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# Bioassay on Metal Silver and Toxicity Studies in Freshwater Fish "*Cyprinus carpio*"

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**Abstract:** ***Silver is a noble metal that has a high electrical conductivity, heat stability and special properties. Silver is present in different forms in the environment especially in the living organisms. Silver has been utilized in jewellery, monetary currency and photography and is known to have an antibacterial effect. The toxic effect or heavy metal poisoning is defined as, "a functional or morphologic change in the body produced by an ingested, injected, inhaled or absorbed drug, chemical, or biological agent". Argyrosis is pathologic bluish-black pigmentation in a tissue resulting from the deposition of an insoluble albuminate of silver. Fish are ideal sentinels for behavioral assays of various stressors and toxic chemical exposure due to their constant, direct contact with the aquatic environment where chemical exposure occurs over the entire body surface. Toxicological studies of the pollutants upon aquatic organisms are very important from the point of environmental consequences. Bioassay can be defined as a test to evaluate the relative potency of a chemical by comparing its effect on a living organism with that of standard preparation. In the present investigation the median lethal concentration (LC 50) of Silver to an Indian major carp Cyprinus carpio for 24 and 96 hours were found to be 1.5 mg/L and 0.75 mg/L.***

**Keywords:** Silver, *Cyprinus carpio*, Bioassay

## 1. Introduction

Silver is a naturally occurring precious metal, most often used as mineral ore in association with other elements. It has been positioned as the $47^{th}$ element in the periodic table, having a atomic weight of 107.8 and two natural isotopes 106.90 Ag and 108.90 Ag with abundance 52 and 48%. Silver nanoparticles in the aquatic environment is estimated to about 0.01 $\mu gL^{-1}$ (Tiede *et al.*, 2009), the discharge of silver nanoparticles undoubtedly increase. Prior to recent nanotechnology, silver has been utilised in jewellery, monetary currency and photography (Chen and Schluesener, 2008), and is known to have an antibacterial effect (Atiyeh *et al.*, 2007). Silver is furthermore, discharged to the aquatic environment from leaching, mining and anthropogenic sources (Purcell and Peters, 1998), and the concentration of silver (excluding nanoparticles) in the aquatic environment is <0.01-100$ngL^{-1}$ (Rozan *et al.*, 1995; Wen *et al.*, 1997). The toxicity of a metal is influenced by several factors like solubility, binding specificity to a biological site. The toxic effect or heavy metal poisoning is defined as, "a functional or morphologic change in the body produced by an ingested, injected, inhaled or absorbed drug, chemical, or biological agent". In contrast, Westhofen *et al.* (1986) observed that the affinity of silver for membrane and neuronal structures and the deposition of silver as an insoluble compound ($Ag^2S$) induce the progression of clinical diseases like generalized argyria. Argyrosis is a pathologic bluish-black pigmentation in a tissue resulting from the deposition of an insoluble albuminate of silver. Even in its bulk form, silver is extremely toxic to fish (Hogstrand *et al.* 1996) algae, some plants, fungi (Eisler *et al.* 1996), crustaceans and bacteria like nitrogen fixing heterotrophic and soil forming chemolithotrophic bacteria (Albright *et al.* 1974).

Recent evidence indicates that fish, an extremely valuable resource, are quickly becoming scarce. One consequence of this scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are safe for fish and other aquatic life. Fish are ideal sentinels for behavioral assays of various stressors and toxic chemical exposure due to their 1) constant, direct contact with the aquatic environment where chemical exposure occurs over the entire body surface, 2) ecological relevance in many natural systems (Little *et al.*, 2001), 3) ease of culture, 4) ability to come into reproductive readiness (Henry and Atchison, 1986) and 5) long history of use in behavioral toxicology. Silver is present in different forms in the environment especially in the living organisms. The most common is metallic silver, silver salts (ionic silver), silver complexes and colloidal silver. Metallic silver dissolves in acids and salts like nitrate and silver nitrate is formed. Aqueous solution of soluble silver nitrate contains silver in the form of hydrated silver cations $Ag(H_2O)_n^+$ which is typical "ionic silver". However, silver cation can be complexed with various organic ligands and even if silver cation is still present in the molecule, the overall charge of the complex can be neutral. Furthermore, highly stable complexes are known which are not dissociated in the solution or biological liquids.

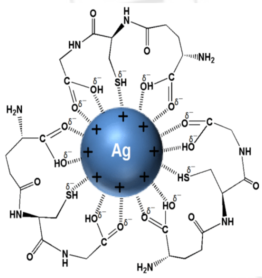
Bioassay can be defined as a test to evaluate the relative potency of a chemical by comparing its effect on a living organism with that of standard preparation. Toxicological studies of the pollutants upon aquatic organisms are very important from the point of environmental consequences. Acute toxicity is an important tool in bioassay studies. The objective of acute toxicity is to determine the concentration of a test material (Toxicant of a metal) on the level of an agent (Temperature and pH) that produces a deleterious effect on a group of test organisms during short term exposure under controlled condition. The effects of pollutants on a population can be better understood and predicted by studying the sublethal effects on an individual. Sublethal effects in fish allow us to define toxicity of the environment and understand the potential danger of pollutants imputs. The most important constant is the LC50 (median lethal concentration which is the metal value causing 50% mortality in fish population being studied. The LC50 derived by observation (ie 50% of the test organisms were cause in a concentration) interpolation on calculation.

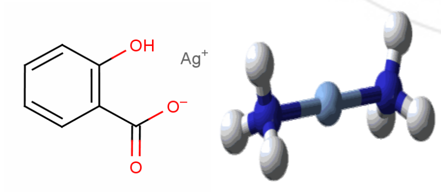
Mortality increased with increasing concentrations of ionic silver. The 24-hour silver ion $LC_{50}$ was estimated to 28 μg $L^{-1}$ (95% CL = 26–31 μg $L^{-1}$) and the estimated 48-hour $LC_{50}$ was 25 μg $L^{-1}$ (95% CL = 23–26 μg $L^{-1}$). In Cyprinus carpio the 48-hour nanosilver $LC_{50}$ value has been found to be 6.05 mg $L^{-1}$ exposed to metal oxide-coated silver nanoparticles with a size of 43.5 and 21 nm in suspension (Griffith *et al*., 2008). In another study on *zebrafish* and in Cyprinus carpio, Choi *et al*. 2010 reported the 24-hour $LC_{50}$ to be 250 mg $L^{-1}$ for 5–20 nm silver nanoparticles, which is far less toxic than the $LC_{50}$ value found by Griffith *et al.*, 2008. In Japanese medaka (*Oryzias latipes*), the 96-hour $LC_{50}$ has been demonstrated to be 34.6 μg $L^{-1}$ for 50 nm uncoated silver particles (Chae *et al*., 2009). In fish researches, few ecotoxicological studies on aquatic organisms have been performed, so in current study conventional median lethal concentration tests were conducted on the Common carp, as they provide insights to the potential toxic effects on aquatic environments and introduce most toxicants material.

## 2. Toxicant

Silver (Ag) was used as a toxicant in the present investigation. Analytical grade of Silver (Ag) was obtained from (CAS no. 7440-22-4, purity> 99%) chem. Industry.com.

## 3. Chemical Structure





**Physical properties of silver**
Chemical Symbol : Ag
Atomic Number : 47
Atomic Weight/Mass : 107.8682 amu
Standard State : Solid, at 298 Degrees Kelvin( 77 degrees Fahrenheit )
CAS ID : 7440-22-4
Group Number : 11
Class : Metal, Transition
Melting Point of Silver : 961.93 °C
Boiling Point of Silver : 2,212.0 °C.

Silver metal and silver dressings, when used in reasonable amounts, has no negative effects on the human body and it has a natural antimicrobial activity towards many pathogens such as bacteria (Hill and Pillsbury, 1939; Zhang and Sun 2007), viruses, fungi, yeast etc. Silver salts have been used for the treatment of mental illness, nicotine addiction, gastroenteritis and infectious disease like syphilis and gonorrhea (Gulbranson *et al*. 2000, Drake *et al*. 2005). New silver coated catheters are used because they stop the infections that effect common place with the old ones. To protect us from food poisoning, silver particles are now being put in cutting boards, table tops, surface disinfectants, and refrigerators. Silver is woven and impregnated into fabrics to kill bacteria that cause body odour.

**Chemical structure**
Silver is a noble metal that has a high electrical conductivity, heat stability and special properties. This has made it popular to use in electric and photochemical industry. It is also been used in jewellry, coins and also in medicine for a long time. Silver has a broad antibacterial effect and due to this, it is widely used in food storage, household products, disinfectants, textiles, medical equipment.

## 4. Materials and Methods

Bioassay are of the two types Continuous flow (Mount and Warner, 1965) and static method (APHA, 2005) the static bioassay method was chosen considering the limitation of laboratory facilities. Healthy specimens of Cyprinus carpio were obtained from Bhavani river, Tamilnadu, India. Fish of the same age and size which hatched from the same lot of eggs were collected, the age of the fish being 2 to 3 month old. They were safely brought to the laboratory in well packed polythene bags containing aerated water and stocked in a large cement (36'x 18'x 19') tanks. Fish were acclimatization for about 20 days before the commencement of the experiment. During the acclimatization period, fish were fed ad libitum with rice bran and ground nut oil cake in the form of dough once in daily. Water was replaced every 24h after feeding in order to maintain a healthy environment for the fish. This ensures sufficient oxygen supply for the fish and the environmental is devoid of any accumulated metabolic waste. The feeding was withheld for 24h before the commencement of the experiment and to keep the specimens more or less in the same metabolic state

## 5. Result

In the present investigation the median lethal concentration (LC 50) of Silver to an Indian major carp *Cyprinus carpio* for 24 hours and 96 hours were found to be 1.5 mg/L and 0.75 mg/L. The main toxic action of silver on aquatic animals during acute and sublethal toxicity leads to mortality due to metal accumulation in fishes through the gills. During acute treatment of silver the fish *Cyprinus carpio* showed behavioral changes such as gastroenteritis and infectious disease like syphilis and gonorrhea. The toxicity of a metal is influenced by several factors like solubility, binding specificity to a biological site. In contrast the changes like the affinity of silver for membrane and neuronal structures and the deposition of silver as an insoluble compound (Ag) induce the progression of clinical diseases like generalized argyria. These changes were observed during the bioassay studies in static flow method.

**Table 1:** Calculation of log concentration / probit regression line for 24h experiment in fish *Cyprinus carpio* were exposed to different concentration of Silver.

| S.No | Conc in PPM Ag | No of Fish Used | % Dead | Log Conc | Emprical Probit | Expected Probit | Working probit | Weight Co-efficient | Weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | X | | Y | Y | | W | WX | Wy | $WX^2$ | $Wy^2$ | WXy |
| 1 | 0.4 | 10 | 35 | 1.544 | 4.8 | 4.75 | 4.65 | 0.62 | 6.2 | 9.572 | 28.83 | 91.638 | 831.16 | 44.513 |
| 2 | 0.9 | 10 | 40 | 1.602 | 5 | 4.9 | 4.8 | 0.63 | 6.3 | 10.092 | 30.24 | 101.86 | 914.45 | 48.444 |
| 3 | 1.5 | 10 | 50 | 1.698 | 5.1 | 5 | 5 | 0.635 | 6.35 | 10.782 | 31.75 | 116.25 | 1008.0 | 53.911 |
| 4 | 2.1 | 10 | 59 | 1.77 | 5.15 | 5.1 | 5.1 | 0.64 | 6.4 | 11.328 | 32.64 | 128.32 | 1065.3 | 57.772 |
| 5 | 2.6 | 10 | 68 | 1.832 | 5.27 | 5.2 | 5.15 | 0.645 | 6.45 | 11.816 | 33.21 | 139.62 | 1103.4 | 60.854 |

X = 1.639
X2 = 3.278
Y = 4.990
Fiducial limits at 5% level = 0.065
b = 0.327
Lower limit m1 = 0.013
Variance 'V' = 0.065
Upper Limit m2 = 0.655
LC50 24 h value = 1.5 ppm
Regression equation Y = 2.013 + 0.210X
Values are means of five individual observations

**Table 2:** Calculation of log concentration / probit regression line for 24h experiment in fish *Cyprinus carpio* were exposed to different concentration of Silver.

| S.No | Conc in PPM Ag | No of Fish Used | % Dead | Log Conc | Emprical Probit | Expected Probit | Working probit | Weight Co-efficient | Weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | X | | Y | Y | | W | WX | Wy | $WX^2$ | $Wy^2$ | WXy |
| 1 | 0.05 | 10 | 20 | 1.301 | 4.3 | 4.2 | 4.2 | 0.58 | 5.8 | 7.545 | 24.36 | 56.939 | 593.409 | 31.692 |
| 2 | 0.35 | 10 | 34 | 1.531 | 4.65 | 4.45 | 4.4 | 0.59 | 5.9 | 9.032 | 25.96 | 81.593 | 673.921 | 39.744 |
| 3 | 0.75 | 10 | 48 | 1.681 | 4.9 | 4.5 | 4.5 | 0.6 | 6 | 10.086 | 27 | 101.727 | 729 | 45.387 |
| 4 | 1.15 | 10 | 52 | 1.732 | 5.1 | 5 | 4.9 | 0.63 | 6.3 | 10.911 | 30.87 | 119.063 | 952.956 | 53.466 |
| 5 | 1.55 | 10 | 56 | 1.748 | 5.15 | 5.1 | 5 | 0.635 | 6.35 | 11.099 | 31.75 | 123.205 | 1008.063 | 55.499 |

X = 1.598
X2 = 2.553
Y = 4. 650
Fiducial limits at 5% level = 0.127
b = 0.232
Lower limit m1 = 0.025
variance 'V' = 0.0464
Upper Limit m2 = 0.510
LC50 96 h value = 0 .75 ppm
Regression equation Y = 1.453 + 0.131X
Values are means of five individual observations

## 6. Discussion

Silver is potentially one of the most toxic metals to freshwater fish and the effects are primarily caused by disturbance of osmoregulation (Lima *et al*., 1982, Nebeker *et al*., 1983, Bianchini *et al*., 2002). Silver exists in a variety of forms, ionic silver is particularly toxic (Morgan and Wood, 2004). Recent studies demonstrate that silver nanoparticles induce embryonic injuries and reduce survival in zebra fish (Danio rerio) (Lee *et al*., 2007, Asharani et al., 2008, Griffith *et al*., 2008; Yeo and Kang, 2008). Concentration of silver has been found to be less than 5 ng $L^{-1}$ in three Connecticut undeveloped headwaters and between 25 and 100 ng $L^{-1}$ in rivers from industrialised and urban areas (Rozan *et al*., 1995). In rivers, particulate silver concentrations range from <0.01 to 62 ng $L^{-1}$ Wen *et al.*, 1997. Generally, the concentration of silver ions is extremely low in surface waters, because ionic silver binds to a variety of negatively charged ligands (Purcell *et al*., 1998 and Hogstrand *et al*.,1998). The concentration of silver nanoparticles from consumer products in the aquatic environment is predicted to be about 0.01 µg $L^{-1}$ Tiede *et al*., 2009. In 2010, the silver concentration in courses of the Rhine, receiving outlets of textiles and plastics containing nanosilver, was estimated to be between 4 and 40 ng silver $L^{-1}$, accounting for 15% of the total silver release (Blaser *et al*.,2008).

Silver atoms forms under various conditions so called clusters which can eventually further aggregate forming silver nanoparticles. Silver clusters can be formed by the action of laser during ablation of metallic silver or silver salts. For example, charged silver clusters like $Ag^{2+}$, $Ag^{3+}$ are formed by laser desorption/ionization of Ag(s) or Ag salts and $Ag_n$ clusters with *n* upto 50-60 were described (Staudt *et al*. 2000). Due to the two isotopes of silver, in mass spectra characteristic isotopic envelops are developed. Evidently, there are three combinations of the silver isotopes yielding three peaks in mass spectrum. Silver cluster $Ag_8$ can be stabilized in erionite channels. Erionite is a kind of zeolite which shows cavities with diameter 0.63nm and length 1.5nm, such elongated cavities are connected by small windows with a diameter 0.25nm.The form of silver affects the uptake and bioavailability. The form depends on the physiochemical condition of the environment and the bioavailability of silver is dependent on its speciation. Silver binds strongly with reduced silver, chloride, thiosulfate and organic material. Silver in reducing condition will be metallic state or in sulphide complexes, which is insoluble in water. In oxidative condition silver is commonly found in complex with bromide, chlorides and iodides. In polluted water silver thiolates have been found. With increased salinity an increase in formation of silver-chloro complexes will happen. Silver uptake decreases with increasing salinity (Ratte 1999).

Ionic silver is also acutely toxic to *zebrafish* at concentrations consistent with a prior estimation for fish *Cyprinus carpio* (Griffith *et al*., 2008) and within the 96-hour $LC_{50}$ range (5–70 µg $L^{-1}$) for other teleosts . Given that tank water contained NaCl, it is excluded that some of the silver existed as silver chloride. In rainbow trout (*Oncorhyncus mykiss*), Hogstrand , Bury *et al*.,1999 found that dissolved silver chloride is at least ten times less toxic than silver ions to fish and particulate silver chloride is nontoxic. Similarly, Bury *et al*., 1999 reported that increasing chloride concentrations decreased silver toxicity in rainbow trout whereas chloride ions did not significantly affect the 96-hour $LC_{50}$ values for fathead minnows (Pimephales promelas). The silver ions were approximately 3.4 times more toxic than the silver nanoparticles by mass of silver added to the tanks. In an in vitro study, PVP-coated nanosilver from the same supplier as the particles was found to be approximately four times less toxic than silver ions (Foldbjerg *et al*., 2009). In current research, the toxicities of $Ag^+$ on *Cyprinus carpio* were evaluated. The results suggested that $Ag^+$ have toxic potential toward *C. carpio* and Ag induced mortality might provoke higher-level consequences, which could comprise a contribution to the knowledge on the aquatic toxicity of AgNPs on aquatic ecosystems, for which little data are available. However, further research on the mechanism behind $Ag^+$ induced damage and mortality that explains the ecotoxicity of Ag in *C. carpio*. Based on the obtained results of this study, it is suggested that the metal exert toxicological or biological responses and they induce mortality responses by repeated water exposure.

In the current study metal silver were exposed to fresh water fish *Cyprinus carpio* and the bioassay (acute and sublethal) studies were determined and were evaluated. The 24h value were found to be 1.5ppm and the 96h value were 0.75ppm where ppm is parts per million and is also recognized as mg/L. The median lethal concentration ie, 1/10 of LC50 value for 24h were taken as sublethal concentration 0.15ppm. In conclusion, this study provides evidence that silver reduce the ability of fish to extract oxygen from the water during progressive oxygen depletion, thereby increasing vulnerability to hypoxia. Also the examined concentrations of silver in the present study is high compared with the 24h acute toxicity , although the fish were exposed to 96h. Such high silver concentrations only occur in the aquatic environment in very rare cases.

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