

**EXPOSURE OF SILVER NANOPARTICLES IN GLYCOGEN
CONTENT TO FRESHWATER FISH “CYPRINUS CARPIO”**¹*Dr. Y. Thangam, ²P. Ranganayaki and ³Dr. M. Rabeeth¹J.K.K. Nataraja College of Arts and Science, PG and Research Department of Zoology
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Arts and Science, PG and
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Zoology Komarapalayam,
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India.**ABSTRACT**

Background: The term nano-silver refers to the nanoparticulate size of the silver nanoparticles particles. Silver to 100µm breaks to silver nanoparticles. Silver nanoparticles in the aquatic environment is estimated to about 0.01 µg/L⁻¹. Nanosilver has a more active surface area and better porosity than commercial silver. Traditional methods in toxicology research and assessments have focused mostly on biochemical agents in glycogen level. Widely used nanoparticles, such as silver nanoparticles, most likely enter the ecosystem and may produce a biochemical and physiological response in many animals, possibly altering their fitness and might ultimately change the glycogen level. Silver nanoparticles used as biomarkers for monitoring environmental quality, water pollution and the health condition of

aquatic organisms. **Objective:** The change leads to toxicity in biochemical level and changes occurs in glycogen content. Blood hepatic glycogen can be utilized as a parameter of stress response, as it is rapid, practicable and quantitative reported that blood glycogen appeared to be sensitive indicator of environmental stress in fish and it has been widely monitored to study stress in fish. **Material & Methods:** Estimation of muscle glycogen was done by anthrone method (samseifter *et al.*, 1949). **Results:** Silver nanoparticles in glycogen content exhibited a significant decrease throughout the study period. **Conclusion:** The action of silver nanoparticles is most likely on the gill surfaces resulting in impaired gas exchange. Nanosilver concentrations and the expected future use of nanosilver undoubtedly result in decreased environmental concentrations. Also the examined concentration of silver

nanoparticles in the present study is low when exposed to fish *Cyprinus carpio* in the environment. The decrease in glycogen content due to silver nanoparticles exposure in fish might be due to an enzyme metabolite named plasmatic pyruvate. Decreased of glycogen level in fish *Cyprinus carpio* exposed to silver nanoparticles may also be due to the absence of metabolite in liver.

KEYWORDS: Silver nanoparticles, *Cyprinus carpio*, glycogen content.

INTRODUCTION

Aquatic pollution on environment has focused interest on health of fish populations. Increasing awareness of the adverse effects of anthropogenic activities shows the possibilities to utilize health parameters for assessment of the quality, of aquatic environment. More contaminants enter the aquatic and marine environment every year due to water pollution monitoring and leads crucial problem. The aquatic environment for biomonitoring using various measurable attributes, to find the toxicity level is the predication of current trend (Pace, 2001). The assessment of environmental disturbances requires the elucidation of stress effects throughout the hierarchy of biological organization, from molecular and cellular levels up to organism and population levels due to the changes in biomarker especially in biochemical parameters (Moore, 2002). To do so, the development of different biomarkers to investigate the in vivo effects of contaminants is a priority requirement to reveal the action mechanisms of toxicants silver nanoparticles in glycogen level (Oliveira Ribeiro *et al.*, 2006). Metals play an important role in aquatic organisms. Among metals silver and silver nanoparticles is a naturally occurring metal. Silver is a naturally occurring precious metal, most often used as mineral ore in association with other elements. It has been positioned as the 47th 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%. Recently nanoparticles in the aquatic environment enhanced different changes in fishes and the changes were embossed in fish physically and biochemically. The change leads to toxicity in biochemical level and changes occurs in glycogen content. Silver to 100 μ m breaks to silver nanoparticles. Silver nanoparticles in the aquatic environment is estimated to about 0.01 μ g L⁻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 $<0.01-100\text{ngL}^{-1}$ (Rozan *et al.*, 1995; Wen *et al.*, 1997).

Nano silver means usually nano-sized particles of nanometer size and the term is connected to colloidal silver or identical. There are now many commercial products, some are named as “NanoSilver”. The term nano-silver refers to the nanoparticulate size of the silver particles of 5-50 nm. Nanosilver has a more active surface area and better porosity than commercial silver (Alt *et al.* 2004). Many of these studies have revealed silver nanoparticles (Ag NPs) to have noticeable toxicity against biochemical parameters and several cell lines as well as a number of aquatic organisms, but the mechanistic basis of these toxic effects is now an area of active research. In particular, the bioavailability of silver ions (Ag^+) from Ag NPs, considered by many as a major factor in Ag-mediated toxicity, remains poorly understood. Such studies underscore the need to understand the transport, uptake and degradation of Ag NPs under biochemical and physiological conditions, to accurately assess the relative benefits and risks of using Ag NPs in commercial products. Traditional methods in toxicology research and assessments have focused mostly on biochemical agents in glycogen level, so determining the toxicological effects of Ag NPs raises several challenges. To observe biochemical (glycogen) changes in the plasma/organs of fish treated with sublethal concentration of silver nanoparticles and so to use these biomarkers for monitoring environmental quality, water pollution and the health condition of aquatic organisms. Silver nanoparticles (AgNPs) are the most commonly used in our planet, including spectrally selective coatings for solar energy absorption, chemical catalysts especially antimicrobial sterilization and, which has many applications made them one of nonmaterial's (Pal, *et al.*, 2007). Widely used nanoparticles, such as silver nanoparticles, most likely enter the ecosystem and may produce a biochemical and physiological response in many animals, possibly altering their fitness and might ultimately change the glycogen level. Open access literature regarding the toxicity of nanoparticles (NPs) is still emerging and gaps still exist in our knowledge of this area (Pal, *et al.*, 2007). Despite the dramatic increase in the use of these NPs, little data is available on their potential harmful effects on the ecosystems. Most researches on the toxicity of NPs come from mammalian studies that have focused on bioassay, biochemical from *in vivo* assays using mammalian cells (Lovern *et al.*, 2007).

Blood hepatic glycogen can be utilized as a parameter of stress response, as it is rapid, practicable and quantitative reported the blood glycogen appeared to be sensitive indicator of

environmental stress in fish and it has been widely monitored to study stress in fish (Teles *et al.*, 2003). In order to meet the high energy demand of stressed animals, glycogen which is rapidly available for energy production may be rapidly catabolized resulting in huge or less of its reserve (Ruparelia *et al.*, 1997). Glycogen is the main source of energy for animals during normal metabolism and their content in muscle of fish exposed to chemical substances may indicate the health condition of the fish. They also reported that during unfavorable environmental situation the normal metabolism is affected which in turn leads to alteration in the glycogen reserve of fish. The significant increase in muscle glycogen content during sublethal lindane treatment may indicate an adaptation of fish to lindane toxicity or impairment in the carbohydrate metabolism (Saravanan *et al.*, 2011). Sastry and Siddiqui (1984) reported that the muscle glycogen level was increased in quinalphos exposed fish indicating a decrease in the rate of glycogenolysis. In the present study also the significant decrease in muscle glycogen during acute and sublethal treatments indicates impairment in carbohydrate metabolism or a decrease in the rate of glycogenolysis. A very small concentration of Nanosilver provides greater effectiveness inside the body of fish *Cyprinus carpio* in the aquatic environment.

MATERIALS AND METHODS

ESTIMATION OF GLYCOGEN

Estimation of muscle glycogen was done by anthrone method (samseifter *et al.*, 1949).

RESULT

Table 1 represents the data on changes in the biochemical profiles of fish *Cyprinus carpio* exposed to concentration of silver nanoparticles in glycogen level to sublethal toxicity. Silver nanoparticles in glycogen level exhibited a significant decrease throughout the study period. The significant decrease in glycogen level was directly proportional to the exposure period showing a percent decrease of -4.18, -3.72, -2.70, -1.19, -1.24 at the end of 7th, 14th, 21st, 28th, 35th, days respectively. There were significant ($P < 0.05$) variation among the treatments.

Table 1. Changes in the glycogen content of *Cyprinus carpio* exposed to sublethal concentration of silver nanoparticles for 35 days.

S.No	EXPOSURE PERIOD	CONTROL	EXPERIMENT	CHANGE %
1	7	114.80 ± 0.046c	110.00 ± 0.300b	-4.18
2	14	113.92 ± 0.270c	109.68 ± 0.209a	-3.72
3	21	118 ± 0.051a	114.81 ± 0.278a	-2.70
4	28	117 ± 0.050 a	115.60 ± 0.278a	-1.19
5	35	115.03 ± 0.057c	113.60 ± 0.398b	-1.24

Values are mean \pm S.E. of five individual observations. (-) Denotes percent decrease over control. ** Significant at 5% level. Means in a column bearing same letter(s) are significantly different according to DMRT ($P > 0.05$).

DISCUSSION

Measurement of biochemical parameters is a commonly used diagnostic tool in aquatic toxicology and biomonitoring (Larsson *et al.*, 1985; Korkmaz *et al.*, 2009). Moreover biochemical and physiological biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to different toxic substances (Theodorakis *et al.*, 1992; Abou El-Naga *et al.*, 2005). Biochemical mechanisms involved in cellular detoxification are particularly relevant in understanding the deleterious effects of metal silver nanoparticles in the environmental pollutants (Lopez *et al.*, 2001) and useful biomarkers of exposure to aquatic pollutants (Monteiro *et al.*, 2010). Glycogen is the main source of energy for animals during normal metabolism and their content in muscle of fish exposed to chemical substances may indicate the health condition of the fish. They also reported that during unfavorable environmental situation the normal metabolism is affected which in turn leads to alteration in the glycogen reserve of fish. The significant increase in muscle glycogen content during sublethal lindane treatment may indicate an adaptation of fish to lindane toxicity or impairment in the carbohydrate metabolism. In freshwater fish species along with depleted hepatic glycogen (Bhattacharya *et al.*, 1987). In order to meet the high energy demand of stressed animals, glycogen which is rapidly available for energy production may be rapidly catabolized resulting in huge or less of its reserve (Ruparelia *et al.*, 1997). Sastry and Siddiqui (1984) reported that the muscle glycogen level was increased in quinalphos exposed fish indicating a decrease in the rate of glycogenolysis. In the present study the significant decrease in muscle glycogen during acute and sublethal treatments indicates impairment in carbohydrate metabolism or a decrease in the rate of glycogenolysis. (Ravichandran *et al.*, 1995) suggested that breakdown of glycogen (glycogenolysis) to cope with the high-energy demand due to stress caused by pollutants.

The engineered nanomaterials comprise of numerous different biochemical and physical forms and some of these materials including carbon nanotubes, carbon spheres called fullerenes (Zhu *et al.* 2006) and nanoparticles made from metals (Griffitt *et al.* 2007), metal oxides (Federici *et al.* 2007) or composites made of several metals (King-Heiden *et al.* 2009) have adverse effects on fish (Smith *et al.* 2007). To study the aquatic toxicity of

fish in glycogen level, fish species has been widely used as an indicator of pollutant and they strongly respond to stress conditions. An earlier report of Ramesh *et al.* (2013) emphasized the toxicological impact of nanoparticles on antioxidant enzymes and DNA strand break using zebra fish and *Cyprinus carpio*. Generally depletion of glycogen level under stress condition may indicate an expression of an initial regulatory step resulting in an increase in the intermediary metabolism. The reduction in glycogen content in *Cyprinus carpio* during acute and sublethal treatment of silver nanoparticles indicates the utilization of stored glycogen to meet the high energy requirement under the silver nanoparticles stress. The reduction of hepatic and muscular glycogen in *Silver catfish* exposed to high nitrate levels at different PH may indicate that stress generated by ammonia toxicity is accompanied by a rapid degradation of tissue glycogen. In fact, carbohydrates stored in muscles are the first nutrients used in response to stress conditions (Vijayavel *et al.*, 2006). Lower glycogen levels in muscle were also observed in freshwater fishes exposed to high NH₃ levels (1-4mg/L). Depletion of glycogen may be due to direct utilization for energy generation, a demand caused by latex – induced hypoxia. Fish white muscles constitute more than 50% of the whole body mass and anaerobic processes require glycogen for their action (Knox *et al.*, 1980). In the present study the significant decrease in muscle glycogen level during sublethal treatment may indicate that stress generated by silver toxicity is accompanied by a rapid degradation of muscle glycogen. The alteration in glycogen content can be understood in two exclusive ways, the glucose can be converted to other tissues, especially in the brain, or it can be converted into glycogen.

In conclusion, this study provides evidence that silver nanoparticles reduce the ability of fish to extract oxygen from the water during progressive oxygen depletion, thereby increasing perch vulnerability to hypoxia. On the other hand, BMR, was affected by nanosilver exposure during normoxia. The action of silver nanoparticles is most likely on the gill surfaces resulting in impaired gas exchange. Nanosilver concentrations and the expected future use of nanosilver undoubtedly result in decreased environmental concentrations. Also the examined concentration of silver nanoparticles in the present study is low when exposed to fish *Cyprinus carpio* in the environment. The decrease in glycogen content due to silver nanoparticles exposure in fish might be due to metabolite named plasmatic pyruvate. Decreased of glycogen level in muscle, in fish *Cyprinus carpio* exposed to silver nanoparticles may also be due to the absence of metabolite in liver.

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