# Determination of lethal concentration ($LC_{50}$) of silver nanoparticles produced by biological and chemical methods in Asian seabass fish

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**Abstract**

Nanotechnology is a technology that originates from the reactions and reactions that occur at the atomic level and is a new revolution for all future sciences. The aim of this study was to investigate the lethal concentration of silver nanoparticles produced by biological method from Sargassum algae and commercial silver nanoparticles produced by chemical method in Asian sea bass fish. The fish were exposed to different concentrations of the two types of nanoparticles in a 30-liter aquarium for 96 h and their mortality was recorded every 24 h. After mortality was recorded, lethal concentration was calculated using probit test in SPSS software. According to the results, the mean lethal concentrations of silver nanoparticles were calculated for biological and chemical nanosilver respectively 19.669 and 1.569 mg/L, respectively. The results showed that with increasing concentration of silver nanoparticles as well as exposure time the percentage of mortality in fish increased. The highest mortality was observed at the highest concentration of silver nanoparticles.

**Keywords:** Toxicity, Biosynthesis, Silver Nanoparticles, Asian Sea Bass

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## Introduction

The term nanotechnology was first coined in 1974 by Japanese scientist Nario Taniguchi under the title "The Main and Basic Concept of Nanotechnology" in a newspaper. The first nanotechnology guidelines were developed by KEric Drexler, who introduced nanotechnology to the public through the publication of The Engines of Creation (Mnyusiwalla *et al*., 2003). Silver-containing nanomaterials have been widely considered in various industries for their outstanding properties such as good conductivity, chemical stability, catalytic and antibacterial activity (Moreno-Garrido *et al*., 2015). Products containing silver nanoparticles are likely to release dissolved silver and silver nanoparticles into the environment, which are likely to be stable and bioaccumulative (Gottschalk and Nowack, 2013). Therefore, the toxicity of silver ions released from silver nanoparticles is a cause for concern because silver ions are typically considered to be the most toxic form of silver. The duration of the lethal concentration test is 96 hours and the mortality rate is calculated at 24, 48, 72 and 96 hours. To determine the toxicity of a new chemical in aquatic life, it is first necessary to estimate the average lethal concentration ($LC_{50}$) of that chemical in the water. This is done through acute toxicity testing on organisms exposed to the chemical (Hedayati and Jahanbakhshi, 2012). The Asian sea bass (*Lates calcarifer*) is euryhaline and belongs to the Latidae family, which is of commercial and fishery importance in many parts of the world and is fully developed in the aquaculture industry (Szűcs *et al*., 2018 ), so due to the development of nanotechnology In the world and the possibility of increasing its entry into aquatic environments, it is necessary to investigate its toxicity on this breeding species.

## Materials and methods

For this study, Asian sea bass fry were prepared from Ramoz Fish Breeding Company in Bushehr and transferred to the Research Laboratory of the Aquatic Health Department of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. After transferring the fish, the operation was initially adapted to laboratory conditions for 2 weeks, during which time 2% of body weight twice (morning and evening) with commercial concentrate food for imported sea bass (Australia) were fed. To determine the lethal concentration ($LC_{50}$) of each nanoparticle, first a pilot study was performed on two types of silver nanoparticles and then based on the pilot study, the required concentrations in the study were determined. The lethal range was determined based on the lowest concentration at which 96 casualties were observed, and the first concentration at 100% mortality. To do this, 5 pieces of fish in each 30-liter aquarium, equipped with an aeration system, are transferred and with ascending concentrations of silver nanoparticles synthesized from sargassum algae and commercial silver nanoparticles manufactured by US Research Nanomaterials, Inc. The United States faced. During the $LC_{50}$ determination test, mortality was monitored continuously, and fish were considered dead when they did not show gill cap movements and response to mechanical stimuli (Bilberg *et al*., 2012). After conducting a preliminary test and determining the lethal range, the $LC_{50}$ determination test was performed according to the standard O.E.C.D method in 1998, stationary for 96 hours (Rasmussen *et al*., 2018). After recording the losses, the $LC_{50}$ was determined at 24, 48, 72 and 96 hours with a 95% confidence interval using SPSS software version 21 and and standard probit test.

## Results

The results related to the number of losses in increasing concentrations of the two types of nanoparticles are given in Tables 1 and 2. As shown in Tables 1 and 2, the concentrations causing zero to 100% of the losses in chemical and biosynthetic nanoparticles were 1 to 8 and 10 to 60, respectively. No losses were observed in the control group that did not contain silver nanoparticles. When exposed to chemically produced silver nanoparticles, the onset of losses was 24 hours at a concentration of 2 mg/L (Table 1), but when exposed to bio-produced silver nanoparticles, the losses started at 24 hours and At a concentration of 40 mg/L (Table 2). As you can see in Tables 1 and 2, with increasing the concentration of silver nanoparticles, the amount of losses in fish has increased. In terms of exposure time, the highest number of casualties was obtained in 96 hours. The results of the lethal concentrations of two types of nanoparticles at different times are given in Figure 1. According to this diagram, the lethal concentrations of silver nanoparticles produced by chemical and biological methods were calculated to be 1.569 and 19.669 mg/L, respectively. As you can see in this diagram, chemically produced nanoparticles are more toxic than biodegradable methods.

**Table 1: Results related to the toxicity of chemically synthesized silver nanoparticles in Asian marine bass fish during exposure to different concentrations of nanoparticles.**

| Chemical nanosilver concentration (ppm) | 24h | 48h | 72h | 96h |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 2 | 3 |
| 2 | 1 | 3 | 5 | 5 |
| 4 | 4 | 5 | 8 | 10 |
| 8 | 6 | 8 | 8 | 10 |

**Table 2: Results related to the toxicity of biosynthesized silver nanoparticles in Asian marine bass fish during exposure to different concentrations of nanoparticles.**

| Biosynthetic nanosilver concentration (ppm) | 24h | 48h | 72h | 96h |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 1 | 2 |
| 20 | 0 | 1 | 2 | 4 |
| 40 | 2 | 4 | 6 | 8 |
| 50 | 6 | 8 | 10 | 10 |

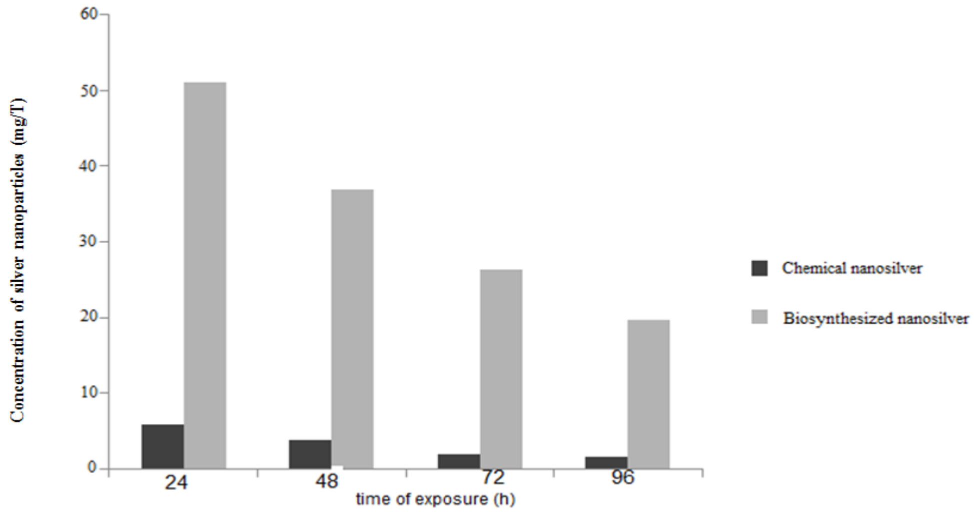


**Figure 1: Lethal concentration ($LC_{50}$) of silver nanoparticles synthesized by chemical and biological methods in Asian sea bass.**

## Discussion

The results showed that with increasing the concentration of silver nanoparticles and increasing the exposure time, the mortality rate increased, because one of the factors affecting aquatic poisoning, in addition to the concentration of toxin, is the duration of exposure to the toxin (Sharifpour *et al*., 2003). According to the results, the average lethal concentration during 96 hours of exposure to chemically and biologically synthesized silver nanoparticles in Asian sea bass (*Lates calcarifer*) was determined to be 1.569 and 19.569 mg/L, respectively, indicating toxicity. Chemically synthesized silver nanoparticles are higher than bio-synthesized silver nanoparticles. In the study of Bita *et al.* (2016), the median lethal concentration of silver nanoparticles synthesized from sargassum algae on common carp (*Cyprinus carpio*) was determined to be 11.34 mg/L (Bita *et el*., 2016). In other stuby, the level of LC50 in common carp in the face of two types of silver nanoparticles under the brand name Nanosil (less than 100 nm) and Nanocid (18 nm) respectively 73.8 and 0.43 mg/L, respectively (Hedayati *et al*., 2012), that one of the reasons for the difference in the 50% lethal concentration was the difference in the size of nanoparticles, in addition, it was found that silver nanoparticles synthesized biologically are less toxic than chemical methods (Bilberg *et al*., 2012). Ostaszewska *et al*. (2016) Reported a median lethal concentration of LC50 of 96 hours during exposure of the Siberian sturgeon (*Acipenser baerii*) to silver nanoparticles at 15.03 mg/ L. Comparing the results of different researchers with our research study shows that the toxicity of nanoparticles produced varies significantly depending on the experimental species, the type of nanoparticle production method, the administration method and the experimental environment in which the organisms are raised (Lekamge *et al*., 2018).

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