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IMPACT OF IRON OXIDE NANOPARTICLES TREATED TANNERY EFFLUENT ON HEMATOLOGICAL, ENZYMATIC AND BIOCHEMICAL CHARACTERISTICS OF TILAPIA *Oreochromis mossambicus*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author MRR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SH managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The present study aimed to know the impact of iron oxide nanoparticles treated tannery effluent on hematological, enzymatic, and biochemical characteristics of Tilapia *Oreochromis mossambicus*. Iron oxide nanoparticles were synthesized and characterized by using Scanning Electron Microscope, Energy Dispersive X-Ray Spectroscopy, X-Ray Powder Diffraction, Fourier Transform Infrared Spectroscopy, and Vibrating Sample Magnetizer. Physico-chemical characteristics of tannery effluent were estimated. Dissimilar strength of Iron oxide nanoparticles such as 50,100,150,200 and 250 ppm of Fe3O4NPs were used for treating tannery effluent. For median lethal (LC50) studies dissimilar strength of iron oxide nanoparticles treated tannery effluent such as 200,225 and 250 ppm were used and seven fishes were maintained in triplicates for 96 hrs. Based on medium lethal studies, 0 (control), 2.32 (low), 4.65 (medium), and 23.27ppm (high) were selected for sub-lethal tests by introducing Tilapia for 14 days. At the end of the 14th day of exposure, fish and blood samples were collected randomly in each concentration along with control for further test such as hematology, enzymes (Aminotransferase, Alanine aminotransferase), and biochemical analysis in muscle, gill, and liver of tilapia. The results indicated that 200 and 250 ppm iron oxide nanoparticles were effective to reduce the toxic substances of tannery effluent. Hematological parameters and enzymatic parameters (Aminotransferase (AST) and Alanine aminotransferase (ALT) of tilapia fish exposed to sub-lethal concentration of Fe_3O_4 Nps are increased in T_1 and

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T_2. Biochemical characteristics such as protein, carbohydrate, and lipid in muscle, gill, and liver of Tilapia are higher in T_1.

Keywords: Biochemical; enzymatic; hematological; iron oxide nanoparticles; tannery effluent; tilapia.

1. INTRODUCTION

Nanoparticles are submicron moieties made of inorganic or organic materials ranging from 1 to 100nm, which may have many novel properties compared to bulk materials [1]. Nanoparticles play a vital role in the production of food products against microbial contamination, enzymes, food additives and also improves the physical, chemical, and nutritional quality of feed [2]. Nanoparticles are commonly used in various fields such as biomedical, physics, chemistry, and material sciences [3]. Among different nanoparticles, iron oxide is now used for the removal or degradation of wide range of chemical pollutants [4]. The accelerated population growth and rapid urbanization are the socio-economic and environmental factors that place stress on deteriorating water, sanitation, and infrastructure in developing countries. The discharge of untreated wastewater containing pollutants and toxic substances into the surrounding surface water sources serves as a direct threat to the macro, microflora, and fauna. Adsorption by nanoparticles is considered one of the most effective, efficient, and economical methods for the removal of pollutants from wastewater. This is because, compared to bulk materials, nonmaterial-based adsorbents are small in size with a larger surface area, which can provide a greater number of active sites for adsorption. Among nano-adsorbents, the utilization of iron oxide nanoparticles has been received much attention due to their unique properties and effectiveness in the adsorption of toxic substances and heavy metals from tannery effluent [5].

The current biomedical research uses the animal models such as roundworms, drosophila, mice, rats, and primates for the study of diseases and their mechanisms. But there is a wide research gap between the use of invertebrate and vertebrate animal models. Fish is used as an animal model in toxicological studies. Research on fish revealed that the nanoparticles are toxic in both high and low concentrations [6]. Acute toxicity tests provide information on the time and concentration causing a significant effect or detectable response in 50% of the exposed population of test organisms [7]. The toxicants in an aquatic ecosystem are taken by fish and transported to the tissues and organs through the blood. Hence hematological and biochemical parameters are widely used as a health indicator in ecotoxicological studies because these parameters react before the toxicants enter into the body of fish. Fish blood is a suitable way to determine and diagnose the toxicity of metal and metal oxide nanoparticles [8,9], and hematological analysis is excellent to assess the stress condition of aquatic organisms [10]. Furthermore, the enzymatic analysis is one of the consequential parameters to know the influence of metal oxide that causes stress to an aquatic organism [11]. Enzymes such as alkaline aminotransferase (ALT), and aspartate aminotransferase (AST) have been used as biomarkers of metal oxides pollution in fish. The work related to the impact of iron oxide nanoparticles treated tannery effluent on hematological, enzymatic, and biochemical characteristics of Tilapia *Oreochromis mossambicus* is wanting. Hence, the present study was carried out.

2. MATERIALS AND METHODS

2.1 Synthesis of Iron Oxide Nanoparticles

The co-precipitation approach was used for the synthesis of iron oxide nanoparticles. The aqueous solution of $FeCl_2$ and $FeCl_3$ has prepared by 1:2 ratio and NaOH (0.1N) with constant stirring and within 30 minutes, the solution gets brownish yellow color and the pH turns 1. In the aqueous solution with constant stirring, within 30 minutes a visible color change was observed. The yellow color aqueous solution turned into greenish-black precipitate and the pH was adjusted to 12. After the precipitation, it was centrifuged at 5000rpm, washed several times with distilled water and ethanol to remove the by-products. The supernatant was then removed and the pellet was dried. After drying, the precipitate was calcinated in a muffle furnace at 300ºC for 3 h. Finally, iron oxide nanoparticles were ground with mortar to be shaped into powder.

2.2 Characterization of Iron Oxide Nanoparticles

The iron oxide nanoparticles are characterized by using Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDAX), Fourier Transform Infrared Spectroscopy(FT-IR), X-ray Diffraction(XRD), and Vibrating Sample Magnetometer(VSM).

2.3 Collection of Fish and Tannery Effluent

For toxicity studies, tilapia (*Oreochromis mossambicus*) fingerlings (4.0±2.5g) were procured from A.M. Aquafarm, Arumbanoor, Madurai, Tamilnadu, India, and shifted to the laboratory in polyethylene pack. Fishes were accustomed in round basins for 15 days at 28±2ºC. During accustomation, fishes were fed with feed composed of fish meal, groundnut oil cake, wheat flour, and rice bran. Tannery effluent samples were collected in plastic containers from the discharged stream of tannery effluent situated in the central part of Dindigul -Bathlakundu Highway, Dindigul, Tamil Nadu, India.

2.3.1 Physico-Chemical characteristics of Tannery Effluent

The Physico-chemical characteristics such as color, odor, pH, electrical conductivity, total solids, total dissolved solids, total suspended solids, hardness, sodium, potassium, calcium, magnesium, sulfate, chloride, dissolved oxygen, dissolved carbon dioxide, BOD, COD, and copper were estimated using standard methods [12].

2.4 Performance of Iron Oxide Nanoparticles on Physico-Chemical characteristics of Tannery Effluent

The performance of iron oxide nanoparticles on the physico-chemical characteristics of tannery effluent was estimated. Iron oxide nanoparticles were used in treating tannery effluent with different concentrations such as 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm of Fe_3O_4NPs.

2.5 Medium Lethal Toxicity Tests (LC_{50} Determination)

Healthy fishes were used for LC_{50} analysis. The acute toxicity test was conducted after the Organization for Economic Cooperation and Development Guidelines [13] under static conditions. Dissimilar strength of iron oxide nanoparticles (200, 225 and 250 ppm) was selected for the median lethal concentration (LC_{50}). Each treatment was run in triplicate and placed under the same conditions. Groups of seven fishes were subjected to various strengths of iron oxide nanoparticles for 96 h at room temperature. Values of mortalities were measured at 24, 48, 72, and 96 h, 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 Probit Analysis.

2.6 Sub – Lethal Test of Tilapia exposed to Iron Oxide particles

Based on medium lethal studies, 2.32 (low-T_1), 4.65(medium –T_2), and 23.27ppm (high-T_3) for 1/100th, 1/50th, and 1/10th of the LC_{50} value(232.7ppm) were selected for sub-lethal tests by introducing seven Tilapia in each treatment for 14 days. This experiment was done in triplicate. A control (T_0) test without test suspension was conducted under the same state. After 14 days, fish and blood samples were collected randomly in each concentration along with the control group for blood profile analysis viz., White Blood Cells (WBC), Red blood cells (RBC), and platelets were counted by hemocytometer method [14], hemoglobin (Hb) were determined by cyanomethemoglobin method [15]. The microhematocrit method was used for the determination of hematocrit (Hct) and the Mean corpuscular hemoglobin concentration (MCHC) was calculated by using the standard calculation method [16]. Enzymes Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated [17]. Total protein,carbohydrate, and lipid content were determined [18,19,20].

Data were statistically analyzed by using the statistical software package IBM SPSS Version 20. The 96hrs LC_{50} values were calculated by using the Probit analysis method in SPSS. The data of toxicological parameters exposed to fish were expressed as an average from triplicate values of each concentration.

3. RESULTS AND DISCUSSION

Iron oxide nanoparticles depend on size, shape, methods, and kind of salt used, pH, and ionic strength [21]. The SEM image confirms that the iron oxide nanoparticles are circular which is analyzed in the scale size range of about 9.84 mm (Fig. 1). The SEM image of synthesized iron oxide nanoparticles has a clear image of the cluster shape ranges from 30 nm to 100 nm [22]. However, the percentage of nanoparticles beyond 100nm is very less. The average percentage of nanoparticles is 66 nm [23].

In EDAX, iron oxide nanoparticles show three peaks and are found between 2 KeV and 10 KeV (Fig. 2). The maximum peak is located on the spectrum at 6.4 KeV coming from iron. The second peak found on 0.3 KeV indicates oxygen and the third peak was found at 2.6 KeV from iron. It confirms that the chemically synthesized iron oxide NPS has substantial peaks of Fe and O. The EDAX spectrum of iron oxide nanoparticles shows three peaks and is found between 2 KeV and 10 KeV [24].

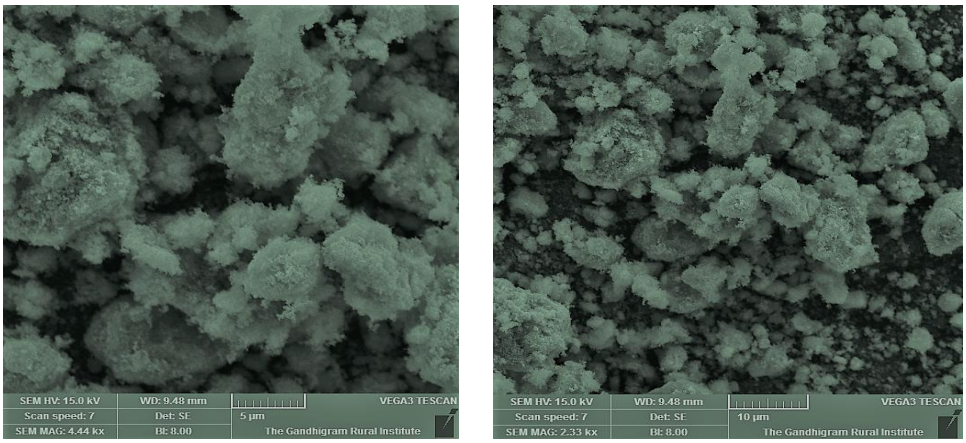


Fig. 1. SEM image of iron oxide nanoparticles

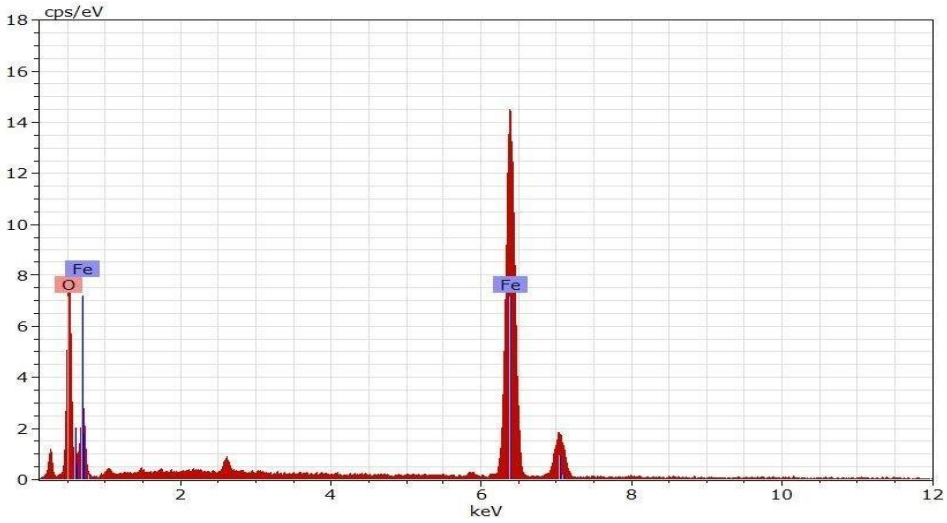


Fig. 2. EDAX Analysis of iron oxide nanoparticles

FTIR measurement was carried out to identify the functional groups (3430.1 – O=C, 2917.4 – C-H, 1632.6 – C=O, 1371.1 – O=C=O, 1082.7 – C-O, 570.04 – Fe3O4) of iron oxide nanoparticles (Fig. 3). The FTIR spectrum of iron oxide nanoparticles was analyzed range from 4000-400 cm-1 [25]. A functional group such as N-H stretching, PO2- symmetric stretch, nucleic acid COO- symmetric stretch and amino acids, C-O asymmetric stretching of glycogen.

The XRD pattern of synthesize iron oxide nanoparticles with diffraction peaks (30.403,31.980, 35.788,43.403,45.653,53.807,57.442 and 62.980cm-1) (Fig. 4). The crystalline form of the piece and the calculated average particle size are 23nm. Similarly, Rouhollah Khodadust [25] reported that the XRD patterns of synthesized Fe3O4 nanoparticles with diffraction six peaks of (30.205, 35.515,43.325,53.711,57.215, and 62.945cm-1). The synthesized iron oxide nanoparticles are crystalline form and further confirmed by XRD and nano-crystal size of 10 to 16 nm. The synthesized Fe_3O_4Nps are crystalline through XRD analysis [26].

The magnetic properties of synthesized nanoparticles in the presence of a magnetic field were measured using a vibrating sample magnetometer. Fig. 5 exhibits that the saturation magnetization (Ms) of iron oxide nanoparticles is 8.865emu/gm. The VSM result revealed that the saturation magnetization (MS) is 1.019 emu/gm [27].

Physico-chemical characteristics of untreated and iron oxide treated tannery effluent are presented in Table 1. The unpleasant odor of tannery effluent was due to microbial growth [28]. Very high EC may be due to a higher concentration of acid-base and salt in water [29]. Also increase in BOD level is a reflection of microbial oxygen demand, leads to depletion of dissolved oxygen [30]. Rajan and Murali [31] reported the physicochemical characteristics of

tannery effluent collected from Common Tannery Effluent treatment located near Senkulam lake in Dindigul and treated with different leaves of plants for the removal of chloride. Similar physicochemical properties of tannery wastewater were also reported [32]. Taju et al. [33] also reported the physic-chemical characters of tannery effluent (PH-7.62, TSS-5435.25, TDS-3983, DO-0.2± 0.01, BOD-515.32, and COD-761.26). The characteristics of tannery effluent such as chloride-1309±495, chromium- 5.33± 2.93, and copper-1.28±0.56. 200ppm and 250 ppm iron oxide nanoparticles exhibit better for decreasing the physicochemical characteristics of tannery effluent [34] (Table 1).

Dissimilar strength of iron oxide nanoparticles (50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm) was used as nano adsorbents in treating tannery effluent to reduce physico-chemical characteristics (Table 1). The maximum adsorption efficiency of iron oxide nanoparticles in treating tannery effluent was observed at low pH and high concentrations of iron oxide nanoparticles (200 and 250 ppm). With the increase of adsorbent dose, the reduction of physico-chemical characteristics of tannery effluent increase due to the increased available binding sites in the nanocomposite for the complexation of metal ions [35]. A faster initial removal rate was possible due to the availability of sufficient vacant adsorbing sites in the adsorbent [36].

The 96 hours LC50 value of effluent-treated iron oxide nanoparticles subjected to tilapia fish was 232.77 ppm. Anupuli Aich et al. [34] evaluated the effect of tannery effluent using Guppy and reported that 96 hour LC50 of Guppy *P. reticulata* was 258.99 ppm. Tilapia was exposed to a period of 14 days in the sub-lethal strength of iron oxide nanoparticles such as low (2.32 ppm), medium (4.65 ppm), and high (23.27 ppm).

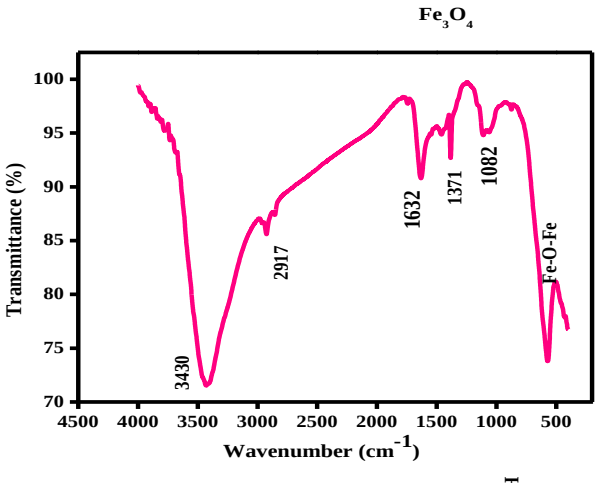


Fig. 3. Fourier Transform Infrared Spectroscopy Analysis of Iron Oxide nanoparticles

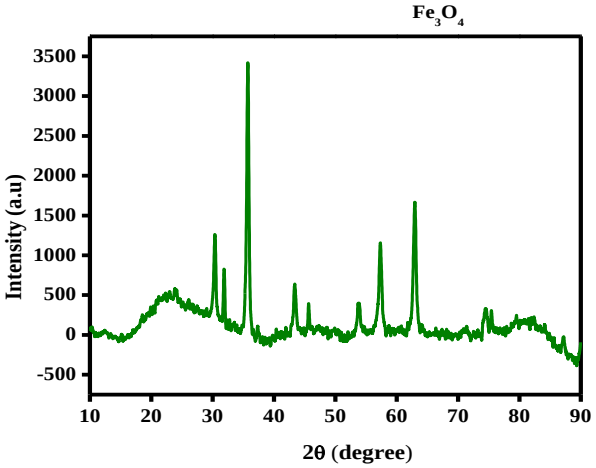


Fig. 4. XRD image of iron oxide nanoparticles

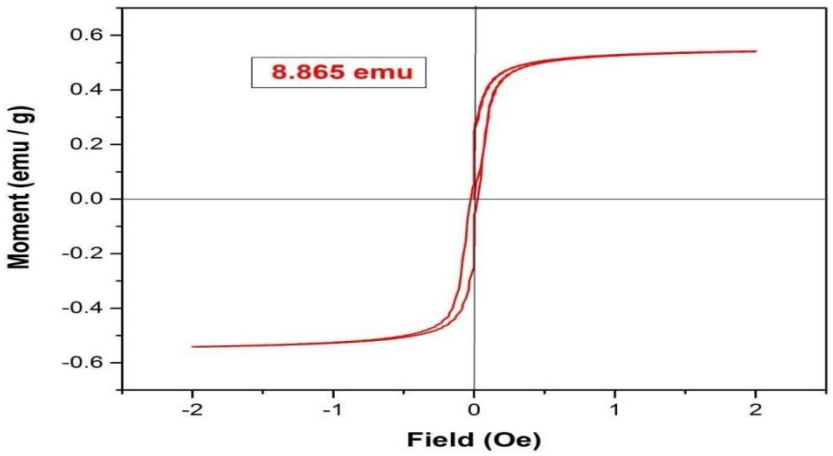


Fig. 5. VSM image of iron oxide nanoparticles

Table 1. Physico-chemical characteristics of untreated and Fe_3O_4 NPs treated tannery effluent

S. No.	Parameters	Unit	Untreated Tannery effluent	Dissimilar strength of iron oxide nanoparticles treated tannery effluent				
				50ppm	100ppm	150ppm	200ppm	250ppm
1.	Colour	-	Dark brown	Dark brown	Light brown	Light green	Light yellow	colourless
2.	Odour	-	Unpleasant smell	-	-	-	-	-
3.	pH	-	8.55	8.30	8.22	7.92	7.54	7.11
4.	Electrical conductivity	Ms/cm	251.24	242.1	226.4	216.28	200,84	182.55
5.	Total solids	mg/l	17444.4	3433	3233	2933	2850	2833
6.	Total dissolved solids	,,	15190.4	2912	2780	2655	2624	2100
7.	Total suspended solids	,,	2254	521	300	278	226	100
8.	Total hardness	,,	5844.8	5200	4400	3200	2800	2000
9.	Dissolved oxygen	,,	3.39	4.04	6.464	6.48	6.52	6.56
10.	Dissolved carbon dioxide	,,	63.96	18	16	14	10	6
11.	Chloride	,,	1840	3834	3550	3266	2840	2414
12.	Magnesium	,,	410.8	290	282	266	250	243
13.	Sulphate	,,	62.12	59	55.6	54.54	54.25	53
14.	BOD*	,,	4890.8	1433	734	406	118.2	80.8
15.	COD**	,,	3.39	20000	12800	11200	9600	5600
16.	Nitrogen	,,	25.2	124	109	90	72	55
17.	Sodium	ppm	1570	2752	2715.4	2533	2032	1667
18.	Potassium	,,	21480	63.82	63.70	54.04	43.12	36.606
19.	calcium	,,	155	908	400	342	301	297.8
20.	Copper	,,	0.016	0.004	0.003	0.003	0.002	0.001

**Biological Oxygen Demand, **Chemical Oxygen Demand. All the values are an average of ten individual observations*

Hematological parameters can be useful for the measurement of physiological disturbances in stressed fish and thus used as a reliable indicator for toxicological research, environmental monitoring, and as indicators of disease and stress [37]. In this study hematological parameters of tilapia fish subjected to sub-lethal concentration of Fe_3O_4 Nps are gradually increased in T1, T2 and suddenly decreased in T3 when compared to control (Table 2). A similar decrease of hematological parameters in *Labeo rohita* and Oreochromis mossambicus subjected to iron and zinc oxide nanoparticles and also indicates a decrease in nonspecific immunity [38,39]. Behera et al. [40] reported that iron nanoparticles could induce the hematological parameters of Indian major carp, *Labeo rohita.* Hematological parameters of common carp

(*Cyprinus carpio*) were gradually increased with different doses of selenium nanoparticles supplemented feed [41]. The reduction in RBC count indicated abnormalities of blood tissue composition and maybe also be related to gills damage which disturbs the respiratory process [42]. Suganthi et al., [43] reported that the total red blood cell (RBC) count in 30, 50 and 70ppm ZnO treated groups was reduced due to the hemolysis of blood cells which reflects changes of Hb and Hct count in O. mossambicus. It is inferred that increased levels of Hct due to polycythemia and decreased level may be due to anemic condition. The change of MCHC reflects erythrocytes swelling which is related to macrocytic anemia. The change in MCHC could be attributed to hemolysis of RBC or anemic condition due to production in the hemopoietic tissues under the action of the accumulated metal oxide nanoparticles [42,44]. The WBC and platelets count increased in T2 and then decreased in T3 of *O.mossambicus* subjected to iron oxide treated tannery effluent. This result proved that WBC count was altered by the toxic effect of iron oxide-treated tannery effluent. Fırat, [45] reported that the decrease in WBC count can be associated with the cortisol hormones which play an important role in the prevention and healing of inflammation on fish by the introduction of toxicants.

The blood enzymes Aminotransferase (AST) and Alanine aminotransferase (ALT) was evaluated in the dissimilar sub-lethal concentration of iron oxide nanoparticles. The enzymes AST and ALT were gradually increased at T1, T2, and suddenly decreased in T3 when compared to control (Fig. 6). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were altered when subjected to a higher concentration of selenium and nano selenium [46]. Alanine aminotransferase(ALT) and aminotransferase(AST)was used as potential marker enzymes for diagnosing liver, muscle, and gill damage of *Poecilia reticulata* subjected to tannery effluent [34]. Ferat and Kargen, [47] reported an increase in the serum ALT and AST activities in O. niloticus exposed to concentrations of Zn, Cd, and Zn+ Cd compared with control. The serum enzymes AST and ALT were significantly increased in zinc

Table 2. Hematological parameters of tilapia

Blood parameters	T0 (Control)	T1 (2.32ppm)	T2 (4.65ppm)	T3 (23.27ppm)
WBC (CELLS/CUMM)	6,900	11,800	35,9000	8,200
RBC (Millions/cmm)	0.33	0.31	0.61	0.51
Hemoglobin(g/dL)	0.3	0.6	0.9	0.4
Hematocrit(PCV) (%)	0.7	1.0	1.8	0.9
MCHC (%)	42.8	60	50	44.4
Platelets Count (Cells/Cumm)	29,000	40,000	59,000	34,000

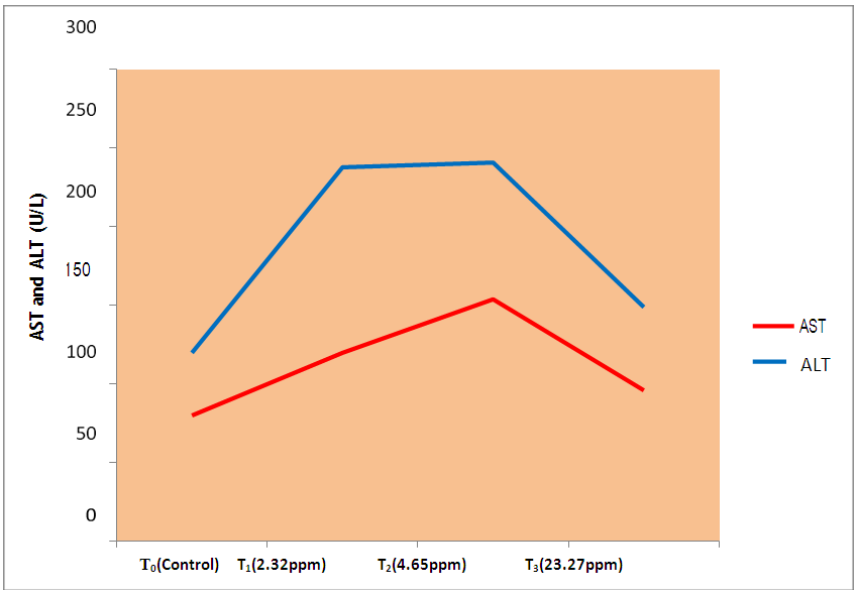
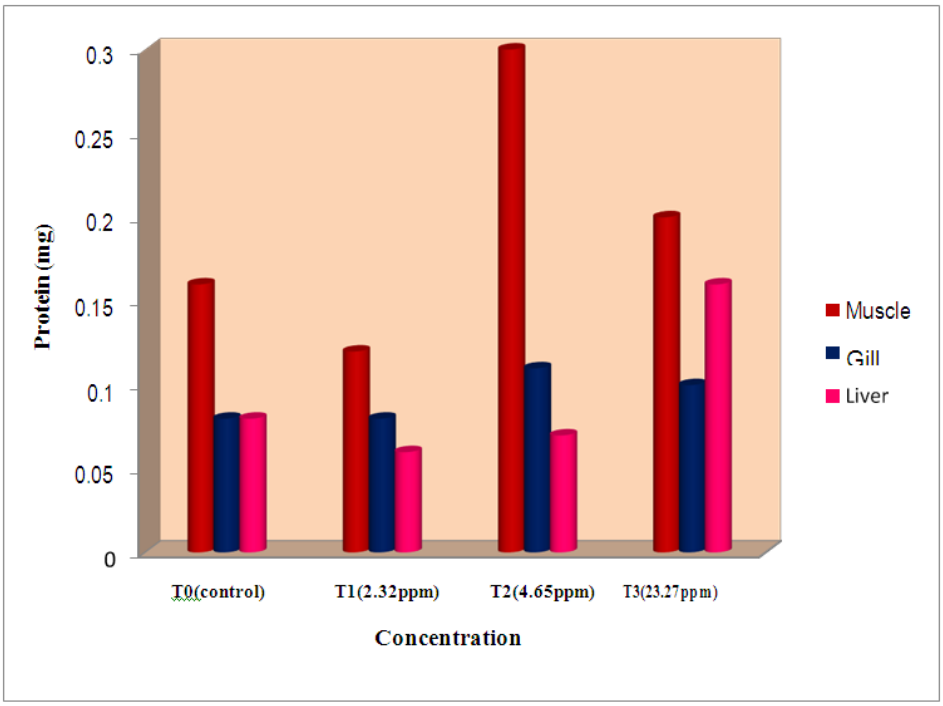


Fig. 6. Aspartate aminotransferase and Alanine transferase of *Oreochromis mossambicus* subjected to dissimilar strength of iron oxide nanoparticles treated tannery effluent

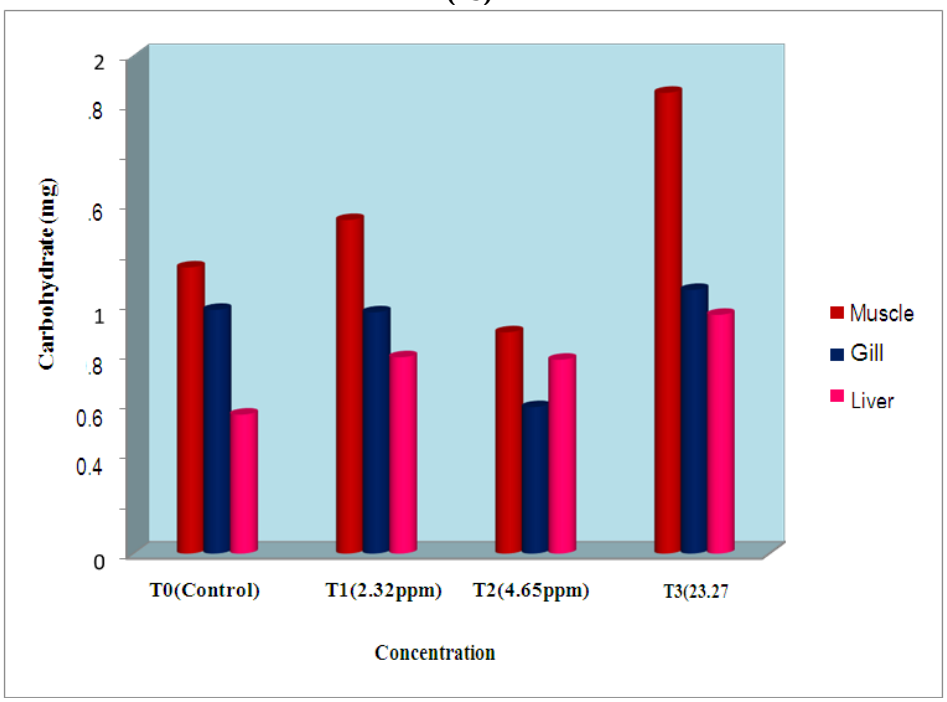
oxide nanoparticles treated Oreochromis niloticus compared to control in exposure period and mentioned that the liver and kidney are target organs with exposure to ZnO NPs [48].

Biochemical characteristics of muscle, gill, and liver of Tilapia are higher in T1 and lower in T2 when compared to control (Fig. 7a, 7b, 7c) Biochemical characteristics are used in ecotoxicological research to assess contaminant impacts on organisms [49]. The biochemical parameters of muscle, gill, and liver of zebrafish *Danio rerio* decrease from control (normal water) when exposed to raw tannery effluent [50].

(7a)



(7b)



(7c)

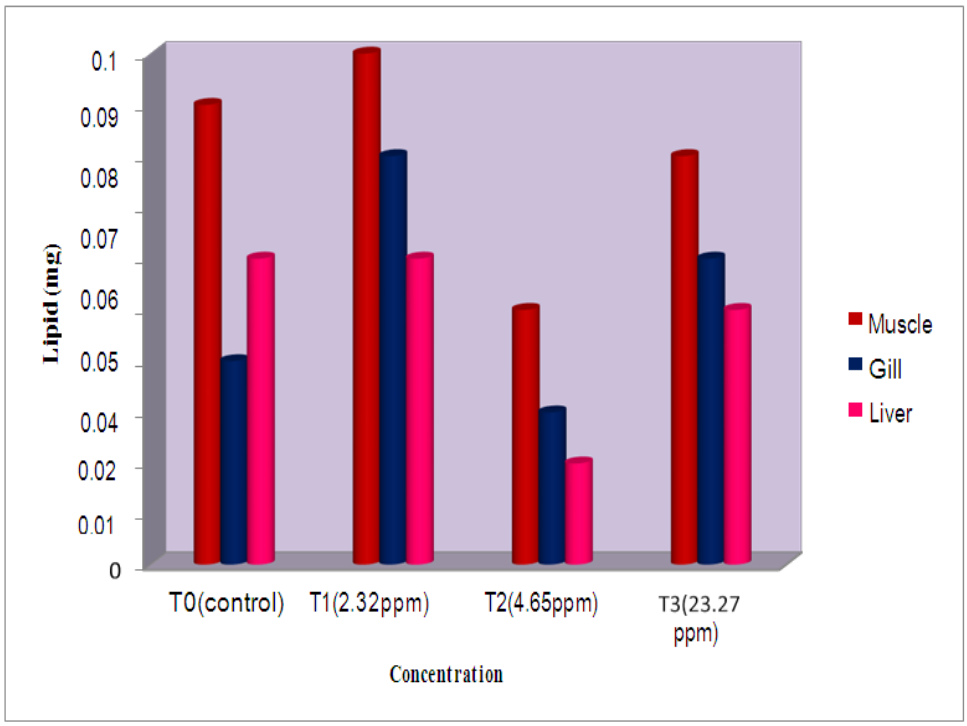


Fig. 7. Biochemical characteristics of muscle, gill and liver of *Oreochromis mossambicus* (7a-Total Protein, 7b-Carbohydrate, 7c -Lipid) exposed to dissimilar strength of iron oxide nanoparticles treated tannery effluent

4. CONCLUSION

It is concluded that iron oxide nanoparticles can be adopted for the successful elimination of toxic substances from tannery effluent and medium(4.65ppm) strength of iron oxide treated tannery effluent is suitable for hematological, enzymatic, and biochemical characteristics of Tilapia.

ETHICAL APPROVAL

The use of fishes as an animal model in the present study has followed the guidelines of the Committee for Control and Supervision of Experiments on Animals [CPCSEA, Ministry of Environment & Forests (Animal Welfare Division), Government of India] on the care and use of animals in scientific research and also accepted by the Ethical Committee of the Gandhigram Rural Institute, Gandhigram, Tamil Nadu, India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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