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Impact of zinc oxide nanoparticles treated dye industry effluent on growth and hematological characteristics of common carp

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Abstract

Zinc oxide nanoparticles were synthesized and characterized by UV-VIS, SEM, EDAX, and FTIR. Different concentrations of zinc oxide nanoparticles such as 20,40,60,80 and 100 mg were prepared for treating dying industry effluent. In this study, different quantities of dye industry effluent on the survival of Common carp as function of exposure time and mortality were analyzed. Based on the acute toxicity test 1/100 (T1), 1/50 (T2), 1/10 (T3) of LC50 value were selected for the sub-lethal test and feed utilization and hematological parameters were estimated after 14 days. The UV-Visible absorption spectra show that ZnO nanoparticles exhibit strong adsorption at 370nm. SEM image shows that the zinc oxide nanoparticles were observed at the wavelength range of 10.81mm (2µm), and 10.8mm (10µm). EDAX spectrum recorded on the zinc oxide nanoparticles is shown as two peaks were located on the spectrum at 1.00 KeV and 8.62 KeV. The FTIR spectrum of zinc oxide nanoparticles was analyzed in the range of 500-4000cm-1. Feed utilization parameters were higher in T3. Haematological parameters such as WBC, Hemoglobin, RBC, Haematocrit and platelets count of Common carp is gradually decreased from T1 to T3 and MVC, MCH and MCHC gradually increased from T1 to T3.

Keywords: Impact; Zinc Oxide Nanoparticles; Dye Industry Effluent; Growth; Hematology; Common Carp

1 Introduction

Pollution occurs as a result of anthropogenic activities and it concentrates the emissions and discharges in areas where people live and work places[1]. 80% of the world's wastewater is dumped largely untreated back into the environment, polluting rivers, lakes and oceans. Many industries release large quantities of effluents into the environment and the dye industry is one among them. The dye industry uses more than 8000 chemicals in various processes of textile manufacture including dyeing and printing. Many of these chemicals are poisonous and damage human health directly or indirectly. Large quantities of water are required for textile processing, dyeing and printing. The daily consumption of dyeing varies from 30-50 liters per kg of cloth depending upon the type of dye used. Out of the 72 chemicals identified in dye industry effluent, 30 chemicals cannot be removed [2]. Effluent from industries is discharged into surface water and may kill aquatic organisms because of the reduction in dissolved oxygen content in that water[3]. Dye industry effluents are treated by various methods such as chemical, physical and biological. Recently different nanoparticles are used for the treatment of industrial effluents and zinc oxide is one among them. Zinc oxide nanoparticles is one of the most important metal oxide nanoparticles, employed in various fields due to its peculiar physical and chemical properties. Treated water can be reutilized in the environment for growing aquatic organisms including fishes. The present study was reported first time on the treatment of dye industry effluent using ZnO nanoparticles and its impact on the growth and haematological characteristics of common carp *Cyprinus carpio* var. communis.

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2 Material and methods

2.1 Synthesis of Zinc oxide nanoparticles

The co-precipitation method was used for the synthesis of zinc oxide nanoparticles. 0.5 M zinc acetate and 0.1M sodium hydroxide were prepared separately in distilled water. Precipitation was achieved by adding NaOH. The precipitating process was continued till milky white colour precipitate was obtained. The solution was incubated in a hot air oven at 100˚C for 2 hours. It was centrifuged at 2000 rpm for 10 minutes and the centrifuging process continued with distilled water and ethanol in trace volume. The pellet was dried and calcinated in a hot air oven at 100˚C for 10 minutes. Finally, zinc oxide nanoparticles were formed.

2.2 Characterization of Zinc oxide nanoparticles

The synthesized nanoparticles were characterized by UV-Vis Spectrophotometer (JASCO-V-530), Scanning Electron Microscope (SEM)(LEO 1455 VP), Energy Dispersive X-ray detection instrument (EDAX)(HORIBA 8121-H), and Fourier Transform Infrared Spectroscopy FTIR(JASCO FTIR-6200).

2.3 Collection and Acclimation of fish

For toxicity studies, Common carp (*Cyprinus carpio*) fingerlings(4.0±2.5g) were collected from Pandian Fish Farm, Dindigul, Tamil Nadu, India and acclimated in the laboratory in plastic aquaria (60cm dia) at 28±2˚C. During laboratory acclimation, the fish were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice bran in the form of dry pellets.

2.4 Collection of Dye industry effluent

Dye industry effluent was collected in plastic containers from the discharged stream of dye industry effluent situated in new yarn dyes, Palayakaadu, Tiruppur, Tamil Nadu, and India.

2.5 Physico-chemical parameters of Dye industry effluent

The physico-chemical parameters such as colour, pH, electrical conductivity, total dissolved solids, hardness, sodium, potassium, calcium, chloride, BOD, COD, copper, nitrogen, and zinc were determined using standard methods [4].

2.6 Role of zinc oxide nanoparticles on physico-chemical parameters of dye industry effluent

The role of zinc oxide nanoparticles on the physico-chemical parameters of dye industry effluent such as color, pH, electrical conductivity, total dissolved solids, hardness, sodium, potassium, calcium, chloride, BOD, COD, copper, nitrogen, and zinc were estimated. Zinc oxide nanoparticles were used as nano absorbents in treating one liter of dye industry effluent with different quantities such as 20mg, 40mg, 60mg, 80mg, and 100 mg of Zinc oxide nanoparticles.

2.7 Acute toxicity test (LC_{50} Determination)

Acute toxicity tests were conducted for 96 hours [5]. The raw dye industry effluent was taken as a control. Different quantities of zinc oxide nanoparticles such as 20mg, 40mg, 60mg, 80mg, and 100 mg were taken. Seven fishes were introduced in each treatment and maintained in triplicate. Values of mortalities were measured at 24, 48, 72 and 96 hours and dead fish were immediately removed to avoid possible deterioration of the water quality. The LC_{50} values were calculated by SPSS software version 20 for-profit analysis.

2.8 Sublethal test of Common carp exposed zinc oxide nanoparticles

Based on the acute toxicity test 1/100 (T_1), 1/50 (T_2), 1/10 (T_3) of LC_{50} value were selected for the sub-lethal test and ten fishes (4.0±2.5g) were exposed for a period of 14 days. The length and weight of the fishes were weighed. This experiment was done in triplicate. A control (T_0) test without test suspension was conducted under the same condition. A fish feed was prepared with a protein level of 40% (Table 1) and was given to the fish twice a day from 09-10AM and 03-04 PM in the form of dry pellets. After feeding time the unfed were collected. The faecal matter was collected before changing the test medium. At the end of the 14^{th} day of the exposure period, the fishes were weighed in live condition and blood samples were collected randomly in each treatment doing with the control group for haematological analysis. Feed utilization parameters were also estimated.

Table 1 Composition of the Experimental Feed (g/100g) of Common carp Fish

Sr. No.	Ingredients	Quantity(g)
1.	Fish meal	36.2
2.	GNOC	36.2
3.	Wheat flour	8.7
4.	Tapioca	8.7
5.	Fish oil	2
6.	Sunflower oil	2
7.	Supplevite mix	4
8.	Sodium chloride	1
9.	Sodium benzoate	1

3 Results and discussion

3.1 Characterization of Zinc oxide nanoparticles

UV-Visible absorption spectroscopy is a widely used technique to examine the optical properties of the nanosized particle. Zinc oxide nanoparticles exhibit a strong absorption band at 370 nm as shown in figure 1. Similarly, UV-Vis spectra of ZnO NPs synthesized using zinc nitrate and zinc acetate were observed at 380nm [6]. Scanning electron microscopy shows that nanoparticles formed of clusters because of the adhesive nature of flower-shaped appearance as shown in figure 2. Jeyabharathi et al[7] reported that SEM images of zinc oxide nanoparticles synthesized using zinc acetate are spherical in shape with more aggregation. Satyanarayana et al[8] reported SEM pictures of ZnO nanoparticles at different magnifications. The presence of oxygen (O) and zinc (Zn) were revealed in synthesized nanoparticles by EDAX spectral analysis. EDAX spectrum recorded on the zinc oxide nanoparticles is shown two peaks located between 0.40 KeV and 9.0 KeV. The two peaks indicating the purity of the zinc oxide nanoparticles were located on the spectrum at 1.00 KeV and 8.62KeV and another peak of O element was located at 0.48 KeV(Fig.3). Chaudhuri and Malodia [9] reported that EDAX analysis was carried out to determine the elemental composition and stereochemistry of the synthesized zinc oxide nanoparticles. FTIR spectroscopy is a useful technique for the analysis of the structure of compounds and to identify the functional groups present in the samples. The FTIR spectra of the synthesized ZnO powders are in the range of 500-4000 cm^{-1}(Fig.4). Maribel Guzman et al[10] reported that the FTIR spectra of nanoparticles show absorption bands at 483 and 507.31cm^{-1} corresponding to the stretching vibrations of the Zn-O bond. Rajan and Rohini [11] reported that the zinc oxide formation was confirmed between 3394.2 and 413.46 cm^{-1}bands.

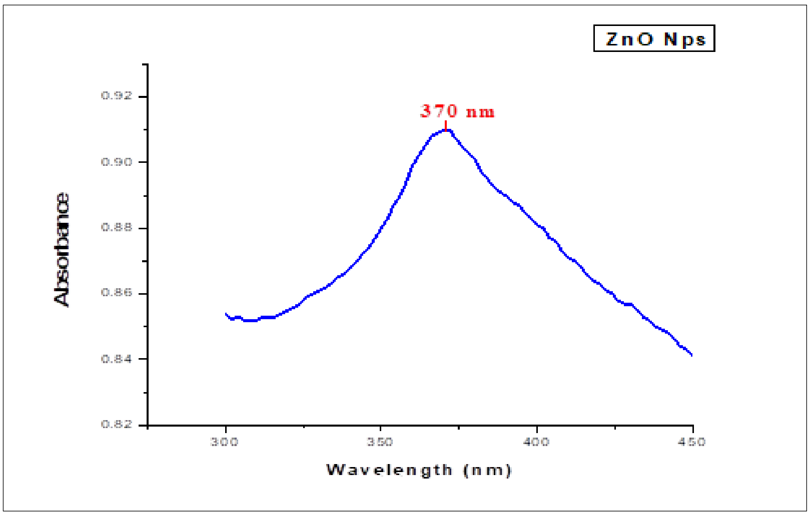
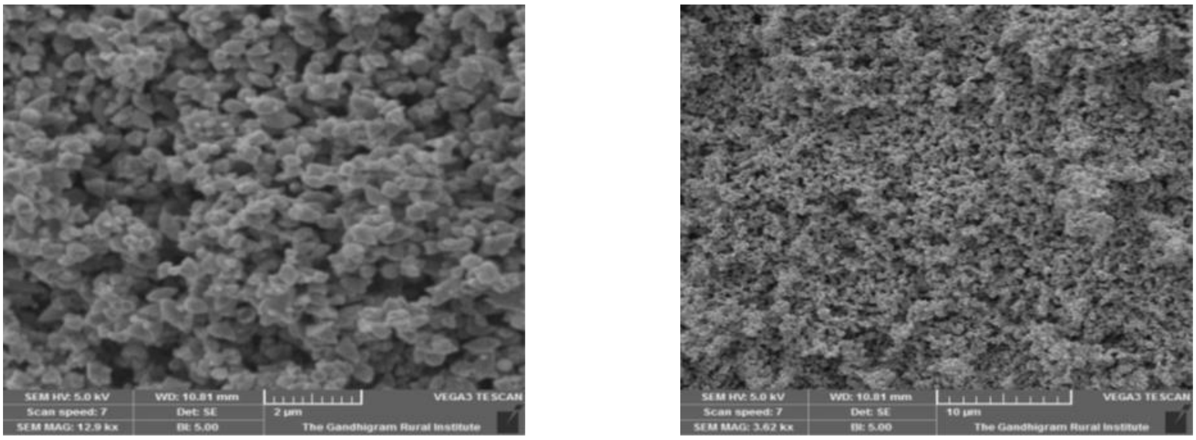


Figure 1 UV-Visible Spectroscopy Analysis of Zinc oxide nanoparticles



(a) **(b)**

Figure 2 SEM Analysis of Zinc oxide nanoparticles (a -2µm; b -10µm)

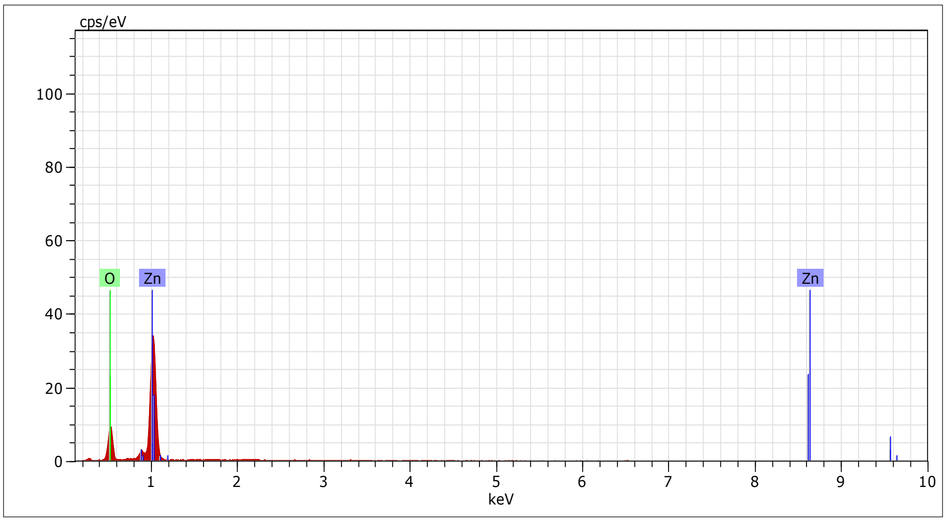


Figure 3 EDAX Image of Zinc oxide nanoparticles

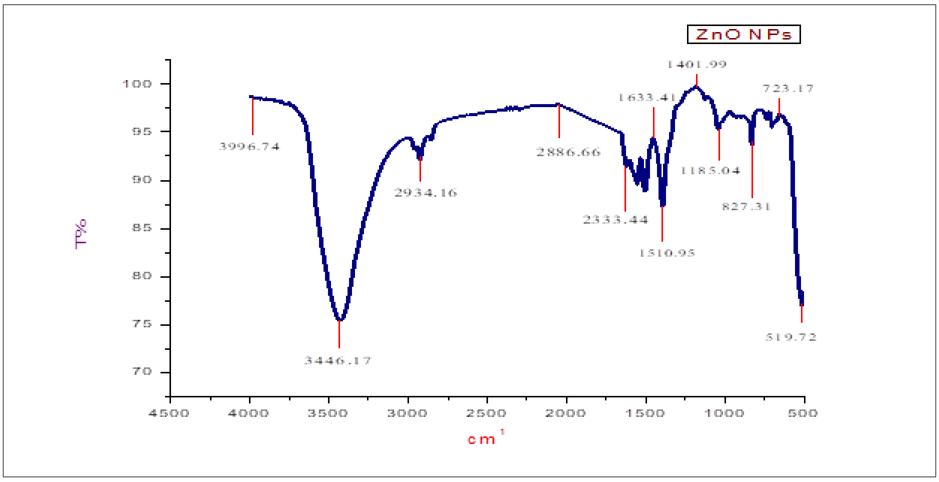


Figure 4 FTIR Image of Zinc oxide nanoparticles

Physico- chemical parameters of untreated and zinc oxide treated dye industry effluent are presented in Table 2. 100mg zinc oxide nanoparticles treated dye industry effluent showed complete removal of colour and reduction of Physico-chemical parameters. Anitha et al [12] reported the removal of colour from textile mill effluents using CuO nanoparticles. Premkumar et al [13] reported the removal of toxic substances from textile dyeing industry effluent using iron oxide nanoparticles. Khousalya and Rajan [14] reported that 500 mg of iron oxide nanoparticles removed the colour and reduced the parameters such as electrical conductivity, COD, calcium and magnesium present in the textile dyeing industry effluent.

Table 2 Physico-chemical parameters of untreated and ZnO Nanoparticles treated Dye Industry effluent

Sr. No.	Parameters	Unit	Untreated Dyeing Industry Effluent	Different quantity of zinc oxide nanoparticles treated dying industry effluent				
				20mg	40mg	60mg	80mg	100mg
1.	Color	-	Dark Red	Red	Light red	Light orange	Light yellow	Colorless
2.	pH	-	7.66	7.42	7.36	7.28	7.14	7.05
3.	Electrical conductivity	Ms/cm	143.4	142.9	138.9	129.7	126	125.1
4.	Total dissolved solids	mg/l	12525	12434	12274	12268	11908	11802
5.	Hardness	„	63.0	62.4	61	57.9	54.5	53.1
6.	Chloride	”	4307	3987	3941	3902	3899.7	3891
7.	BOD*		34	32	30	25	24	20
8.	COD**	„	25600	24800	20150	18740	15490	13600
9.	Sodium	ppm	0.60	0.5	0.4	0.35	0.30	0.25
10.	Potassium	„	0.30	0.29	0.28	0.25	0.24	0.22
11.	Calcium	„	0.48	0.42	0.40	0.35	0.30	0.28

The raw dye industry effluent was taken as a control in this study of different concentrations of zinc oxide treated dye industry effluent in LC50 (20,40,60,80 and 100mg), 80 and 100mg were found effective in decreasing the toxic substances. Hence three concentrations of zinc oxide nanoparticles treated dye industry effluent in sub-lethal concentrations such as low, medium, and high (1/100, 1/50 and 1/10) were selected for the introduction of Common carp. Taju et al [15] reported the wastewater generated from a common effluent treatment plant (CEFT) contains BOD, COD, TDS and a variety of toxic heavy metals especially chromium, which makes it potentially toxic to other living beings.

Preliminary toxicity tests were carried out to find the median lethal tolerance limit of Common carp. The mortality or survival rate of Common carp *Cyprinus carpio* in dye industry effluent (control) and different concentrations of zinc oxide nanoparticles (80, 90, and 100 mg) was recorded after 96 hours. The 96 hours LC_{50} value of effluent treated iron zinc oxide nanoparticles exposed to Common carp was 53.77 ppm in different concentrations of zinc oxide nanoparticles for LC50 determination. The survival studies revealed that the calculated (Probit analysis) LC_{50} of Common carp was 55.00(Table 3). Beyond this limit, mortality occurred and the mortality increased with increased concentration of dye industry effluent. Lin-Peng Yu, et al [16] reported that the 96 h LC_{50} value of the ZnO suspension exposed to zebrafish was 3.969 mg/L. A similar report was given on electroplating industry effluent[17].

Table 3 Probit Analysis (LC_{50}) of Zinc oxide Nanoparticles treated Dye Industry Effluent exposed to Common carp

Probit	95% Confidence limits for concentration		
	Estimate	**Lower Bound**	**Upper Bound**
LC1	13.037	8768.195	35.314
LC10	31.883	4807.272	45.855
LC15	36.305	3878.325	48.648
LC20	39.819	3140.266	51.106
LC25	42.833	2507.397	53.535
LC30	45.541	1939.556	56.211
LC35	48.049	1414.272	59.596
LC40	50.430	917.931	64.910
LC45	52.733	445.254	77.591
LC50	55.000	917.931	64.910

3.2 Behavioral Studies

Behavioural changes are the most sensitive indication of potential toxic effects [18]. In the present study, Common carp exposed to zinc oxide treated dye industry effluent shows behavioural changes (Table 4). Charjan and Kulkarani [19] reported that *Channa orientalis* exposed to sublethal concentration of Zinc sulphate exhibited abnormal behavioural responses such as rapid movement, faster opercular activity, surfacing and gulping air, erratic swimming with jerky movements, hyperexcitability, convulsions. Suganthi et al., [20] reported that the behaviour patterns are disturbed when the concentration such as irregular jerky movements, continuous opercular movement and spitting water, and dorsal and anal fin movements are reduced in *O. mossambicus* treated with ZnO NPs. Similar behavioural changes of Zebrafish *Danio rerio* grown in iron oxide nanoparticles treated tannery effluent [21].

Table 4 Behavioural changes of Common carp exposed Zinc oxide Nanoparticles treated Dye industry effluent

S. No	Activity	Observation
1.	Circular swimming	Yes
2.	Jerk movement	Yes
3.	Surface respiration	Yes
4.	Aggressive movement	Yes
5.	Mortality	Yes
6.	Breathing movement	Surface

3.3 Growth Studies

The average initial condition factor of koi carp reared in different feeds was 2.39. The final Condition factor was gradually increased in all concentrations and the average was 2.72. Srinivasan et al [22] reported an increase in the condition factor of *Macrobrachium rosenbergii* post-larvae fed with 40g/ kg-1 of iron oxide nanoparticles in the feed. The different feed utilization and growth parameters are presented in table 5. Feed consumption (FC) of *Cyprinus carpio* is higher in T_3 containing 60mg of zinc oxide nanoparticle treated dye industry effluent. But the decrease in feed consumption was reported in *Cyprinus carpio* reared in Tannery effluent [23]. Feed conversion efficiency (FCE) of *Cyprinus carpio* was higher in T2. The feed conversion efficiency is gradually decreased when the concentration of effluent increases. The feed conversion efficiency of Catla decreased when exposed to sublethal concentrations of dye industry effluent [24]. Ramesh Francis et al [25] reported reduced feed conversion efficiency in freshwater fish *Clarias* *batrachus* exposed to Sago effluent. The feed conversion ratio was increased with an increase in the quantity of zinc oxide nanoparticles treated dye industry effluent. Rajan and Palpandi[26] reported that the feed conversion ratio was decreased when Cyprinus carpio is reared in dye industry effluent. Growth, gross growth efficiency and net growth efficiency of Common carp were gradually increased in zinc oxide treated dye industry effluent. Rajan and Palpandi [26] reported a similar increase in growth and gross and net growth efficiency when Common carp was reared in dye industry effluent. But Ramachandran and Rajan[17] reported decreased net growth efficiency in *Cyprinus carpio* reared in different concentrations of electroplating industry effluent.

Table 5 Feed utilization and Growth parameters of common carp in relation to different quantities of Zinc oxide nanoparticles treated dye industry effluent. Each value is the average (± SD) performance five individuals in triplicates reared for 14 days

Parameters	**Treatments**			
	T_0 (Control)	**T_1 (20 mg)**	**T_2 (40 mg)**	**T_3 (60 mg)**
Feed consumption (g/g live wt/30 days)	$1.85±0.24^a$	$0.10±0.05^b$	$2.03±0.2^c$	$2.16±0.46^d$
Feed conversion Efficiency	0.09±0.06	0.06±0.05	1.20±0.03	0.16±0.01
Feed conversion Ratio	4.38±0.61	5.26±0.23	6.25±0.52	6.3±0.7
Growth	$0.25±0.01^a$	$0.26±0.01^b$	$0.33±0.01^c$	$0.45±0.01^d$
Gross Growth Efficiency(%)	$28.7±2.9^a$	$36.5±2.1^b$	$28.8±3.2^c$	$42.6±1.5^d$
Net Growth Efficiency (%)	$29.9±2.89^a$	$39.2±2.2^b$	$45.8±1.7^c$	$48.9±4.5^d$

Feed Consumption	**Growth**	**Gross Growth Efficiency**	**Net Growth Efficiency**
a vs b (P>0.05) S	a vs b (P>0.05) S	a vs b (P>0.05) S	a vs b (P>0.05) S
a vs c (p>0.05) S	a vs c (p>0.05) S	a vs c (p>0.05) S	a vs c (p>0.05) S
a vs d (P>0.05) S	a vs d (P>0.05) S	a vs d (P>0.05) S	a vs d (P>0.05) S

3.4 Haematological Studies

The haematological parameters of Common carp is presented in Table 6. The WBC, Hemoglobin, RBC, Haematocrit and platelet count of Common carp is gradually decreased from T_1 to T_3.MVC, MCH and MCHC gradually increased from T_1 to T_3. Haematological analysis acts as a rapid and economical method for assessing the metal oxide toxicity of fish. Shah and Altindag [27] reported that the haematological parameters such as haematocrit, Hb, RBC and WBC are used to assess the functional status of the oxygen-carrying capacity of the bloodstream and have been used as indicators of metal pollution in the aquatic environment. Haematological parameters are very helpful in the judgement of the health condition of fish species. In the present study haematological parameters such as WBC, Hemoglobin, RBC, Haematocrit and platelet count of *Common* carp is gradually decreased from T_1 to T_3 and MVC, MCH and MCHC gradually increased from T_1 to T_3 when exposed to zinc oxide nanoparticles treated dye industry effluent. WBC count was altered by toxic effect of ZnO that causes stress on the cell production activity of the spleen. Nussey et al[28] reported a similar result in *O. mossambicus* exposed to sublethal zinc concentration. The reduction in RBC count clearly indicated abnormalities of blood tissue composition and maybe also related to gills damage which disturbs the respiratory process [29]. Similarly, Suganthi et al.,[20] reported that the total red blood cell (RBC) count in 30, 50 and 70ppm ZnO treated groups was reduced due to the haemolysis of blood cells which reflects changes of Hb and Hct count in *O. mossambicus*. On other hand, Younis et al [30] reported that RBC and Hb content was increased with increasing concentration of ZnCl2 sequentially for short- and long-term exposure. In the present study, hematocrit (Hct) was significantly decreased on 14th day with increased concentration compared to control. The changes of MCH, MCV and MCHC reflect erythrocyte swelling which is related to macrocytic anaemia. Abdel-Khalek et al [29] reported that the change in MCH and MCHC could be attributed to hemolysis of RBC or anaemic conditions due to production in the hemopoietic tissues under the action of the accumulated metal oxide nanoparticles. Celik et al [31] reported that the RBC and Hct count of *O. mossambicus* exposed to zinc was significantly decreased in low and high concentrations with a significant increase of MCV, MCH and MCHC values on the 14th day which are similar to the present result.

Table 6 Haematological parameters of Common carp

Blood Parameters	T_0	T_1	T_2	T_3
WBC (cells/cumm)	10,000	17,600	13,200	11,700
Hemoglobin (gm/dI)	0.4	1.0	1.8	1.0
RBC count (millions/cumm)	0.3	0.54	0.3	0.2
Haematocrit (PCV)(%)	0.0	2.86	0.56	0.4
MCV	0.1	1.0	0.19	2.56
MCH	0.0	1.76	3.02	6.09
MCHC	0.2	3.5	4.0	4.2
Platelets count (Lakhs/cumm)	62,000	64,000	58,000	46,000

4 Conclusion

Zinc oxide nanoparticles were synthesized and characterized by UV-Vis, SEM, EDAX and FT-IR. The UV-Visible absorption spectra show that ZnO nanoparticles exhibit strong adsorption at 370nm. Scanning electron microscopy shows that ZnO nanoparticles formed of clusters because of the adhesive nature of flower-shaped appearance. EDAX spectrum recorded on the zinc oxide nanoparticles is shown two peaks located between 0.40 and 9.0 KeV. The FTIR spectra of the synthesized ZnO powders in the range of 500-4000 cm^{-1}. 100mg zinc oxide nanoparticles treated dye industry effluent showed better adsorption efficiency by decreasing the Physico-chemical parameters. 60mg zinc oxide nanoparticles treated dye industry effluent was suitable for the growth of Common carp. Most of the haematological parameters of Common carp increased with an increase in the quantity of zinc oxide treated dye industry effluent. Zinc oxide nanoparticles will be used for the complete removal of colour, and reduction of certain Physico-chemical characteristics of dye industry effluent and the treated effluent will be used for the growth of Common carp.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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