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ORIGINAL ARTICLE

BIOCHEMICAL ANALYSIS OF SUBLETHAL TOXICITY OF SILVER NANOPARTICLES ON THE GILLS OF THE FRESHWATER CAT FISH *Clarias batrachus* (Linn. 1755)

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ABSTRACT

Fish are more sensitive to many toxicants and are a convenient specimen for aquatic toxicity assays. Heavy metals are produced from a variety of natural and anthropogenic sources. Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level; They offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats. In the present decades, the nano particles are being synthesized through chemical, physical and biological methods and chemical reduction is more suitable method due to less cost of chemical, ease of control and less byproducts. The extensive use of silver nanoproducts increases the discharge into aquatic ecosystem. It further contaminated the environment through cement manufacturing, weathering of rocks, burning of fuel, processing of ores and leaching and anthropogenic activities. The present investigation has been conducted to understand the effect of sublethal concentration of silver nanoparticles (16.825 ppm) on total protein, glucose, glycogen and total lipids in gills of *Clarias batrachus* for a period of 5, 10, 20, 30, and 45 days. The result showed that all the parameters under investigation had a decreasing trend from control to various exposure periods.

Keywords: Gill, Nnano toxicity, *Clarias batrachus,* Biochemistry

1.INTRODUCTION

The effect of toxic wastes upon fish and other aquatic forms have stimulated perhaps more interest and research in recent years than any other sub-field of water pollution biology and the necessity of determining the toxicity of wastes to fish and other aquatic forms has resulted in development of the toxicity bioassays (Banerjee and Mukherjee, 1994) into a useful accepted, although far from perfected tool in water quality management. The interrelationships between the fish and the elements of its abiotic and biotic environment are interdependent and any changes in one system of relationships inevitably produce changes in the other. As the nature of interaction of fish with any particular elements of its environment and the effect of total environment, natural or polluted, on fish, depends to a great extent upon the conditions of the fish itself (Radhakrishnan and Hemalatha, 2008). Contamination of freshwater bodies by heavy metals is an area of current concern over the years. The danger of heavy metals is aggravated by their almost indefinite persistence in the environment because they cannot be destroyed biologically but only transformed from oxidation state or by forming organic complex to another which bind to cell membranes affecting the intracellular transport processes in the living forms. Silver nitrate is the most dominant procured compound to synthesized Ag-NPs. (Awasthi *et al.*, 2013; Taju *et al.*, 2014). Rain is liable to release the silver in the water reservoirs or ground water. In the aquatic environment, it exists in four oxidation states (Ag, Ag^{+}, Ag^{2+} and Ag^{3+}) with Ag and Ag^{+} most common form (Levard *et al.*, 2012). In recent studies, the researchers have focused on toxicity of Ag-NPs in aquatic organisms (Khan *et al.* 2015a; Monfared and Soltani 2013; Rajkumar *et al.* 2015; Reddy *et al.* 2013; Taju *et al.* 2014). Numbers of toxicological studies have been performed but it show huge variations due to lack of proper particles characterization (Gliga *et al.* 2014). In recent studies, the

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researchers have focused on toxicity of Ag-NPs in aquatic organisms (Khan et al. 2015a; Monfared and Soltani 2013; Rajkumar et al. 2015; Reddy et al. 2013; Taju et al. 2014). But the data on biochemical studies are scanty, hence the present study has been designed to understand the sub lethal toxicity of silver nano particles on the gill of the catfish *Clarias batrachus.*

2.MATERIALS AND METHODS

Collection and maintenance of fish: The fresh water fish *Clarias batrachus* (14 ± 2 cm length and 30 ± 2 g weight) were collected locally from Chidambaram, Cuddalore, district and were brought to the laboratory and kept in a tank size of 60 x 30 x 30 (l x b x h) cm, filled with tap water for acclimatization for about two weeks. During the acclimatization the fish were fed with minced goat liver on every alternate days. Water in the tank was renewed, three or four times in a week and aerated to ensure sufficient oxygen supply. For the fish used in experiments, feeding was stopped two days before the start of the experiments to reduce the quantum of excretory products in the tank.

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Experimental Protocol: Prior to the commencement of the experiment the median lethal concentration (LC_{50}) for 96 h was calculated by trimmed Spearman Karber method (Hamilton *et al.,* 1972) and was found to be 168.25 ppm at 95% confidence limit. For the analysis of sublethal toxicity five groups of 10 fish each were exposed separately to silver nanoparticle (16.825ppm; solution prepared in tap water. The experimental medium was prepared by dissolving AgNPs (13 ppm) in tap water having dissolved oxygen 6 ppm, pH 7.5, water hardness 40.44 mg/L and water temperature 28 ± 2 °C (APHA, 2008). Each group was exposed to 50 L of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaria containing 50 L tap water without the addition of AgNPs as controls. Feeding was allowed in the experimental as well as control groups everyday for a period of 3 h before the renewal of the media throughout the tenure of the experiment.

Biochemical Analysis: After the expiry of 5, 10, 20, 30 and 45 days of exposure five fish each from the respective experimental and control groups were sacrificed. Gills were dissected out along with control groups and were subjected to biochemical analysis. The total protein contents in the tissues was estimated by the method of Lowry *et al.* (1951). Lipid content was estimated by the semi micro determination method of Pande *et al.* (1963) and the estimation of glucose and glycogen was carried out following the methods of Kemp and Kits Van Heijhigen (1954**).**

Statistical Analysis: The data obtained were subjected to standard statistical analysis for each sampling time and their respective control groups in different groups. Duncan's multiple range test (Bruning and Kintz, 1968) was performed to determine whether the parameters altered significantly by exposure periods.

3.RESULTS

Total Protein: The control gill recorded 24.14 ± 0.00 mg/g of protein. Continuous decrease in protein content occurred in the gill as 21.11 ± 0.01, 18.00 ± 0.00, 14.12 ± 0.02, 10.15 ± 0.01 and 8.16 ± 0.04 mg/g of protein after10, 20, 30,40 and 50 days respectively (Fig.1).

Glucose: The control gill registered 0.84 ± 0.01 mg/g of glucose. The value showed a decreasing trend as 0.60 ± 0.02, 0.51 ± 0.34, 0.40 ± 0.14, 0.36 ± 1.25 and 0.24 ± 0.00 mg/g for 10, 20, 30, 40, 50 and 60 days of exposure periods (Fig.1).

Glycogen: The gill of control fish recorded 4.68 ± 0.04 mg/g of glycogen. On exposure to sublethal concentration of AgNPs gradual decrease has been noted as 4.03 ± 0.02, 3.25 ± 0.62, 2.01 ± 0.21, 3.75 ± 0.10 and 0.66 ± 0.20 mg/g respectively for10, 20, 30,40 and 50 days respectively (Fig.1).

Total Lipids: The control gill recorded 0.154 ± 0.02 mg/g of total lipid. After sublethal exposure to AgNPs significant decrease in lipid content were noted as 0.104 ± 0.02, 0.96 ± 0.01, 0.66 ± 0.72, 0.51 ± 0.68 and 0.40 ± 0.00 mg/g for 10, 20, 30, 40 and 50 days respectively (Fig.1).

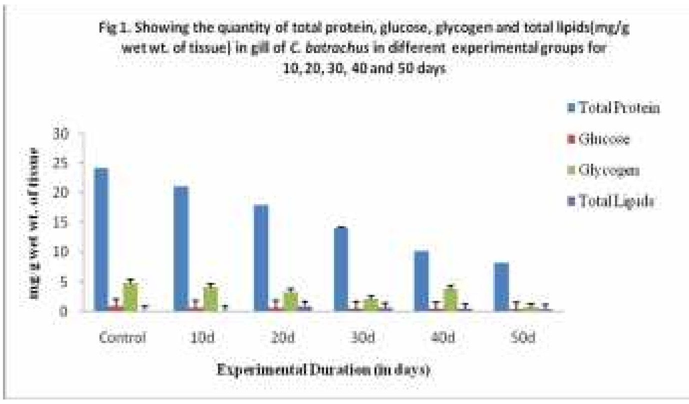

Fig 1. Showing the quantity of total protein, glucose, glycogen and total lipids(mg/g wet wt. of tissue) in gill of *C. batrachus* in different experimental groups for 10,20,30, 40 and 50 days

4.DISCUSSION

Proteins are involved in major physiological events and therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are highly sensitive to heavy metal poisoning (Jacobs *et al.,* 1977). The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride of the lower exposure period (Shakoori *et al.,* 1994) due to their utilization in the formation of mucoproteins which are eliminated in the form of mucus. Further, direct and /or indirect utilization of proteins and lipids for energy need was also reported (Nagai and Ikeda., 1971). Protein is important organic substances required in tissue building and repair. Under extreme stress conditions, proteins supply energy in metabolic pathway and biochemical reactions (Winer 1971). Torreblanca *et al.,* 1991 observed decrease in protein content in the fish *Fundulus rolitus and* stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source. A number of workers have reported depletion in the protein level in different tissues/organs of experimental animals under the stress of various metals, pesticides, chemicals etc, (Mastan 2008). This is concomitant with the metabolic profile that does not indicate a metabolic demand that is able to induce substantial shift from carbohydrate to protein catabolism

(Hori *et al.,* 2006). The depletion of protein also suggests increased proteolysis and possible utilization of the product of their degradation for metabolic processes (Bhilave *et al.*, 2008). The depletion of total protein content may be due to break down of protein into free amino acid under the effect of mercury chloride at the lower exposure period (Shakoori *et al.*, 1994). Hence high activity of protease, a lysosomal enzyme, in the organs caused by mercury to lysosomes (Martin Deva Prasath and Arivoli., 2008). Elevated protease activity induced proteolysis, the intensity increased with the increase in exposure periods in the present study may be the increase in free amino acid pool due to increased proteolysis would act as an osmotic and ionic effectors to bring the electrostatic equilibrium between the external medium (Schmidt Nielson, 1975; Jurss, 1980). Besides, free amino acids would also serve as precursors for energy production under stress, and for the synthesis of required proteins to face the metal challenge (Sreedevi *et al.*, 1992).

The changes in glycogen and glucose content were found to be decreased in the gill after sublethal AgNPs treatment. The decreased glycogen concentration in the test fish could be due to its enhanced utilization as an immediate source to meet the energy demand under heavy metal stress. Fish are largely used in evaluation in the quality of aquatic systems and some of their physiologic changes can be considered as biologic markers of environmental pollution (Dautremepuits *et al.,* 2004). Depleted glycogen level under heavy metal stress was reported in common carp *Cyprinus carpio* (Vinodhini and Narayanan, 2008) and this supports the reduction of hepatic synthesis of detoxifying enzymes requires high energy levels (Begum and Vijayaraghavan. 1995, Hori *et al.,* 2006). Reduction of carbohydrate rates in the reproduction and other tissues indicated the possibility of active glycogenolysis. Tissue acidosis due to reduced oxygen transport must have also favored the process of glycogenolysis in tissues (Senthilkumar *et al.,* 2007). In the present study the depletion of glycogen in the gill suggest that alterations in the chemical composition of the aquatic environments affect the behavior and biochemical system of fish (Radhaiah, *et al.,* 1987) Changes in enzyme activities and alterations in glycogen, glucose and lipid metabolism in fish due to lindane intoxication have also been studied by Ferrando and Moliner 1991, Gopal *et al.,* 1993. Due to natural, geochemical and anthropogenic factors, the infiltration of toxic heavy metals into aquatic ecosystems is in the increase. These toxic heavy metals in aquatic ecosystems are carried via the food chain to the upper trophic levels and create important ecological problems (Cicik and Engin 2005). Fish population is generally considered very sensitive to all kinds of environmental changes to which it is exposed as they are exclusively aquatic with external mode of fertilization (Martin Deva Prasath and Arivoli, 2008). It is presumed that the biochemical changes in fresh water fishes are mostly to cope with usual environmental stressors, including hypoxia (Raja and Kulkarni 2008). It was shown that glucose levels in fish were affected by many stress factors, including heavy metals (Canli, 1995). The results of the study reveal significant effect of heavy metals on carbohydrate metabolism. Glucose content in all the tissues under study was found to decrease continuously throughout the exposure period. Depletion in glucose in the present study may be due to its rapid utilization to meet the demands under toxic manifestation (Bhilave *et al.,* 2008). In conclusion the disturbance in the carbohydrate metabolism was considered as one of the most outstanding biological lesions due to the action of heavy metal (De Bruin, 1976). The decrease in carbohydrate content in the gill may be due to glucose utilization to meet excess energy demand imposed by severe anaerobic stress of mercury intoxication (Margarat and Jegadeesan, 1999). Also, the condition and response of the test organism to the amount of metal penetrating into the body, the degree of retention and the rate of excretion influence the toxic effect of heavy metal.

In relation to the total lipid in this study it was found that there was significant decrease of lipid in the heavy metal intoxicated groups as compared to control. Lipids are also the storage form of energy like glycogen. The lipid levels also decreased in the gill of the fish exposed to the sub-lethal concentration of AgNPs. Earlier studies have also shown that lipid and protein concentration of vital organs like brain, gill, liver and muscle depleted in fishes exposed to heavy metal chromium (Ambrose *et al.,* 1994 and Deepali Sexena and Madhu Tripathi, 2007), Copper (Senthil kumar *et al.,* 2007), Mercury chloride (Martin Deva Prasath and Arivoli, 2008), Cadmium (Levesque *et al.,* 2002, Fabien Pierron *et al.,* 2007). According to them decrease in tissue lipid might be partly due to their utilization with the formation of lipoproteins which are important cellular constituents of cell membranes and cell organelles present in cytoplasm (Harper *et al.,* 1977). Lipids are also the storage form of energy like glycogen. The lipid levels also decreased in the tissues of the fish decreased in the sub-lethal low and high concentration of present study may also be attributed to its utilization in cell repair and tissue organization. Lipids act as reversed depot of energy from where the energy is supplied as and when required (Katti and Sathyanesan, 1983; Bhilave *et al.,* 2008). In stress condition induced by heavy metal, the lipid content depleted to meet the energy demands. In the present investigation, stress imposed by sub lethal concentration of AgNPs to *Clarias batrachus* resulted in decrease in lipid content in the tissues, thereby indicating high-energy demand.

5.REFERENCES

Ambrose, T., A.K.L. Cyril, S.Vincent and L. Roselyn 1994. Biochemical responses of *Cyprinus carpio* communis to the toxicity of tannery effulent. *J. Ecobiol.,* **3:** 213-216.

APHA, 2008. Standard methods for the examination of water and wastewater. American Public Helth Association Washingten D.C.

Awasthi KK, Awasthi A, Bhoot N, John P, Sharma SK, Awasthi K. 2013. Antimicrobial properties of electro-chemically stabilized organo-metallic thin films. Adv Electrochem 1:42–47.

Banerjee, T.K. and Mukherjee, D. 1994. Melanophore indexing: A Quick bioassay technique for detection heavy metal toxicity. *Curr. Sci.***67(1):P** 48-49.

Begum, G and S. Vijayaraghavan 1995. Carbohydrate metabolism in hepatic tissue of fresh water *Clarias* *batrachus* L. during dimethoate exposure. *Fd. Chem.Toxic*., **33:**423-426.

Bhilave, M.P., D.V. Muley and Deshpande, V.Y. 2008. Biochemical changes in the fish *Cirrhinus mrigala* after Acute and Chronic exposure of heavy metals . *Nat. Environ. Poll. Tech.* **7(1):** 65-71.

Bruning, J.L. and Kintz, B.L. 1968. Computational Handbook of Statistics, Foresman and Company, Dallas, USA, p. 308.

Bu- Olayan A.H. and Al-Yakoob S. 1998. Lead, Nickel and Vanadium in sea food an exposure assessment for Kuwait consumers. Sci Environ. 2-3, 81-86.

Canli, M. 1995. Effects of Mercury, Chromium and Nickel on some blood parameters in the carp *Cyprinus carpio*. *Tr.J. Zool.,* **19:**305-311.

Cicik, B. and. Engin, K 2005. The effects of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio*(L., 1978). *Turk. J. Vet. Anim. Sci.,* **29:**113-117.

Dautremepuits, C., S.Betoulle., S.Paris-palacios, and.Vernet. G 2004. Immunol-related perturbations induced by copper and chiston in carp *Cyprinus carpio*. *Arch. Environ. Contam. Toxicol.,* **47:**37 0-378.

De Bruin, A. 1976. Biochemical toxicology of environmental agents, Elsevier. North Holland Biochemical press. N. Y. USA.

Deepali Saxena and Madhu Tripathi 2007.Hexavalent chromium induces biochemical alterations in air – breathing fish, *Channa punctatus*. *J. Ecophysiol. Occup. Hlth.***7:**171-175.

Fabien Pierron, Magalie Baudrimont, Angélique Bossy, Jean-Paul Bourdineaud, Daniel Brèthes, Pierre Elie and Jean-Charles Massabuau 2007. Impairment of lipid storage by cadmium in the European eel (*Anguilla anguilla*). *Aquatic Toxicology.* **81(3):** 304-311.

Ferrando, M.D and E. Andreu-Moliner 1991a. Changes in selected biochemical parameters in the brain of fish, *Anguilla anguilla(L.)* exposed to lindane. *Bull. Environ.Contam.Toxicol.,* **47:**459-646.

Gliga AR, Skoglund S, Wallinder IO, Fadeel B, Karlsson HL (2014. Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. Part Fibre Toxicol 11:1–17.

Gopal, K. M.D. Ram and Agarwal, D. 1993 Some physiological consequences to fresh water, *Channa punctatus*. After exposure to lindane. *Bull. Environ. Toxicol.,* **50:**187-191.

Habig WH, Pabst MJ, Jakoby WB 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Hamilton, M.A.; Russo, R.C.;Thurston, R.V. 1977. Trimmed Spearman Karber method for estimating median lethal concentrations in toxicity bioassays.Environ. Sci. Tech., 11: 714-719.

Harper, H.A., V.W. Rodwell and Mayes, P.A. 1977. Review of physiological chemistry. Lange medical publications, Los Atlos, California.

Hori, T.S.F., I.M. Avilez, L.K. Inoue and Moraes, G. 2006 Metabolical changes induced by chronic phenol exposure in matinxa *Brycon cephalus* (Teleostei:Characidae) juveniles. *Comp. Biochem. Physiol.* **143(1):**67-72.

Hoshina M, Marin-Morales M 2010. Evaluation of the genotoxicity of petroleum refinery effluents using the comet assay in Oreochromis niloticus (Nile tilapia). J Braz Soc Ecotoxicol 5:75–79

Jacobs, J.M., N.Carmicheal and Cavanagh, , J.B. 1977. Ultra structural changes in the nervous system of rabbits poisoned with methyl mercury. *Toxicol. Appl. Pharmocol*. **39:**249-261.

Jurss, 1980.The effect of changes in external salinity on the free amino acids and amino transferases of white muscle from fasted salmo gairdneri. *Comp.Biochem. Physiol.* **65:**501-504.

Katti, S.R. and Sathyanesan, A.G. 1983. Lead nitrate induced changes in lipid and cholesterol levels in the fresh water fish *Clarias batrachus*. *Toxicol. Lett.* **19(1-2):** 93-96.

Kemp, A. and J.M. Kits Van Heijhigeri, 1954. A colorimetric method for the determination of glycogen in tissue. *Biochem. J.,* **56:** 646-648.

Khan MS, Jabeen F, Qureshi NA, Asghar MS, Shakeel M, Noureen, A 2015b. Toxicity of silver nanoparticles in fish: a critical review. J Bio Environ Sci 6:211–227

Lowry, O.H., N.J. Rosebrough, A.L. Farr and Randall. R.J. 1951. Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.,* **193:** 265-275.

Levard C, Hotze EM, Lowry GV and Brown, GE. 2012. Environmental transformations of silver nanoparticles: impact on stability and toxicity. Environ Sci Technol 46:6900–6914.

Margarat, A. and. Jagadeesan, G 1999. Antidotal recovery ion carbohydrate Metabolism of mercury intoxicated mice *Mus musculus* (L.):*Uttarpradesh. J.Zoo.,* **19:**196-198.

Martin Deva Prasath, P. and Arivoli, S. 2008. Biochemical study of fresh water fish *Catla catla* with reference to mercury chloride. *Iran. J. Environ. Health. Sci. Eng.,* **5(2):**109-116.

Mastan, S.A. 2008. Copper induced changes in protein level of certain tissues o*f Heteropneutes fossilis. Journal of Herbal medicine and Toxicology.* **2(2):**33-34.

Monfared AL, Soltani, S 2013. Effects of silver nanoparticles administration on the liver of rainbow trout (Oncorhynchus mykiss): histological and biochemical studies. Eur J Exp Biol3:285–289

Nagai, M. and Ikeda, S.1971. Carbohydrate metabolism in fish. I. Effects of starvation and dietary composition on blood glucose level and hepatopancreatic glycogen and lipid content in *Cyprinus carpio*. *Bull. Japan soc. Fish.* **37:**404-409.

Pande, S.V., R. Parvinkhan and Venkitasubramanian, T.A. 1963. Microdetermination of lipids and serum total fatty acids. *Analyt. Biochem.*, **6:** 415-423.

Radhaiah, V. M. Girija and Rao, K.J. 1987. Changes in selected biochemical parameters in the kidney and blood of the fish, *Tilapia mossanbica*. Exposed to heptachlor. *Bull. Environ. Contam. Toxicol.,* **39:**1006-1011.

Radhakrishnan, M.V., and Hemalatha, S.2008. Morphometric analysis of sublethal toxicity of Cadmium to gills of freshwater mural *Channa striatus* (Bloch). *J. Annamalai University*, **44:** 85-88.

Raja, I.A. and Kulkarni, K.M. 2008. Tissue biochemical adjustments in the Air Breathing fresh water fish *Channa punctatus* exposed to environmental hypoxia. *Environment &Ecology*. **26(2):** 669-672.

Rajkumar, K, Kanipandian N, and Thirumurugan, R 2015. Toxicityassessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish Labeo rohita. Appl Nanosci 1–11

Reddy, TK, Reddy, SJ, Prasad, T. 2013. Effect of silver nanoparticles on energy metabolism in selected tissues of Aeromonas hydrophila infected Indian major. Carp Catla catla IOSR J Pharm 3:49–55.

Schmidt Nielson, B. 1975. Osmoregulation: Effect of salinity and heavy metal. *Fed. Proc.* **33:**2137-2146.

Senthilkumar, P. K. Samyappan, S. Jayakumar. and M. Deecaraman 2007. Effect of heavy metal copper on the Nutritive value in a fresh water field crab, *Spiralothelphusa hydrodroma*. *Res. J. Agric.& Biol. Sci.,* **3(6):**775-781.

Shakoori, A.R. M. Javed Iqbal, A. Latif mughal, A. Syed shahid. 1994. Biochemical changes induced by inorganic mercury on the blood, liver and muscles of fresh *Ctenopharyngodon idella*. *J. Ecotoxicol. Environ. Monit.* **4:**81-92.

Sreedevi, P., B. Sivaramkrishna, A. Suresh, and Radhakrishnaiah, K(1992. Effect of nickel on some aspects of protein metabolism in the gill and kidney of the fresh water fish, *Cyprinus carpio*. *Environ. Pollut.* **77:**59-63.

Taju G, Majeed SA, Nambi K, Hameed AS 2014. In vitro assay for the toxicity of silver nanoparticles using heart and gill cell lines of Catla catla and gill cell line of Labeo rohita. Comp Biochem Physiol C Pharmacol Toxicol 161:41–52.

Torreblanca, A. J. Del Ramo. and J. Diaz Mayans 1991 Effects of cadmium on the biochemical composition of the fresh water Cray fish *Procambarus clarkia*, *Bull. Environ. Contam.Toxicol.* **47:**933-938.

Vinodhini, R. and M. Narayanan 2008. Effect of heavy metals on the level of vitamin E, total lipid and glycogen reserves in the liver of common carp (*Cyprinus carpio* L.). *Mj. Int. J.Sci.Tech.,* **2(02):**391-399.

Winer, B.J. 1971. Statistical principles in Experimental Design, 2nd Edition. McGraw- Hill, New York.

Zaki, M.S, Sharaf, N.E and Osfor, M.H 2010. Effect of vanadium toxicity in *Clarias lazera*. J Am Sci 6: 291-296.

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