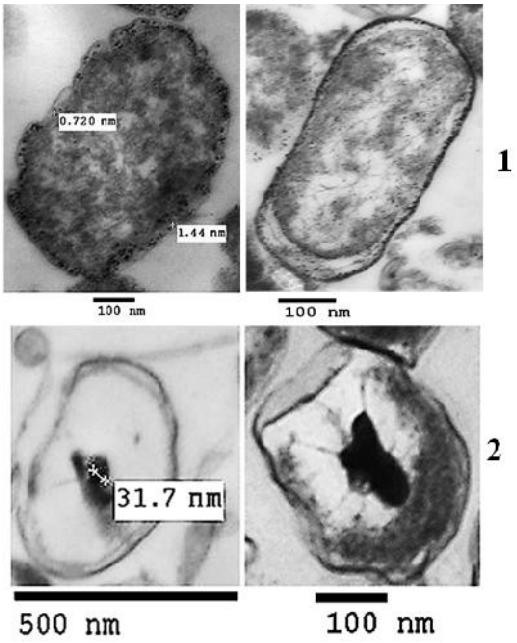

Figure 5 TEM of biosynthesized NPs; 1) AgNPs, and 2) NiNPs.

Biocide activity of biosynthesized nanoparticles

Different types of microbial forms as bacteria (Gram-positive, Gram-negative), mold and yeast were used in determination of NPs antimicrobial activity.

Quantitative assay (MIC) for planktonic bacteria, yeast and molds

Generally, AgNPs were effective than NiNPs (Table 2). In addition, salts of metals exhibit lower activity than their nano forms. However, Gram-negative bacteria were resisting to higher concentrations with all examined antimicrobial agents. That could be explained by the variation of cell wall polarity as a result of cell wall composition (Gordon *et al*., 2011). In addition, lipopolysaccharide of gram negative pathogens suggested sequestering the NPs preventing them from easier penetration. Moreover, the antagonistic effect of all examined agents was tested under aerobic and anaerobic conditions. NPs exhibited inhibition to *C. perfrengense* under anaerobic incubation. That emphasizing that the biosynthesized NPs were potent inhibitors under oxic and anoxic conditions. On opposes, Sharma *et al*. (2009) reported that the antibacterial activity of Ag^{+} ion under anaerobic conditions was found less potent than in oxygen rich environment. Both AgNPs and NiNPs showed high antifungal activity.

Although, fungal cell has ergosterol and can keep their strongly membrane potential ability, perturbation of the membrane lipid bilayers, dissipating the electrical potential of the membrane and inhibition of bud growth were reported as antifungal effect of NPs (Noorbakhsh, 2011; Nasrollahi *et al*., 2011).

Effect of NPs on biofilm

The inhibitory effect of NPs and the other examined antimicrobial agents on both *S. aureus* (G^{+ve}) and *P. aeruginosa* (G^{-ve}) biofilms were presented in Table (3). Generally, there were common features between planktonic bacteria and their biofilms. In compare to *S. aureus*, *P. aeruginosa* biofilm was less susceptible to all treatments and concentrations. AgNPs causes the highest reduction in the biofilm biomass with 93 and 86 % inhibition at 0.3 mg/ml for *S. aureus* and *P. aeruginosa*, respectively. NiNPs exhibited the lowest inhibitory effect due to its relative larger dimensions and somewhat aggregation than AgNPs. That's make their penetration more difficult and required in high concentration or extent exposure time to achieve the same result as AgNPs. NPs inactivate biofilms through a biosorption-dependent manner (Markowska *et al*., 2013) or porins that are water-filled channels allow the exchange and transport of low-molecular weight compounds with the environment and so allow NPs to exert their antimicrobial effect (Franci *et al*., 2015).

Table (3) NPs effect on biofilm biomass from *S. aureus* and *P. aeruginosa*

Biofilm Type			***S. aureus***		***P. aeruginosa***	
Treatment (mg/ml)			**OD**	**Inhibition %**	**OD**	**Inhibition %**
Antimicrobial Agent	Positive control		2.195	0.00	2.136	0
Metal Nanoparticles	SONPs	0.15	0.563	74.35	0.943	55.85
		0.3	0.133	93.94	0.281	86.84
	NONPs	0.15	1.027	53.21	2.00	6.37
		0.3	0.527	75.99	1.706	20.13
Metal Salts	Ag	0.15	1.252	42.96	0.504	76.40
		0.3	0.735	66.51	0.35	83.61
	Ni	0.15	1.415	35.54	0.956	55.24
		0.3	0.707	67.79	0.298	86.05
Antibiotics	Rifamycin	0.15	0.312	85.79	1.125	47.33
		0.3	0.281	87.20	0.843	60.53
	Tetracycline	0.15	1.525	30.52	1.022	52.15
		0.3	0.495	77.45	0.617	71.11
	Streptomycin	0.15	1.147	47.74	1.567	26.64
		0.3	1.13	48.52	1.49	30.24
Conventional Disinfectant	Chlorine	0.15	1.367	37.72	0.711	66.71
		0.3	0.308	85.97	0.322	84.93

There were multi-disruptive mechanisms of nanoparticles could be exerted at various stages during biofilm growth. By which, it could influence at the initial phase (the planktonic form).

Table 2 MIC of antimicrobial agents against various microbial types; A) NPs and metal ions, B) antibiotics and chlorine

Microorganism		**Antibiotics (mg/ml)**			**Chlorine**
Class	**Strain**	**Rif.**	**Tetracy.**	**Strept.**	**mg/ml**
Gram +ve Bacteria	*B. cereus ATCC -7464*	0.064	0.064	0.032	0.15
	S. aureus ATCC -25923	0.032	0.016	0.128	0.15
	C.perfringens ATCC -13124	0.016	0.016	0.064	0.075
Gram -ve Bacteria	*E.coli ATCC -25922*	0.032	0.032	0.128	0.075
	Salmonella typhi ATTC -700931	0.064	0.064	0.128	0.3
	P.aeruginosa ATCC -27853	0.064	0.128	0.128	0.3
	Enterococcus faecalis ATCC -29212	0.032	0.032	0.032	0.075
Mold & Yeast	*A. brasiliensis ATCC -16404*	------	------	------	0.15
	C. Albicans ATCC -10231	------	------	------	0.15

Also, the aggregated state, or sessile, in which cells are closely bound and firmly attached to one another and to a solid surface through disrupting exopolysaccharides production, which are essential for biofilm formation and enhanced quorum quenching activity (Franci *et al.*, 2015). Saeb *et al*., (2014) and Ibrahim (2015) have reported that the efficiency of silver nanoparticles enhanced in combination of standard antibiotics and so overcoming troubleshooting of antibiotic/heavy metal resistance. In the same way conjugation of more than type of nanoparticles enhance antimicrobial activity.

Effect of MONPs of algae

Chlorella vulgaris is a single-cell, photosynthetic, fresh water green algae, (Oukarroum *et al.*, 2012) that can create green and opaque water problems in aquaria (eutrophication with other algal bloom) due to high nitrate and phosphate levels with direct sunlight. So, it was selected to determine the toxicity of NPs in comparable to metal salts and chlorine. Such effects were represented in Table (4). As indicated, all NPs exhibited the most lethal effect on survival and morphological characteristics of algae in compare to their precursor. In compare to all examined antimicrobial agents, AgNPs had the strongest antagonistic effect. Visual differences of algae growth was illustrated in Figure (6), where, algal growth turned yellowish-green in color in compare to the respective controls which remained green and flourished well in the culture medium. On 7th day of exposure to NPs (0.15 mg/ml), extensive damage of chloroplasts was observed leading to their granulation and contraction in algal cells. Such phenomena similar to that reported in filaments of *Pithophora sp.* (Dash *et al*., 2012), where, chloroplast became fragmented and subsequently got disintegrated.

Table 4 Effect of 0.15 mg/ ml of NPs on *Chlorella vulgaris*

Treatment (mg/ml)			Count CFU/ ml	Inhibition %
	Positive control		11* 10^4	0
Metal Nanoparticles	AgNPs	0.15	1.375 * 10^4	87.5
	NiNPs	0.15	1.8 * 10^4	80
Metal Salts	$AgNO_3$	0.15	2.3 * 104	79
	Ni $(NO_3)_2$	0.15	13.2 * 10^4	-20
Conventional Disinfectant	Chlorine	0.15	5.6 * 10^4	49
		0.3	3.5 * 10^4	68

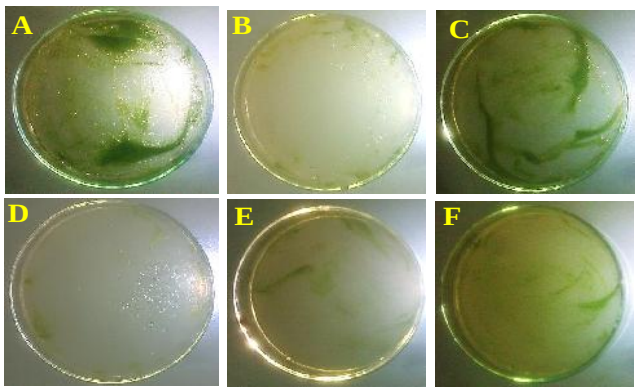

Figure 6 Visual differences representing qualitatively effect of NPs on *Chlorella vulgaris* growth under different treatment conditions as follows; A) control (no treatment), B) treated with $AgNO_3$, C) treated with $Ni(NO_3)_2$, D) treated with AgNPs, E) treated with NiNPs, and F) treated with 50 mg/ml chlorine.

On the other hands, 0.15 mg/ml of nickel salts enhance the *Chlorella vulgaris* biomass with 20% increase of the control. As reported by Lustigman *et al*. (1995), metals as nickel ions at small concentrations are indispensable microelement for microalgae and diatom cells to perform cellular functions (respiration, photosynthesis, and oxygen transport or cell proliferation).

Many parameters considered and suggested in the manner by which metal oxide nanoparticles influence their toxicity. These include; size (Gong *et al*., 2011; Mazumder *et al*., 2013), shape (Pal *et al*. 2007), chemical composition of functionalizing agents (Gong *et al*., 2011), dissolution (Franci *et al.*, 2015), concentration (2007; Kim *et al*., 2007), surface charge (Franci *et al.*, 2015).

It is known that positive zeta potential NPs exhibited stronger antagonistic effect in compare to the negative one (Franci *et al.*, 2015). In the present study, NPs synthesized by MMT has negative zeta potential and considerable antimicrobial effect. Such could be explained by Columbia's law. In which, describing the electrostatic interaction between electrically similar charged particles. Columbia's law assumed that, two objects with different dielectrics come into contact; the result is that one of the objects gains a net negative charge and the other a net positive charge. Once charged, the objects then display some unusual behavior. Among such unusual behavior is that attraction occurs between strong negative and weak negative charges objects. This appears to fully violate Coulomb's Law which predicts that such objects should repel. If the like-charges are of a similar strength then they do indeed repel. But attraction appears to occur when the like-charges are largely different in magnitude.

Application of NPs to disinfect real water samples

Chlorination is the most common conventional method used worldwide for the disinfection of wastewater from pathogens before discharge into receiving streams, rivers or oceans. Effective chlorine disinfection depends on the correct combination of pH, chlorine concentration and contact time as well as the levels of ammonia and suspended solids (Tree *et al.*, 2003; Pant and Mittal, 2007). While, such method suffering from some drawbacks as reaction of free chlorine with various constituents in natural water to form harmful disinfection byproducts (DBPs) such as chlorophenols, trihalomethanes (THMs) and haloacetic acid (HAAs) that are carcinogens and harmful for the environment even at low concentrations (Pant and Mittal, 2007). To overcome drawbacks of chlorination, nanotechnology application in environmental field attract great interest.

In the present study, the biosynthesized AgNPs and NiNPs were applied as chlorine alternatives in disinfection of water and wastewater samples collected from different sources. The quality criteria of them were determined through chemical analysis of some parameters as indicated in Table (5).

Generally, There was reduction in the bacterial count by treatment of NPs as indicated in Tables (6- 7). The reduction

percentage in microbial count was higher in fresh water than salted one. Also, the inhibition effect increases with more contact time and concentration applied.

concentrations and contact time intervals. That suggested indication on presence of microbial load that resist metals biocide effect. **3)**, the antimicrobial potential of

Table 5 Chemical analysis of water samples from different source

Sample Type	Sample Name	Analysis Parameter											
		pH	Na^+ mg /l	K^+ mg /l	Cl^- mg /l	Salinity psu	TDS mg /l	EC µs /mc	PO_4^- mg /l	SO_4^- mg /l	NH_4^- mg /l	NO_3^- mg /l	NO_2^- mg /l
Fresh water	Almahmoudia Canal	8.04	49.1	0	920	0.04	71	112	0.97	78.5	0.023	1.135	0.042
Agricultural wastewater	Bahig Canal	8.34	12.9	54.7	1050	3.58	4029	6565	0	2240	2.15	2.905	0.061
Industrial wastewater	Tolombat -Almax	8.1	177	20	1513	17.61	5570	6875	0.185	3210	0.018	0	0.046
Lake water	Mariout Lake	7.59	132	30	2009	42.94	2130	6354	3.53	5430	0.515	0	0.355
Sea water	Al Max sea	7.68	144	24.7	2132	47.72	4870	8280	0.14	3160	0.037	0	0.045
Salt mine water	Elmahahat	7.51	181	46	3271	50.1	9696	14920	0.132	3330	0	0	0
Municipal wastewater	Burgelarab sewage plant	8.15	106	1.31	1170	2.28	1995	2948	8.81	211	4	0.392	0.533
	Sewage -21 k-region	7.84	133	1.53	1487	5.11	2785	4526	3.9	1965	1.046	2.46	2.029

Table 6 Effect of AgNPs on real water samples

Sample Type	Sample Name	Control Count Cfu/ml	Disinfection by AgNPs Count (CFU/ml)					
			0.1 mg/ml			0.3 mg/ml		
			30 min	60 min	2 h	30 min	60 min	2 h
Fresh water	Almahmoudia Canal	530	28	8	0	6	3	0
Agricultural wastewater	Bahig Canal	656	21	15	1	2	0	0
Industerial wastewater	Tolombat -Almax	233	182	133	52	98	50	3
Lake water	Mariout Lake	383	147	87	37	28	17	0
Sea water	Al Max sea	630	120	9	0	8	0	0
Salt mine water	Elmahahat	291	196	180	151	133	109	59
Municipal wastewater	Borg el arab sewage plant	$74.56 * 10^3$	480	318	75	200	111	51
	Sewage -21 k-region	4370	155	68	6	37	3	0

Table 7 Disinfection of real water samples from different sources by NiNPs

Sample Type	Sample Name	control Count CFU/ml	Disinfection by NiNPs Count (CFU/ml)					
			0.3 mg/ml			0.6 mg/ml		
			30 min	60 min	2 h	30 min	60 min	2 h
Fresh water	Almahmoudia Canal	530	254	193	96	122	89	57
Agricultural wastewater	Bahig Canal	656	384	292	203	258	202	130
Industrial wastewater	Tolombat -Almax	233	193	176	94	160	75	37
Lake water	Mariout Lake	383	284	187	100	188	111	74
Sea water	Al Max sea	630	389	297	177	218	123	74
Salt mine water	Elmahahat	291	239	204	171	158	130	101
Municipal wastewater	Burgelarab sewage plant	$74.56 * 10^3$	1840	1012	712	942	420	180
	Sewage -21 k-region	4370	987	803	414	685	394	166

As illustrated in Table (8), chlorine was the superior in their disinfection as it was strong oxidizing agent. Where, reduction percentage of microbial count reached to 100% at 60 min contact time and 5 mg/L concentration.

NPs even chlorine influenced greatly with high reduction in microbial count in fresh water samples, agricultural wastewater and municipal wastewater.

Table 8 Disinfection of real water samples from different sources by chlorine

Sample Type	Sample Name	Control Count CFU/ml	Disinfection by Chlorine Count (CFU/ml)					
			5 mg/l			10 mg/l		
			30 min	60 min	2 h	30 min	60 min	2 h
Fresh water	Almahmoudia Canal	530	13	3	0	0	0	0
Agricultural wastewater	Bahig Canal	656	30	0	0	0	0	0
Industrial wastewater	Tolombat -Almax	233	155	59	0	0	0	0
Lake water	Mariout Lake	383	120	53	9	19	4	0
Sea water	Al Max sea	630	102	19	0	0	0	0
Salt mine water	Elmahahat	291	58	21	10	35	11	2
Municipal wastewater	Burgelarab sewage plant	$74.56 * 10^3$	156	48	17	18	5	0
	Sewage -21 k-region	4370	25	13	0	0	0	0

There were number of general observations out of Tables (6, 7 and 8), **1)**, AgNPs have the strongest antimicrobial potential due to their particle size that were smaller than NiNPs, enabling them for easier penetration to microbial niches. **2)**, Tolombat El max water sample exhibited the lowest reduction percentage after various treatments with different

While, decrease in such potential in industrial wastewater and salted water (Malahaate < Mariout Lake < Al Max sea). As documented by Pant and Mittal (2007), the disinfection efficiency was highly influenced due to the presence of suspended solids and soluble organic compounds. This may be due to the presence of high suspended solids that hampers the

way of NPs to microbial cells either by entrapping between or adhere on the particles. That phenomenon exactly occurred with chlorine when used in disinfection of wastewater, with high suspended solids (Pant and Mittal, 2007). In addition, Hazani *et al*. (2013) reported that aggregation of nano-particles depends on pH, ionic strength, ionic composition, concentration and composition of natural organic matter, and other characteristics of the aqueous media. So, large aggregation of NPs would be formed in salted and industrial wastewater. Handy *et al*. (2008) reported that aggregation of nanoparticles in sea-water is more likely than in fresh water.

In the same way, increase concentration of electrolyte ions as Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} and Cl^{-} would influence adversely on antimicrobial properties of NPs. Zhang *et al.* (2013) mentioned that after adding AgNPs into NaCl and $CaCl_2$ solutions, the Cl^{-} ion presents could react with the Ag^{+} released from AgNPs and form precipitation. Additionally, cations (Na^{+} and Ca^{2+}) present in the electrolyte solution could adsorb onto the surface of the nanoparticles and neutralize the surface charge of the AgNPs which could lower the efficiency of interaction process between nanoparticles and microbial surface.

Finally, increasing in contact time or NPs concentration or both, leads to increase in reduction percentage. The dose required from disinfectant depends upon a number of parameters like number of bacteria, pH of the wastewater, temperature and turbidity (Pant and Mittal, 2007).

CONCLUSION

Out of this study, strain MMT has the ability to synthesized silver and nickel oxide nanoparticles. The biosynthesized NPs were characterized by UV-vis, EDX, XRD, zeta potential and TEM. The biosynthesized NPs exhibited antimicrobial activity against aerobic, anaerobic, Gram positive, Gram negative, mold, yeast, biofilm and algae. In addition, NPs were examined as chlorine alternatives in water and wastewater disinfection. The biocide activity increased with increasing applicable dose and contact time depending on water characters and microbial load.

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