



The Impact of Colloidal Silver on Natural Communities of Juvenile Fishes After Short-term Exposure

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ABSTRACT: Many consumer products, such as sports clothes, cosmetics, and even food containers contain tiny silver particles. This could end up in the water, possibly harming the aquatic life. Recognizing the significance of the life stages of fish in aquatic ecosystems as well as the food web concern, the effect of colloidal nanosilver in consumer products was tested in juvenile fishes of a pond ecosystem. Fishes were exposed to colloidal nanosilver at different concentrations of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, and 5µg/ml. The estimated LC₅₀ and LC₉₀ values were 3.16 and 6.70 µg/ml respectively for the juvenile fishes, revealing higher sensitivity even in lower concentrations. It is also established that with the increase in concentration of colloidal silver solution mortality of fishes also increases from different Experimental trials. It was found that there was a positive relationship between mortality and concentration levels. Thus, colloidal nanosilver can cause toxic effects which means that the release of colloidal nanosilver into the aquatic environment or its direct application as an antimicrobial agent in consumer products should be reduced or no longer be allowed.

Keywords: Antimicrobial, Ecotoxicity, Nanosilver, LC₅₀, LC₉₀.

INTRODUCTION

The increasing applications of silver nanomaterials and their inevitable release posed great potential risks to aquatic organisms and ecosystems. Considerable attention has been attracted on their behaviors and transformations, which were critically important for their subsequent biological toxicities and ecological effects. Due to their superior antimicrobial activities and advanced catalytic properties, nano-Ag has been widely applied in the medical, food industry, environmental remediation, and energy fields (Reddy *et al.*, 2016). Nanosilver has unique physiochemical properties and strong antimicrobial activities. Although silver ion release is likely the main antimicrobial mechanism of nanosilver, the contributions of silver nanoparticles and reactive oxygen species generation to the overall toxicity of nanosilver must not be neglected. Several research directions are proposed to better understand the dissolution kinetics of nanosilver and its antimicrobial mechanisms under various aquatic environmental conditions. Because of its strong antimicrobial activities, silver is regulated by various environmental regulations as a toxic heavy metal. The extensive application leads to an inevitable release of nano silver into the environment during manufacturing,

transportation, use, and disposal, which has triggered much interest on their exposure risks to organisms and humans (Schäfer *et al.*, 2011). It has also been shown that silver nanoparticles can pose significant burdens to the environment from life cycle emissions associated with their production, but these impacts must be considered in the context of actual products that contain nanosilver. The increasing use of silver nanoparticles in consumer products as antimicrobial agents has prompted extensive research toward the evaluation of their potential release to the environment and subsequent ecotoxicity to aquatic organisms. Colloidal silver can have detrimental effects on plants, animals, and microorganisms, once silver nanoparticles enter the environment, it is very difficult to detect and measure them Nawaz *et al.* (2019).

In the present investigation attempts to study the detrimental effects of nanosilver particles on the freshwater juvenile fishes in a selected pond. It also aims to assess how the fish is affected by different concentrations of colloidal silver. Different concentrations of colloidal nanosilver were used to test how the juvenile fishes react to it. The juvenile fishes were small, easy to handle, and easily available. Detailed studies regarding the effect of nanosilver

particles on aquatic life were found to be less. Hence the present study was carried out to assess the detrimental effect of nanosilver particles present in various consumer products on organisms.

MATERIALS AND METHODS

For the present study, the effect of colloidal nanosilver in consumer products on pond life, the juvenile fishes were collected from a pond near Peyadu, Thiruvananthapuram district, Kerala, India. They were collected using net and cloth. The collected samples were checked to sort out healthy juvenile fishes (which showed active movements) and those that were almost identical in size were selected and transferred into separate plastic storage bottles with proper aeration. An adequate amount of pond water was also collected for the experiment in the laboratory. The substance used for the study was 250ppm colloidal silver solution [Natural Path Silver Wings colloidal silver mineral supplement, 250 ppm). Different concentrations of nanosilver solutions were prepared. The concentrations selected for the study were 1 µg/ml, 2 µg/ml, 3 µg/ml and 4 µg/ml, 5 µg/ml. A total of 180 juvenile fishes collected from the pond were added to 6 beakers with 30 fishes each. Experiment was monitored for half an hour, one hour, one and a half hour, two hour, two and a half hour, three hour, three and a half hour and four hour. The live and dead juvenile fishes in each beaker were counted and recorded.

RESULTS AND DISCUSSION

The mortality rates of the juvenile fishes collected fish and the corresponding concentrations of colloidal nanosilver are represented in Table 1. In the concentration of 1 µg/ml of colloidal silver, up to one hour no mortality was observed. After 1.30 hours two fish were died and 28 were alive. No more mortality was observed in 2 hours, 2.30 hours, 3 hours, 3.30 hours and 4 hours of exposure time (Table 1). In the concentration of 2µg/ml of colloidal silver there were no mortality within the first hour. However, after 1.30 hours, six fishes were dead and 24 remained alive. After 2 hours, 2 more mortalities were recorded, leaving 22

alive. At the 2.30 hours of exposure time, two more fishes were dead, with 20 remaining alive. After 3 hours, four more fishes showed mortality, leaving 16 alive. After 3.30 hours, 2 more deaths were observed with only 14 fish remaining alive. Finally, at the 4 hours of exposure, a total of 22 fishes were dead, leaving only 8 fish alive (Table 1). In the concentration of 3µg/ml of colloidal silver, complete mortality was observed after 2.30 hours of exposure (Table 1). In the concentration of 4µg/ml and 5µg/ml of colloidal silver, complete mortality was observed in the 4.00 hours of exposure (Table 1). The probability of mortality at different concentrations of colloidal silver during different exposure time is given in Fig. 1.

The results of LC₁₀, LC₃₀, LC₉₀, LC₇₀, and LC₉₀ are represented in Table 2. Based on the results the LC50 value calculated was 3.16 mg/L. The LC10, LC30, LC70 and LC90 values obtained in the present study were 1.48 mg/L, 2.14 mg/L, 6.30 mg/L and 6.70 mg/L respectively. The obtained results indicated that a lower concentration of zinc oxide nanoparticles is needed to kill 50% of the fish population and it belongs to the low toxicity category (Helfrich *et al.*, 2009). In the present study, the observed percentage of mortality of juvenile fish for different concentration of colloidal silver was found to be high and maximum for concentrations 3 to 5 µg/ml. It is also evident from the present study that as the concentration increases the rate of mortality also increases. Similar results were reported by Reed *et al.* (2016) ; Shaw and Handy (2011).

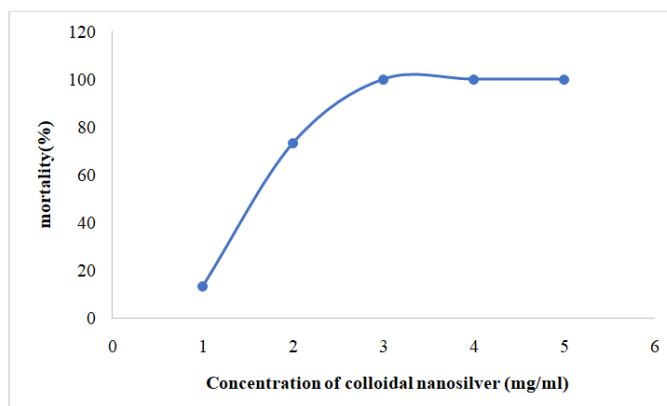
Silver nano particles are likely to enter the aquatic environment because of their multiple uses Shaw and Handy (2011). The pond from where the water is collected and used for the present study is also used for many domestic purposes like washing clothes, bathing etc. According to Benn and Westerhoff (2008); Westerhoff *et al.* (2015) the nanosilver leeches out of clothes and escapes through the waste water treatment systems into nearby lakes, rivers and streams, it could damage aquatic ecosystems. According to Oberdörster (1996) once the silver ions get into the gills of fish, it is a pretty efficient killer.

Table 1: Mortality rate of juvenile fishes exposed to different concentrations of colloidal silver.

Time	Control		Different Concentrations of Colloidal Silver Solution									
			1 µg/ml		2 µg/ml		3 µg/ml		4 µg/ml		5 µg/ml	
	live	Dead	live	Dead	live	Dead	Live	Dead	live	Dead	Live	Dead
0.00m	30	0	30	0	30	0	30	0	30	0	30	0
30m	30	0	30	0	30	0	26	4	28	2	30	0
1.00hr	30	0	30	0	30	0	22	4	28	0	28	2
1.30hr	30	0	28	2	24	6	18	4	26	2	18	10
2.00hr	30	0	28	0	22	2	8	10	18	8	16	2
2.30hr	30	0	26	2	20	2	0	8	10	8	12	4
3.00hr	30	0	26	0	16	4	0	0	6	4	6	8
3.30hr	30	0	26	0	14	2	0	0	4	2	4	2
4.00hr	30	0	26	0	8	6	0	0	0	4	0	4

Table 2: Lethal concentrations of colloidal silver for juvenile fishes exposed to colloidal silver.

Lethal concentration	LC ₁₀	LC ₃₀	LC ₅₀	LC ₇₀	LC ₉₀
Values (µg/ml)	1.48	2.14	3.16	6.30	6.70

**Fig. 1.** The probability of mortality at different concentrations of colloidal silver during different exposure time.

There are many studies evaluating the behavior of silver nano textiles during washing Geranio *et al.* (2009). The biological effects and the routes of uptake of silver nano particles to organisms are evaluated by Fabrega *et al.* (2011). Mechanisms of toxicity of nano silver particles are still poorly understood although it seems clear that in some cases nanoscale-specific properties may cause biouptake and toxicity over and above that caused by the dissolved Ag ion Johari *et al.* (2013). The antimicrobial effects of minute silver particles are reported by Nowack (2010). In the present study also the mechanism of toxicity is not clear and it may be due to the adverse effect of nano particles to the important biomolecules like proteins and nucleic acids. According to Arvidsson *et al.* (2012) silver nanoparticles can have a severe environmental impact if their utilization in clothing continues to increase. According to Donaldson and Stone (2003) silver nanoparticles are more dangerous for aquatic ecosystems than silver ions. Hence from the present study, it is revealed that nano particles in different consumer product are toxic to aquatic life.

The present study also attempts the estimation of LC₅₀ and LC₉₀ values. It is very important to calculate the extent of toxicity in bioassay studies. In to the present studies the LC₅₀ (3.16µg/ml) and LC₉₀ (6.70µg/ml) values were reached in relatively lower concentrations. It is evident that there was a positive relationship between mortality and concentration levels of colloidal silver solutions. Toxicity studies to estimate LC₅₀ and LC₉₀ values indicated that the rate of mortality increases with increase in concentration Nawaz *et al.* (2019); Reed *et al.* (2016).

It also revealed that the toxicity potential of colloidal nanosilver solution is very low in the present study. Mortality rate in the present study serve as direct evidence for the negative influence of colloidal silver solution on the aquatic ecosystem. Hence, the toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems.

CONCLUSIONS

It has long been known that, in the form of free ions, silver particles can be highly toxic to aquatic organisms. There is a lack of detailed knowledge about the doses required to trigger a response and how the organisms deal with this kind of stress. In the past, silver mostly found its way into the environment in the vicinity of silver mines or via wastewater emanating from the photo industry. More recently, silver nanoparticles have become commonplace in many applications as ingredients in cosmetics, food packaging, disinfectants, and functional clothing. As per the comparative study of different concentrations of colloidal nanosilver in each experiment trails, it is evident that with the increase in concentration of colloidal silver solution, mortality of fishes also increases.

FUTURE SCOPE

Colloidal nanosilver can cause toxic effects and its release into the aquatic environment is harmful for the aquatic life. It may leads to biodiversity depletion and hence its direct application as an antimicrobial agent in consumer products should be reduced or no longer be allowed.

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Conflict of Interest. None.

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