

## Effects of Sulfur Compounds on Oreochromis Niloticus Blood Chemistry

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### Abstract

Sulphur is a non-metallic chemical element and most public water supplies contain sulfate concentrations of less than 500 mg/L. Sulfuric acid is corrosive and toxic by ingestion, inhalation or contact with skin and eyes. Animals were divided into six groups exposed to zero, 30, 50, 70, 100 and 140 ml / 100 L water of sulfuric acid for 32 days, the study concluded that the increase of sulfuric acid concentration in the water have a clear indication of the negative impact on the metabolism in fish and on various vital elements of the fish's body, under study, and these effects depends on the concentration and duration of exposure.

**Key words:** Fish metabolism, Oreochromis niloticus, Sulfuric acid .

### 1. Introduction

Water pollution is one of the principal environmental and public health problems; Egypt and the Middle East region are facing [3,7]. Water pollution does not only greatly damage the aquatic ecosystems but even the terrestrial organisms and ecosystems are severely damaged and threatened [4]. Sulfuric acid is a highly corrosive strong mineral acid with the molecular formula H<sub>2</sub>SO<sub>4</sub> from strong mineral acids, which dissolve in water in all concentrations. It is one of the first acids that have been identified and used in many vital industries, however, it has a negative impact on the environment may cause acid rain and pollution of water, which in turn effects on aquatic organisms generally and on fish especially, is the most important tilapia [22]. [6] reported that, accumulation of sulfur and nitrogen in forest soils; as sulfate is released from the soil in response to decreases in the deposition of sulfur, it continues to acidify adjacent streams and lakes. The recovery of surface waters in response to emission controls has therefore been delayed and will not be complete until the sulfur left by a long legacy of acidic deposition is released from soil [11,18]. [8] confirmed that, most public water supplies contain sulfate concentrations of less than 500 mg/L, but sulfate levels in water around 250 mg/L and above are detectable due to an off odor and taste, and this generally causes those exposed to water with higher concentrations of sulfate to switch to bottled water sources for drinking. Still, adaptation to water with high sulfate content is known to occur. Extremely high sulfate concentrations in water have been recorded; for example, 1,500 mg/L in a coal mine in Pennsylvania and 63,000 mg/L in a zinc mine in Idaho [21]. Sulfuric acid is used in industrial applications and other processes where workplace exposures can occur. Consumers might come in contact with sulfuric acid contained in lead-cell batteries and should avoid exposure as sulfuric acid can cause severe health effects. H<sub>2</sub>SO<sub>4</sub> is dangerous to human health. Sulfuric acid solutions, particularly

the more concentrated ones, are rapidly destructive to all body tissues, causing severe burns which may result in scarring. Most sulfuric acid is consumed in manufacturing processes or recovered and re-used. H<sub>2</sub>SO<sub>4</sub> can make its way into the environment through unintentional releases (spills) and industrial or consumer discharges. H<sub>2</sub>SO<sub>4</sub> will not bio-accumulate and is not biodegradable. Based on ecotoxicological testing performed on fish and freshwater invertebrates, H<sub>2</sub>SO<sub>4</sub> in higher concentrations can be harmful to aquatic life [24]. The present work aimed to evaluate the harmful effects of sulfuric acid on blood chemistry in Nile Tilapia Oreochromis niloticus with different concentrations.

### 2. Materials and methods

#### 2.1 Samples

##### 2.1.1 Fish

A total number of 240 apparently healthy Nile Tilapia [niloticus] with average body weight of fish (50 ± 5 g) were obtained from Birsik fish farm. Fish were transported alive to the wet laboratory in fiber glass tanks containing water enriched by pump air (2/3) and supported by oxygen tubes at Faculty of veterinary medicine, department of Fish disease and management the period from mid-May to first July 2015.

##### 2.1.2 Aquaria

Fish were kept in twelve (12) glass aquaria (90 × 50 × 35) each containing 20 fish. These aquaria were used for holding the experimental fish throughout the period of present study, supplied with chlorine free tap water according to [10]. The continuous aeration was maintained in each aquarium using an electric air pumping. Water temperature was kept at 25 ± 2 c<sup>0</sup>. Fish were acclimated for two weeks prior to the experiment.

##### 2.1.3 Fish diets

Fish were fed on a commercial fish diet containing 25% crude fish protein. The diet was

daily provided at 4% of body weight as described by [30]. The daily amount of food was offered on two occasions over the day (at 9 AM and 4PM).

### 2.1.4 Sulfuric acid

5 different concentrations of liquid sulphuric acid prepared from analar sulphuric acid with high concentration (98%). Sulphuric acid were obtained from private chemical products company. The concentration is

G1 - Zero ml. / 100 L water (control group)

G2 - 30 ml. / 100 L water

G3 - 50 ml. / 100 L water -

G4 - 70 ml. / 100 L water -

G5 - 100ml. / 100 L water

G6 - 140ml. / 100L water

For fish groups: The sulphuric acid diluted in amount of aquarium water the added to the rest of water in the aquarium in zero day and partially exchange amount of water in each aquarium daily with keeping the same concentration of the suggested sulphuric acid concentration. Each concentration distributed in two aquaria from the twelve aquaria. The exposer time was 32 days.

### 2.1.5 Blood sampling

Blood sample were collected every 4 days for 32 days of experiment from the caudal blood vessel of fish (4fish/time) to each aquaria using disposable syringes for biochemical assays. Serum separation was done for biochemical determination according to [17].

### 2.2 Biochemical analysis

Samples from blood of the studied fish were subjected for sulfuric acid analysis of serum cholesterol and triglyceride [26] (SPECTRUM kit, Egypt), total lipids [15]. ALT and AST activities were calorimetrically assayed following the method of [23], using commercial kits produced by Biosystem Lab.

### 2.3 Statistical analysis

The data were computed, expressed as means + standard error and statistically analyzed [24].

## 3. Results

### 3.1 Biochemical parameters

Tables (1-4) present the changes in ALT, AST, Cholesterol and Triglycerides contents in the blood of *O. niloticus* collected from Birsik fish farm during mid-May to first July.

**Table (1)** Effect of different concentration of sulfuric acid at different days of experiment on ALT level in blood of *O. niloticus*

Group	Period (day)								
	0	4	8	12	16	20	24	28	32
G1 (control)	16.57 ±1.11 <sup>aA</sup>	18.60 ±1.36 <sup>aAB</sup>	16.67 ±1.76 <sup>aA</sup>	16.17 ±1.69 <sup>aA</sup>	22.00 ±1.36 <sup>aAB</sup>	19.83 ±1.74 <sup>aAB</sup>	18.32 ±2.03 <sup>aAB</sup>	22.32 ±3.48 <sup>aB</sup>	23.00 ±2.08 <sup>aB</sup>
G2 (30)	16.57 ±1.11 <sup>aA</sup>	22.00 ±1.15 <sup>aAB</sup>	26.67 ±1.2 <sup>bb</sup>	40.33 ±1.45 <sup>cCD</sup>	45.00 ±2.51 <sup>bd</sup>	36.17 ±1.09 <sup>cC</sup>	22.00 ±3.06 <sup>aA</sup>	27.30 ±2.19 <sup>bb</sup>	30.83 ±1.44 <sup>bb</sup>
G3 (50)	16.57 ±1.11 <sup>aA</sup>	19.63 ±2.33 <sup>aA</sup>	19.31 ±1.88 <sup>aA</sup>	32.32 ±1.20 <sup>bb</sup>	71.00 ±1.15 <sup>cE</sup>	60.90 ±2.52 <sup>dD</sup>	38.03 ±4.04 <sup>cC</sup>	36.00 ±6.01 <sup>bc</sup>	38.17 ±1.85 <sup>cC</sup>
G4 (70)	16.57 ±1.11 <sup>aA</sup>	16.07 ±2.54 <sup>aA</sup>	25.30 ±1.84 <sup>bb</sup>	50.67 ±1.44 <sup>dD</sup>	71.47 ±2.29 <sup>cE</sup>	29.67 ±0.88 <sup>bb</sup>	28.33 ±1.20 <sup>bb</sup>	37.33 ±4.06 <sup>cC</sup>	41.38 ±1.88 <sup>cC</sup>
G5 (106)	19.33 ±1.88 <sup>aA</sup>	40.67 ±2.88 <sup>bb</sup>	74.33 ±1.76 <sup>cCD</sup>	72.70 ±2.65 <sup>cC</sup>	97.67 ±3.17 <sup>dE</sup>	91.33 ±1.20 <sup>eE</sup>	72.00 ±3.02 <sup>dC</sup>	77.67 ±2.96 <sup>dCD</sup>	79.67 ±2.85 <sup>dD</sup>
G6 (140)	18.23 ±1.62 <sup>aA</sup>	65.27 ±1.16 <sup>cB</sup>	77.00 ±2.08 <sup>cC</sup>	83.1 ±2.43 <sup>dD</sup>	68.47 ±3.8 <sup>cB</sup>	99.67 ±2.33 <sup>fE</sup>	164.67 ±3.84 <sup>eF</sup>	184.33 ±2.33 <sup>eG</sup>	203.67 ±4.18 <sup>eH</sup>

SE: Standard error. a, b & c: There is no significant difference ( $P > 0.05$ ) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference ( $P > 0.05$ ) between any two means; within the same row have the same superscript letter.

**Table (2)** Effect of different concentration Sulfuric acid at different days of experiment on AST level in blood of *O. niloticus*.

Group	Period (day)								
	0	4	8	12	16	20	24	28	32
G1	19.00 ±1.58aA	20.33 ±1.45aA	23.57 ±1.84aA	24.33 ±2.48aA	23.33 ±2.06aA	20.33 ±1.45a	27.67 ±2.85aB	29.00 ±1.58aB	28.33 ±1.88aB
G2	19.67 ±1.20aA	42.39 ±2.33cC	50.33 ±1.20cD	35.67 ±3.18bcB	38.17 ±2.17bB	40.33 ±1.20bBC	40.67 ±1.45bC	41.93 ±1.18bC	42.33 ±2.19bC
G3	21.00 ±1.15aA	44.33 ±2.29cC	74.33 ±2.40dG	61.33 ±4.13eE	76.33 ±2.91cG	67.87 ±1.44cFG	41.00 ±2.89bBC	38.00 ±1.15bBC	52.67 ±3.67cD
G4	19.33 ±1.88aA	45.00 ±2.58cC	35.00 ±1.06bBC	41.33 ±3.70cC	88.33 ±3.88dDE	85.00 ±1.53dD	94.50 ±1.26cE	94.33 ±2.40cE	118.00 ±3.37dF
G5	20.00 ±0.58aA	26.53 ±1.79bA	106.00 ±5.50fB	105.33 ±2.60eB	102.67 ±3.78eB	134.00 ±2.08fC	141.00 ±1.53dC	142.33 ±1.20dC	170.67 ±4.84eD
G6	19.67 ±1.67aA	42.67 ±3.84cB	80.00 ±4.01eC	98.33 ±3.18dD	105.90 ±3.99eE	124.37 ±3.26eF	172.67 ±4.44eG	187.33 ±3.12eH	223.33 ±4.13fI

**Table (3)** Effect of different concentration Sulfuric acid at different days of experiment on Cholesterol level in blood of *O. niloticus*.

Group	Period (day)								
	0	4	8	12	16	20	24	28	32
G1	134.33 ±2.96 <sup>bCD</sup>	124.13 ±2.03 <sup>bcB</sup>	110.67 ±1.20 <sup>bA</sup>	114.00 ±1.73 <sup>aA</sup>	124.00 ±3.46 <sup>aB</sup>	135.00 ±2.08 <sup>aCD</sup>	140.62 ±2.91 <sup>bD</sup>	143.00 ±2.50 <sup>aD</sup>	147.29 ±4.87 <sup>aD</sup>
G2	113.70 ±2.73 <sup>aB</sup>	105.00 ±2.65 <sup>aAB</sup>	101.30 ±6.49 <sup>aA</sup>	117.70 ±8.21 <sup>aBC</sup>	123.77 ±8.29 <sup>aCD</sup>	136.00 ±2.52 <sup>aAB</sup>	131.00 ±5.77 <sup>aD</sup>	152.67 ±4.60 <sup>bEF</sup>	161.00 ±1.53 <sup>bF</sup>
G3	119.34 ±2.96 <sup>aBC</sup>	109.67 ±2.96 <sup>aA</sup>	131.00 ±1.15 <sup>cC</sup>	142.00 ±1.53 <sup>cD</sup>	127.67 ±4.48 <sup>aC</sup>	152.33 ±0.88 <sup>bEF</sup>	153.00 ±2.31 <sup>cEF</sup>	159.00 ±1.53 <sup>cF</sup>	161.87 ±3.44 <sup>bF</sup>
G4	130.00 ±3.61 <sup>bA</sup>	127.67 ±1.76 <sup>cA</sup>	152.00 ±1.53 <sup>dB</sup>	155.00 ±2.52 <sup>dB</sup>	180.00 ±6.08 <sup>dD</sup>	179.13 ±5.26 <sup>cD</sup>	169.00 ±3.51 <sup>dC</sup>	171.07 ±3.16 <sup>dCD</sup>	180.66 ±4.78 <sup>cD</sup>
G5	134.29 ±2.96 <sup>bA</sup>	133.23 ±2.85 <sup>cA</sup>	127.67 ±1.45 <sup>cA</sup>	144.00 ±1.15 <sup>cB</sup>	163.67 ±5.24 <sup>cC</sup>	178.47 ±7.22 <sup>cD</sup>	172.59 ±5.90 <sup>dDE</sup>	181.69 ±6.89 <sup>eE</sup>	179.74 ±5.85 <sup>cE</sup>
G6	134.00 ±2.65 <sup>bA</sup>	134.33 ±2.96 <sup>cA</sup>	151.00 ±0.58 <sup>dB</sup>	136.32 ±2.19 <sup>bcA</sup>	147.00 ±3.06 <sup>bB</sup>	154.00 ±2.52 <sup>bC</sup>	181.60 ±6.29 <sup>eD</sup>	185.00 ±5.03 <sup>eD</sup>	206.30 ±4.70 <sup>dE</sup>

**Table (4)** Effect of different concentration Sulfuric acid at different days of experiment on triglyceride level in blood of *O. niloticus*

Group	Period (day)								
	0	4	8	12	16	20	24	28	32
G1	74.67 ±1.86 <sup>aA</sup>	76.17 ±2.33 <sup>bA</sup>	80.33 ±2.84 <sup>cAB</sup>	76.83 ±2.13 <sup>bA</sup>	78.00 ±2.58 <sup>bAB</sup>	87.33 ±1.76 <sup>aB</sup>	98.00 ±1.53 <sup>aC</sup>	100.14 ±2.93 <sup>aC</sup>	100.21 ±2.33 <sup>aC</sup>
G2	75.00 ±1.53 <sup>aBC</sup>	60.67 ±2.73 <sup>aA</sup>	70.53 ±2.84 <sup>bB</sup>	92.90 ±3.08 <sup>cD</sup>	80.33 ±3.91 <sup>bC</sup>	106.00 ±3.09 <sup>bEF</sup>	110.33 ±3.36 <sup>bF</sup>	136.67 ±4.27 <sup>cG</sup>	133.00 ±3.77 <sup>bG</sup>
G3	74.67 ±1.86 <sup>aB</sup>	65.33 ±1.76 <sup>abA</sup>	67.70 ±2.86 <sup>bA</sup>	74.07 ±1.49 <sup>bAB</sup>	76.00 ±2.65 <sup>abBC</sup>	85.00 ±2.06 <sup>aC</sup>	99.67 ±2.88 <sup>aD</sup>	125.00 ±3.54 <sup>bE</sup>	142.67 ±3.17 <sup>cF</sup>
G4	74.76 ±1.86 <sup>aB</sup>	64.41 ±1.45 <sup>abAB</sup>	57.90 ±2.47 <sup>aA</sup>	64.83 ±3.03 <sup>abAB</sup>	70.61 ±2.51 <sup>aB</sup>	84.63 ±3.76 <sup>aC</sup>	98.82 ±2.10 <sup>aD</sup>	107.17 ±1.86 <sup>aE</sup>	143.15 ±2.74 <sup>cF</sup>
G5	72.40 ±2.19 <sup>aAB</sup>	70.15 ±3.24 <sup>bA</sup>	80.00 ±2.08 <sup>cB</sup>	133.51 ±1.45 <sup>dB</sup>	142.57 ±3.18 <sup>cC</sup>	156.47 ±2.96 <sup>cD</sup>	177.31 ±3.26 <sup>cE</sup>	201.63 ±4.45 <sup>dF</sup>	197.87 ±3.85 <sup>dF</sup>
G6	74.17 ±1.86 <sup>aA</sup>	75.53 ±2.15 <sup>bA</sup>	135.00 ±3.51 <sup>dB</sup>	144.00 ±2.65 <sup>eB</sup>	179.63 ±4.86 <sup>dD</sup>	186.09 ±3.88 <sup>dD</sup>	196.18 ±3.76 <sup>dC</sup>	199.36 ±4.88 <sup>dC</sup>	210.00 ±4.51 <sup>eD</sup>

### 3.1.1 Liver function profile

Tables (1 & 2) and Figures (1&2) show the mean values of serum aspartate aminotransferase (AST (U/l)) and alanine aminotransferase (ALT (U/l)) level of *O. niloticus* (G2-G6) treated groups, compared to normal control group (G1). The present work showing that, no significant difference between any treated groups with different concentration of sulfuric compared with control group at zero day. Where there is significant increase ( $P>0.05$ ) between all treated groups (G2-G6) for all periods (4-32) days compared with the control group. Also there is significance difference between treated groups (G2-G6) ( $P>0.05$ ) through the period of exposure.

### 3.1.2 Cholesterol & Triglyceride

The table No (3&4) and figures (3&4) show that significant difference between control and all treated groups (G1-G6) from zero day to 4<sup>th</sup> day period which the values increased significantly ( $P>0.05$ ). Where there is significant increase ( $P>0.05$ ) between all treated groups (G2-G6) for all periods (8-32) days compared with the control

group according to the increasing to the sulfuric acid concentration.

## 4. Discussion

Pollution of aquatic habitats is a major problem in Egypt. In recent years, more toxic compounds were detected in aquatic ecosystem [13]. Fishes are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health [20]. Fishes are used as a bio indicator of aquatic ecosystem for estimation any pollutants and risk potential for human consumption [2]. Liver function tests are usually recognized as the reliable indicator of liver metabolism [27]. The raised enzymatic activity in the liver may be because of induction of enzyme synthesis [25,14,16], while their low levels could either be due to enzymatic inhibition [9]; [19] or due to liver damage without any regeneration.

The results of this study revealed that, the serum enzymes AST and ALT levels were increased with the increasing of concentration sulfuric acid in water. Increased serum transaminases may reflect hepatic toxicity which leads to extensive liberation of the enzymes into the blood circulation [5].

Moreover, serum ALT and AST activities are considered as a sensitive indicator to evaluate hepatocellular and myocardial damage [1].

From the present results, it can be concluded that there is a positive relationship between the concentration of sulfuric acid and the values of AST and ALT enzymes in the end of experiment, the serum enzymes AST and ALT levels were increased with the increasing of concentration sulfuric acid in water. These results were in agreement with [12,29]; they said that increased serum enzymes AST and ALT levels may be due to necrotic changes occurring in liver with liberation of cholesterol as a byproduct of cell destruction. Also, the present work indicated that, cholesterol and triglyceride levels increased and decreased at different periods of the experiment. This attributed may be due to the high response to sulfuric and the decrease of destruction of hepatocyte. Or may be to reflect the presence of a marked hypercholesterolemia [28].

The study concluded that the increase of sulfuric acid concentration in the water has a clear indication of the negative impact on the metabolism in fish and on various vital elements of the fish's body, under study, and these effects depend on the concentration and duration of exposure.

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