

Toxicity of Silver Nanoparticles in Aquatic Ecosystems: Salinity as the Main Cause in Reducing Toxicity

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ABSTRACT

Background: In recent years, silver nanoparticles due to their antimicrobial properties, have formed about 56% of nanoparticles global production. Since the released nanoparticles ultimately enter water ecosystems, their maximum toxic effects are magnified in aquatic ecosystems. The aim of this study is to show how salinity can decrease the toxic effects of silver nanoparticles on exposed rainbow trout fry (*Oncorhynchus mykiss*), as a model in aquatic eco-toxicological studies.

Methods: The effects of colloidal silver nanoparticles on rainbow trout fry (n=540, 10-week-old; 1000 ± 35.0 mg) were examined in two different salinities (6±0.3ppt, and 12±0.2 ppt) in comparison with de-chlorinated tap water (0.4ppt). Median lethal concentration (LC₅₀) of various concentrations of the Ag-NPs (ranging from 0.25 up to 80 ppm) was determined through a 6-day static-renewal exposure of tested fish fry to the salinities.

Results: LC₅₀ of the colloidal Ag-NPs for rainbow trout fry in 12±0.2 ppt salinity was almost 20 and 2 times greater than 0.4 and 6±0.3ppt salinities, respectively.

Conclusion: The release of silver nanoparticles into fresh water ecosystems can lead to more biological, physical, and chemical irrecoverable impacts on the ecosystems and their fishes in comparison with saline water ecosystems.

Keywords: LC₅₀, Rainbow Trout, Silver Nanoparticle, Toxicity, Water Salinity.

INTRODUCTION

Nanoparticles (NPs) have at least one dimension of 100 nm or less and nanotechnology industry is a rapidly growing science producing nano-sized particles (1,2). The NPs have always existed in our environment, from both natural and anthropogenic sources (3,4). But engineered materials on nanoscale, due to their unusual chemical and physical properties, are increasingly used in a wide range of industries. As it is estimated, by 2014, more than 15% of all products in the global market will have some kind of nanotechnology incorporated into their manufacturing processes (e.g., electronics, engineering, medicine, wound dressing, socks and other textiles, air filter, tooth paste, and etc.) (5,3,6).

Although the applications of NPs are growing in every field, concerns about their environmental and health impacts remain unresolved (7,8). Given the increasing

production of NPs of all types, however, the potential for their release in the environment can be transported to aquatic ecosystems and may induce malignant impacts on aquatic biota (1,3). Therefore, the aquatic ecotoxicology of engineered NPs (aquatic nanoecotoxicology) is a relatively new and evolving field.

In recent years, silver nanoparticles (Ag-NPs) due to their antimicrobial properties have been produced a lot so that 56% of NPs global production is dedicated to Ag-NPs (9,10). The limited information that is slowly emerging demonstrates that Ag-NPs cause cytotoxicity, oxidative stress, and inflammatory responses in fish and other aquatic organisms (1,11, 12). In some studies, it has been shown that the size of NPs is determinant in their uptake and thereby toxicity is reported to be reciprocally proportional to size (13). For instance, a very small concentration of silver in nanosilver provides greater effectiveness inside the body

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than bulk silver solutions in the colloidal form of many times greater concentrations (14).

It has been shown that silver ion (Ag^+) toxicity for fish is significantly less toxic in salt water than in fresh water (15). This difference is due to the high ionic strength that creates links between free silver ions and anions (e.g. silver chloride) in the salt water (16) and also the competition for gill binding sites between Ag^+ and other cations, such as Ca^{2+} , Na^+ , and Mg^{2+} , which results in decreased Ag^+ interactions at the gill and, in turn, its toxic effect (17). In freshwater, silver toxicity is induced by ionic Ag^+ which targets specific sites in fish gills. On the basolateral gill membrane, Ag^+ inhibits Na^+/K^+ -ATPase activity, decreasing the active uptake of both Na^+ and Cl^- . Thus, with the majority of ionoregulation occurring at the gills, this organ is considered the main site of acute toxic action of Ag^+ in freshwater fish (18).

Since the released nanoparticles eventually enter water ecosystems, their maximum toxicity effects could be magnified in aquatic ecosystems. On the other hand, Marinella Farré et al have demonstrated that to date there has been no information and database available on the toxic effects of Ag-NPs on marine environments animals, in comparison with freshwater ecosystems (4). Therefore, the main objective of this study was to investigate how salinity can alter the toxicity effects of colloidal silver nanoparticles in saline waters. Accordingly, rainbow trout fry (*Oncorhynchus mykiss*), as a model in aquatic ecotoxicological studies (19), was used in our study.

MATERIALS AND METHODS

Characterization of colloidal silver nanoparticle

The colloidal silver nanoparticles (Ag-NPs), brand L (Nanocid brand), were purchased from Pars Nano Nasb Co. Ltd (Tehran, Iran). This product is enrolled by United States Patent Application No: [20090013825](#) (20). Before utilizing the colloidal silver nanoparticles, the surface charge (zeta potential) of the colloidal silver nanoparticle was assayed by Zeta potential analyzer (Zetasizer-Malvern Instruments Inc, UK, Model: 3000HS_a). Also, measurements of the actual size and shape of silver nanoparticle in

stock solution were carried out using motioned zetasizer and transmission electron microscope (TEM). The concentration of Ag in Ag-NPs stock solution was assessed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Model: 3410 ARL, Switzerland). Also, colloidal Ag-NPs was added to tap water to determine whether the substance used had yellow/brown nanosized particles, scientifically well-established characteristic of silver nanoparticles solution, was formed (21).

Water and Fish Testing

Waters used in this experiment included de-chlorinated tap water (0.4ppt), and two different saline water containing 6 ± 0.3 , 12 ± 0.2 ppt salinity.

The entire stock of rainbow trout fry ($n=540$, 10-week-old; 1000 ± 35.0 mg) used for this experiment was already hatched with de-chlorinated tap water (0.4 ppt salinity).

In order to adapt the fish to the above mentioned waters, before conducting the experiment they were separated into three equal groups ($n=180$) (19) in 300-L fiberglass tanks. So, for at least one week two of the groups transferred to the waters with 6 ± 0.3 and 12 ± 0.2 ppt salinities and the third group was kept in the de-chlorinated tap water. Fish were hand-fed to three times daily on commercial trout food until 24 hours prior to use. The feeding was stopped during the test period (19). Also, some physical and chemical parameters of the waters used including total ammonium, magnesium, total hardness, total alkalinity, calcium hardness, and chloride were measured using photometer (Palintest, UK, Model: 8000). Sodium was measured using atomic absorption. In addition, pH, dissolved oxygen, and water temperature were recorded daily using digital pH meter and DO meter.

Toxicity testing

Before starting the experiment, range-finding tests in different concentrations in all of the above mentioned waters were performed because they enabled us to choose the appropriate concentration range for the definitive test (19). The main toxicity study involved 6-day static-renewal exposure. Geometric series of colloidal Ag-NPs concentrations were chosen for toxicity testing in three replicates. Ten

healthy fries were randomly selected from the stocks and transferred to circular fiberglass tanks, containing 20 liters, vigorously aerated to maintain the dissolved oxygen concentration close to saturation. Light intensity at the water surface varied between 10 and 100 lx and the photoperiod was 12:12 (light: dark) (19). Constant conditions were maintained as much as possible throughout the experiment.

Concentrations of the colloidal Ag-NPs added to the circular fiberglass tanks containing 0.4, 6 ± 0.3 , and 12 ± 0.2 ppt salinity waters were (0.25, 0.5, 1, 2, and 4 ppm), (1, 5, 10, 20, and 40 ppm) and (5, 10, 20, 40, and 80 ppm), respectively. Then adequate mixing of them was achieved by well aeration. Behavioral observations and mortality of fish were noted after 24, 48, 72, 96, 120, and 144 hours once daily throughout the experiment every day at the same time. Also, at the end of the experiment, statistical analysis of lethal concentration (LC10, 50, and 90) values and the 95% confidence

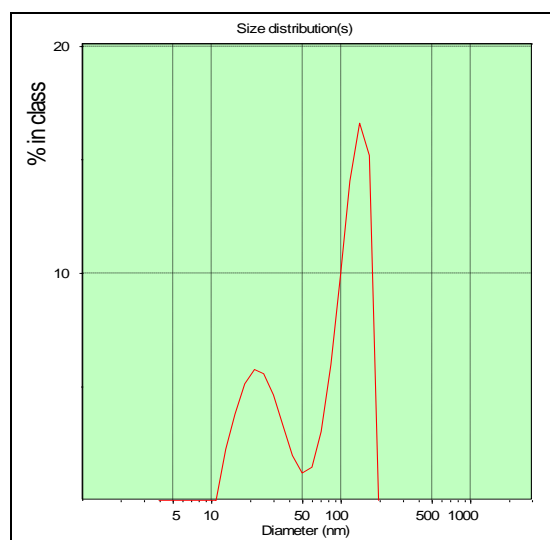


Figure 1. Size distribution in stock solution of colloidal silver nanoparticles (Ag-NPs) determined by Zeta Sizer.

Water quality

The means of de-chlorinated tap chemical values (0.4), 6 ± 0.3 , and 12 ± 0.2 ppt waters are shown in Table 1.

intervals of the Ag-NPs were estimated by using EPA Probit analysis program (version 1.5).

RESULTS

Ag-NPs Particle characterization

Zetasizer results (Figure 1) showed that Ag-NPs in colloidal solution mainly ranged in size from 3.9 to 193.8 nm and totally three classes of particles were distinguishable: 10-50nm (33.6%), 50-100nm (20.5%), and 100-165nm (45.9%). Also, the zeta average (mean particles size) of the silver particles in Ag-NPs colloid was 54.8 nm. In addition, TEM results of nanocid (Ag-NPs) from Pars Nano Nasb Co. Ltd. revealed that their size was about 25.90 ± 8.44 nm (Figure 2). Zeta potential of Ag-NPs solution had an average of $+1.03\pm 0.13$ mV. The actual concentration of Ag in Ag-NPs colloid was measured of $3980\mu\text{g}/\text{ml}$.

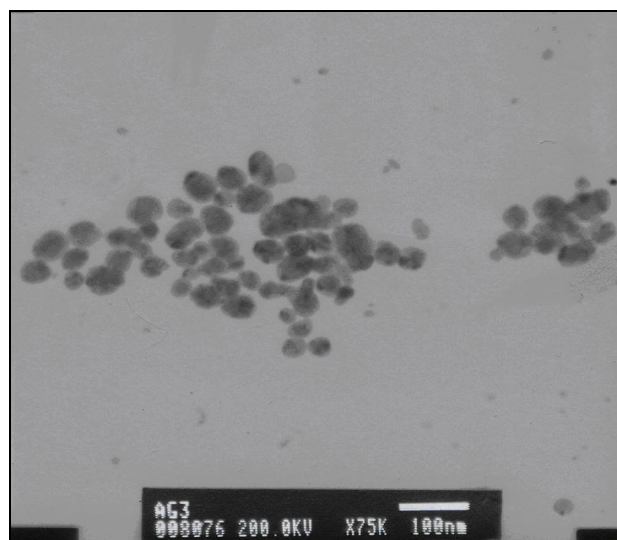


Figure 2. TEM photograph of Ag-NPs obtained from Pars Nano Nasb Co. Ltd.

During the experiment, the means of temperature, dissolved oxygen, and pH of the water in all of the fiberglass tanks were 14-15(°C), $9\pm 0.3\text{mg}/\text{l}^{-1}$, and 7.9-8.2, respectively.

Table 1. Some chemical properties of the experimental waters (0.4, 6± 0.3, and 12± 0.2 ppt) used in experiment

Salinity (ppt)	Magnesium (mgL ⁻¹)	Total alkalinity (mgL ⁻¹)	Total Ammonium	Chloride (mgL ⁻¹)	Sodium (mgL ⁻¹)	CaCO ₃ (mgL ⁻¹)	HCO ₃ ⁻ (mgL ⁻¹)
0.4	41	326	0.1	1.7	13.8	102	320
6 ± 0.3	51	254.5	0.2	3.9	1857.5	138	290
12 ± 0.2	72	183	0.5	5.2	3084.8	176	231

Lethal concentrations (LC) and observed effects of Ag-NPs on fish and water

In fry rainbow trout, following 6 days of colloidal silver nanoparticle exposure in waters with 0.4, 6±0.3, and 12±0.2 ppt salinities showed that increasing salinity significantly reduced the

acute toxicity of colloidal Ag-NPs to fry rainbow trout. As amounts of median lethal concentrations (LC50) caused by colloidal Ag-NPs in 0.4, 6±0.3, and 12±0.2 ppt salinity waters at 96, 120, and 144 h were (2.08, 1.85, and 1.66 ppm), (19.58, 16.25, and 15.03 ppm) and (41.79, 40.09, and 32.88 ppm), respectively (Table 2).

Table 2. Magnitudes of lethal concentrations (LC10, 50, and 90) in 0.4, 6±0.3, and 12±0.2 ppt salinity waters caused by colloidal silver nanoparticles in rainbow trout (*Oncorhynchus mykiss*)

Toxicity	LC ₁₀ (ppm)						LC ₅₀ (ppm)						LC ₉₀ (ppm)					
	24	48	72	96	120	144	24	48	72	96	120	144	24	48	72	96	120	144
0.4	-	-	1.11	0.76	0.65	0.45	-	-	2.21	2.08	1.85	1.66	-	-	4.41	5.65	5.23	6.11
6 ± 0.3	21.69	14.41	9.28	6.23	5.15	5.01	34.27	23.18	21.41	19.58	16.25	15.03	54.16	37.28	49.39	61.46	51.48	45.08
12 ± 0.2	34.02	33.16	25.70	25.70	24.71	17.33	51.03	48.61	41.79	41.79	40.09	32.88	77.59	71.27	67.95	67.95	65.05	62.37

Raising the salinity from 0.4 to 6±0.3 increased 144-h LC50 from 1.66 to 15.03 ppm. Moreover, at 12±0.2 ppt, another significant decrease in mortality, 144-h LC50 32.88, was observed between the 6±0.3 and 12±0.2 ppt (Figure 3). The 96 hour median lethal concentration (LC50) of colloidal Ag-NPs in 12±0.2 ppt salinity was almost 20 and 2 times greater than 0.4 and 6±0.3ppt salinities, respectively. However, there were significant differences in acute toxicity of colloidal Ag-NPs among the fish residing in 0.4, 6 ± 0.3, and 12 ± 0.2 ppt salinities (Figure 3).

Lethal concentrations (LC10, 50 and 90) of colloidal Ag-NPs in 0.4, 6±0.3, and 12±0.2 ppt salinity waters on rainbow trout fry (Figure 3).

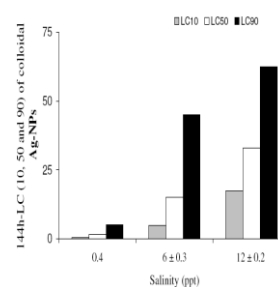


Figure 3. Lethal concentrations (LC₁₀, LC₅₀ and LC₉₀) of colloidal Ag-NPs in 0.4, 6±0.3, and 12±0.2 ppt salinity waters on rainbow trout fry

Furthermore, maximum allowable toxicant concentration (MATC), lowest observed effect concentration (LOEC), and NO observed effect

concentration (NOEC) of the colloidal Ag-NPs were calculated from the LC (19) (Table 3).

Table 3. Magnitude of MATC, NOEC, and LOEC of colloidal Ag-NPs during 96 hours for experimental rainbow trout fry in 0.4, 6 ±0.3, and 12±0.2 ppt salinity waters.

Salinity (ppt)	LOEC (mg l ⁻¹)	NOEC (mg l ⁻¹)	MATC (mg l ⁻¹)
0.4	0.76	0	0.2
6 ± 0.3	6.23	1	1.96
12 ± 0.2	25.70	10	4.18

Inhabitant fries exposed to colloidal Ag-NPs in 6±0.3 and 12±0.2 ppt salinities showed different responses and behaviors compared to those exposed to analogous levels of colloidal Ag-NPs in 0.4 ppt. As a significant increase in mucous production (slimy coating) and respiratory problems were observed in resident fry group in water with 0.4 ppt salinity compared with other groups. This mucus production with Ag-NPs aggregation was found most clearly and resembled in the gill. Also, we observed significant changes in fish (fry) behavior when exposed to 20 and 40 ppm of the Ag-NPs in waters with 6±0.3 and 12±0.2 ppt salinities, respectively. As our observations included repeated collisions with the tanks, swimming in circles at the water surface, losing a herd swimming, increasing the size of eyes and also having black skin. But the control groups appeared normal throughout the test period. Moreover, after the addition of colloidal Ag-NPs to the waters with 6±0.3 and 12±0.2 ppt salinities, different colors were observed in the water. With the appearance of water in the tanks with 6±0.3 and 12±0.2 ppt salinities, the same concentration of colloidal silver nanoparticles was added to them which were brownish and black, respectively. Also, fast coagulation and aggregation of colloidal Ag-NPs in waters with 6±0.3 and 12±0.2 ppt salinities, in doses higher than 10 ppm of Ag-NPs, occurred within an hour after adding the Ag-NPs stock to these waters. Finally, different concentrations of sediment with black and brownish colors on the bottom of the tanks were observed in 6±0.3 and 12±0.2 ppt salinities, respectively. In the final days of experiment these waters were transparent.

DISCUSSION

The purpose of this study was to evaluate the relationship between salinity and toxicity of silver nanoparticles in the rainbow trout as a model in the water ecosystem. Increasing salinity reduced the acute toxicity sign of silver nanoparticles on rainbow trout fry, so that the amounts of toxicity of silver nanoparticles in 12 ±0.2 ppt water salinity according to 96h-LC50 were 20 and 2 times greater than 6±0.3ppt and fresh water, respectively.

Kittler et al (2010) showed that a large portion of the toxicity of silver nanoparticles is created by silver ions emitted from the surface of these nano-materials (22). So it seems that mechanisms of toxicity by silver nanoparticles are largely similar to the mechanism of silver ion toxicity. Several chemical and physiological factors can affect the toxicity levels of silver ions. Some chemical parameters of water, such as dissolved organic matter (23), calcium carbonate (24), thiosulfate (25), and chloride (18), can reduce the toxicity of silver ions in aquatic environments. It has been reported that salinity is the most important chemical factor in reducing silver toxicity (17) due to the high tensile forces, particularly Cl⁻ ion, which reduced the toxicity of silver ion greatly (3,26). A relatively large amount of information has been reported on the reduced toxicity of silver ions with increased salinity in fish simultaneously (17, 27, 28, 29, 30). By increasing Cl⁻ followed by increased salinity, the relative contribution of each silver-chloro compounds (AgCl_(aq), AgCl, AgCl²⁻, and AgCl³⁻) increases in the saline water (27, 29, 31). Silver-chloro compounds, with the exception of soluble form (AgCl_(aq)), in comparison with the silver ions is less toxic.

Other silver-chloro compounds after formation in salt water environments can be deposited and taken out of the water column and available aquatic organisms and ultimately reach the surface sediments (27). When a lot of silver enters the brackish water suddenly, a substance called cerargyrite ($\text{AgCl}_{(s)}$) is formed (26, 23, 32, 33). Cerargyrite is a brown and insoluble material that has 3.75% of the silver ions and 7.24% of the chloride ions in its structure.

During this study it was shown that one hour after entering the different colloidal silver nanoparticles doses into the 6 ± 0.3 and 12 ± 0.2 ppt waters, the brownish and black sediments were formed at doses higher than 10 ppm. Thus it seems that in 6 ± 0.3 ppt and 12 ± 0.2 ppt waters at high doses, large amounts of colloidal silver nanoparticles were formed in the cerargyrite that got out of the water column. In the fresh water environments, due to low ion chlorine, chloride-silver forms were not present. Therefore, after entering silver ions into the fresh water ecosystems, they remained free ion silver (Ag^+), the most toxic silver type, in the water column and cause toxicity in aquatic organisms (32). In various studies, the toxicity mechanisms caused by silver compounds in fresh and salt water ecosystems have been studied (19, 23, 25, 27, 34, 35). In fresh water, the silver ions with impaired of Na^+/K^+ ATPase activity (17, 25, 34) and reduced gill carbonic anhydrase enzyme led to the inhibition of chloride and sodium ions absorption by the gills and acidosis of fish blood (26, 31). In addition, the silver ion reducing

activity of liver amino-transferase, a family of enzymes involved in nitrogen metabolism, increased plasma ammonia and finally led to toxicity in fish (26). Due to the combination of divalent cations such as Ca^{2+} and Mg^{2+} with bicarbonate ions (HCO_3^-) carbonate pellets, Ca-Mg-carbonate mineral, are formed in the intestines of marine fish or fish acclimated to salt water (35, 36). Some studies have shown that the carbonate pellets are involved in the detoxification of various pollutants such as silver ions (17, 37). Also, in the present study, the formation of carbonate pellets in the intestines of the fry acclimated to 6 ± 0.3 and 12 ± 0.2 ppt waters probably led to the sublethal toxicity of silver nanoparticles. Thus, considering that the toxicity of silver nanoparticles is largely related to silver ions generated from its surface and noticing the existence of a negative correlation between increasing salinity and toxicity of silver ions, the results of this study can confirm the inverse relationship between the amount of water salinity and toxicity of silver nanoparticles in rainbow trout fry. According to the toxicity classification of chemical materials (U.S.EPA) (38) (Table 4) and also based on data obtained in this study (based on LC_{50} 96 hours), it is suggested that silver nanoparticles in fresh water be classified as moderately toxic substances and 6 ± 0.3 and 12 ± 0.2 ppt water salinities be classified as slightly toxic substances for rainbow trout fry.

Table 4. Ecotoxicity categories of materials for aquatic organisms according to U.S.EPA

Concentration (ppm)	Toxicity Category
<0.1	very highly toxic
0.1 - 1	highly toxic
>1 - 10	moderately toxic
>10 - 100	slightly toxic
>100	practically nontoxic

While the maximum acceptable toxicant concentration of colloidal Ag-NPs is 10% of the LC_{50} 96h, the acute risk criteria for aquatic species are 1/10 the LC_{50} and 1/2 the LC_{50} for the sensitive species in aquatic ecosystems.

Below 1/10 the LC_{50} , the risk is seen acceptable. Between 1/10 LC_{50} and 1/2 the LC_{50} the risk is assumed to be mitigated by restricted use status. Moreover, above 1/2 LC_{50} , the risk is assumed unacceptable.

CONCLUSION

Silver nanoparticles have higher toxicity effects on fresh water than salt water, so release of silver nanoparticles into fresh water ecosystems, can lead to more biological, physical, and chemical irrecoverable effects on the ecosystems and their fishes in comparison with saline water ecosystems.

Due to lack of comprehensive studies on the physiological processes related to the toxicity of silver nanoparticles in salt and fresh waters, definitive and explicit comments on the mechanism of toxicity of silver nanoparticles in fresh water and salt waters are not plausible. Thus, identifying factors and physiological mechanisms affecting the relationship between the chemical properties of water and toxicity of nanoparticles, such as silver nanoparticles, in various aquatic organisms should be considered more.

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