Comparative toxicities of bulk and nanoforms of TiO2 and CuOin the fresh water fish Rasboradandia(Ham.1872)

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Abstract- The acute toxicity of nano-scaled metal oxides CuO and TiO2 were examined and compared to that of their respective bulk (micro-scaled) counterparts in the fresh water fish Rasboradandia for 96 h. Nanoparticles with different sizes, viz. of CuO of 25 nm, TiO2 of20nm were evaluated for their toxicity by estimating LC50 at 96 hrs. Median lethal concentrations (LC50)that were determined by probit analysis was found to be 97.02 ppm for CuOnp, 518.3 ppm for TiO2np and 2263 and 599 ppm for their bulk forms respectively. The results proved that nanoparticles showed acute toxicity to fishes when exposed for 96 hrs and this could reveal the fact that exposure of nanoparticles may result in adverse toxic effects to fishes that eventually affects the health status of the aquatic ecosystem.
Keywords –Nanoparticles, LC50, Copper oxide, Titanium dioxide, Rasboradandia

I. INTRODUCTION

Nanotechnology has emerged as a fast growing sector impacting key economical fields and providing new engineered nano-enabled products, constituted by nanoparticles (NP), with novel and unique functions that reach the market every day. The application of nanotechnology finds wide use in almost all fields.NP with a size between 1 and100 nm ,possess unique physicochemical properties, but the same properties can be problematic in a toxicological perspective. Therefore, increased focus on various toxicological issues related to nanoparticles is now critical. This size related-properties results in larger reactivity and higher mobility (Rauscher et al., 2014), leading to numerous applications in medical diagnostics, electronics, computers, cosmetics and environmental remediation. (Cha and Myung, 2007; Bouret al.,2015).The major concern about metal oxide np is that because of their chemistry, size, and nonbiodegradable nature, they will rapidly distribute throughout the environment with unknown consequences. Until now little is known about the potential toxicity of metal oxide np.The rapid expansion of np increases the chance for release into aquatic environments. It is also important to distinguish effects of np from its bulk forms when assessing the toxicity of metal oxide np (Wang et al., 2009).

CuOnp is widely used as additives in lubricants, polymers/plastics, metallic coating, inks etc. chemical industry, electronics, biomedicine, bio remediation etc. TiO2 is an opacifier which is used in paints, paper, plastic, and many cosmetics products (Jiang, 2009; Klaineet al., 2008). This np will inevitably enter the environment and cause significant adverse effects to aquatic organisms. Therefore, there is a need to develop rapid and sensitive screening methods to monitor the potential impact of toxicants; besides water qualities. Previous work have investigated the toxicity of ZnO and Al2O3 np to plants (Lin and Xing, 2007, 2008), ZnO, CuO and TiO2np to bacteria (Reddy et al., 2007; Heinlaanet al., 2008; Huang et al.,2008), ZnOnp to freshwater microalga (Franklin et al., 2007), and ZnO,TiO2, SiO2 and C60np to D. magna (Adams et al., 2006; Lovern et al.,2007). Very few studies are available on the toxicity of metal oxide np to fresh water fish especially in tropical environments (Griffith et al.,2008). Hence, the present study has been proposed to determine the acute toxicity of CuO and TiO2np to the freshwater fish R. dandiaand to compare the toxicities between the np and its bulk counter parts.

II. MATERIALS AND METHODS

2.1Test chemicals and species–

Healthy fishes (length 8 ± 2 cm) were collected locally from Trivandrum and were confined to large glass aquaria bearing tap water for a period of two weeks in the laboratory for acclimation. They were kept in batches (10 each) in 50L tanks filled with dechlorinated tap water. The fishes were fed twice daily and the water was renewed every day by routine cleaning of aquaria to remove fecal matter or death fish (if any). Prior to the commencement of the experiment,feeding was stopped. The physico–chemical characteristics of the water was analysed as per standard procedures (APHA, 2008) .

The np used in this study were analytical grade of 99% purity. Chemicals were dry powder, and the average particle size of the four chemicals used in the study was 20 nm and 200 nm for TiO2 np and bulk forms and 25 nm, and 350 nm of CuOnp and bulk forms respectively. All the chemicals were used without further purification. Test suspensions of 1000 ppm were dispersed in millipore water by sonication prior to use.The NP suspensions were sonicated for 30 min using Ultrasonic probe sonicator, HielscherUSA,UP100H(100watts, at a frequency of 30 kHz)prior to the toxicity testing. The hydrodynamic size of the np in the suspensions was measured using TEM.

To determine the acute toxicity effects of selected nanoparticles, the median lethal concentration or LC50 values for 96 h were determined by probit analysis, with a confidencelevel of 0.05 (Finney, 1971). In order to assess LC50 of the nanoparticles, the fishes were not fed a day prior to and during the test period to reduce fecal and excess food contaminating the test solution. For the analysis of acute toxicity, 10 fishes each were exposed separately to different concentrations of the chemical and mortality was recorded after 96 hours of exposure. Control groups were also maintained without the addition of chemicals.

A wide range of concentrations were prepared for each metal oxide used in the study. The concentrations were selected based on the results of range finding tests, so that the concentrations of test chemicals were increased in geometric proportion series in each group as follows: Group I: CuO-bulk at seven different concentrations, ie., 10, 20, 30, 40, 50, 60 and 70 ppm for 96 h, Group II: CuOnp at eight different concentrations, ie., 5, 25, 50, 75, 100, 125, 150 and 175 ppm for 96 h, Group III: TiO_2-bulk at nine different concentrations ie., 25, 50, 75, 100, 125, 150, 175, 200 and 225 ppm for 96 h, and Group IV: 200,300,400,500,600,700,800 ppm for TiO_2 np. The movement and the behaviour along with the mortality of fishes were continuously monitored throughout the study. Fishes without any movement for a long period were considered as dead and were removed from the tanks immediately to prevent contamination.

2.2 Statistical analysis

All experiments were performed in triplicates for the accuracy of the results. Total number of animal used in the experiments, the exposure concentrations and the mortality rate in each experiment were fit to a probit model using log10 concentration transformation using the statistical package SPSS 22.0. The correlation between concentration on Y-axis and mortality on X-axis and the best-fit line was obtained by plotting graph using MS Excel 2007

III. EXPERIMENT AND RESULT

Physico-chemical features of the test water were analysed as per APHA guidelines and recorded as:

Water temperature- (26± 2 °C)

Dissolved Oxygen (mg/L)- 6.9;

Total Hardness (mg/L)- 225;

pH ±7.4

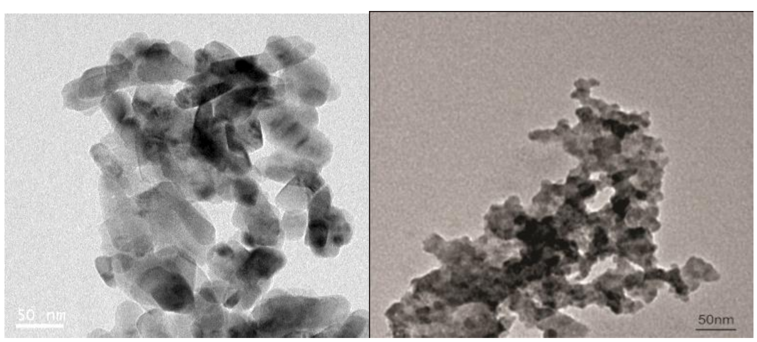

Figure.1. TEM images of TiO_2 and CuO nanoparticles

The shape and size distribution of the CuO and TiO_2 nanoparticles were evaluated by TEM (Fig. 1). The average size of the particles was found to be 25 and 20 nm respectively.

Fishes exposed to xenobiotics were divided into 4 groups and mortality of the fishes in each group was continuously monitored throughout the experiment. It was observed that in Group I of CuO bulk exposed fishes no mortality was observed at 500ppm for 96 h duration. At 1000, 1500 ppm, fishes showed 20 and 30 % mortality, respectively. However, at 4000 ppm total mortality of fishes was observed at 96 h (Table 1). In Group II of np treated fishes, no mortality was observed at 5, and 25 ppm concentrations up to 96 h. Cumulative mortality of 10 and 30% were observed at 50 and 75ppm concentrations, and 100% mortality was recorded at 175 ppm.(Table 3).

TiO_2 bulk exposed fishes (Group III) showed no mortality at 200 ppm and 20 and 90% mortalities were recorded at 400 and 900 ppm respectively at 96 hrs. In Group IV of TiO_2 np treated fishes, no mortality was observed at 200 ppm, up to 96 h and 100 % mortality was recorded at 900 ppm.

Acute LC50 values at 96 hrs were recorded as 2263 ppm for CuO bulk, 97.02 ppm for CuO np,599 ppm for TiO2 bulk and 518 ppm for TiO2 np (Tables 2,4 and 6; Figs. 3-5). The results of probit analysis indicated that the percentage of mortalities were positively correlated (r = 0.97, 0.78, 0.81 and 0.94) with the concentrations of CuO bulk, CuOnp, TiO2 bulk and TiO2-NPs, with r values 0.97, 0.78, 0.81 and 0.94 respectively.

Table 1: Percentage of fish mortality when exposed to different concentrations of CuO bulk for 96 h

Concentrations(ppm)	Total (No. of animals)	Mortality (%)	Hour of exposure
500	10.00	0	96 h
1000	10.00	20	96 h
1500	10.00	30	96 h
2000	10.00	50	96 h
2500	10.00	60	96 h
3000	10.00	70	96 h
3500	10.00	80	96 h
4000	10.00	90	96 h

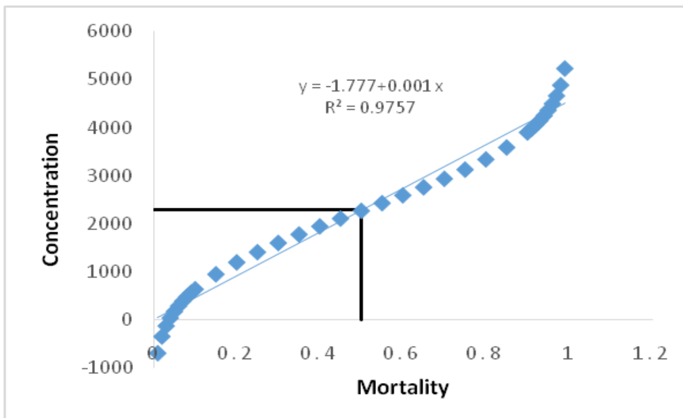

Figure .2 Results of the probit analysis showing the relationship of concentrations of CuO bulk to mortality of Rasboradandia

Table 2: Probit analysis of 95% confidence limits for effective concentrations of CuO bulk in Rasboradandia

		95% Confidence Limits	
Prob	Concentration (ppm)	Lower	Upper
0.01	33	4.009	38.270
0.05	168.5	12.282	35.097
0.1	631.160	265.273	407.573
0.5	2263	2097.062	2428.038
0.75	3122	2927.592	3361.822
0.99	5225	4786.337	5823.971

Table 3: Percentage of fish mortality when exposed to different concentrations of CuOnp at 96 h

Concentrations	Total (No. of animals)	Mortality (%)	Hour of mortality
5	10.00	0	96 h
25	10.00	0	96 h
50	10.00	10	96 h
75	10.00	30	96 h
100	10.00	50	96 h
125	10.00	80	96 h
150	10.00	90	96 h
175	10.00	100	96 h

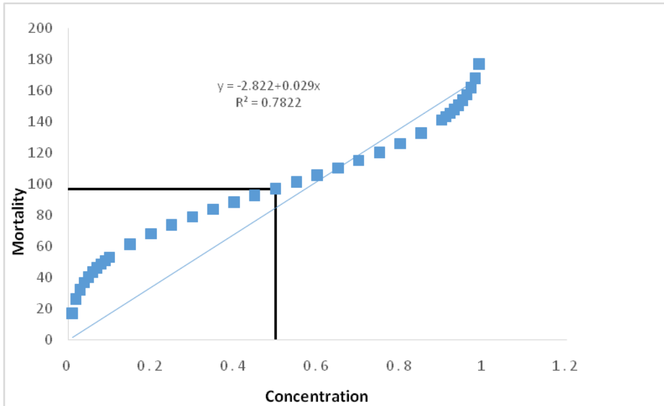

Figure 3. Results of the probit analysis showing the relationship of concentrations of CuOnp to mortality of Rasboradandia

Table 4: Probit analysis of 95% confidence limits for effective concentrations of CuOnp in Rasboradandia

		95% Confidence Limits	
Prob	Concentration (ppm)	Lower	Upper
0.01	17.043	1.751	28.655
0.05	40.473	28.892	49.456
0.1	52.963	43.222	60.683
0.5	97.021	91.452	102.609
0.75	120.210	114.101	127.410
0.99	176.999	165.294	192.422

Table 5: Percentage of fish mortality when exposed to different concentrations of TiO2 bulk of 96 h

Concentrations	Total (No. of animals)	Mortality (%)	Hour of exposure
200	10.00	0	96 h
300	10.00	20	96 h
400	10.00	30	96 h
500	10.00	50	96 h
600	10.00	60	96 h
700	10.00	70	96 h
800	10.00	80	96 h
900	10.00	90	96 h

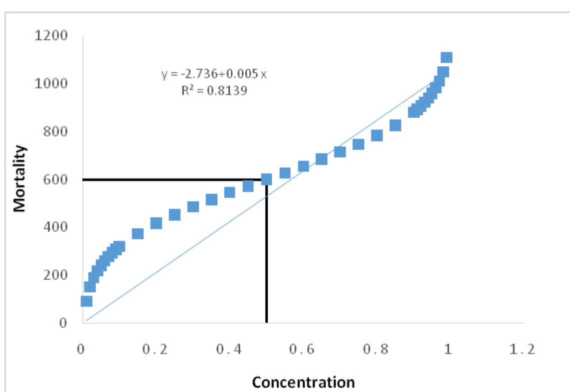

Figure 4. Results of the probit analysis showing the relationship of concentrations of TiO2 bulk to mortality of Rasboradandia

Table 6: Probit analysis of 95% confidence limits for effective concentrations of TiO2 bulk in Rasboradandia

		95% Confidence Limits	
Prob	Concentration (ppm)	Lower	Upper

0.01	89.869	5.130	1610.035
0.05	239.239	139.215	292.649
0.1	318.867	261.386	363.585
0.5	599.757	570.289	630.040
0.75	747.591	712.114	791.029
0.99	1110	1034.321	1210.431

Table 7: Percentage of fish mortality when exposed to different concentrations of TiO2np at 96 h

Concentrations	Total (No. of animals)	Mortality (%)	Hour of exposure
200	10.00	0	96 h
300	10.00	20	96 h
400	10.00	40	96 h
500	10.00	50	96 h
600	10.00	60	96 h
700	10.00	80	96 h
800	10.00	90	96 h
900	10.00	100	96 h

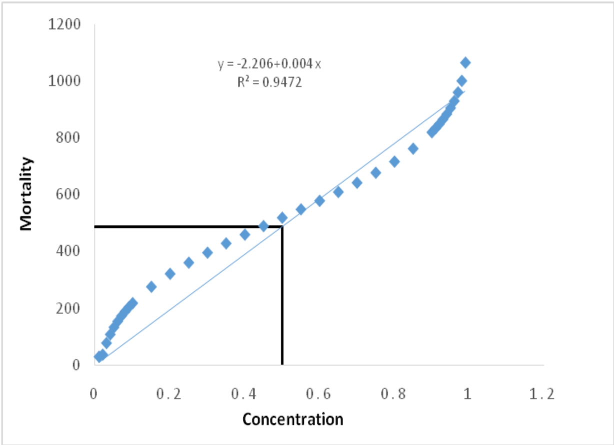

Figure 5. Results of the probit analysis showing the relationship of concentrations of TiO2np to mortality of Rasboradandia

Table 8: Probit analysis of 95% confidence limits for effective concentrations of TiO2np in Rasboradandia

		95% Confidence Limits	
Prob	Concentration (ppm)	Lower	Upper
0.01	28.349	13.633	53.502
0.05	131.817	49.366	193.877
0.1	217.201	148.952	269.345
0.5	518.395	486.513	549.292
0.75	676.915	642.037	718.766
0.99	1065	988.231	1168.510

IV. DISCUSSION

Engineered nanoparticles may present living systems with a uniquely novel challenge, since such materials were not generally encountered by living organisms during the course of biological evolution (Dowling, 2004; Colvin, 2003, 2004; Howard, 2004; Moore, 2002; Warheit,2004).Metal-based nanotechnologies are increasingly used for environmental remediation and several industrial processes; however, toxicological impacts of metal np on the aquatic ecosystem remain poorly understood (Chen et al., 2012).Determination of the LC50 values is highly useful in the evaluation of safe levels or tolerance levels of pollutants (Prenteraet al., 2004)

The present study was aimed to assess the acute toxicity of different nanoparticles in the freshwater fish, Rasboradandia. Results of 96-h bioassay showed that CuOnp had the lowest LC50 value and thus, it is the most toxic metal oxide form among the tested ones in this study. The median lethal concentrations of CuOnp, CuO bulk, TiO2-np and TiO2-bulk were 2263, 97.02, 599.7, 518.3 ppm, respectively. Besides, 100% mortality of fishes was observed at 120 ppm concentration of CuOnp whereas, in TiO2 np exposure, 100% mortality rate was observed only at 900 ppm concentration after 96hrs. Therefore, the nanoparticles CuO is comparatively more toxic to the fish than TiO2 np. TiO2np of less than 25 nm was reported to cause higher growth inhibition of algae (Desmodesmussubspicatus) and greater immobilization of Daphnia magna than those bigger than 100 nm (Hund-Rinke and Simon, 2006). In addition, exposure to 0.22 μm filtered TiO2np caused higher mortality among D.magna than un-filtered ones, indicating that toxicity might be directly related to the size of the dispersed np(Lovern and Klaper, 2006).The toxicities of oxide np have been reported for mammalian cell lines (Chang et al., 2007), bacteria (Huang et al., 2008), and crustaceans (Heinlaanet al., 2008). Heinlaanet al., (2008) reported that nanoCuO showed more toxicity than bulk CuO to Crustaceans. The difference in toxicity might be due to the difference in chemical composition and size of the nanoparticles as reported in these studies.

Hall et al.,2009 reported very high LC50 values of >500 mg/L were observed for fathead minnow(Pimephalespromelas) after 48hourrsof exposure to TiO2np, whereas in Oreochromismykiss 96 hr LC50 of TiO2npwas>100 mg/l (Warheitet al., 2007) and no effects on Daniorerio were observed below 500 mg/L (Zhu et al., 2008).These observations agree with the results of the present study which recorded an LC 50 value of 518.3 ppm for TiO2np at 96 hours in Rasboradandia. Sub lethal effects of nanosized TiO2 have been reported on juvenile rainbow trout (Oncorhynchusmykiss) after 14 days waterborne exposure (Federiciet al., 2007) and 8 weeks exposure through fish food (Ramsdenet al., 2009). In the above mentioned studies, the authors assessed TiO2np of different sizes and with or without different stabilization agents, which seems to be the reason for the differing acute toxicity.

Comparison of LC50 at 96 hrs indicates that the toxicity of nanoparticles and their bulk forms are in the order of CuOnp> TiO2np>TiO2 bulk >CuO bulk. When compared to their bulk counterparts, CuOnp were found to be more toxic at lower concentration whereas TiO2np and bulk form produced mortality at a similar concentration. Although the np of CuO and TiO2 used in the study were almost similar in size they differ very much in their toxicity. A key difference between bulk and nanoscale materials is the much higher surface area of a given mass or volume of nanoparticles (NPs), compared to an equivalent weight or volume of bulk material particles. This increased surface area enhances certain properties of the materials.(Clemente et al.,2013)

TiO2 nanoparticles were not more toxic compared to larger particles as observed by when rats were exposed to TiO2np and that the toxicity was not dependent on particle size/surface area, but rather the surface reactivity (Warheitet al.,2006, 2007). However, Adams et al., (2006) reported no difference between TiO2, SiO2, ZnOnp and bulk toxicity to bacteria because they formed similar size aggregates. Franklin et al,. (2007) also reported similar toxicity between ZnOnp and bulk forms to freshwater microalgae. Therefore np toxicity, caused by size or by composition, differ very much in their toxic effects.TiO2np seems to be less toxic than other metal oxide np, TiO2 exists in different structural forms with different properties and consequent environmental impacts. Among them, anatase and rutile are considered the most likely to be found in the environment (Ju-Nam and Lead,2008).In the present study np selected was anatase form considering its diverse array of industrial and medical applications.Toxicities of TiO2npanatase have been reported to be biologically more active in terms of cytotoxicity or DNA damage (Affuanet al., 2009). Their toxic effects were reported in zebra fish embryos after exposure to 500mg/l of anatase TiO2 (Zhu et al.,2008).

Hence nanoparticles remain unpredictable in their toxicities when exposed to the environment. NP are behaving very differently from their bulk particles of the same chemical composition (Wiggintonet al., 2007).This may be attributed to high reactivity, small size, and high surface area per unit volume of np. Direct np toxicity results from the chemical composition and surface reactivity (Navarro et al., 2008).

V.CONCLUSION

The widespread application of manufactured metal oxide NPs arises great concern about their safety for human and aquatic organisms. The high toxicity of nanoparticles shows that the nano level gives rise to specific concern. But today there is a lack of information regarding if nanoparticles of different composition are more toxic than larger particles when assessing differenttoxicological outcomes. Hence acute toxicity study of different nanoforms needs much attention

VI. REFERENCES

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