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Comparison of Total Protein Content in the Muscles of *Catla catla* on Exposure to Ag and ZnO Nanoparticles and their Metal Ions

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Abstract
Growing applications of nanotechnology necessitates understanding of nanotoxicity. The toxicity of nanoparticles has gained special attraction amongst various toxicological studies, but restricted data are available on its toxic effects on aquatic organisms. An attempt has been made in present investigation to determine toxicity of NPs of Ag and ZnO and their metal ions to major carp catla. Protein content in muscle tissues of the fingerlings varied with concentration of the selected toxicants and exposure time (5–15 days); however, significant decrease was observed in silver and ZnO NPs exposed fingerlings as compared to control. This depletion in the total protein might be due to the diversification of energy to accomplish the impending energy demands when fingerlings are under toxic stress.

Keywords: *NPs, metal ions, protein, muscles, catla*

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INTRODUCTION

Nanotechnology is growing fast with industrialization [1]. Nanoparticles are known to be used in many physical, biological and pharmaceutical applications in recent years. NPs and materials having them have different or superior properties as compared to their conventional 'bulk' (micro-size) counterparts. These advanced properties are due to increased relative surface area which gives them higher reactivity [2]. In bulk materials the surface atoms constitute only a few percent of the total number of atoms, while in NPs most of the atoms lay close to or at the surface [3]. NPs of some metals such as copper oxide, zinc oxide and especially nanosilver are intentionally used to inhibit the undesirable growth of bacteria, fungi and algae. Use of NPs in industries can cause release of them from consumer and household products into waste streams and further into the aquatic environment which may pose threat to its inhabitants.

Silver is generally used in its nitrate form to induce antimicrobial effect, but when silver NPs are used, it increases the surface area available to the microbes. Though Ag NPs have many antimicrobial applications, the action of this metal on microbes is not fully known. It has been hypothesized that Ag NPs can cause cell lysis [4]. Long-term exposure to silver and its compounds may increase blood concentration to levels which can have toxic effects. It may also cause hazardous health effects such as induction of sarcomas, anemia, and enlargement of the heart [5].

Zinc is a crucial element for many metabolic functions in organisms as they may be adapted to the prevailing Zn concentration. Tolerance of zinc in any organism in the field may depend on the local natural background concentration of it [6,7]. ZnO nanoparticles are used in many products. ZnO NPs have also antimicrobial properties and are used in sunscreens, cosmetics and coatings as pigments and adhesive, (e.g., sunscreen, coatings, and paints) [8]. ZnO NPs are also widely incorporated in commercially available merchandise. Some studies involving marine and freshwater organisms have suggested that the dissolution of the Zn ions from ZnO nanoparticles can account for their toxicity [9–12].

Silver and ZnO NPs are reaching to the aquatic environment through different commercial and consumer applications. So, it is important to investigate possible toxic effects of them. Such toxicity studies are also done in this field. Toxicity of metal oxide NPS has been reported in fresh water crustaceans, daphnia magna and zebra fish. ZnO NPs significantly increased mortality and decreased hatchability of zebra fish embryos [13]. *In vivo* inhibition or induction of oxidative stress biomarkers is a good ecotoxicological tool to assess the effects of xenobiotics in organisms [14]. In recent years, the culture of fish species has been expanding due to an increasing demand from consumers, in urban areas. In aquatic ecosystem generally fish population is considered as very sensitive to all kinds of environmental changes. Certain stages of them such as fingerling in the life cycle of fresh water fish are more susceptible to environmental and pollution stresses. Fish is an excellent and relatively a cheaper protein source of high biological value. Therefore, its use may help bridge the protein gap because of its multifarious economic advantages and nutritional significance [15]. The present study is carried out for comparison, of the toxic effects of NPs of Ag and ZnO and their metal ions toward fresh water fish catla. Protein content in muscles was addressed in fingerling of this fish.

MATERIAL AND METHODS

Fingerlings of *Catla catla* were obtained from a Government fish farm, Surat, Gujarat. Mean weight and length of the selected fish (n=100) were 5–7 g and 6.0–7.0 cm, respectively. Fish were acclimatized in aerated dechlorinated tap water at 28ºC. They were fed twice daily with commercial feed. Fingerlings were kept in two glass aquaria with capacity of 100 l each for 15 days prior to experiments. Fish were starved for 48 h prior to and during the experiments. All aquaria were aerated during the experiments except for the period of dosing. The water quality parameters were carried out by standard methods [16]. They were checked regularly and were ensured to be in permissible range.

Preparation of Nanoparticle Suspensions

ZnO NPs were purchased from Sigma Aldrich USA. Nanoparticles suspension of the desired concentration was prepared by mixing preweighed ZnO nanoparticles in deionized water. The dispersed nanoparticles were ultrasonicated for 30 min. Ag NPs were synthesized by bulk-solution synthetic method [17]. $AgNO_3$ and $ZnSO_47H_2O$ were purchased from Eagle chemicals India. Stock solution was prepared by taking the appropriate amount of the chemical weighed and refrigerated. Exposure media was changed at an interval of 24 h in Ag NPs and Ag^+ exposure study and aquarium were covered with a black paper around their surface as it is a photo reactive metal.

Experimental Set up and Exposure

Experiments were conducted under semi-static conditions in aquaria after acclimatization. According to obtained LC_{50} values of fingerlings, two sublethal concentrations of Ag^+ and Zn^{2+} metal ions and their NPs were selected. For the experimental studies fingerlings in group of 10 per aquaria were randomly taken out. Aquaria for the exposure study were of 10 liter capacity. Fingerlings were exposed to 0.01 ppm for Ag NPs and Ag^+ (lower concentration) and 0.03 ppm for Ag^+ and Ag NPs (higher concentration), 5 ppm (lower concentration) and 10 ppm (higher concentration) for both ZnO NPs and Zn^{2+}. Experiment was performed after 5, 10 and 15 days. Triplicates were performed for each concentration. A group of 10 fingerlings were taken out as a control group study in dechlorinated water.

Protein Estimation

The total protein was estimated separately in muscle tissue of exposed fingerlings [18]. It is highly sensitive and can detect protein levels as low as 5 μg/ml. 100 mg of muscles free from scale and skin was taken from dissected fish and homogenates was prepared. Absorbance was measured at 660 nm using spectrophotometer (Elico SL 150). The amount of protein in given sample compared the obtained values with standard graph. All the data obtained were subjected to the statistical analysis. The significance of the data among control and exposed fishes were derived at 5% level using the student's t test.

RESULTS

The values obtained from study of physicochemical parameters of water samples during this investigation are presented in

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Table 1. Not much variation was seen in these parameters after the addition of Ag NPs, ZnO NPs and their metal ions.

***Table 1:** Water Quality Parameter.*

Parameter	Values
pH	7 ±0.5
Temperature	28 ±1°C
Dissolved oxygen	7.44±0.3 mg/L
Hardness	140±7 mg/L
Alkalinity	111±2 mg/L
Free CO_2	Absent
Inorganic phosphorus	0.1±0.01 mg/L

Protein Content

The most commonly used nanomaterial in consumer product is silver. A large part of toxicants in the form of heavy metals and NPs caused threat to human health and environment.

Depletion in the protein content in the muscle of the *Catla catla* exposed to Ag and ZnO NPs are presented in Figures 1 and 2. During this study muscle samples collected from all the exposed fingerling showed remarkable alterations in their protein content. Results show that it was decreased in all exposure period in both lower and higher concentrations of both Ag and ZnO NPs. Protein content in control fingerlings showed normal values while there was marked depletion noticed in exposed group to both the concentration of Ag^+ and Zn^{2+}. It was gradually decreased in the lower concentration of Ag^+ while in higher concentration protein content decreased after 5 days and slightly increased after 10 and 15 days exposure (Figures 3 and 4).

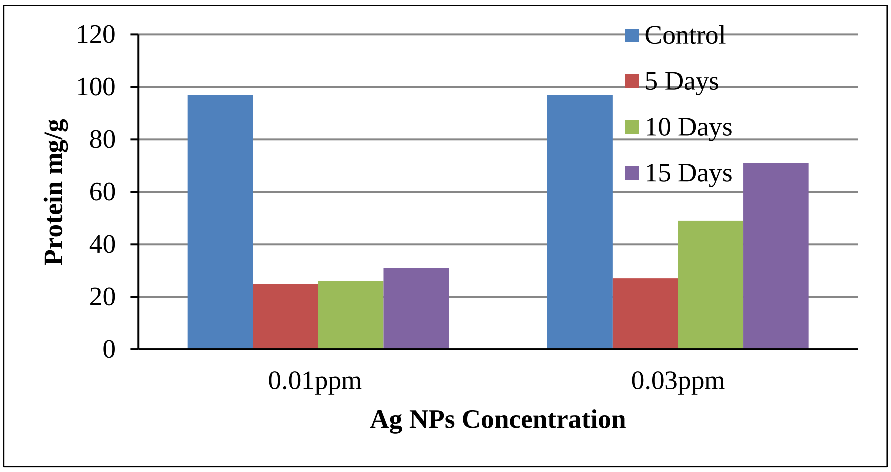

***Fig. 1:** Depletion in Protein Content in the Ag NPs Exposed and Control Fingerlings. Asterisks (*) Indicate Significant (p<0.05) Difference Compared to Control.*

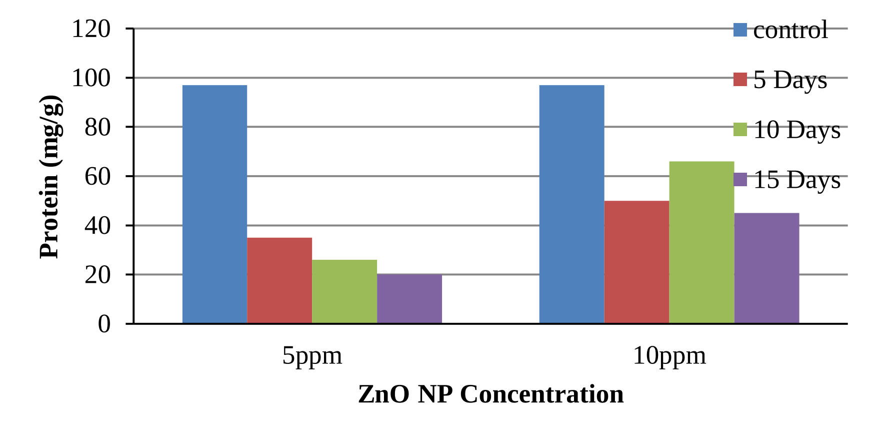

***Fig. 2:** Depletion in Protein Content in the ZnO NPs Exposed and Control Fingerlings. Asterisks (*) Indicate Significant (p<0.05) Difference Compared to Control.*

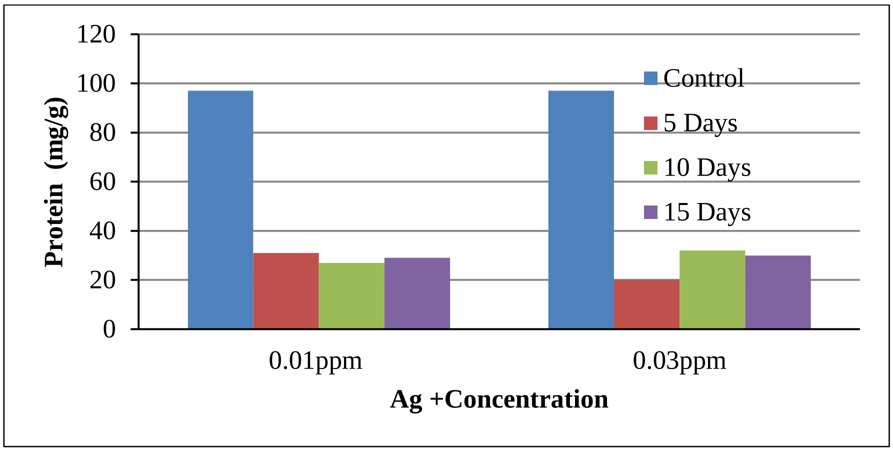

Fig. 3: *Depletion in Protein Content in the Ag^{+} Exposed and Control Fingerlings. Asterisks (*) Indicate Significant ($p<0.05$) Difference Compared to Control.*

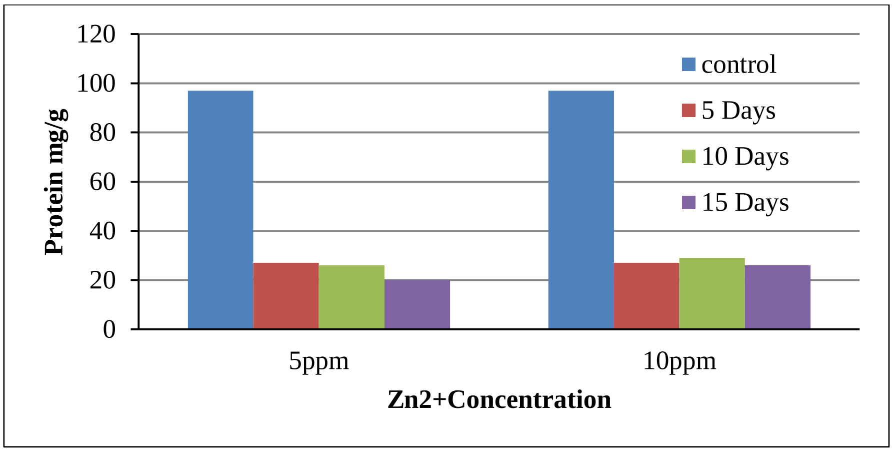

Fig. 4: *Depletion in Protein Content in the Zn^{2+} Exposed and Control Fingerlings. Asterisks (*) Indicate Significant ($p<0.05$) Difference Compared to Control.*

DISCUSSION

The assessment of the protein content can be considered as a diagnostic tool because they are involved in major physiological events of organism [19]. Proteins are highly sensitive to heavy metal poisoning. Depletion of protein content has been observed in the muscle, intestine and brain of the fish *Catla catla* as a result of mercury chloride toxicity [20]. Gradually depletion in protein content in muscles of exposed fingerlings during this study was similar and supportive to results obtained in *Heterocephatycthys thermalis* and *Cyprinus carpio* due to cypermethrin intoxication [21,22]. The decreased levels of total protein content during this study in muscles of fingerlings were similar to that observed in *Catla catla* exposed to fenvalerate [23]. When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted [24]. The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of toxicants at the lower exposure period [25]. Increased protein content after 5 days in Ag NPs and Ag^{+} in both concentrations, as well as in higher concentration of ZnO NPs observed in this study may be due to increased concentration of total free amino acids in muscles of fingerlings. The increase in total free amino acids recorded in muscles of exposed fingerlings was attributed to increases in individual amino acids. During the study period depletion in the total protein might be due to the diversification of energy to accomplish the impending energy demands when fingerlings are under toxic stress. This reduction also may be due to reduced protein synthesis due to higher affinity of metal compounds towards different amino acid residues of proteins.

CONCLUSION

The results suggest that the protein content of fish greatly varies during different seasons. This might be due to changes in environmental conditions and toxic response of applied metal ions and NPs. Among these two metals and their NPs, Ag was found to be more toxic than zinc. This study provides important information on variations in protein content of fish species studied in order to take precautions in using products having NPs. Biochemical studies of fish tissue are of considerable interest for their specificity in relation to the food values of the fish. Thus it could be suggested that precautionary measures should be taken against the discharge and usage of these metals and nanoparticles. Safety measures should be taken for treated effluent of these NPs and metals before releasing in the fresh water bodies.

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