Original Research Paper Nanotoxicology

Impact of Iron Oxide Nanoparticles on Behavioural Changes in Fresh Water Fish Labeo Rohita

V.Keerthika Department of Biology, Gandhigram Rural Institute- Deemed University, Gandhigram -634302, Tamil Nadu, India

R.Ramesh Department of Biology, Gandhigram Rural Institute- Deemed University, Gandhigram -634302, Tamil Nadu, India

*** M.R.Rajan** Department of Biology, Gandhigram Rural Institute- Deemed University, Gandhigram -634302, Tamil Nadu, India
* Corresponding Author

ABSTRACT

The increasing production and use of iron oxide NPs will inevitably result in a greater exposure risk for both people and the environment. Thus, it has become essential to assess the potential health and environmental effects of iron oxide NPs on humans, non-human biota, and ecosystems. Currently, Iron Oxide nanoparticles (Fe3O4) are increasingly used for environmental remediation; however, toxicological impacts of iron oxide nanoparticles on the aquatic ecosystem remain poorly understood. Hence, the present study endeavoured to study the effects of sub-lethal concentrations of iron oxide nanoparticles on behavioural parameters of Labeo rohita. Abnormal behavioural changes such as Bottom resting, Surface respiration and Jerk movement were affected by Iron oxide NPs at sub lethal exposure levels. This study highlights the potential ecotoxicological effects of Fe3O4 nanoparticles release in aquatic environments and may serve to encourage regulatory agencies in India to more carefully monitor and regulate the industrial use and disposal of Fe3O4 nanoparticles.

KEYWORDS Impact, iron oxide nanoparticles, Behavior, Labeo rohita

INTRODUCTION

Nanotechnology has gained a great deal of public interest due to the needs and applications of nanomaterials in many areas of human endeavor such as industry, agriculture, business, medicine, public health and many others. It is estimated that nanotechnology will have a good impact on the industrial revolution and is projected to become a $1 trillion market in 2015 and will provide employment to 2 million workers.(Roco, 2005). Metal oxide nanoparticles (NP) are one of the most broadly applied groups of nanomaterials. Although bulk materials of metal oxide have been massively produced for long time, with the development of nanotechnology, nanoparticulate versions of these metal oxides have been produced and applied to a wide range of commercial and industrial products in recent years. Among the metal oxide nanoparticles, Iron oxide nanoparticles (NPs), with the main forms being of magnetite (Fe_3O_4) and hematite (a-Fe_2O_3 and c-Fe_2O_3), have attracted extensive interest for application because of their super paramagnetic properties and high catalytic abilities (Huber,2005 and Kadar *et al.*,2010).The research on health risks and ecological impacts of iron oxide nanomaterials is very limited because iron oxide nanomaterials are generally regarded as non- or low-toxic materials (Karlsson et al., 2008, 2009; Soenen and De Cuyper, 2010). However, recent studies have revealed that iron oxide nanomaterials pose a potential health risk. Super paramagnetic iron oxide nanoparticles show cytotoxicity (Mahmoudi et al., 2010). Zhu et al. (2009a, 2011) also found that ferric oxide nanoparticles had potential lung and systemic cumulative toxicity in rats, and intravascular iron oxide nanoparticles may induce human endothelial inflammation and dysfunction. Moreover, iron oxide nanomaterials could serve as significant carriers of toxic chemicals (Guan et al., 2008; Tang et al., 2009) and increase exposures to adsorbed pollutants. However, to date, very few studies have investigated on the ecotoxicity of iron oxide (Fe_3O_4) Nanoparticles particularly in aquatic systems. The study related to the short-term sub lethal exposure of iron oxide nanoparticles on Behavioural changes in an Indian major carp Labeo rohita is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

Ferrous chloride ($Fecl_2$) and Sodium hydroxide (NaOH) was purchased from Loba chemicals, India. All the reagents used for the synthesis Fe_3O_4 were analytical grade and used without further purification. All the glass wares were washed thrice with deionized water and dried before use.

Experimental Animals

Healthy adults of *Labeo rohita* were procured from SGC fish farm, E. Puthupatti, Theni,Tamilnadu, India and acclimatized to laboratory conditions for about 15 days before the commencement of the experiment. Adult fishes of both sexes with an average weight of 10.6 g and average length of 15.00 cm were selected for the study .The fishes were maintained in standard laboratory conditions.

Synthesis and Characterization of Iron oxide nanoparticles

Iron oxide nanoparticles were synthesized by Precipitation method .For synthesis, 0.03 mole of $FeCl_2$ (5. 96g) were dissolved into 150mL of distilled water and stirred vigorously using magnetic stirrer for 20 minutes. Precipitation was achieved by adding 100ml of 1 M NaOH solution in drop wise under vigorous stirring. The initial pH was observed as 3 and it was increased to pH 12 using 1M NaOH. Then precipitating process was continued until dark black color precipitate obtained. Then the Fe3O4 precipitate was taken into centrifuge tube and centrifuged at 1500 rpm for 20 minutes. The centrifuging process continued with water and two times with ethanol. Then the precipitate was dried. Finally, iron oxide nanoparticles (Fe_3O_4) were obtained. The morphology and composition of Fe_3O_4 nanoparticles were examined by Scanning Electron Micros-

copy (SEM) using a LEO 1455 VP equipped with energy dispersive. The FTIR spectra of synthesized iron oxide nanoparticles was analyzed for knowing the possible functional groups. The measurement was carried out by JASCO (FTIR-6200) spectrum.

Determination of Iron oxide Nanoparticles Toxicity

To estimate the lethality of iron oxide nanoparticles, a stock solution mg/ml was prepared, and a series of three iron oxide nanoparticles target concentrations (i.e. 100, 1500, 3000 ppm) were prepared. A control was al so maintained in tap water. Ten healthy fish, with an average length of 15.0 cm and average weight of 10.6 g were selected and introduced into each trough. Triplicates were maintained for experimental and control. The manifestation and survival time of fish was observed in each concentration for seven days. There was no mortality of fish exposed to 100 and 1500 ppm of Fe_3O_4 nanoparticles. However, mortality was observed at 3000ppm .

Sublethal toxicity studies (1/10th and 1/100th of 96h LC 50 of Fe_3O_4)

To assess the sub lethal toxicity Fe_3O_4 NPs, 30 healthy fish were selected from the stock and divided into three groups (one control and two experiments) and then introduced into three separate plastic troughs (10 fish in each tank). 1/10th and 1/100th of LC 50 value of Fe_3O_4 NP (300ppm and 30 ppm) was added directly into two experimental plastic troughs after removal of the same volume of water. Experiment was conducted for a period of 7 days. At the end of 7^{th} day of exposed fish were randomly collected from control and experiment trough for the study of behavioural changes. No mortality was observed during the sub lethal exposure period.

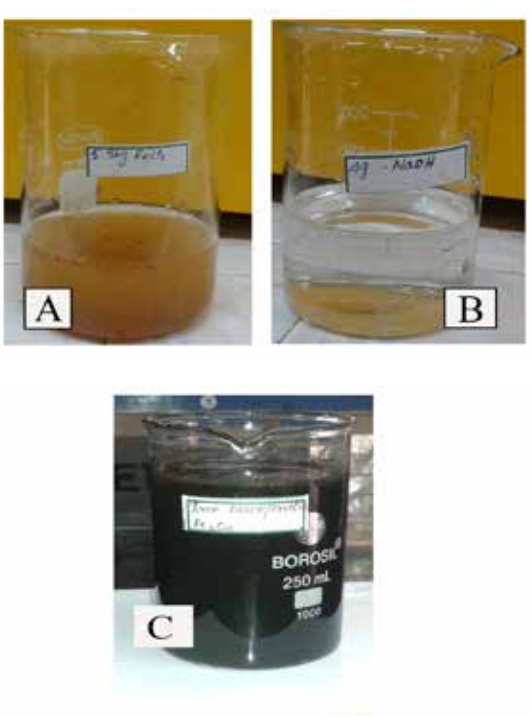
Behavioural analysis

Behavioral analysis of the fish was conducted between 10 am and 5 pm . Fish locomotion and abnormal behavioral changes were recorded during the experiment.

RESULTS AND DISCUSSION:

Synthesized Iron oxide nanoparticles by Precipitation method is presented in Fig.1

Fig.1 Synthesis of Iron oxide nanoparticles



The basic Behavioural changes of Labeo rohita exposed to the iron oxide nanoparticles are

Presented in Table 1.

Table 1. Basic Behavioural changes of Labeo rohita

S. No	Activity	Yes	No
1	Circular swimming		✓
2	Jerk movement	✓	
3	Bottom resting	✓	
4	Surface respiration	✓	
5	Aggressive movement		✓
6	Excess of mucous secretion		✓
7	Mortality observation		✓
8	Behavior observation	Good	
9	Breathing movement	Good	

The behavioral responses of fish were observed daily and control group showed normal behavior during experimental period. First abnormal behavioral changes were noticed in fish exposed to sub lethal concentrations after the start of experiment. Behavior is an appropriate indices in toxicological studies because behavioral changes in fishes are good indexes of organism response toward aquatic pollutants. Fishes showed behavioral abnormalities in exposure to chemical pollutants . Chen et al (2011) reported the behavioral effects of titanium dioxide nanoparticles on larval zebra fish (Danio rerio). Little and Finger(1990) reported the swimming behavior of fish exposed to sub lethal toxicity. The assessment of fish behaviour is a promising tool for ecotoxicological studies, as it reveals toxicant induced complex physiological consequences at the individual level and has the potential to complement conventional toxicological methods with higher ecological relevance (Scott and Sloman, 2004 and Zala and Penn, 2004).

Conclusion

It can be concluded that Iron oxide nanoparticles can cause behavioral disturbances in fishes at sub-lethal concentrations. These changes are potentially disruptive to the survivability of *Labeo rohita*. This fact should be taken into consideration when Iron oxide nanoparticles is used for environmental applications.

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