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Effect of Copper on the Humoral and Biochemical Indices of the Teleost fish, *Anabas testudineus* (Bloch, 1792)

Bhaskaran Nair Girish KUMAR[1] and Sivasankaran Bijoy NANDAN[2,*]

[1]*Department of Zoology, NSS Hindu College, Changanacherry, Mahatma Gandhi University, Kottayam, Kerala, India*
[2]*Department of Marine Biology, Microbiology & Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin, Kerala, India*

([*]Corresponding author's e-mail: bijoynandan@yahoo.co.in, msmgirish9@gmail.com)

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Abstract

The effects of sub-lethal concentrations of copper and the ameliorating capacity of vitamin C on the humoral and biochemical parameters in the teleost fish, *Anabas testudineus*, were investigated. The 96 h LC_{50} value of copper for *A. testudineus* was determined by Probit method and was found to be 1.74 mg/L. The blood of fish from two sub-lethal concentrations along with vitamin supplemented media and control medium, were analysed. The haemoglobin (Hb), haematocrit (Hct), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were reduced ($p > 0.0001$) in copper exposed fish compared to the control. The leucocyte (WBC) count increased ($p < 0.0001$) with dose and duration. Immature and amitotic RBCs, hypoxia, hypochromia, and crenation were observed in highest nominal concentrations and last exposures. The plasma glucose, cortisol, GOT (Glutamate oxalate transaminase), GPT (Glutamate pyruvate transaminase), ALP (Alkaline phosphatase) and LDH (lactate dehydrogenase) increased ($p < 0.0001$), and serum protein decreased. However, on day 28, the Hb, RBC count, MCH, MCHC, and the oxygen carrying capacity increased, and glucose, cortisol and LDH decreased, markedly. The administration of vitamin C improved the blood parameters, illustrating its prophylactic capacity to overwhelm the trace metal stress.

Keywords: Copper, vitamin C, antioxidant capacity, haematological and biochemical parameters, *Anabas testudineus*

Introduction

Trace metals are considered to be one of the main causes of pollution in aquatic ecosystems at present, having the highest environmental stress index, often in excess of the recommended threshold limit [1]. Trace metal and pesticide contamination of aquatic ecosystems has increased in the last few decades, due to their extensive use in agricultural, chemical and industrial processes that are becoming threats to living organisms. Fish are more frequently exposed to these pollutants because it is believed that, regardless of where pollution occurs, it will eventually end up in the aquatic environment.

Copper is relatively abundant and found at varying concentrations in nearly all uncontaminated aquatic and terrestrial ecosystems [2]. Copper sulphate is widely used as an algaecide for controlling phytoplankton in fish ponds and lakes, as well as a herbicide in aquatic weed control, and has many industrial applications, including the preparation of Bordeaux mixture (a fungicide). Intensive industrial developments in the last few decades have increased the concentration of copper in aquatic ecosystems and these affected fish and caused deterioration in the natural resources. For instance, a typical freshwater

ecosystem, Periyar, the longest river in Kerala, is gradually undergoing eco-degradation, with a burden of copper in water and sediments as 0. 075 - 2.59 mg/L and 0.055 - 4.32 µg/g respectively, which is far beyond the permissible limit suggested by the US Environmental Protection Agency (USEPA), the World Health Organization (WHO) and the Bureau of Indian Standards (BIS) [3]. Copper is an essential micronutrient for all organisms, and in the case of fish, is acquired by the gills from the surrounding water, as well as through diet by the digestive tract.

Copper is well known for its high reactivity with H_2O_2 and potential to undergo redox reactions to form reactive oxygen species (ROS) that may cause irreversible cellular damage and death. High concentrations of copper cause haematological and physiological changes leading to retarded growth and inhibition of spawning [4]. There is not much scientific information available on the toxic effects of trace metals on fisheries. The quantum of work in fishes, especially *Anabas testudineus*, in relation to copper is relatively low. It is in this context that the present study was undertaken.

Materials and methods

A static, renewal bioassay method was adopted for the determination of 96 h. LC_{50} [5]. *Anabas testudineus*, abundantly seen in ponds, streams, lakes and rivers in Kerala, was selected for the study. The fish is grey-green on the dorsal and lateral sides and yellowish green on the ventral side. The body is covered with large overlapping cycloid scales and has labyrinthiform organs for aerial breathing, hence the fish being known by the trade name "climbing perch".

Before the start of the experiment, fish were acclimatized for a period of one month in 200 L tanks disinfected with potassium permanganate solution. The temperature in the tank during the experiment was maintained at 27.1 ± 2.4 °C, pH 7.2 ± 0.07 with dissolved oxygen at 7.74 ± 0.34 mg/L. The oxygen saturation was maintained by aerating the tank using an aquarium pump. Healthy and active fish irrespective of sex with a weight of 45 - 50 gm and length 8 - 10 cm were selected for the experiment. Fish were fed once daily with a commercial feed and the water was changed 1 h after feeding. A stock solution of copper was prepared from hydrated copper sulphate ($CuSO_4$. $5H_2O$) and added subsequently to the water in experimental tanks to obtain desired test concentrations. Prior to the toxicity experiment, a range finding test was carried out. The acute toxic levels of copper were determined by a static renewal test using 12 fish randomly selected from the holding tank and transferred to experimental tanks containing 20 L of dechlorinated tap water. They were observed regularly and the mortality was recorded daily for a period of 96 h. Probit values were plotted on probit paper and the 96 h LC_{50} with 95 % confidence limit was calculated [6] following the computerized statistical package, SPSS 16. 0. Based on LC_{50} values, 2 sub-lethal concentrations of copper,$1/5^{th}$ and $1/10^{th}$ of 96 h LC_{50} (0. 34 mg/L and 0.17 mg/L), were added to each experimental tank. Replicates were run for each concentration. Another set of sub-lethal concentrations supplemented with Vitamin C (Ascorbic acid, 2.5 mg/L) were also run parallel, to evaluate the prophylactic and curative effect of vitamin C against copper intoxication. The medium was renewed every 24 h.

On the 7^{th}, 14^{th}, 21^{st} and 28^{th} days of exposure, fish were caught, anaesthetized, and blood samples for haematological studies were collected in small vials pre-rinsed with heparin by puncturing the caudal peduncle. Blood was treated with EDTA to prevent coagulation. All the haematological analyses were performed using standard techniques. RBC and WBC counts were performed haemocytometrically with a Neubauer chamber [7,8]. Hb was determined by cyanmethaemoglobin method [9]. The Hct was determined by Wintrobe's method [10]. The erythrocyte indices, MCV, MCH, and MCHC, were calculated following standard formulae [11]. The serum LDH was determined as per standard methods [12]. The serum cortisol was determined by an enzyme linked immuno fluorescent assay (ELFA) [13]. Serum GOT, GPT, and alkaline phosphatase (ALP) were determined by kinetic method [14-16]. The serum protein and plasma glucose were determined as per standard methods [17,18].

Result and discussion

The humoral response of *Anabas testudineus* on exposure to sub-lethal concentrations of copper depicted significant decrease ($p < 0.0001$) in RBC count, Hb content and oxygen carrying capacity up to the 21st day of exposure, followed by gradual increase on day 28. The microscopic examination of RBCs in both sub-lethal concentrations after the third week of exposure showed discrete pathological deviations such as crenation, enlarged RBC nucleus (**Figure 1**), hypochromia, immature RBCs (**Figure 2**) and reactive lymphocytes (**Figure 3**). The most conspicuous morphological alteration observed in fish exposed to 0. 34 mg/L copper on 28th day was an occurrence of many amitotic RBCs (RBCs with double nuclei) (**Figure 4**). The supplementation of vitamin C in both sub-lethal concentrations enhanced the RBC count significantly ($p < 0.0001$). The fish exposed to 0.17 mg/L copper supplemented with vitamin C showed signs of recovery from crenation (**Figure 5**) compared to the fish exposed to copper. Such morphological and pathological changes were not observed in fish from copper and vitamin free control medium (**Figure 6**).

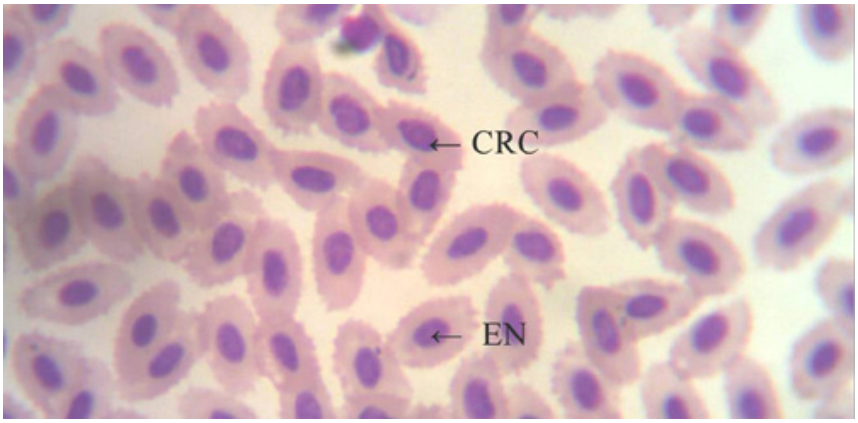

Figure 1 Blood of *A. testudineus* exposed to 0.17 mg/L copper for 21 days showing enlargement of RBC nucleus (EN) and crenation of RBCs (CRC). Giemsa stained (100×).

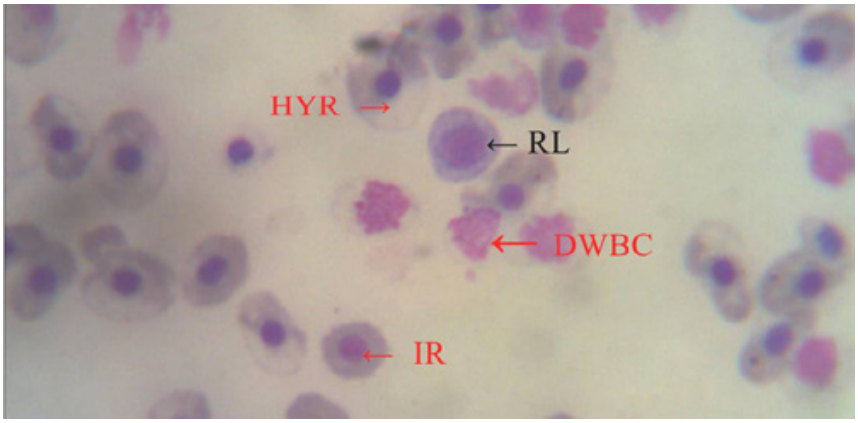

Figure 2 Blood of *A. testudineus* exposed to 0.34 mg/L copper for 21 days showing hypochromic RBCs (HYR), immature RBCs (IR), reactive lymphocytes (RL) and degenerated WBCs (DWBC). Giemsa stained (100×).

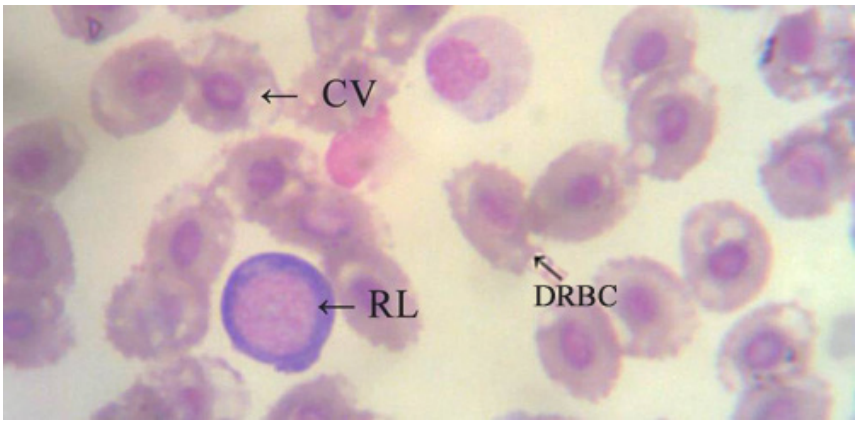

Figure 3 Blood of *A. testudineus* exposed to 0.17 mg/L copper for 21 days showing cytoplasmic vacuolization (CV) of RBCs and reactive lymphocytes (RL) and Degenerated RBCs (DRBC). Giemsa stained (100×).

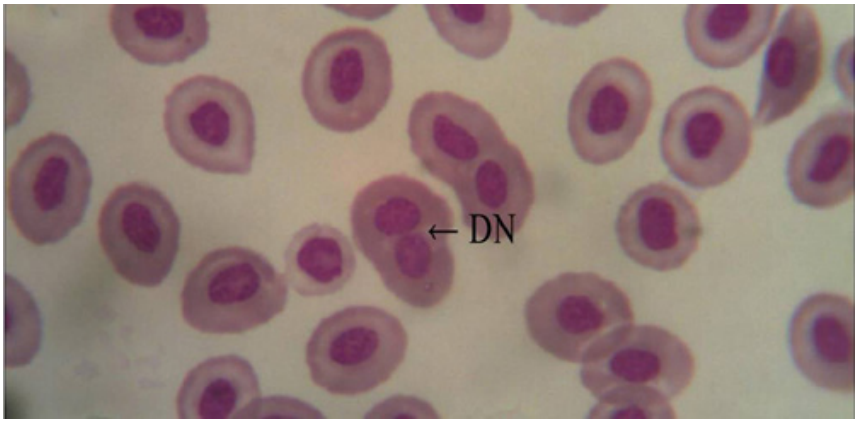

Figure 4 RBCs with double nuclei (DN) or amitotic erythrocytes of *Anabas testudineus* exposed to 0.34 mg/L of copper for 28 days. Giemsa stained (100×).

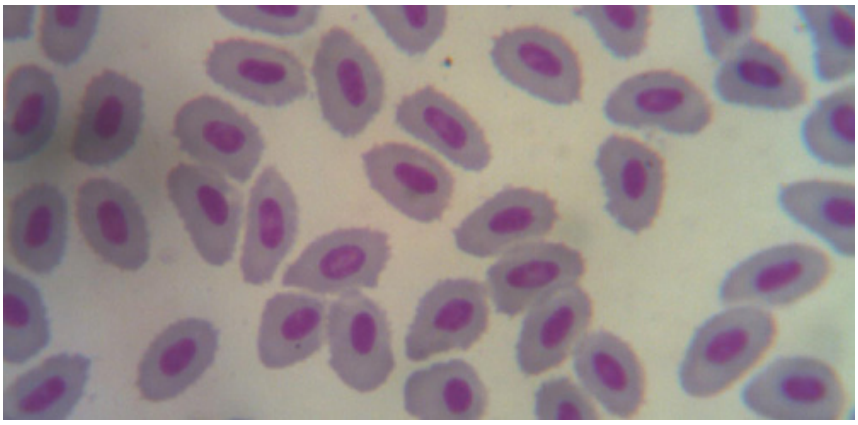

Figure 5 Blood of *A. testudineus* exposed to 0.17 mg/L copper +2.5 mg/L vitamin C for 21 days showing recovery of RBCs from crenation. Giemsa stained (100×).

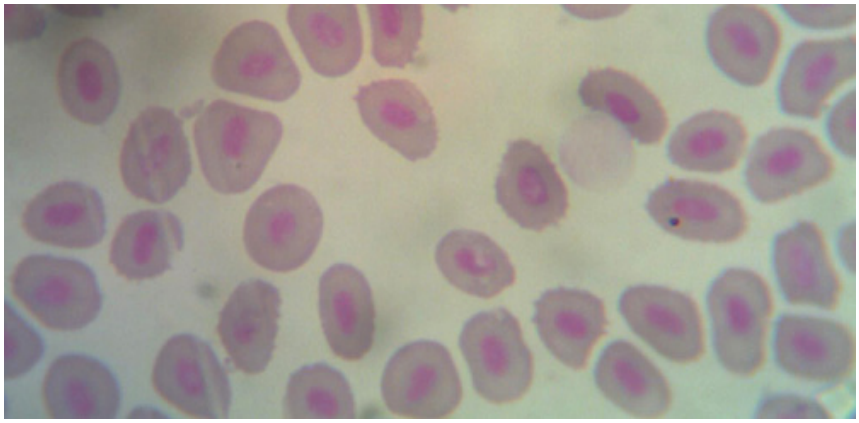

Figure 6 Blood of *Anabas testudineus* showing normal RBCs in copper and vitamin free control medium after 28 days of exposure. Giemsa stained (100×).

The Hct value decreased significantly ($p < 0.0001$) in both sub-lethal concentrations compared to the control. The haematological indices MCH and MCHC decreased up to day 21, followed by an increase on day 28. The MCV value showed prominent decrease in both tested concentrations at all exposures compared to the control. The administration of vitamin C brought the RBC count, MCH, MCHC, MCV and Hct values towards the control values. The WBC count increased significantly ($p < 0.0001$) in fish exposed to increasing copper concentrations as well as duration of exposure. As a response to metal stress, numerous reactive lymphocytes (stress lymphocytes) were detected in the blood of fish in higher copper concentrations (**Figure 3**). A significant reduction ($p < 0.0001$) in serum protein was noticed, with an increase in copper concentration as well as duration of exposure in both sub-lethal concentrations compared to the control. The decrease in serum protein level was more pronounced and significant in higher nominal concentration and longer exposure than in lower concentration and shorter exposure. Vitamin C administration markedly increased the serum protein level closer to the control value.

On the 7^{th}, 14^{th} and 21^{st} days of exposure, plasma glucose, serum LDH and cortisol level increased significantly ($p < 0.0001$) with increase in copper concentrations. However, on the 28^{th} day of exposure, serum LDH, cortisol and glucose level decreased markedly, but the values stood much higher than the control value. The serum ALP activity also increased significantly ($p < 0.0001$) in all the tested concentrations and for all durations of exposure. Vitamin C administration brought the serum cortisol, LDH and ALP level towards the control values. The serum GOT and GPT activity were high in both tested concentrations and was found to be statistically significant ($p < 0.0001$) as per the results of ANOVA. The increase in serum GOT and GPT activity in tested concentrations was parallel to the increase in concentration of copper and the period of exposure. In vitamin C supplemented fish, serum GOT and GPT activity declined in all concentrations in relation to increase in dose and duration. The alterations in the humoral and biochemical parameters in experimental and control fish are presented in **Tables 1** and **2**.

Table 1 Variation in hematological parameters of *Anabas testudineus* on exposure to copper with and without supplementation of vitamin C.

Hematological Parameter	Duration of exposure	Control	Sub-lethal Concentrations			
			0.34 mg Cu./L	0.34 mg Cu./L+2.5 mg Vitamin C	0.17 mg Cu./L	0.17 mg Cu./L+2.5 mg vitamin C
Haemoglobin (Hb) - (g%)	7 days	14.818 ±0.025	8.748±0.011*	9.31± 0.003*	10.09±0.008*	11.24 ±0.007*
	14 days	14.622 ±0.011	7.844 ±0.004*	8.26 ±0.008*	8.68 ±0.008*	9.2 ±0.003*
	21 days	13.94 ±0.024	6.317 ±0.003*	7.12±0.004*	9.54 ±0.005*	8.15 ±0.006*
	28 days	13.762 ±0.013	8.32 ±0.004*	9.24 ±0.006*	9.164 ±0.004*	10.02 ±0.006*
Haematocrit (Hct) - (%)	7 days	39.925 ± 0.029	27.374 ± 0.036*	28.795 ± 0.030*	30.741 ± 0.007*	32.797 ± 0.058*
	14 days	36.608 ± 0.043	24.16 ± 0.027*	25.861 ± 0.02*	27.148 ± 0.009*	28.235 ± 0.037*
	21 days	38.771 ± 0.03	22.122 ± 0.010*	23.147 ± 0.006*	24.602 ± 0.082*	25.677 ± 0.048*
	28 days	37.078 ± 0.05	18.772 ± 0.005*	14.625 ± 0.053*	20.23 ± 0.035*	20.697 ± 0.07*
Erythrocyte (RBC) - (10^6/mm^3)	7 days	3.121 ± 0.004	2.42 ± 0.004*	2.53 ± 0.004*	2.61 ± 0.007*	2.734 ± 0.004*
	14 days	3.101 ± 0.001	2.195 ± 0.004*	2.301 ± 0.002*	2.418 ± 0.005*	2.527 ± 0.006*
	21 days	2.994 ± 0.002	1.87 ± 0.006*	2.092 ± 0.004*	2.181 ± 0.005*	2.31 ± 0.006*
	28 days	2.965 ± 0.002	2.322 ± 0.007*	2.31 ± 0.004*	2.534 ± 0.004*	2.682 ± 0.005*
Leucocyte (WBC) - (10^4/mm^3)	7 days	7.82 ± 0.01	9.35 ± 0.003*	10.30 ± 0.01*	8.85 ± 0.03*	9.26 ± 0.01*
	14 days	7.75 ± 0.01	10.24 ± 0.09*	10.88 ± 0.06*	9.35 ± 0.06*	9.69 ± 0.02*
	21 days	7.78 ± 0.005	10.77 ± 0.04*	10.43 ± 0.006*	10.20 ± 0.03*	10.70 ± 0.01*
	28 days	7.76 ± 0.01	11.34 ± 0.02*	11.72 ± 0.02*	10.53 ± 0.002*	10.88 ± 0.01*
Oxygen carrying capacity (ml O_2/g Hb)	7 days	18.52±0.032	12.221 ± 1.288*	11.637 ± 0.004*	12.612 ± 0.010*	14.04 ± 0.009*
	14 days	18.278 ± 0.013	9.805 ± 0.005*	10.324 ± 0.005*	10.849 ± 0.011*	11.499 ± 0.003*
	21 days	17.419 ± 0.033	7.896 ± 0.004*	8.901 ± 0.006*	9.424 ± 0.006*	10.187 ± 0.008*
	28 days	17.203 ± 0.017	10.399 ± 0.006*	11.549 ± 0.008*	11.455 ± 0.005*	12.524 ± 0.008*
Mean corpuscular volume(MCV) - (fL)	7 days	127.962 ± 0.18	113.112 ± 0.14*	113.817 ± 0.21*	117.782 ± 0.33*	119.938 ± 0.236*
	14 days	127.707 ± 0.15	114.584 ± 0.23*	112.365 ± 0.09*	112.248 ± 0.264*	111.73 ± 0.287*
	21 days	129.45 ± 0.14	118.344 ± 0.03*	110.6 ± 0.23*	112.782 ± 0.402*	112.782 ± 0.402*
	28 days	125.017 ± 0.21	80.817 ± 0.262*	84.96 ± 0.35*	79.821 ± 0.156*	77.142 ± 0.035*
Mean corpuscular Haemoglobin - MCH (pg)	7 days	47.46 ± 0.052	36.145 ± 0.039*	36.792 ± 0.067*	38.655 ± 0.114*	41.104 ± 0.063*
	14 days	47.145 ± 0.039	35.721 ± 0.072*	35.885 ± 0.045*	35.844 ± 0.074*	36.4 ± 0.096*
	21 days	46.717 ± 0.061	33.788 ± 0.093*	34.015 ± 0.091*	34.562 ± 0.079*	35.277 ± 0.111*
	28 days	46.4 ± 0.026	35.815 ± 0.105*	39.998 ± 0.057*	36.157 ± 0.053*	37.344 ± 0.090*
Mean corpuscular Haemoglobin Concentration MCHC - (g/dl)	7 days	37.11 ± 0.060	31.942 ± 0.015*	32.318 ± 0.036*	32.817 ± 0.029*	34.272 ± 0.072*
	14 days	36.914 ± 0.045	31.152 ± 0.021*	31.941 ± 0.31*	31.967 ± 0.36*	32.578 ± 0.051*
	21 days	35.931 ± 0.060	28.541 ± 0.013*	30.752 ± 0.022*	30.66 ± 0.109*	31.728 ± 0.071*
	28 days	37.112 ± 0.070	42.074 ± 0.016*	47.078 ± 0.150*	45.297 ± 0.078*	48.73 ± 0.178*

Each value is the average of seven observations ± SE. *All values are significant at ($p < 0.0001$).

Table 2 Variations in biochemical parameters of *Anabas testudineus* on exposure to copper with and without supplementation of vitamin C.

Biochemical Parameter	Duration of Exposure	Control	Sub-lethal Concentrations			
			0.34mg Cu./L	0.34mg Cu./L+2.5mg Vitamin C	0.17mg Cu./L	0.17Cu./L+2.5 mg vitamin C
Glucose - (mg%)	7 days	101.27 ± 0.66	125.04 ± 0.71*	118.55 ± 0.17*	117.37 ± 0.35*	110.61 ± 0.35*
	14 days	109.22 ± 0.39	139.24 ± 0.31*	129.68 ± 0.13*	129.75 ± 0.16*	122.58 ± 0.10*
	21 days	105.16 ± 0.22	147.44 ± 0.36*	132.97 ± 0.19*	138.84 ± 0.19*	124.6 ± 0.18*
	28 days	105.77 ± 0.20	136.9 ± 0.28*	128.74 ± 0.28*	129.48 ± 0.52*	121.38 ± 0.27*
Gluatamate Pyruvate transaminase (GPT) - (IU/L)	7 days	51.95 ± 0.12	90.57 ± 0.38*	76.2 ± 0.06*	77.80 ± 1.01*	66.77 ± 0.74*
	14 days	52.78 ± 0.27	108 ± 0.11*	90.21 ± 0.19*	92.65 ± 0.15*	78.48 ± 0.14*
	21 days	54.14 ± 0.23	121.22 ± 0.24*	112.24 ± 0.19*	106.48 ± 0.17*	92.58 ± 0.27*
	28 days	54.88 ± 0.07	139.75 ± 0.11*	124.64 ± 0.31*	118 ± 0.25*	114.14 ± 0.35*
Glumate Oxalate transaminase (GOT) - (IU/L)	7 days	118.37 ± 0.26	245.22 ± 0.57*	201.84 ± 0.26*	140.85 ± 0.26*	129.95 ± 0.22*
	14 days	118.78 ± 0.32	291.88 ± 0.16*	168.85 ± 0.35*	161.07 ± 0.25*	147.65 ± 0.54*
	21 days	116.98 ± 0.16	308.72 ± 0.34*	178.45 ± 0.46*	177.24 ± 2.00*	151.28 ± 0.20*
	28 days	123.04 ± 0.21	321.25 ± 0.16*	198 ± 0.36*	191.95 ± 1.89*	165.34 ± 0.19*
Alakaline Phosphate (ALP) - (IU/L)	7 days	12.08 ± 0.06	22.09 ± 0.05*	18.71 ± 0.10*	17.99 ± 0.12*	14.82 ± 0.07*
	14 days	12.55 ± 0.04	28.12 ± 0.08*	23.34 ± 0.14*	23.98 ± 0.19*	19.34 ± 0.26*
	21 days	12.71 ± 0.05	36.37 ± 0.20*	28. ± 0.14*	29.32 ± 0.27*	23.4 ± 0.17*
	28 days	12.32 ± 0.09	48.12 ± 0.13*	32.9 ± 0.14*	35.57 ± 0.12*	26.8 ± 0.03*
Protein - (g %)	7 days	7.32 ± 0.05	4.66 ± 0.12*	5.16 ± 0.01*	5.11 ± 0.02*	5.99 ± 0.01*
	14 days	7.33 ± 0.04	4.06 ± 0.02*	5.99 ± 0.04*	4.52 ± 0.003*	6.49 ± 0.01*
	21 days	7.39 ± 0.007	3.48 ± 0.009*	4.40 ± 0.008*	4.12 ± 0.003*	5.59 ± 0.004*
	28 days	7.36 ± 0.01	3.03 ± 0.02*	4.16 ± 0.01*	3.61 ± 0.002*	4.91 ± 0.003*
Lactate dehydrogenase (LDH) (IU/L)	7 days	269.72 ± 2.09	408.31±1.46*	371.97 ± 0.56*	323.08 ± 6.80*	310.24 ± 0.43*
	14 days	268.37±0.27	433.2 ± 1.43*	387 ± 0.86*	347.54 ± 0.63*	317.95 ± 0.42*
	21 days	269.65 ± 0.25	456.85 ± 0.64*	416.51 ± 1.00*	372.72 ± 0.92*	334.81 ± 0.75*
	28 days	274.15 ± 4.03	422.07 ± 0.28*	382.55 ± 0.56*	349.1 ± 0.92*	291.22 ± 0.65*
Cortisol (microgram/dl)	7 days	130.31 ± 0.17	190.51 ± 0.29*	174.97 ± 0.43*	165.35 ± 0.97*	158. ± 0.61*
	14 days	136.85 ± 0.31	229.94 ± 0.25*	193.11 ± 0.68*	194.78 ± 0.26*	176.07 ± 1.19*
	21 days	132.41 ± 0.17	252.65 ± 0.37*	213.24 ± 0.37*	233.12 ± 0.28*	201.34 ± 0.20*
	28 days	129.58 ± 0.25	201.12 ± 1.27*	166.27 ± 0.56*	172.47 ± 0.29*	156.11 ± 0.53*

Each value is the average of seven observations ± SE. *All values are significant at ($p < 0.0001$)

The reduction in RBC, Hb, Hct, MCV and MCH in *Anabas testudineus* in both sub-lethal concentrations of copper might be due to the destruction of mature RBCs, or the inhibition of erythropoiesis due to degeneration of erythropoietic tissues in the kidney and spleen leading to haemolytic anaemia [19], or inhibition of aerobic oxidation that curtails the de novo synthesis of Hb [20], as in *Etroplus maculatus* on exposure to lindane.

The enlargement of RBC nuclei in fish exposed to 0.17 mg/L copper on day 21 in the present study may be the initial sign of disintegration of the nuclei followed by gradual shrinkage. The increase in population of immature RBCs might be as compensation for the increased degeneration of cells. The morphological changes in RBCs may be taken as a serious indication of heavy metal intoxication in fish. The reduced oxygen carrying capacity in the present study up to the day 21 may be attributed to the fall in RBC count, intense haemolysis, and haemodilution induced by the copper stress. It can be inferred that the improvement of RBC count, Hb content, MCH, MCHC and oxygen carrying capacity on day 28 might be due to the gradual shift from anaerobiosis to aerobiosis as a sign of compensatory homeostatic adjustment of test organisms. Amitotic RBCs and immature round RBCs in highest nominal concentration in the present study concur with similar observations in *Alburnus alburnus*, *Scardinus erythrophtalmus* and *Perca fluviatilis* [21]. Vitamin C administration was found effective in enhancing Hb, Hct, RBC count, oxygen carrying capacity, and haematological indices, compared to those exposed to copper, elucidating the curative role of vitamin C against metal intoxication.

The increase in WBC count in the present study may be due to the innate immune response to overwhelm the tissue and organ level toxic atrocities induced by the metal. The stress lymphocytes and degenerated WBCs observed in fish exposed to 0.34 mg/L copper on the 21st day of exposure (**Figure 2**) may be interpreted as a symptom of heavy metal toxicosis. The hypersensitivity of WBCs to copper and the enhancement of antibody production to cope with the heavy metal stress in *Channa punctatus* corroborate the present study [22]. A WBC count closer to the control value in vitamin treated fish highlights the therapeutic role of ascorbic acid.

Glucose is one of the most sensitive indices of oxidative stress, and its high as well as low concentration in blood has been considered as a reliable indicator of stress in fish. The increase in plasma glucose in copper exposed fish over the control in the present study might be due to disorders in carbohydrate metabolism elicited by the physical and chemical stress, and the fish might be using the energy reserves such as liver and muscle glycogen extensively to overwhelm the energy crisis induced by copper. The manifestation of hyperglycemia in exposed fish might be due to the increased rate of glycogenolysis or glucogenesis as an inductive response of circulating glucocorticoids and catecholamines [23] or the transportation of glucose through the blood, probably from the liver to the muscles, as a toxic response of fish to copper to cope with the high energy demand experienced in the muscles [24]. The fall in plasma glucose and increase in oxygen carrying capacity in fish on day 28 in both sub-lethal concentrations may be interpreted as the efforts on the part of fish to recover from the toxic effect of copper and glycogenolytic reversal.

Serum cortisol, in addition to glucose, is also taken to be one of the most important stress indicators in fish. The increased trend of serum cortisol in the present study up to day 21suggests that copper elicits the stress even up to the third week of exposure, and fish did not acclimate to copper during this period. It could be assumed that the hypothalamo-hypophyseal-inter renal (HHI) coupling was not impaired during this period, since the ability of the head kidney to secrete cortisol was not significantly affected by copper exposure. A reduction in uptake, phosphorylation, and consumption of glucose by the extra hepatic tissue and increase in the liver glycogenolysis induced by the elevation of cortisol, resulting in increased blood glucose level, has been reported in fish [25]. The decrease in cortisol and glucose on the 28thday of exposure in the present study is in conformity with earlier studies; the fish might have evolved a gradual adaptation to copper on long term exposure, or the metal might have caused exhaustion of HHI axis and atrophy of inter renal tissue, causing a decreased cortisol level [26]. In metal poisoned fish supplemented with vitamin C plasma glucose and serum, cortisol levels were brought near to control values, highlighting its capacity to reduce copper toxicity in fish and enhancement of fish tolerance against heavy metal contamination.

The decrease in serum protein in fish exposed to copper in the preset study agrees with similar findings in *Catla catla* on exposure to copper cyanide; namely, the fall in serum protein might be the result of intense breakdown of protein into amino acids, to be fed in to the Kreb's cycle to cope with the energy crisis manifested by copper intoxication [27], impaired protein synthesis, the functional deterioration of the liver, or excessive loss of protein caused by nephrosis. Decrease in serum protein, due to increased protein breakdown by hypersecretion of corticosteroid hormones, providing amino acids for gluconeogenesis to produce more glucose to compensate the increased energy demand under stress situations, was observed [28] in *Oreochromis niloticus*. In the present study, vitamin C administration brought the serum protein level near to the control value.

Among transaminases, GOT is the principal enzyme that interferes with the TCA cycle in a major way. A rise in its activity indicates the occurrence of greater energy demand, which is normally associated with synthetic activities of the cell. GOT is concerned with the molecular rearrangement of amino acids linked to the citric acid cycle, providing sufficient reduction equivalents like NADH and NADP into mitochondria, or to synthesize ATP to meet the high energy crisis [29]. GPT is more predominant in organs concerned with intense glycogenesis, such as the liver. GOT and GPT are cellular metabolic enzymes, usually found in small concentrations in plasma derived probably from the regular physiological shedding of cells. The exorbitant increase of these enzymes in the blood stream of exposed fish could be due to the detrimental effect of copper on the hepatic parenchyma cells. The increase in enzymatic activity in exposed fish in this study might be due to leakage of these tissue specific enzymes from the damaged hepatic cells into the blood circulation, or increased synthesis of these enzymes, or induction of these enzymes, and it could be regarded as a strong indication of hepatotoxicity [30]. The synthesis of more lactic acid in the muscle and other tissues due to copper induced hypoxia, and the subsequent transportation to the liver through the blood, might be the reason for the increase in LDH activity in the present study. The LDH enzyme is present in most of animal tissues, and is involved in the inter conversion of pyruvic acid to lactic acid and acts as a vital enzyme between the glycolytic pathway and the tricarboxylic acid cycle. The hyperglycemia, reduced Hb, fall in RBC count, decreased Hct, reduced oxygen carrying capacity of blood, and increased LDH activity with increasing copper concentrations and duration of exposure, could be reliable indicators of a metabolic shift from aerobiosis to anaerobiosis as a toxic response of fish in metallic stress.

Vitamin C is among the most important biological antioxidants. The majority of animals synthesize vitamin C from D-glucose, but most fish are incapable of self-synthesis. Therefore, it could be recommended that vitamin C administration is imperative in reducing copper toxicity in fish. It is also suggested that the antioxidant activity of vitamin C makes it a hunter of free radicals, preventing the autointoxication of immunological cells such as macrophages, which are the first processors of the information about alien bodies, and maximizing the defensive capacity of fish and capability of controlling the oxidizing reactions of fatty acids, thus keeping cellular respiration and avoiding cell death. Vitamin C has proven its efficiency in toxicity reduction, prevention of diseases and enhancement of fish tolerance against environmental stress. The alterations in serum parameters may be the result of target organ damage, such as the liver, gill and kidney, and the dysfunction induced by the toxicants and these parameters can be used as a rapid and reliable indicator of environmental contamination.

Conclusions

The lentic ecosystems and the diverse organisms inhabiting them are subjected to the menace of pollution at an alarming rate. In the present study, the obvious changes in haematological and biochemical parameters of fish in sub-lethal concentrations of copper compared to those in control medium confirms the detrimental effect of copper. However, supplementation of vitamin C was found effective in reducing the tissue level atrocities caused by the trace metal copper and the restoration of the normal physiology of fish.

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