Bactericidal efficiency of Silver nanoparticle against water contaminants isolated from fish farms water with special reference of some physicochemical parameters of water

Reem Dosoky, Saber Kotb and Mohamed Farghali

Animal hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt
saberkotb@yahoo.com

Abstract: The bactericidal efficiency of AgNP was evaluated against *Total bacterial Counts (TBC*), *Total Coliform Counts (TCC)* and *Total Faecal Streptococcal Counts (TFS*) of water samples collected from fish farms water. Our finding showed that the highest concentration of Ag nanoparticle exhibited highest bactericidal efficiency against *TBC* where after 2 hours contact time, 0.1, 0.05 and 0.01 mg/L Ag nanoparticle was sufficient to inhibit (85.33 %, 71.93 % and 62.19 %) of *TBC* in fish farms water. Moreover, the results showed that the lowest mean of *TCC* was at 0.1 ppm of AgNP after 2 hrs. contact time (144.21 ± 99.94), where its antibacterial activity reached to 92.48 % and this percentage of *TCC* inhibition was higher than the other 2 concentrations at the same times (58.34 % for 0.05 ppm and 31.01 % for 0.01 ppm at 2 hrs.). Furthermore**,** the results showed that the lowest mean of *TFS* was the mean of 0.1 ppm of AgNP after 2 hrs. contact time (155.50 ± 60.86) followed by 0.1 ppm after 1 hr. contact time (212.46 ± 97.46). Moreover, the highest concentration (0.1 ppm) produced highest antibacterial activity against *TFS* and its efficiency reached to 90.48 % followed by 0.05 ppm, which resulted in 87.82 % inhibition of *TFS* after 2hrs.The mean value of 0.1 ppm at 1hr. nearly equal in their inhibition to 0.05 at 2hrs., while the inhibition of 0.1 at 5 min. was higher than 0.01 at 2 hrs. contact time. Also, our results revealed that there were significant positive correlations between water pH, water hardness, chemical oxygen demand (COD) and *TBC, TCC, TFS* count, this means that when water pH, water hardness, COD increased there were increase in the bacterial count (decreased AgNP efficiency), while there were significant negative correlations between water temperature and *TBC, TCC, TFS*, this means that when the water temperature increased there was decrease in the bacterial count (increased AgNP efficiency) and vice versa. Silver nanoparticles proved good efficiency against *Faecal bacterial* indicators and *TBC* of water, so we recommend using the silver nanoparticles in the field of fish farms water treatment. To obtain a good efficiency of silver nanoparticles, the fish farms water must be treated to remove water hardness and organic matter before the applications of AgNP.

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1. Introduction

Microbial quality of farmed fish is largely affected by the quality of the water in which they were cultivated (**Ekpo*etal.*, 2010**). Good water quality is needed for maintaining viable aquaculture production. While, poor water quality can result in low profit, low product quality and potential human health risks. Production is reduced when the water contain contaminants that can impair development, growth, reproduction, or even cause mortality to the cultured species. Some contaminants can accumulate to the point where it threatens human health even in low quantities and cause no obvious adverse effects.

Detection of organisms normally present in the feces of humans and other warm-blooded animals is used as indicators of fecal pollution as well as water treatment and disinfection efficacy. Indicator bacteria such as *Total Coliform Bacteria, Faecal Coliform, and Faecal Streptococci* are widely used for assessment of fecal pollution and possible water quality deterioration in fresh water sources (**APHA, 2005**).

Ag-NP applications have been extensively studied as disinfectants in medical institutions, and an increasing amount of research has been carried out on Ag-NP applications in the food industry and for drinking water treatment and distribution systems **(Kumar** and **Raza 2009; Zhao *et al.*, 2010)**. The use of Nano silver particles in water treatment is relatively new and has recently become of interest **(Jain and Pradeep, 2005).**Most research has focused on the impact of Ag-NPs on individual or certain types of bacteria cultivated under laboratory conditions. However, the impact of Ag-NPs on natural water microorganisms is not well understood.

In aquatic systems, it is of particular importance to identify the main water constituents as abundant cations such as calcium, hardness as well as natural organic matter (NOM). Since Silver release is a very complicated process that depends on physicochemical composition of the water such as temperature, pH, and organic matter **(Kulthong *et al.*, 2010)**. The characteristics of the environmental medium in which

nano-Ag exposure occurs can affect the properties of nano-Ag that ultimately influence nano-Ag dissolution, bioavailability, and reactivity and its fate in the environment, all of which can affect its toxicity **(Gao *et al.*, 2009; Dasariand Hwang, 2010; Liu** and **Hurt, 2010).**

Aim of the Work

The present study was conducted to evaluate the disinfection efficiency of silver nanoparticles (AgNPs) against water contaminants isolated from Fish farms water. Moreover, evaluate the effect of some physicochemical properties of water such as water temperature, pH, water hardness and organic matter (NOM) on the bactericidal efficiency of AgNPs.

2. Material and Methods

2.1. Sampling

Water samples collection was carried out in accordance to the Standard Methods for the Examination of Water and Wastewater **(APHA, 2005).**

A total numbers of twenty seven water samples were collected from five fish farms, the first farm located in Al-Ghorieb Village -Sahel-Sleem City and it belong to faculty of Agriculture Assiut University, Egypt while the other four farms located in El-Saleba Village-Samalout City-Al-Minya Governorate, Egypt. All farms are of closed fish farms system.

Al-Ghorieb fish farm area is about 6 carats, it is of concrete type floor and walls, and it received water source from ground water by using dug well, in this farm only African Sharp tooth catfish (***Clariasgariepinus***) was reared.

Al-Saliba fish farms located in Samalout City, Al-Minya Governorate, 125 kilometers north to Assiut city. All fish farms at Al-Saliba Villages received its water source via tubular system from Bahr Yousf, which is the branch of Al-Ibrahimeya Canal. All fish farms of Al-Saliba were of earthy soil and only Nile tilapia **(*Oreochromisniloticus*)** was reared. The areas of Al-Saliba four fish farms were 2.6 acres, 1.5 acres, 16 carats and 8 carats.

2.2. Preparation of silver nanoparticles (AgNPs)

Stable AgNPs less than 100 nm were synthesized in a typical one-step protocol according to **Vigneshwaran *et al.* (2006)**. After preparation of silver nanoparticles, the size of silver nanoparticles was measured by Transmission electron microscopy (TEM) Model JEOL-JEM- 100CX II in Electron Microscopy Unit, Assiut University. Total concentration of AgNP stock was measured by Graphite Furnace Atomic Absorption Model 210VGP in Faculty of Science, Assiut University.

2.3. Disinfection experiments

In the lab, water samples were mixed and thoroughly shaken before use to re-suspend any sediment, then sample was divided into two parts, one part for disinfection experiment and the another part for selected physicochemical analysis.

2.3.1. Application of silver nanoparticles

For each water sample, disinfection assays were carried out in four sterile conical flasks of 500 ml capacity, each flask containing 250 ml of water sample, silver nanoparticles suspension was aseptically added to three flasks by using micropipette to obtain a final treatment of 0.01, 0.05 and 0.1 mg/L. Each AgNP treatment was thoroughly mixed and allowed to interact with bacterial communities in collected water samples for a five contact times 5, 15, 30, 60and 120 minutes. The remaining water sample in fourth flask was the negative control (water sample without any AgNPs) which, represent the count before treatment, where each treatment has two control negative tests, one at the beginning of contact time and the other with the last contact time then we take the mean values.

2.3.2. Examination of Viability of bacteria before and after application of silver nanoparticles.

Viability of bacteria was examined using different bacteriological tests after 5 minutes, 15 minutes, 30 minutes, 1hour, and 2hours contact times. After the end of each contact time, sufficient amount of mixture of water sample and silver nanoparticles was transferred aseptically into sterile bottle and silver nanoparticles was quenched by adding 5 g/L sodium thiosulfate ($Na_2S_2O_3$) to stop the antimicrobial reaction between AgNPs and bacteria as described in the European quality standards (**NEN, 1997)**.

2.3.2.1. Enumeration of total viable bacteria using Pour plate method was used for the enumeration colony-forming units (CFU /ml) on Plate Count Agar according to **(APHA, 2005)**.

2.3.2.2. Detection and counting of some classical Bacterial Indicators.

The *Total Coliforms (TCC)* and *Faecal Streptococci (TFS)* were determined using the Most Probable Number (MPN) Method (**APHA, 2005**).

3.3- Evaluation of disinfection efficacy of AgNP

Disinfection efficiency of AgNP was obtained by comparing the counting of bacteria before and after treatment for each contact time of water sample to determine if there were differences in treatments.

Percent of disinfection efficacy was calculated with the following equation.

$$\% \text{ Disinfection efficacy} = \frac{(C_0 - C) \times 100}{C_0}$$

Where C_0 is the initial bacterial count in raw water (control negative), C is count of bacteria after a certain contact time of the treated water **(Li *et al.*, 2006)**.

2.4. Physico-chemical analysis of water samples.

Water samples were shaken before use to re-suspend any sediment. The physicochemical analysis were carried out included the determination of temperature, pH, total hardness and Chemical Oxygen Demand (COD).

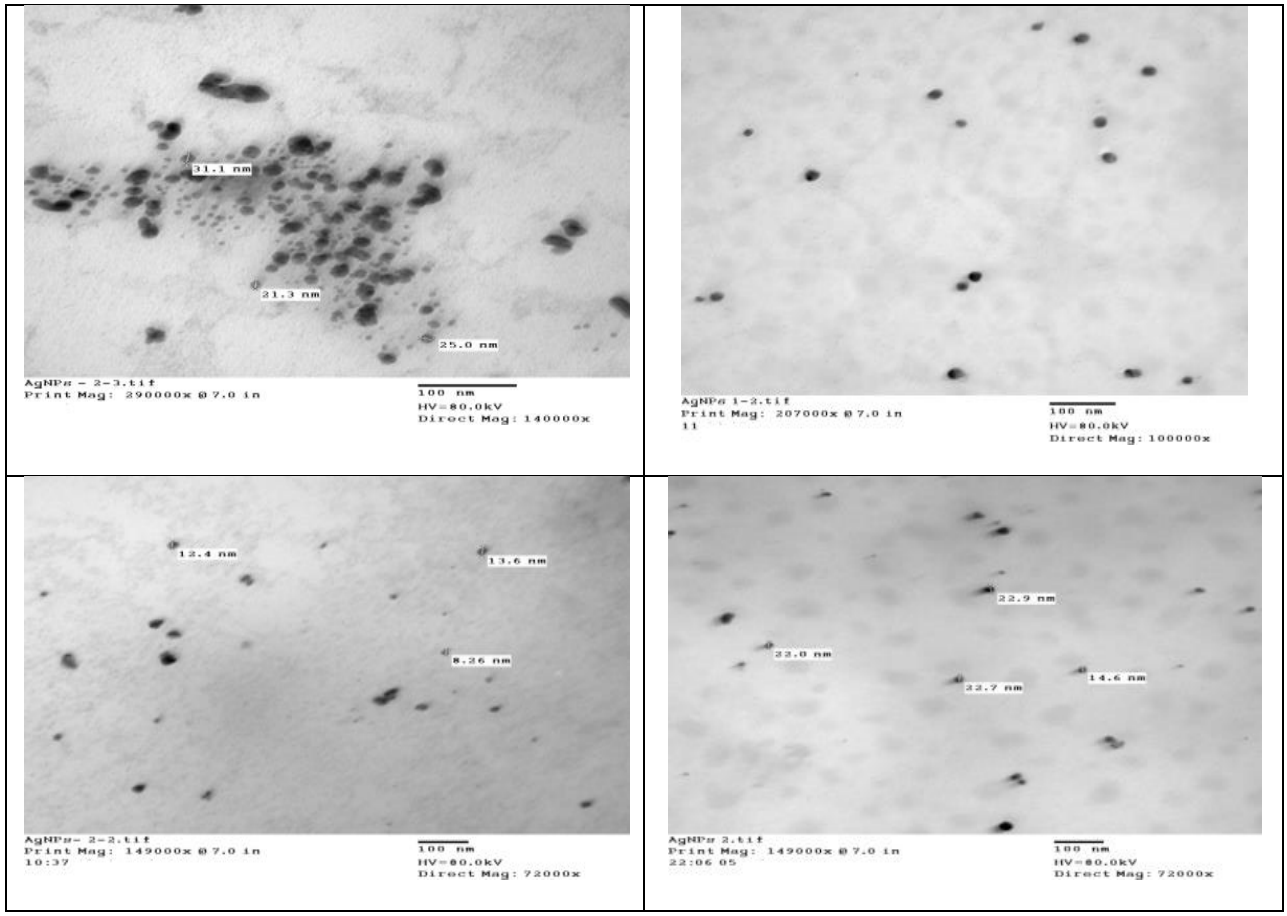

Figure (1): Transmission Electron Microscopy (TEM) images of AgNPs. TEM images of AgNP showed spherical shapes of nanoparticles and their sizes ranged between 8.26- 31.1

2.4.1. Water temperature

For each water sample, water temperature was estimated at the time of application of each concentration of AgNP by using ordinary mercuric thermometer ranged from 0-100 °C.

2.4.2. Water Hydrogen ion concentration (pH)

Water pH was estimated by using pH meter model JWNWAY 3505 at the Central Lab, Faculty of Veterinary Medicine, Assiut University.

2.4.3. Water Hardness:

Lovibond Microprocessor Multidirect Photometer, Animal Hygiene Department, Faculty of Veterinary Medicine, Assiut University, was used to estimate total water hardness using HARDCHECK P/TOTAL HARDNESS.

2.4.4. Chemical Oxygen Demand (COD)

Lovibond Microprocessor Multidirect Photometer was used to estimate COD with Vario Tube Test 0 – 1500 and 0 – 1500 mg/l O_2.

2.5. Statistical analysis:

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

4. Results and Discussion

1. Effect of silver nanoparticles (AgNP) against Total bacterial count (TBC) of fish farm water samples.

The statistical analysis of **table (1)** showed that at the 1st (0.1ppm) and 3rd (0.01) used concentration, the *TBC* was significantly reduced in all AgNP-exposed samples when compared with the control group, moreover at the 1st concentration there were significant differences between the mean value at 2hrs. and the mean value at 5 min. (P<0.05), while at the 2nd concentration (0.05 ppm), there was only significant difference between the mean value of 2hrs. contact time and the mean value of the control group *(P< 0.05)*, moreover the analysis of variance showed no significant differences between the mean values of different five contact times (5 min., 15 min., 30 min., 1hr. and 2hrs.) and each other at the 2nd and 3rd concentrations.

Our obtained results showed that the lowest mean value of *TBC* was after applications of 0.1 ppm of AgNP after 2 hrs. contact time (1519.61 ± 416.57) followed by 0.05 ppm after 2 hrs. (2034.21 ± 566.34) and then 0.1 ppm after 1 hr. contact time (2339.49 ± 706.29) (**Table 1)**.

The results of **table (1)** showed that there was variation between the efficiency of silver nanoparticles at different concentrations, where the highest concentration produced highest antibacterial activity against *TBC* of fish farms water samples and its efficiency reached to 85.33 %, 77.41% and %74.23 % after 2hrs., 1hr. and 30 min. contact times, respectively and these percentage of *TBC* inhibition was greater than the other 2 concentrations at the same times (71.93 %, 67.50 % for 0.05 ppm at 2hrs.and 1hr., respectively and 62.19 %, 60.84% for 0.01 ppm at 2 hrs. and 1hr., respectively).

Data presented in **table (2)** showed the effect of different contact times of AgNP on the overall mean of *TBC* of fish farms water samples. The statistical analysis of **table (2)** showed that there were significant differences between all contact times and the control groups at all water sources (*P*<0.05), but no significant differences in between the five contact times.

The reduction percentage of *TBC* after 5 min., 15 min., 30 min., 1 hr. and 2 hrs. contact times were (52.18 %, 53.24 %, 64.50 %, 68.63 % and 73.17 %) for fish farms water **(Table 2)**.

Data in **table (2)** showed that when the time was increased to two hours at 0.1, 0.05 and 0.01 mg/L Ag nano particle was sufficient to inhibit 85.33 %, 71.93 % and 62.19 %, in fish farm water samples.

From tables (1 and 2), we could observe that the survival rate of *TBC* decreased with the increase in the concentration of AgNP, moreover, the bactericidal efficiency of AgNP increased with the increase of contact times with bacteria in all concentrations, our findings was agreed with the results of **Pranab *et al.* (2011)** and **Akmaz *et al.* (2013)**, however our finding disagreed with the results of **Bradford *et al.* (2009)**.

2- Effect of AgNP on Total Coliform count (*TCC*) after application of Silver nanoparticles.

The statistical analysis of **table (1)** showed that at the 1st used concentration (0.1 ppm) of AgNP, there was only significant differences between the mean of 2 hrs., 1hr. contact times and the control group *(P< 0.05)*, as well as no significant differences between the mean of different five contact times and each others, while at the 2nd concentration (0.05 ppm) and the 3rd used concentration (0.01ppm) of AgNPs, the analysis of variance showed no significant differences between the different five contact times (5 min., 15 min., 30 min., 1hr. and 2hrs.) and the control group as well as between the mean of different five contact times and each other (**Table 1)**.

The results of **table (1)** showed that there were variations between the efficiency of silver nanoparticles at different concentrations, where the highest concentration caused the highest antibacterial activity against *TCC* of fish farms water samples and its efficiency reached to 92.48 % at 2 hrs. Furthermore, the significant differences between the mean values were only between the 2 hrs., 1 hr. and the control group of 0.1 ppm of AgNP and their percentage of *TCC* inhibition was higher than the other 2 concentrations at the same times (58.34 % for 0.05 ppm and 31.01 % for 0.01 ppm at 2 hrs.).

Data presented in **table (2)** showed the effect of different contact times of AgNP on the overall mean of *TCC* of fish farms water samples. The statistical analysis of **table (2)** showed that there were significant differences between all contact times and the control groups (*P*<0.05) of *TCC* in fish farms water, while there was no significant differences between all contact times and each other. Moreover, the reduction percentage of *TCC* after 5 min., 15 min., 30 min., 1 hr. and 2 hrs. contact times were (43.92 %, 43.59 %, 57.59 %, 46.25 % and 64.75 %) in fish farms water samples, respectively **(Table 2)**.

From **tables (1 and 2),** we could observe that the silver nanoparticles exhibited highest bactericidal efficiency against *TCC* in fish farms water. Moreover, the 0.1 ppm concentration of AgNP with the increase in contact times exhibited the highest bactericidal efficiency against *TCC* at the water samples, followed by 0.05 and 0.01, this observation indicated that the efficiency of silver nanoparticles was not only depend on the concentrations of AgNP, but also with how long the AgNP was in contact with bacteria.

The bactericidal efficiency of AgNPs increased with the increase of its concentrations and contact times with bacteria, this conclusion was agreed with the results of **Tuana *et al.* (2011); Nawaz *et al.* (2012)** and **Perez (2012)**, who all proved that there were a positive correlation between the elevated concentration of AgNPs and the inhibition of E-coli.

3- Effect of AgNP on Total Faecal Streptococcal Count (*TFS*).

The statistical analysis of **table (1)** revealed that, at the 1st used concentration, there were significant differences between the mean values of *TFS* at 5 min., 1 hr., 2 hrs. and the control group *(P< 0.05)*, however there were no significant differences between 15 min., 30 min. and the control group, as well as no significant differences were in between the five different contact times. While at the 2nd concentration (0.05 ppm), the analysis of variance showed that *TFS* was significantly reduced in all AgNP-exposed samples when compared with the control group *(P< 0.05)*, however the analysis of variance showed no significant differences in between the contact times (2 hrs., 1 hr., 30 min., 15 min., 5min.). On other hand, at the 3rd used concentration (0.01), the analysis of variance showed no significant differences between the mean of different five contact times (5 min., 15 min., 30 min., 1 hr. and 2 hrs.) and the control group, as well as no significant differences between the different five contact times (5 min., 15 min., 30 min., 1 hr. and 2 hrs.) and each other.

Data in **table (1)** showed that, there were variations between the efficiency of silver nanoparticles at different concentrations, where the highest concentration (0.1 ppm) produced highest antibacterial activity against *TFS* of fish farms water samples and its efficiency reached to 90.48 %after 2 hrs. contact time followed by 0.05 ppm, which resulted in 87.82 % inhibition of *TFS*, moreover the percentage of *TFS* inhibition at 0.1 ppm was higher than the other two concentrations at the same times (87.82 % for 0.05 ppm and 31.41 % for 0.01 ppm at 2 hrs.), the mean value of 0.1 ppm at 1hr. nearly equal in their inhibition to 0.05 at 2hrs., while the inhibition of 0.1 at 5 min. was higher than 0.01 at 2 hrs. contact time.

Table (2) showed the effect of different contact times of AgNP on the overall mean of *Total Faecal Streptococcal Count* of water samples collected from fish farms water. The statistical analysis of **table (2)** showed that there were significant differences between all contact times and the control groups at all water samples (P<0.05), however the analysis of variance of *TFS* showed no significant differences between all contact times and each other at the water fish farm.

From **tables (1 and 2)**, we could summarize that the bactericidal efficiency of AgNPs increased with the increase of its concentrations and contact times with bacteria.

From all the previous data in tables, it's easy to observe that there was re-activation of bacteria in some data which elevate the mean of *TBC, TCC* and *TFS* at certain times and concentrations in the experiment.

Concerning the inhibition percentage of *TBC* at the lowest concentration in fish farms water, the efficiency of AgNPs at last contact time (2 hrs.) was more or less not exceed the inhibition at the 1st contact time (5 min.) with some fluctuations in between the time of contact **(table, 1)**.

In *Total Coliform (TCC)*, the results revealed that there was re-activation of the bacteria at lower treatments (0.05ppm and 0.01ppm), where at both treatments the efficiency of AgNPs at last contact time (2 hrs.) was slightly exceeds the inhibition at the first contact time (5 min.) with some fluctuations in between the times of contact **(tables 1&2)**.The *TCC* re-activation at the lower concentration was agreed with those of **Nawaz *et al.* (2012*)***.

The effect of AgNPs on the *TFS* in water of fish farms takes special trend in which the fluctuations in total numbers not only occurred on the time of contact but also in between the three different used concentration **(Tables 1& 2)**.

From the previous mentioned data, it was clear that, the reactivation of *TBC* and *TCC* was appeared in the lower concentration of silver nanoparticles. On other hand *TFS* showed different manner in its reactivation, where the reactivation appeared at some times of higher and lower concentrations of fish farms water. This reactivation of bacterial growth and decrease efficiency of silver nanoparticles may be due to the presence of high amount of organic matter and water hardness. This finding was agreed with **Dankovich and Gray (2011)** and **Kim *et al.* (2007)**.

From the fore mentioned discussed data it was clear that the bactericidal efficiency of silver nanoparticles increased with the increase in its concentration and contact time with the bacteria, this findings may be attributed to the treated bacterial cells were significantly changed and showed major damage, which was characterized by the formation of "pits" in their cell walls, which exhibits a significant increase in permeability, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane and, finally, causing cell death. The concentration of the nanoparticles gradually decreases, allowing resumed growth of bacterial cells. This process is governed by the interaction of these particles with intracellular substances of the destroyed cells, causing their coagulation and removal from the liquid system (**Sondi** and **Salopek-Sondi 2004*)*.**

4. Effect of some physico-chemical parameters on bioavailability of silver nanoparticles against microbial contamination of fish farm water.

The characteristics of the environmental medium in which nano-Ag exposed affect its antimicrobial activities. For example, changes in the pH, ionic strength, temperature, quantity of natural organic matter (NOM), light availability, can significantly affect nano-Ag dissolution, bioavailability, and

reactivity, all of which can affect toxicity **(Morones *et al.*, 2005; Lok *et al.*, 2007; Carlson *et al.*, 2008).**

Data in **table (3)** illustrated that the mean values of water pH, temperature, hardness and COD were 8.49, 15.08°C, 295.41 mg/l and 330.81mg/ l of fish farm water samples, respectively. This result of water pH was lied within the safe limit suggested by **Pillay and Kutty (2005)**, while the higher water hardness was recorded in the present study was more than that recorded by **Stoskopf (1992)**, who reported that the minimum amount of water hardness which is proper for fish growing in fish farming is 100 mg/L. Furthermore the COD value of fish farms water was higher than the permissible limit of irrigations approved by **WEF (Water Environment Federation), (1998).** The results of physicochemical parameters of fish farms water were more or less agreed with the findings of **El-Nemaki *et al.* (2008); Osman *et al.* (2010a); Abumourad *et al.* (2013)**.

Data in **table (3)** revealed that there were significant positive correlations between water pH, hardness, COD and *TBC*, *TCC*, *TFS*, this means that when water pH, hardness, COD increased, there were increases in the bacterial count (decreased AgNP efficiency), while there were significant negative correlations between water temperature and *TBC*, *TCC*, *TFS*, this means that when the water temperature increased there was decrease in the bacterial count (increased AgNP efficiency) and vice versa.

From all our findings, we could conclude that the increase in organic matter, water hardness and pH decreased the disinfection efficiency of silver nanoparticles, however the increase in water temperature increased the efficiency of silver nanoparticles. Higher antibacterial effect of silver nanoparticles was detected at higher temperature, this agreed more or less with those of **Pal *et al.* (2009)** and **Pathak and Gopal (2012),** but it was in disagreement with the results of **Kim *et al.* (2011)**, while higher antibacterial effect of silver nanoparticles was detected at low pH was more or less disagreed with the findings of **Kim *et al.* (2011)** and those of **Pathak** and **Gopal (2012)**. The anti-bacterial performance of AgNP at selected natural water conditions decreased in the presence water hardness was in agreement with the results of **Zhang *et al.* (2011); Pathak and Gopal (2012)** and **Zhang and Oyanedel-Craver (2012).** The anti-bacterial performance of AgNP at selected water conditions decreased in the presence of natural organic matter, this finding was in agreement with the results of **Zhang *et al.* (2011); Nawaz *et al.* (2012); Zhang** and **Oyanedel-Craver (2012)**.

The bacterial re-activation of *TBC*, *TCC* and *TFS* in fish farms water, may be attributed to the wide variation water hardness, ionic composition, and natural organic matter, all this would induce widely varying aggregation states of silver nanoparticles; thus, resulting in reducing their surface area, reducing the cell-particle interaction, membrane penetration and the rate of silver ion release, all of this resulting in widely varying antimicrobial activities and toxicities **(Liu** and **Hurt, 2010 & Zhang *et al.*, 2011).** Furthermore, the halting of bacterial cell replication process for some time without permanent damage **(Nawaz *et al.*, 2012)**, where the Ag- DNA bound increased to the maximum value during inhibition process and then decline to a low level **(Modak and Fox, 1973)**, in addition to the uptake of silver by live and dead cell decreases the concentration of silver, therefore the antimicrobial activity of silver reduced **(Holt and Bard, 2005)**.

The increase of water hardness decreased the toxicity and bioavailability of silver ions in water (**Nichols *et al.*, 2006*)***.** The presence of divalent cations such as Ca^{2+} (ionic calcium) increased the rate and extent of nanoparticles aggregations and could affect the stable size of the clusters formation **(Cumberland** and **Lead, 2009; Zhang *et al.*, 2011 and Jooa *et al.*, 2013)**. Moreover, elevation of water hardness increased aggregation as a result of a- specific sorption and/or compression of the electrical double layer (EDL) on the surface of a particle **(Handy *et al.*, 2008)**. b-the attractive interaction between divalent cations and negative charged AgNP led to higher aggregation and large particles formation **(Jin *et al.*, 2010)** c- metal ion competition for binding sites on cell surfaces **(Ratte, 1999).**

The presence high amount of organic matter in fish farms water may be lead to increase the interaction between AgNP with organic matter, where the organic matter can absorbed on the surface of AgNP and can rapidly coat the nanoparticles surfaces, creating a physical barrier that prevents interaction between silver nanoparticles and bacterial cells and thus reduced their toxicity **(Baalousha et al., 2008**; **Bradford *et al.*, 2009; Fabrega *et al.*, 2009).** Furthermore, the presence of high-molecular-weight natural organic matter (NOM) compounds in water formed larger nanoparticle clusters and resulting in decrease in their bioavailability and enhanced their deposition into sediments **(Navarro *et al.*, 2008a)**.

Organic materials in water could complex with free silver ions making it unavailable for uptake by bacteria **(Luoma, 2008)**. The high contents of natural organic matter (NOM) could inhibit AgNP dissociation; this resulted in small silver ion release in water, which decrease antimicrobial property **(Liu and Hurt, 2010; Jin *et al.*, 2010).**

Table (1) Mean values of microbial contamination of fish farms water samples after treatment by Ag-NPs.

Treatment	Contact time	*Total Bacterial Count* (Mean± S.E/ml)	Inhibition %	*Total Coliform Count* (Mean± S.E/100ml)	Inhibition %	*Total Fecal Streptococcal Count* (Mean± S.E 100/ml	Inhibition %
0.1 ppm	Control	10357.31 ± 2443.72 [a]		1917.63 ± 584.52 [a]		1633.83 ± 626.28[ab]	
	5 Min.	5145.88 ± 1355.36 [b]	**50.32 %**	1079.54 ± 474.78[abc]	**43.70 %**	685.17 ± 268.15 [cd]	**58.06 %**
	15 Min.	4092.38 ± 1205.00[bc]	**60.49 %**	892.83 ± 479.98[abc]	**53.44 %**	783.00 ± 457.00[bcd]	**52.08 %**
	30 Min.	2669.06 ± 778.11[bc]	**74.23 %**	833.21 ± 481.90[abc]	**56.55 %**	741.38 ± 454.70[bcd]	**54.62 %**
	1 Hr.	2339.49 ± 706.29[bc]	**77.41 %**	735.17 ± 459.39[bc]	**61.66 %**	212.46 ± 97.46 [cd]	**87.00 %**
	2 Hr.	1519.61 ± 416.57 [c]	**85.33 %**	144.21 ± 99.94 [c]	**92.48 %**	155.50 ± 60.86 [d]	**90.48 %**
0.05 ppm	Control	7246.64 ± 2040.03 [ab]		1758.17 ± 589.59[ab]		1969.46 ± 737.33 [a]	
	5 Min.	4199.58 ± 1559.29[bc]	**42.05 %**	752.08 ± 206.59[bc]	**57.22 %**	375.96 ± 112.85 [cd]	**80.91 %**
	15 Min.	3362.44 ± 960.59[bc]	**53.60 %**	904.88 ± 480.66[abc]	**48.53 %**	855.75 ± 480.53[bcd]	**56.55 %**
	30 Min.	3211.28 ± 910.63[bc]	**55.69 %**	672.88 ± 454.41[bc]	**61.73 %**	707.29 ± 457.29 [cd]	**64.09 %**
	1 Hr.	2354.81 ± 749.01[bc]	**67.50 %**	1039.17 ± 627.58 [abc]	**40.89 %**	703.04 ± 453.76 [cd]	**64.30 %**
	2 Hr.	2034.21 ± 566.34 [c]	**71.93 %**	732.50 ± 456.36[bc]	**58.34 %**	239.96 ± 105.33 [cd]	**87.82 %**
0.01 ppm	Control	10639.13 ± 4930.99 [a]		1242.21 ± 438.65[abc]		1164.58 ± 478.66 [abc]	
	5 Min.	4160.78 ± 1609.41[bc]	**60.89 %**	926.13 ± 445.99[abc]	**25.45 %**	308.50 ± 120.41 [cd]	**73.51 %**
	15 Min.	5751.46 ± 2816.34[bc]	**45.94 %**	976.42 ± 449.74[abc]	**21.40 %**	263.17 ± 96.87 [cd]	**77.40 %**
	30 Min.	4144.74 ± 1382.54[bc]	**61.04 %**	579.83 ± 122.13 [c]	**53.32 %**	368.33 ± 190.64 [cd]	**68.37 %**
	1 Hr.	4166.02 ± 1325.97[bc]	**60.84 %**	869.08 ± 457.54[abc]	**30.04 %**	431.13 ± 196.16 [cd]	**62.98 %**
	2 Hr.	4022.92 ± 1400.32[bc]	**62.19 %**	857.04 ± 456.31[abc]	**31.01 %**	798.83 ± 482.44 [bcd]	**31.41 %**

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

Table (2) Effect of different contact times of Ag-NP on microbial contaminants of fish farms water samples

Contact time	*Mean of Total Bacterial Count* (*TBC*)		*Mean of Total Coliform Count* (*TCC*)		*Mean of Total Faecal Streptococcal Count* (*TFS*)	
	Mean ± SE/ ml	Inhibition %	Mean± SE/100 ml	Inhibition %	Mean± SE/100 ml	Inhibition %
Control	9414.00 ± 1937.28[a]		1639.30 ±310.45[a]		1589.30 ± 356.84[a]	
5Min.	4502.00 ± 862.28[b]	**52.18 %**	919.30 ± 225.12[b]	**43.92 %**	456.50 ±105.28[b]	**71.28 %**
15Min.	4402.00 ±1061.60[b]	**53.24 %**	924.70 ± 267.74[b]	**43.59 %**	634.00 ± 222.44[b]	**60.11 %**
30Min.	3342.00 ± 605.43[b]	**64.50 %**	695.30 ± 221.67 [b]	**57.59 %**	605.70 ± 221.88 [b]	**61.89 %**
1Hr.	2953.00 ± 560.95[b]	**68.63 %**	881.10 ± 296.89[b]	**46.25 %**	448.90 ± 167.27[b]	**71.75%**
2Hrs.	2526.00 ± 530.59[b]	**73.17 %**	577.90 ± 217.74[b]	**64.75 %**	398.10 ± 166.97[b]	**74.95 %**

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

Table (3): Statistical correlations between the selected physico-chemical parameters and the selected bacterial tests.

Microorganisms		pH	Temperature (°C)	Water hardness(mg/l)	COD (mg/l)
	Mean ± SE	8.49 ± 0.02	15.08 ± 0.17	295.41 ± 6.84	330.81 ± 21.90
Total Bacterial Count (*TBC*)	r -values	0.24591	-0.20739	0.37734	0.13701
	p- values	<.0001	<.0001	<.0001	<.0001
Total Coliform Count (*TCC*)	r- values	0.21525	-0.20884	0.10284	0.13304
	p- values	<.0001	<.0001	0.0002	<.0001
Total Faecal Streptococcal Count (*TFC*)	r- values	0.215	-0.22251	0.11493	0.1045
	p- values	<.0001	<.0001	<.0001	0.0002

P- Value considered statistically significant when P< 0.05.

5. Conclusion.

Silver nanoparticles proved good efficiency against *Faecal bacterial* indicators and *TBC* of water, so we recommend using the silver nanoparticles in the field of water treatment. To obtain a good efficiency of silver nanoparticles, the fish farm water must be treated to remove water hardness and organic matter before the applications of AgNP. This result suggested that silver nanoparticles could be used in fish farms as a good disinfectant and further studies are needed on the possibilities of the application of silver nanoparticles in form of filter or net system in fish farms as alternative antibacterial agents for the disease free fish culture systems.

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