IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 12 Ver. I (Dec. 2016), PP 55-60
www.iosrjournals.org

Synthesis of Calcium Oxide Nanoparticles and Its Mortality Study on Fresh Water Fish Cyprinus Carpio

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Abstract: *Calcium oxide (CaO) nanoparticle (NP) was synthesized by solution combustion method using urea as fuel. The CaO nanoparticle was characterized by X-ray diffraction (XRD), Scanning Electron Microscope (SEM) analysis and UV-absorption spectral studies. The average crystallite size was found to be ≈36nm and the band gap energy of CaO nanoparticle was 4.9 eV. The toxicity tests were performed on fresh water fish Cyprinus carpio for synthesized CaO NP at different concentrations ranging from 200 to 280 mg/L for 4 days. Bioassays were repeated three times and the data obtained were statistically evaluated using Finney's Probit Analysis. Toxicity test performed revealed that LC_{50} value for Cyprinus carpio was 234.9mg/L with 95% of confidence limits for 226.819- 242.624mg/L.*

Keywords - *Calcium oxide, Cyprinus carpio, LC_{50}, SEM, Toxicity, XRD.*

I. Introduction

Various metal oxide nanoparticles (NP) have been used in various fields, ranging from catalysis, opto-electronic materials to sensors, environmental remediation, and biomedicine [1]. Various NP are known to present in our environment, from both natural and anthropogenic sources [2, 3]. However, the synthesized materials on nano scale are increasingly used in wide range by industries due to their unusual chemical and physical properties.

Thus, given the increasing production of NP, their release into the environment can be transported to aquatic ecosystems that may induce malignant impacts on aquatic biota [4, 5]. Further, the toxicity of metal oxide NP on organisms has become a concern to aquatic eco-toxicology and hence has become relatively new and evolving research field.

Recently, copper oxide (CuO) and zinc oxide (ZnO) NP have been shown to have negative effects on the survival and growth of organisms [6]; these studies suggest that the release of NP might have negative impacts on both organisms and the environment.

Calcium oxide (CaO) is an important inorganic compound, which is used across various industries as catalyst, toxic-waste remediation agent, adsorbent, *etc*. [7-10].

In an aquatic ecosystem, Fish forms an important class of life form on the basis of their use as nutritive food and are also a useful indicator of pollution [11]. Among the aquatic organisms, the pollutants have a very adverse effect on the fish, and hence fishes are considered to be the most relevant organism for assessing pollution in the aquatic ecosystems [12]. Many studies have been demonstrated heavy metal toxicity in this aquatic life form. [13-16].

With this context, present study is focused on the characterization and toxicity determination of synthesized CaO nanoparticle in *Cyprinus carpio*, which is in continuation of our previous work on synthesis of nanoparticles and their application to colour removal of industrial effluents, where the toxicity of the nanoparticles could also play a significant role [17, 18].

II. Materials and Methods

2.1 Chemicals and reagents

All the chemicals used in present study were of analytical grade. Calcium nitrate ($Ca(NO_3)_2.4H_2O$; 99%) and urea (NH_2CONH_2; 99.5%) purchased from Hi-Media chemicals, Mumbai, India and distilled water.

2.2 Synthesis of nanoparticles

Calcium oxide nanoparticles were prepared by solution combustion method using $Ca(NO_3)_2.4H_2O$ (14.16g) and NH_2CONH_2 (6.0g) dissolved in 100cm^3 silica crucible using distilled water. The solution thus obtained was treated at 500°C for calcinations [17, 18]. Resultant product obtained was further characterized using XRD, SEM and UV-absorption spectroscopy.

2.3 Collection and Maintenance of fish

The common edible fish *Cyprinus carpio* were procured from the State Fisheries Department, Bhadra Reservoir Project, Bhadravati, Karnataka, India. The fish were brought to the laboratory in aerated polythene bags. The length of the fish 10-12cm and body size 18.5 to 20.5g. The fish *Cyprinus carpio* were acclimatized to the laboratory condition. During the period of acclimatization in a large aerated tanks, the fish were fed daily with standard fish pellets and allowed to acclimate for12 days. Feeding was stopped one day prior to the experiment. Water was renewed every day to provide freshwater, rich in oxygen. If mortality exceeds more than 5% during the acclimatization, the entire batch of fish was discarded. The water used for acclimatization and conducting experiments was clear un-chlorinated ground water. The containers used for the test media of 20 L capacity were filled with dechlorinated water, where maximum 10 fish were used per each concentration of the calcium oxide nanoparticles. Control group was simultaneously maintained along with experimental groups with same conditions as of experimental group except for presence of CaO-NP.

2.4 Physiochemical parameters of water

During the test physiochemical parameters of water such as, pH, dissolved Oxygen, total hardness, temperature *etc*. were measured according to procedure described earlier (APHA, 2005) and results were recorded as follows, temperature 26±2 °C, pH 7.5±0.2 at 26ºC, dissolved oxygen 8±0.5 mg/L, carbon dioxide 6.3±0.5 mg/L, total hardness 24±2 mg as $CaCO_3$/L, salinity 0.01ppm.

2.5 Acute toxicity and LC_{50} determination

The test organisms (i.e. fish) were randomly distributed in aquaria. The amount of CaO-NP to be added in each aquarium was calculated accurately with reference to the volume of each aquarium. The fish, in batches of 10, were exposed to varying concentrations of CaO-NP with 20 liters of water using three replicates for each concentration. There was simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the nanoparticle keeping all other conditions constant. The experiments were performed in triplicates. The mortality rate in the control group did not exceed 5% and 95% of the fish looked healthy throughout the experiment,

Acute toxicity tests were carried out for a period of 96 h, and dead fish were removed as and when observed. Acute toxic effects of metal oxide to the fish were determined by the use of Finney Probit Analysis (Finney 1971).

2.6 Statistical analyses

Percent mortality was calculated and the values were transformed into probit scale and analyzed as per Finney, 1971. Regression lines of probit against logarithmic transformation of concentrations were obtained. Slope function (S) and confidential limits (upper and lower) of the regression line with Chi-square test (EPA, 1999) were calculated and LC50 value of Calcium oxide nanoparticles were calculated with the help of probit analysis (SPSS software).

III. Results

1.1 Characterizations of NP

The SEM images of synthesized CaO NP are shown in Fig. 1. The SEM images in figure shows the NP possess crystal like structures.

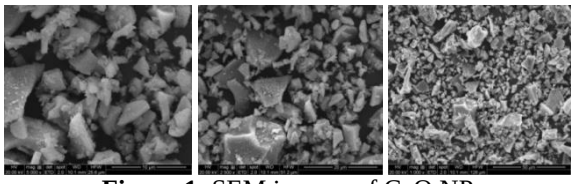


Figure 1: SEM images of CaO NP

The crystalline structure of CaO NP was examined by XRD (Fig. 2). The crystallite size based on X-ray peak broadening was estimated using Debye-Scherrer's equation (1) (El-Trass *et al*., 2012): [23].

Debye Scherrer's formula $D= (K\lambda/\beta \cos \theta)$ -------- Eq. 1

Where *k* is an empirical constant equal to 0.9, λ is the wavelength of the X-ray source (1.5405 Å), β is the full width at half maximum of the diffraction peak, and θ is the angular position of the peak. The average value calculated for the crystallite size for CaO NP is 35.94 nm.

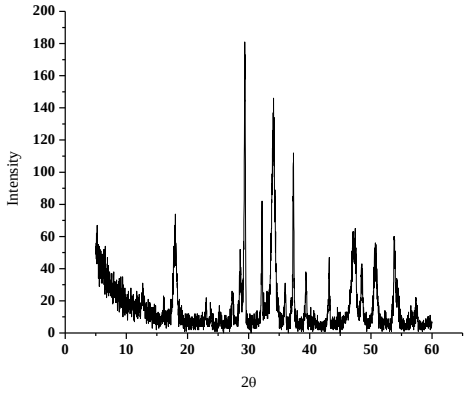


Figure 2: XRD of CaO NP

The UV-absorbance spectra of synthesized CaO are presented in Fig. 3. The absorbance spectrum of synthesized CaO was recorded using UV-VIS spectrophotometer (Ocean Optics DH-2000) over the wavelength range 200-1200nm.The band gap energy of the CaO nanoparticle was calculated using the Planck's equation as follows.

$E = hC/\lambda$

h = Planck's constant,

C = Velocity of light,

λ = wavelength,

$h = 4.135\times10^{-15}$ eV,

$C = 3\times10^{8}$ m/s,

$\lambda = ---\times10^{-9}$nm

Band gap energy (eV) = $4.135\times10^{-15}\times3\times10^{8}\times10^{9}$

Band gap energy (eV) = 1240/wavelength (nm)

The band gap energy of CaO particle was 4.94 eV.

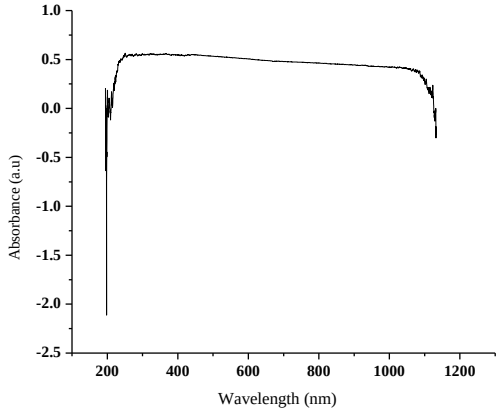


Figure 3: UV-absorption spectra of CaO NP

Table -1: Mortality of C. carpio at 96h after treatment of different concentration of CaO NP

Number	Conc. In mg/l	Log conc.	No. of Subjects	Observed Responses	Corrected % Mortality	Expected Responses	Residual	Probability
1	200	2.301	10	0	0	0.543	-0.543	0.054
2	210	2.322	10	2	20	1.319	0.681	0.132
3	220	2.342	10	3	30	2.567	0.433	0.257
4	230	2.362	10	4	40	4.167	-0.167	0.417
5	240	2.38	10	6	60	5.848	0.152	0.585
6	250	2.398	10	7	70	7.328	-0.328	0.733
7	260	2.415	10	8	80	8.443	-0.443	0.844
8	270	2.431	10	9	90	9.176	-0.176	0.918
9	280	2.447	10	10	100	9.601	0.399	0.96

Table -2: The relation between the calcium oxide concentration and the mortality rate of C. carpio

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Probit a	concentration	22.964	4.117	5.578	0.0	14.895	31.033
	Intercept	-54.445	9.779	-5.567	0.0	-64.225	-44.666

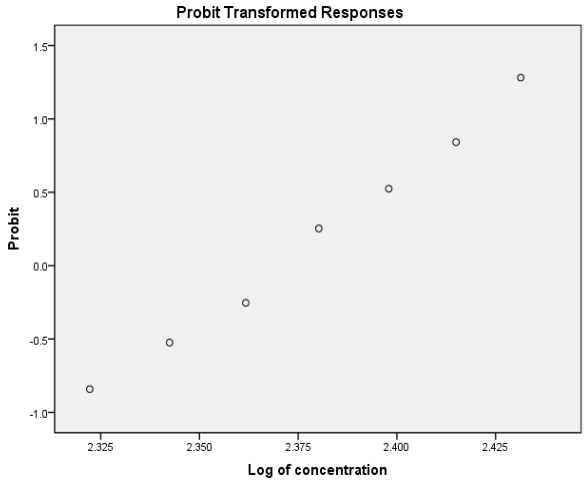
a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Test of probit analysis

Probit	Chi-Square	df[a]	Sig.
Pearson Goodness-of-Fit Test	1.761	7	.972[b]

a. Statistics based on individual cases differ from statistics based on aggregated cases.
b. Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits.

The graph shows linear relationship between probit response and log concentration of Calcium oxide-NP on *C. carpio.*



Graph -1: Probit vs. Log concentration

Table -3: Estimated CaO NP concentration values and confidence limits

Probability	Concentration (ppm)	95% Confidence Limits for concentration	
		Lower Bound	Upper Bound
0.01(LC1)	186.03	162.197	199.26
0.05	199.186	179.857	210.015
0.1(LC10)	206.575	189.917	216.129
0.2	215.892	202.594	224.059
0.3	222.869	211.907	230.337
0.4	229.009	219.77	236.304
0.50 (LC50)	234.901	226.819	242.624
0.6	240.945	233.397	249.857
0.7	247.583	239.883	258.654
0.8	255.585	246.938	270.178
0.9(LC90)	267.111	256.248	287.932
0.95	277.021	263.816	303.912
0.98	288.615	272.378	323.225
0.99 (LC99)	296.612	278.159	336.876

Table -4: Acute toxicity of CaO NP on C. carpio for 96h

point	96h LC5 (mg/l)	96h LC10 (mg/l)	96h LC50 (mg/l)	96h LC99 (mg/l)	S.E
	199.18	206.575	234.901	296.612	4.117

IV. Discussion

At present, nanoparticles are beginning to influence our lives in many ways and understanding the environmental health and safety aspect of nano materials has become a crucial issue. The aim of the present study was to assess the acute toxicity of CaO nanoparticles to fish *C. Carpio* using evaluation of the 96- hour LC50 values. Aquatic toxicity tests may provide insights to the relative sensitivity of *C. Carpio* to CaO NP, which may also provide suitable data on the impact of nanoparticles on aquatic environment, as these species hold important positions in aquatic ecosystems. A significant increase in mortality was observed in *C. Carpio* exposed to acute dose of CaO NP. Jevgenij *et. al.*, reported that calcium oxide-NP, caused cumulative mortality was exposed at 96hr time period at 260ppm with zebra fish (Danio rerio) [27].

In determining the toxicity of a new chemical to fish, an acute toxicity test is first conducted to estimate the median lethal concentration (LC50) of the chemical in water to which organisms are exposed [28]. The relationship between the degree of response of test organisms and the quantity of exposure to the chemical almost always assumes a concentration-response form [29], As in our results the *y*-axis represents percentage mortality and the *x* -axis represents concentration of CaO NP. Both variables increased with distance from origin. Variability in acute toxicity even in a single species and single toxicant depends upon the size, age and condition of the tested species along with other experimental factors. The differences in acute toxicity even may be due to changes in water quality and test species [30]. In the present study, LC50 values indicated that CaO NP is toxic to the experimental fish, *C. Carpio*.

Heavy metals are common pollutants and restricted to frequent inhalation in aquatic organism due to their larger size. However, nano size of these heavy metals may cause inhalation and bioaccumulation very frequently as research is now showing that when normally harmless bulk materials are made into nano particles they tend to become toxic [31, 32]. Penetration of nanoparticles into the aquatic environment is fraught with numerous consequences, as yet unpredictable because of insufficient information.

Several workers have proposed that the size effect seems more important to nanoparticle toxicity than the actual composition of the material [33, 34]. No studies have yet demonstrated that NP accumulation in cells causes cytotoxicity. However, NP has been reported to induce toxicity in cell membranes [35].

V. Conclusion

In our research, the toxicities of CaO NP on *C. carpio* were evaluated. The results suggested that CaO NP may have cumulative toxic potential toward these species and CaO NP-induced mortality might provoke higher-level consequences, which could comprise a contribution to the knowledge on the aquatic toxicity of CaO NP on aquatic ecosystems, for which there are only little data are available [27].

In the present study, the LC50 values and behavior change indicated that the CaO NP was cumulative toxic to fishes. The results of these studies may provide guidance for the selection of acute toxicity to be considered in field bio-monitoring efforts which designed to detect the bioavailability of CaO NP and early warning indicators of this calcium oxide nanoparticle in *C. Carpio*.

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