Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

Int. J. Modn. Res. Revs.

Volume 2, Issue 8, pp 246-250, August, 2014

ORIGINAL ARTICLE

TOXICITY EVALUATION OF Mg DOPED ZnS NANOPARTICLES AND *Bougainvillae glabra* FLOWER EXTRACT ON FRESH WATER FISH *Oreochromis mossambicus*

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Article History: *Received 12th July,2014, Accepted 3rd August, 2014, Published 8th August,2014*

ABSTRACT

Nanoparticles are used in various fields but their usage causes many ill effects to the humans and animals. The toxicity nature of nanoparticles needs to be evaluated for their optimal use. In the present study, a detailed work has been carried out on toxicity, protein changes and FTIR spectrum analysis in liver and kidney of fresh water fish *Oreochromis mossambicus*. The LC_{50}value of Mg doped ZnS nanoparticle showed higher toxic nature on fresh water fish *Oreochromis mossambicus*. The protein content both in liver and kidney decreased in nanoparticle treated *Oreochromis mossambicus*. The protein content both in liver and kidney has recovered near to normal level in nanoparticle+flower extract treated *Oreochromis mossambicus*. The FTIR analysis showed very few variations of peak values in nanoparticle + extract treated *Oreochromis mossambicus*. The FTIR analysis denotes the denaturation of protein and other molecules in liver and kidney of nanoparticle treated *Oreochromis mossambicus*. According to our investigations the toxic nature of Mg doped ZnS nanoparticles can be rectified by the treatment of *Bougainvillea glabra* flower extract. *Bougainvillea glabra* flower extract is the potential antioxidant and can be used for detoxifying medications.

Key words: Mg, ZnS nanoparticles, *Oreochromis mossambicus*, FTIR, Toxicity, Protein.

1.INTRODUCTION

Nanotechnology is a rapidly growing industry which is currently considered as inevitable technology for human beings. The nanotechnology has wide range of benefits like biomedical, electronic, energy production and environmental sectors. The unusual behaviors' of nanoparticles are useful in some applications and causes some potential impact on the environment. The aquatic environment is at risk of exposure to nanoparticles and they cause severe ill effects on aquatic organisms. The present study involves in the evaluation of metal nanoparticles against fresh water fish *Oreochromis mossambicus*. This fresh water fish is very common in the fresh water bodies such as lakes, river and wells of Tamilnadu. It will be a useful study to conduct the nanoparticle toxicity on these fishes.The *Oreochromis mossambicus* fish is anative of Southern Africa. It is a popular fish for aquaculture. It is now found in tropical and subtropical habitats around the globe. The *Oreochromis mossambicus* is laterally compressed and has a deep body with long dorsal fins, the front part of which has spines. Coloration is typically yellow, although this is variable, and there may be weak banding.

The toxicity of nanoparticle may vary with their size, structure and composition of nanoparticles (Klaine *et al*., 2008). It has been demonstrated that the size plays a dominant role in metabolism of NPs. Small semiconductor NPs, namely quantum dots (QDs) can be cleared by renal filtration and urinary excretion (Choi *et al*., 2007). It is suggested that QDs smaller than 5.5 nm can be rapidly and efficiently metabolized by renal clearance and QDs larger than 15 nm can prevent the renal excretion and can be accumulated in the liver and spleen (Cho *et al*., 2010; Fischer *et al*., 2006; Zhou *et al*., 2011)

Acute toxicity occurs at nanoparticle concentrations in the high mg/L range (Kashiwada, 2006). The toxicity the nanoparticle is depends on the physico-chemical characteristics of the nanoparticles. In adult fish, nanoparticles are accumulated in gills and intestine (Smith *et al*., 2007). Aggressive behavior was observed in reaction to exposure to of carbon nanotubes in rainbow trout (Smith *et al*., 2007). Gill injury occurred after exposure to copper nanoparticles (Griffitt *et al*., 2007). Global gene expression analysis in gills of zebrafish demonstrated that the exposure to silver, copper and TiO2 nanoparticles or their soluble

metals produced a distinct gene expression profile (Griffitt *et al*., 2009).

Silver nanoparticles led to cellular and DNA damage and oxidative stress in medaka (Chae *et al*., 2009). The nanosilver cause's oxidative stress and apoptosis are on the liver of zebra fishes. Toxicity of different nanoparticles are studied by various investigators such as copper nanoparticles (Griffitt *et al*., 2007) and nickel nanoparticles (Ipsas *et al*., 2009). Most of the studies are present in the silver and gold nanoparticles. But the studies on the other metallic nanoparticles are very less in number. In the present study the LC_{50}, protein level in liver and kidney and FTIR studies were carried out to analyses the toxic nature of Mg doped ZnS nanoparticle.

2.MATERIALS AND METHODS

Collection of stock fish

The fingerlings of the fish *Oreochromis mossambicus* were brought from a private aqua pond in Thiruvellarai, Trichy district.

Acclimatization

The fingerlings of *Oreochromis mossambicus* were brought to the laboratory and introduced in plastic aquaria. The fish were acclimatized in the laboratory for a period of 15 days. During the period of acclimatization, the fish were fed with standard cattle feed. The aquarium water was changed and the faecal matters of the fishes and the excess feed were removed every day morning and then fed. The stock fish were kept under nature light and dark conditions (LD: 12: 12) and normal environmental temperature of 28 ± 2 ºC. To fulfill the oxygen demand, an aerator was fixed in the tank.

Experimental set up

After an acclimatization period of 15 days, 30 fished were selected for the experiment and they were allowed individually into a plastic container of 10 litre capacity.

Selection and acclimatization of test animals for LC_{50}

The fish *Oreochromis mossambicus* is more sensitive to toxicants and was acclimatized to the laboratory conditions for 15 days in the conditions roughly similar to their original habitat. During the acclimatization period, organism showing any signs of disease was discarded. They were fed with standard cattle feed. Feeding was stopped 24 hours before the commencement of the test and they were not fed during the period of experiment.

Preparation of various doses of Mg doped ZnS nanoparticles

Various doses of Mg doped ZnS nanoparticles were prepared by using distilled water. The Mg doped ZnS nanoparticles were thoroughly mixed with distilled water. The various concentration of Mg doped ZnS nanoparticles were injected to six individuals of *Oreochromis mossambicus* were allowed separately in the plastic containers. A control was kept having only well water. The fishes used in the current investigation were smaller in size upto 10cm length and were almost similar in weight.

Behavior of the fishes was noted closely for the first two hours. Dead fishes were removed. Numbers of dead fishes were counted at particular intervals till 96 hours. The dead fishes were counted from 1,6,8,12,24,36,48,72 and 96 hours to get a clear idea about the intensity of the toxicity. First, a group of 20 individuals of *Oreochromis mossambicus* were injected to wide range Mg doped ZnS nanoparticles. From the results, the narrow range of doses to determine the $LC_{50}$was arrived. The doses chosen were 50, 75, 100,125 and 150mg/l for Mg doped ZnS nanoparticles.

Six individuals were exposed for each concentration. The mortality rates were recorded and the probit regression lines were plotted with the help of Finney's probit analysis (1964).

The values were plotted. The probit mortality in the y-axis and the log concentration of the effluent in the x-axis. A straight line was drawn linking maximum number of points. To determine the LC_{50}, a line was extrapolated from 50% mortality. The intersecting point of this line in the straight line already drawn was taken as the LC_{50}.

Estimation of total protein (Lowry *et al*., 1951)

Proteins react with Folin-Ciocalteau reagent to give a coloured complex. The colour so formed was due to the reaction of alkaline copper with protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic aminoacids present.

Sample Preparation for FTIR studies

The selected tissues were dried in a hot air oven for 12 h to remove water content in the samples. The dried samples were then ground in an agate mortar and pestle in order to obtain tissue powder. The tissue powder was mixed with completely dried potassium bromide at a ratio of 1:100, and then the mixture was subjected to a pressure of 5 tons for 5 min in an evacuated dye to produce a clear transparent KBr disc of 13-mm diameter and 1-mm thickness for use in FTIR spectrometer.

Statistical analysis

Statistical analysis was performed using SPSS 11.5 software. The data were statistically analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) and were expressed as mean ± S.D. The values were considered statistically significant if the *p*-value was less than 0.05.

3.OBSERVATIONS AND DISCUSSION

The mortality of *Oreochromis mossambicus* in different doses of Mg doped ZnS nanoparticles was studied. The doses chosen were 50, 75, 100, 125 and 150mg/l for Mg doped ZnS nanoparticles. The lethal concentration 50 (LC_{50}) value determined as 154.8 mg/l for of Mg doped ZnS nanoparticles (Table 1). The toxicity of Mg doped ZnS nanoparticles is higher in the fresh water fish *Oreochromis mossambicus*. The value of LC_{50} showed the toxic nature of Mg doped ZnS nanoparticles on fresh water fish *Oreochromis mossambicus*.

When the level of toxicants exceeds the assimilatory levels of these regulatory mechanisms leads to biochemical changes and finally results in death (Aruna and Gopal, 1987). The fish *Oreochromis mossambicus* exposed to higher concentrations of textile dyeing effluent exhibited erratic movements become sluggish gradually sank to the bottom and died (Baskaran *et al.*, 1989). Sub-lethal concentration of a synthetic pyrethroid produced a decline of protein and lipid levels (Jebakumar, 1990). Copper, zinc and chloride are highly poisonous and could kill fishes at 0.1- 0.2 ppm in fresh water (Rastogi and Jayaraj, 1997).

Table 1 Analysis of lethal toxicity of Mg doped ZnS nanoparticle on fresh water fish *Oreochromis mossambicus*

Concentration	No. of fishes	Observed mortality (%)	Expected mortality (%)	LC_{50}	95% confidence limit	
					Lower	Upper
50	10	20	17.9			
75	10	30	34.6			
100	10	60	55.1			
125	10	70	74.3	154.8	144.7	168.6
150	10	90	86.0			

Chi Square= 3.549

Table 2 Estimation of protein in liver and kidney of fresh water fish *Oreochromis mossambicus*

GROUP	LIVER	KIDNEY
Control	33.2 ± 1.8 [c]	54.6±1.4 [c]
Nano alone	14.3 ± 1.0 [a]	28.7±0.8 [a]
Nano + Extract	20.9 ± 1.5 [b]	40.4±1.1 [b]
Extract	35.0 ± 1.3 [c]	56.9±0.9 [d]

Table 3 FTIR analysis of liver and kidneyof Mg doped ZnS nanoparticle treated *Oreochromis mossambicus*

Control	LIVER Nano	Nano+ Extract	Extract
1742.41 (str) C=O saturated aldehyde	**1647.52** (var) C=C symmetry reduces intensity	**1653.37** (var) C=C symmetry reduces intensity	**1713.47** (str) C=O saturated ketone
722.53 strong-medium C-H bending & ring puckering	**612.17** strong C-H (deformation bending)	**691.25** strong-medium C-H bending &ring puckering	**535.75** Weak S-S disulphide
	KIDNEY		
3100.89 (med) C-H & CH2 stretching	**2925.10** (str) CH3,CH2& CH stretching	**2924.48** CH3,CH2& CH stretching	**3085.63** (med) C-H & CH2 stretching
1647.70 (var) C=C symmetry reduces intensity	**1744.01** ester (strong) C=O stretching	**1744.10** ester (strong) C=O stretching	**1654.64** (Var) C=C (symmetry reduces intensity)

The protein content of liver steadily decreases control to the Mg doped ZnS nanoparticles treated fishes. The control *Oreochromis mossambicus* has the protein level of 33.2 ± 1.8 mg/g wet weight of tissue. The Mg doped ZnS nanoparticle treated fishes shown highly decreased protein content as 14.3 ± 1.0mg/g wet weight of tissue. The Mg doped ZnS nanoparticles + flower extract treated *Oreochromis mossambicus* has near normal level of protein as 20.9 ± 1.5mg/g wet weight of tissue. The flower extract treated *Oreochromis mossambicus* has near normal level of protein as 35.0 ± 1.3 mg/g wet weight of tissue (Table 2).

Fig. 1. FTIR analysis of liver of control fish *Oreochromis mossambicus*

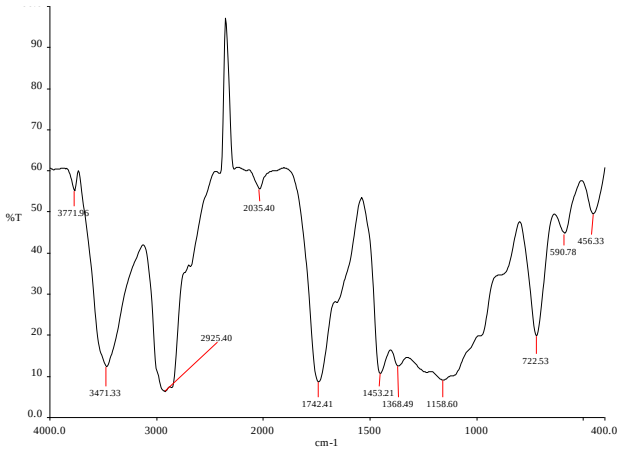


Fig. 2. FTIR analysis of liver of nanoparticle treated fish *Oreochromis mossambicus*

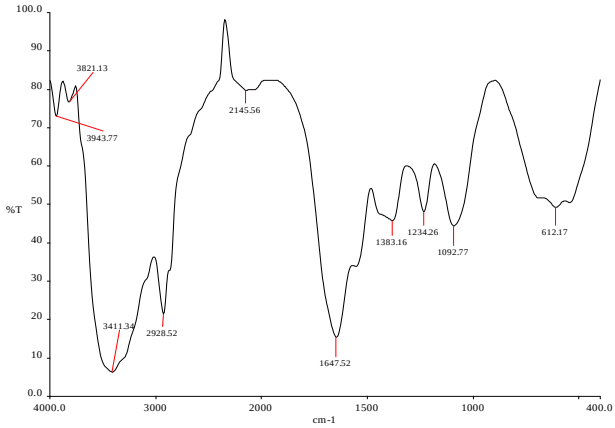


Fig. 3. FTIR analysis of liver of nanoparticle + extract treated fish *Oreochromis mossambicus*

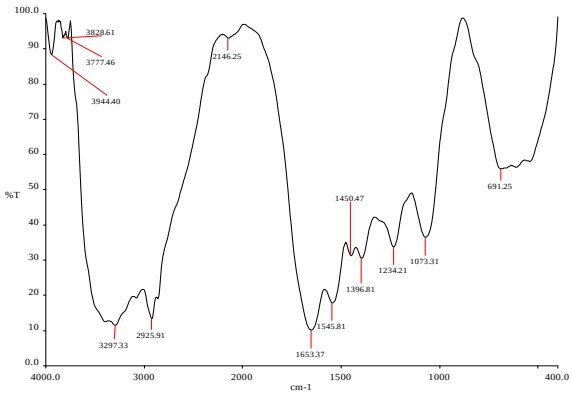


Fig. 4. FTIR analysis of liver of extract treated fish *Oreochromis mossambicus*

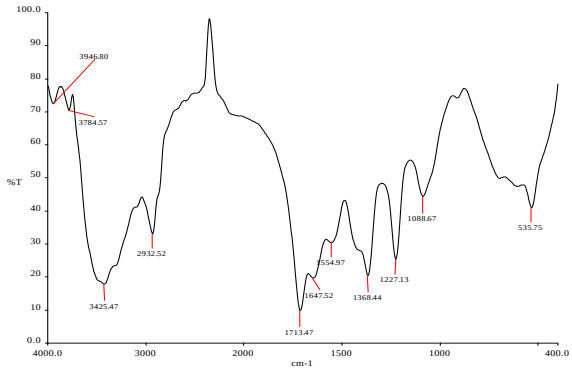


Fig. 5. FTIR analysis of kidney of control fish *Oreochromis mossambicus*

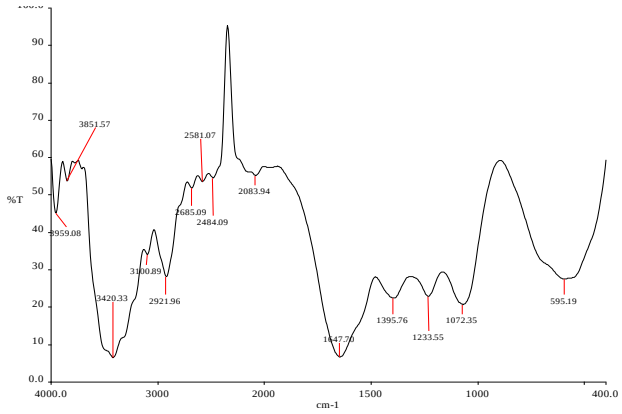


Fig. 6. FTIR analysis of kidney of nanoparticle treated fish *Oreochromis mossambicus*

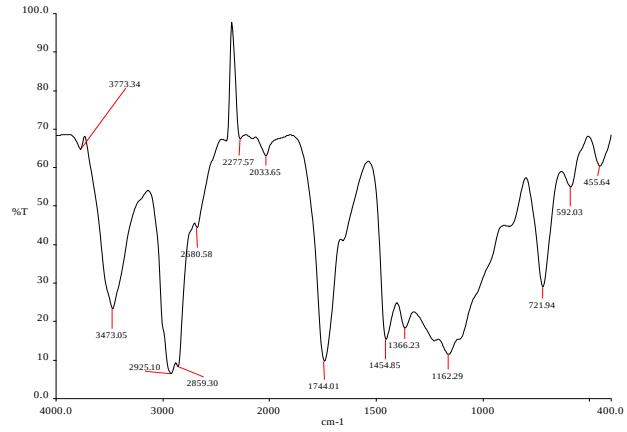


Fig.7. FTIR analysis of kidney of nanoparticle + extract treated fish *Oreochromis mossambicus*

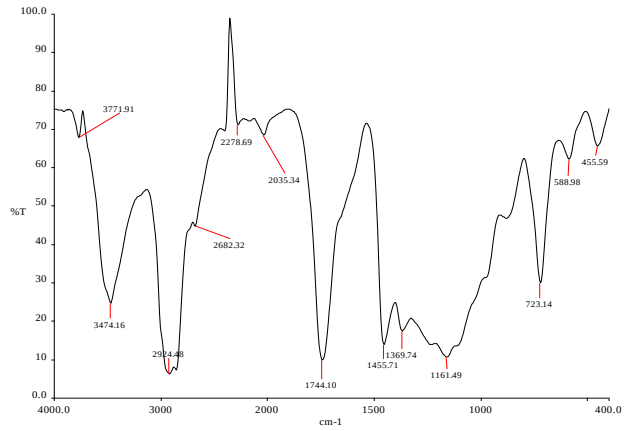
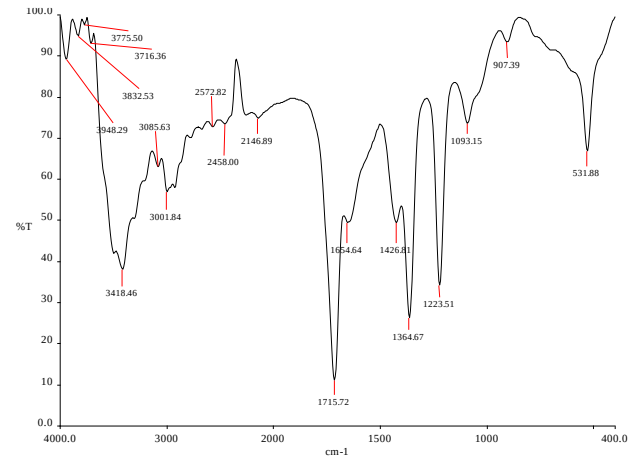


Fig. 8. FTIR analysis of kidney of extract treated fish *Oreochromis mossambicus*



The protein content of kidney decreases control to the Mg doped ZnS nanoparticles treated fishes. The control *Oreochromis mossambicus* has the protein level of 54.6±1.4mg/g wet weight of tissue. The Mg doped ZnS nanoparticles treated fishes shown highly decreased protein content as 28.7±0.8 mg/g wet weight of tissue. The Mg doped ZnS nanoparticles + flower extract treated *Oreochromis mossambicus* has near normal level of protein as 40.4±1.1 mg/g wet weight of tissue. The flower extract treated *Oreochromis mossambicus* has near normal level of protein as 56.9±0.9 mg/g wet weight of tissue (Table 2). The liver and kidney of fresh water fish *Oreochromis mossambicus* were selected for the FTIR analysis. The results were shown in table 3 and Fig.1-8. The level of protein change in liver and kidney were estimated in control and Mg doped ZnS nanoparticles treated fresh water fish *Oreochromis mossambicus*. In the present study, the protein levels are declined in both liver and kidney of nanoparticle treated fishes. The present investigation also coincides with other researchers. Boopathy Raja and Elanchezhiyan, (2011) reported that after treatment with *Bougainvillea glabra* flower extract, the amount of protein decreasedin both muscle and intestine were restored to near normal.Reactive oxygen species (ROS) can affect tissues by damaging proteins, lipids, and nucleic acid, resulting in metabolic dysfunction and cell death (Valdivia *et al*. 2007).

The decreased amount of protein level both in liver and kidney were restored to normal after treated with *Bougainvillea glabra* flower extract. Moreover the *Bougainvillea glabra* flower extract alone treated *Oreochromis mossambicus* does not showed variations in protein level on liver and kidney. The recovery from the toxicity of Mg doped ZnS nanoparticles in *Bougainvillea glabra* flower extract treated fishes denotes the detoxification potential of herbal extracts. According to our investigations the toxic nature of Mg doped ZnS nanoparticles can be rectified by the treatment of *Bougainvillea glabra* flower extract. *Bougainvillea glabra* flower extract is the potential antioxidant and can be used for detoxifying medications.

4.ACKNOWLEDGEMENT

The authors are very grateful to the authorities of Nehru Memorial College, and Head, Department of Zoology, Nehru Memorial College, Puthanampatti, Trichy, for providing all necessary facilities for the completion of this work.

5.REFERENCES

Aruna, D. and Gopal, V. 1987. Toxic effect of sublethal level of mercury on haematological parameters. *Indian. J. Environ Hlth*. 29: 52-56.

Baskaran, P., Palanisamy, S and Arunachalam, S. 1989. Effects of textile dye effluent on feeding energetics, body composition and oxygen uptake of the fresh water fish *Oreochromis mossambicus*. *J. Ecobiol*., 1: 203-214.

Boopathy Raja, A. and Elanchezhiyan, C. 2011. Analysis of toxic nature of Mg Doped ZnS nanoparticle and detoxification activity of flower extract of *Bouigainvillea glabra*. *Int. J.of Cur.Res*, Vol.3, (8), 235-239.

Chae, Y.J., Pham, C.H., Lee, J., Bae, E., Yi, J. and Gu, M.B. 2009. Evaluation of the toxic impact of silver

nanoparticles on *Japanese medaka* (*Oryzias latipes*), *Aquat. Toxicol.* 94, 320-327.

Cho, W.S., Cho, M., Jeong, J., Choi, M., Han, B.S. and Shin, H.S. 2010.Size-dependent tissue kinetics of PEG-coated gold nanoparticles.*Toxicol Appl Pharmacol* ; 245:116-23.

Choi, H.S., Liu, W., Misra, P., Tanaka, E., Zimmer, J.P., and Ipe, B.I. 2007. Renal clearance of quantum dots. *Nat biotechnol*, 25:1165-70.

Finney, D.J. 1964. Probit analysis.A Statistical treatment of the sigmoid response curve. Cambridge Univ. Press.London.

Fischer HC, Liu J, Pang KS, Chang W.C.W. 2006. Pharmacokinetics of nanoscale quantum dots: in vivo distribution, sequetration, and clearance in the rat. Adv Func Mater 16:1299-1305.

Griffitt, R.J., Hyndman, K., Denslow, N.D., Barber, D.S. 2009. Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. *Toxicol. Sci.* 107, 404-415.

Griffitt, R.J., Weil, R., Hyndman, K.A., Denslow, N.D., Powers, K., Taylor, D., Barber, D.S. 2007. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). *Environ. Sci. Technol.* 41, 8178-8186.

Ipsas, C., 2009. Toxicity and developmental defects of different sizes and shape nickel nanoparticles in zebrafish. *Environ. Sci. Technol.* 43, 6349–6356.

Jebakumar, S.R.D., S.D.J. Flora, R.M. Ganesan. 1990. Effect of short term sub lethal exposure of cypermethrin on the organic constituents of the freshwater fish *Cyprinus carpio*. *J. Environ. Biol.*, 4: 203-209

Kashiwada, S., 2006. Distribution of nanoparticels in see-trough medaka (*Oryzias latipes*). Environ. Health Perspect. 114, 1697-1702.

Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.H., Lyon, D.Y., Mahendra, S., McLaughlin, M.J., Lead, J.R. 2008. Nanomaterials in the environment: behavior, fate, bioavailability and effects. *Environ. Toxicol. Chem.* 27, 1825–1851.

Liu, H.C.L., Pang, K.S., and Chan, W.C.W. 2006. Pharmacokinetics of nanoscale quantum dots: *in vivo* distribution, sequestration, and clearance in the rat. Adv Funct Mater; 16: 1299-305.

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

Rastogi, V.B and Jayaraj, M.S. 1997. Animal ecology and distribution of animals" KedemathRamnath, Meerut.

Smith, C.J., Shaw, B.J., Handy, R.D. 2007. Toxicity of single walled carbon nanotubes to rainbow trout (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. *Aquat. Toxicol.* 82, 94–109.

Valdivia, P. A., Zenteno-Savín, T., Gardner, S. C. and Alonso Aguirre, A. 2007.Basic oxidative stress metabolites in eastern Pacific green turtles (*Chelonia mydas* agassizii).*Comp. Biochem. Physiol.* 146C, 111-117.

Zhou, C., Long, M., Qin, Y., Sun, X., and Zheng, J. 2011. Luminescent gold nanoparticles with efficient renal clearance. *Angew Chem Int* Ed; 123:3226-30.

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