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Biochemical changes in fish *Heteropneustes fossilis* (Bloch) on exposure to fly ash

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Abstract

Air breathing fish Heteropneustes fossilis (Bloch) were exposed to different concentration of fly ash to study the biochemical changes in blood. The $LC_{50\ value}$ of fly ash to fishes was estimated. Than fishes were exposed to sub lethal concentration of fly ash (200g/l, 400g/land 600g/l) for of seven days. A control group of fish with normal water was maintained. The biochemical parameters like plasma glucose, protein, triglyceride, cholesterol, creatinine and urea were estimated. There has been significant decline in plasma glucose and protein level where as cholesterol and triglyceride level increased significantly. The blood urea nitrogen and creatinine also increased significantly from the control value indicating kidney dysfunction.

Keywords: Fly ash, Biochemical, Fish

Introduction

Thermal power station uses coal as source of energy which after combustion produces fly ash. Fly ash that is produced from the power station due to burning of coal enters to the aquatic ecosystem there by effecting the flora and fauna[1]. Fly ash contains many heavy metals which inflict stress on the aquatic organism[2]. Fishes occupy an important position in aquatic food chain. Fishes are being continuously exposed to pollutants in aquatic ecosystem due to aquatic pollution which leads to physiological changes[3]. India is a major fly ash producing countries among in the world when compared to the developing countries. Major amount of fly ash is deposited on land or water. With the increasing number of thermal power station, fly ash mound keeps on increasing. Hindalco thermal power plant located near Hirakud reservoir produces fly ash which contains heavy metals like Mn,Co,Pb,Zn,Cd, Ni etc[4]. Heavy metals are the major pollutants of aquatic body and gets accumulated in fish through food chain[5]. Fly ash contains toxic heavy metals that can pollute the drinking water system[6]. Composition of fly are mostly small, glassy, hollow particles with a range of 0.01 to 100 μm and specific gravity 2.1 to 2.6 [7]. Fly ash is generally considered as aferro-aluminosilicate mineral, with Al, Si, Fe, Ca, K and Na are the dominant elements[8] whereas other elements includes As, B, Ca, Mo, S, Se and Sr are also the constituents of fly ash[9]. The pH of ashes vary from 4.5 to 12 which is depends on sulfur content of the coal. High-Sulfur containing coals normally produce acidic ashes and low-Sulfur, containing coals produces alkaline ashes[7].

The present study was aimed to know the effect of fly ash on fish physiology. To understand the stress caused due to fly ash, effects on the biochemical changes in fish *Heteropneustses fossilis* (Bloch) was carried out.

Materials and methods

Live and healthy fresh water fish *Heteropneustes fossilis* varying length 14-16cm and weighing 30-40gm were collected from the pond and acclimatized in laboratory condition for a period of one week. Care was taken to avoid stress and injury during capture and transportation. During the period of acclimation fishes were given commercial fish food. They were fed twice a day. Before the start of the experiment injured or inactive fishes were removed from the aquarium. After the determination of $L.C._{50}$ value[4], three aquariums with 80litre capacity was set with different concentration fly ash (200g/dl, 400g/dl, 600g/dl) collected from the Hindalco Power Plant located near Hirakud reservoir. One aquarium was used with normal water as control (0%). Ten number of fish irrespective sex was released to the experiment aquaria for a period of one week. After the exposure period blood sample was collected for investigation. Serum protein was analysed by electrophoresis of the "biuret" complex. The protein reacts with the copper sulfate to give that violet "buiret" colour. The light intensity of the violet colour of is proportional to the concentration of proteins [10]. Serum glucose was estimated when excess of blood is placed on the reagent bonded to the paper strip. The strip is exposed to glucose oxidase. After a specified times the colour is spectrophotometrically analysed[11]. Cholesterol in serum was determined by reacting it with sulfuric acid and acetic anhydride to produce the intense blue colour (Liberman colorimeteric method) CHOD/PAP method. Definitive methods like isotope dilution mass spectrometry define calibration point for the assays[12]. Creatinine was determined by the Jaffe reaction using

alkaline picrate and byanisotope dilution-mass spectrometry (IDMS) using Trinder's reaction acceptor[13]. Whereas serum urea (BUN) was quantified by photometry of the yellow chromogen and by colorimetric interference[14]. The estimation of triglyceride was done by electrophoresis and apolipo protein measurement and the methods described by Sullivan et al.[15]. The results obtained is presented as mean ± standard deviation. Difference between the were done by Analysis of Variance Analysis (ANOVA). Statistical analysis of data were done with the help of SPSS version 16.0.

Results and discussion

Serum analysis of fish *Heteropneustes fossilis* were done to ascertain the physiological condition of both control and exposed fish. The biochemical parameters like glucose, protein, cholesterol and triglycerides were analysed to understand the metabolism of the fish. Moreover, biochemical parameters like blood urea nitrogen (BUN) and creatinine indicate the functioning of kidney of fish under normal as well as stressful condition. All the results obtained is presented in Table-1.

Serum glucose level of control fish was found to be 90.2±3.12 mg/dl. When exposed to different concentration of fly ash, there has been a significant decrease in glucose content in all experimental fish. However, the decrease in glucose content was independent of fly ash concentration. The amount was recorded as 39.3±1.34mg/dl, 39.1±1.06mg/dl, 35.4±1.43mg/dl in 200g/l, 400g/l, and 600g/l concentration of fly ash exposed fish respectively (Figure-1). Serum protein level of control fish was found to be 8.2±0.42g/dl. When exposed to different concentration of fly ash, there has been a significant decrease in protein content in all experimental fish. However, the decrease in protein content was independent of fly ash concentration. The amount was recorded as 6.1±0.32 g/dl, 6.3±0.40g/dl, 6.4±0.38 (g/dl) in 200g/l, 400g/l, and 600g/l concentration of fly ash exposed fish respectively (Figure-2).

Unlike the sugar and protein metabolism, the fat metabolism shows a different kind of trend under stress. Both cholesterol and triglycerides showed increase in their level from the control value. The cholesterol content of serum of control fish was found to be 211.1±3.72mg/dl whereas triglyceride level was 102.2 ±2.78mg/dl. On exposure to different concentration of fly ash the cholesterol level was found to be 340.3±5.96mg/dl, 289.2±2.97mg/dl, 244.5±2.46mg/dl in 200g/l, 400g/l, and 600g/l concentration of fly ash exposed fish respectively (Figure-3). The triglyceride content of serum of increased to 110.5±3.03mg/dl in 200g/l, 109.5±1.81mg/dl in 400g/l, and 154.1±3.45mg/dl in 600g/l concentration of fly ash exposed fish (Figure-4).

Functioning of kidney is known from the concentration of blood urea nitrogen (BUN) and creatinine level of fish. These are the nitrogenous waste product which increases in case of malfunctioning of kidney. The BUN of normal fish was found to be 19.2±0.63mg/dl whereas the creatinine level was 0.86± 0.04mg/dl).On exposure to different concentration of fly ash there has been elevation in both urea and creatinine level which indicates kidney damage. The BUN increased to 88.4±2.99 (mg/dl), 66.6±1.71 (mg/dl), 41.4±0.97 in 200g/l, 400g/l and 600 g/l respectively (Figure-5). The creatinine level increased to 2.02±0.08mg/dl, 1.9±0.10mg/dl, 1.6±0.46mg/dl respectively (Figure-6).

Serum glucose level of fish exposed to fly ash for a period of one week was found to decrease from the control fish. The decrease of glucose content was independent of fly ash concentration. Carbohydrates are the main source of energy specially the nervous tissue[16]. Serum glucose level is a physiological indicator and used as bio monitoring by many authors[17]. In stressful condition serum glucose level depleted in response to energy demand of the fish[18]. Depletion in blood glucose level was reported in *Channa punctatus* under the stress of industrial effluent[19]. The decrease in the glucose level of *Heteropneustes fossilis* is also in line of the above findings. There is also report of alteration in glucose level due to hypoxic condition of fish[20].

Proteins which comprise mainly of nitrogenous constituent are the important biomolecule and play a major role in health of the fish. The quantitative assessment of proteins indicates the metabolic condition of fish under normal as well as stress condition. In this experiment the serum protein level of fish decreased significantly in all short term and long term exposed fish. As heavy metals are known to inflict depletion in protein level in fishes. Many workers have reported increased amino acid level of fish due to proteolysis as a response to stress. During toxic stress amino acid is used as an alternate mode energy utilization through TCA cycle[21]. In this case also the increased in creatinine level and decrease in protein content of serum can be correlated to stressful condition of fish. Decrease in protein may be due to tissue repair like lipoprotein of cell membrane[22]. Loss of protein may be due to defective protein synthesis or increase in degradation which was observed in fish *Heteropneustes fossilis* on exposure to Nickel[23].

Unlike the sugar and protein metabolism, the fat metabolism shows different kind of trend under stress. Both cholesterol and triglycerides of fly ash exposed fish *Heteropneustes fossilis* showed increase in their level from the control value. Lipids like triglyceride available in blood as lipoprotein. There was also a case of hypercholestermiain treated fish. Fly ash contains heavy metals like Mn,Zn,Pb,Cr,Cu,Ni,Co as its major constituent[4]. Alteration in biochemical parameters was observed in fish *Cyprinus carpio* due to chromium pollution[24].There was an increase in triglycerides in *Channa punctatus* when exposed to metal stress which may be due to high oxidative effect[25]. Increase in lipid content like triglyceride and cholesterol may be a case of lipogenesis due to environmental stress. Increase oxidative stress decreases the fatty acid oxidation in liver which in turn leads to increase in triglyceride contents in blood

[26].Many authors have reported liver damage in fishes due to environmental pollution[27].

Functioning of kidney is known from mainly the concentration of blood urea nitrogen (BUN) and creatinine level in blood of fish. These are the nitrogenous waste product which increases in case of malfunctioning of kidney. The elevation in the BUN and creatinine level in the blood indicates renal dysfunction. Urea is a by-product of amino acid deamination whereas creatinine is produced due to metabolism of muscle creatine. Both BUN and creatinine are normally excreted by kidney. Heavy metals like zinc are known to have damaged kidney of fishes leading to elevated creatinine and BUN[28]. Lead in aquatic environment is known elevate blood urea level in fish *Clarias gariepinus*[29]. In our findings, zinc is also distributed in fly ash which might be the reasons for the elevation of the above parameters. Hypertrophy of epithelial cells and glomerular necrosis due to chromium toxicity is also reported in fish *Channa punctatus*[30]. Chromium in the environment effects the kidney leading to necrosis and fibrosis of tubular lumen in fish *Onchorynchus tshawytscha*[31]. Bioaccumulation of metals like Cd, Cu, Zn, and Pb in fish has affected the kidney leading to metabolic disorders[32]. Organs like kidney accumulate heavy metals in fish *Channa punctatus* in rivulet receiving thermal plant waste water containing fly ash[33]. In this piece of work also the depletion of protein as well as increase in BUN and creatinine indicates kidney damage. This may be due to presence of above said heavy metals in the fly ash.

Table-1: Biochemical changes in fish *Heteropneustes fossilis* (Bloch) on exposure to fly ash.

Parameters	Control		200 g/l		400 g/l		600 g/l		F value	Significance Level
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Glucose (mg/dl)	90.2	3.12	39.3	1.34	39.1	1.06	35.4	1.43	1864.23	0.00-E
Protein (g/dl)	8.2	0.42	6.1	0.32	6.3	0.40	6.4	0.38	102.91	0.00-E
Triglyceride (mg/dl)	102.2	2.78	110.5	3.03	109.8	1.81	154.1	3.45	694.82	0.00-E
Cholesterol (mg/dl)	211.1	3.72	340.3	5.96	189.2	2.97	244.5	2.46	2764.01	0.00-E
Urea (mg/dl)	19.2	0.63	88.4	2.99	66.6	1.71	41.4	0.97	2739.27	0.00-E
Creatinine (mg/dl)	0.86	0.04	2.02	0.08	1.9	0.10	1.6	0.16	426.126	0.00-E

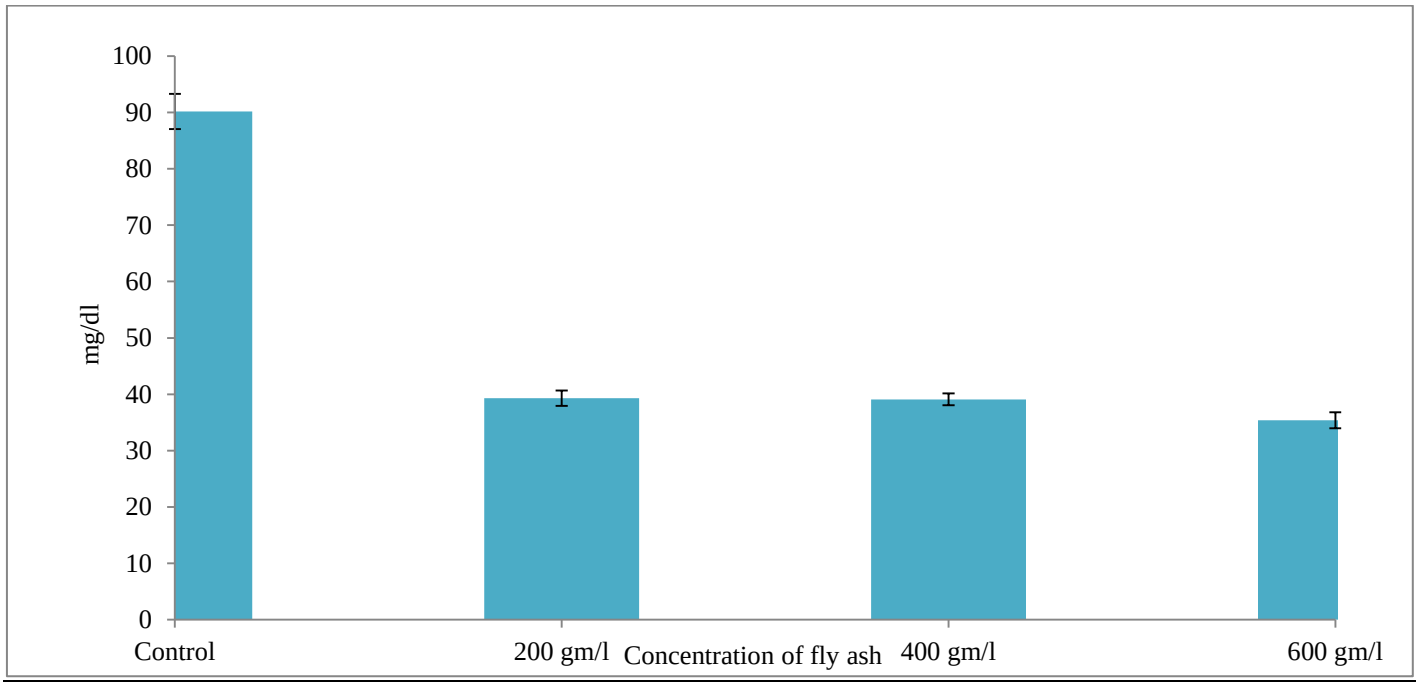

Figure-1: Changes in glucose content of fish *Heteropneustes fossilis* (Bloch) on exposure to Fly Ash.

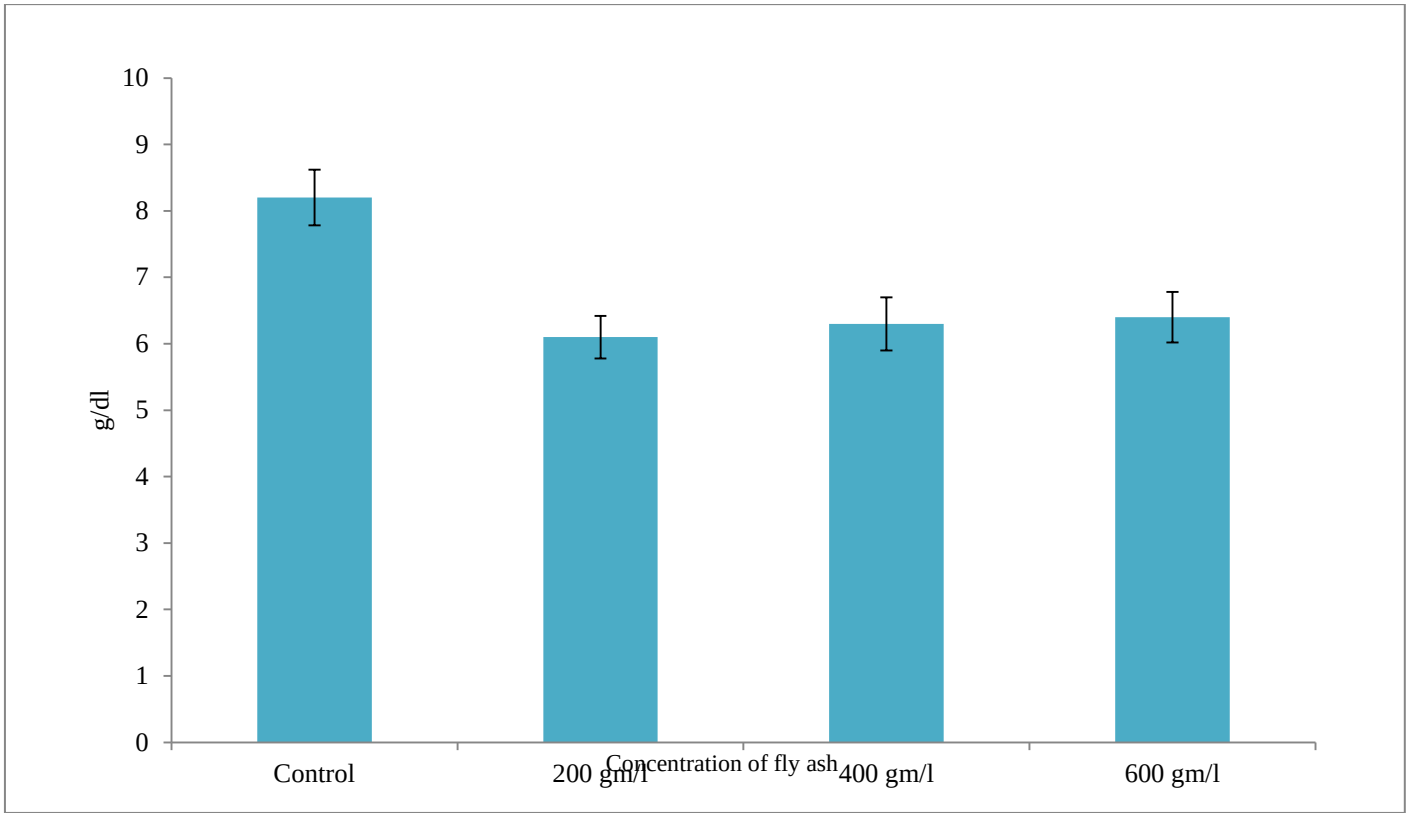

Figure-2: Changes in Protein content of fish *Heteropneustes fossilis* (Bloch) on exposure to Fly Ash.

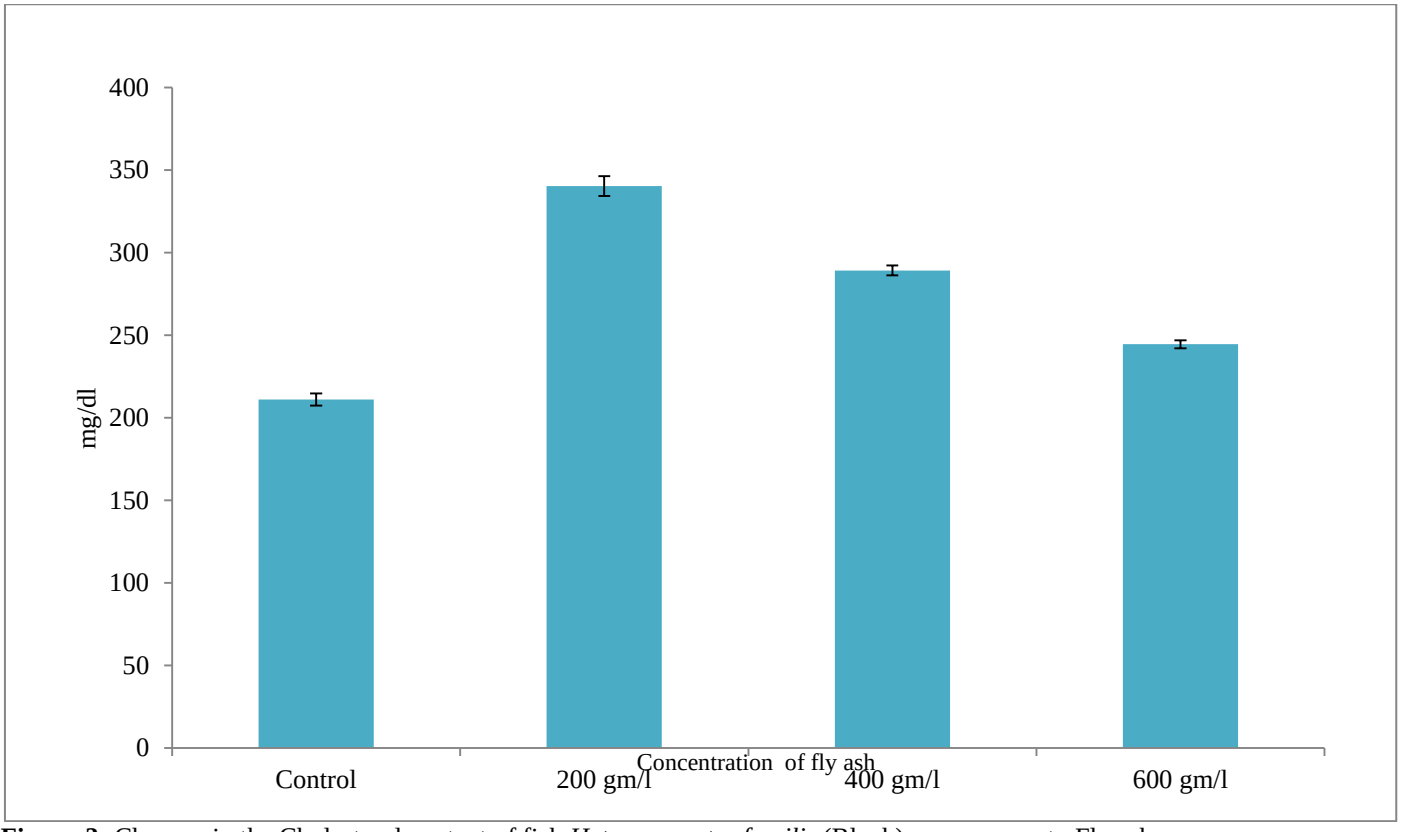

Figure-3: Changes in the Cholesterol content of fish *Heteropneustes fossilis* (Bloch) on exposure to Fly ash.

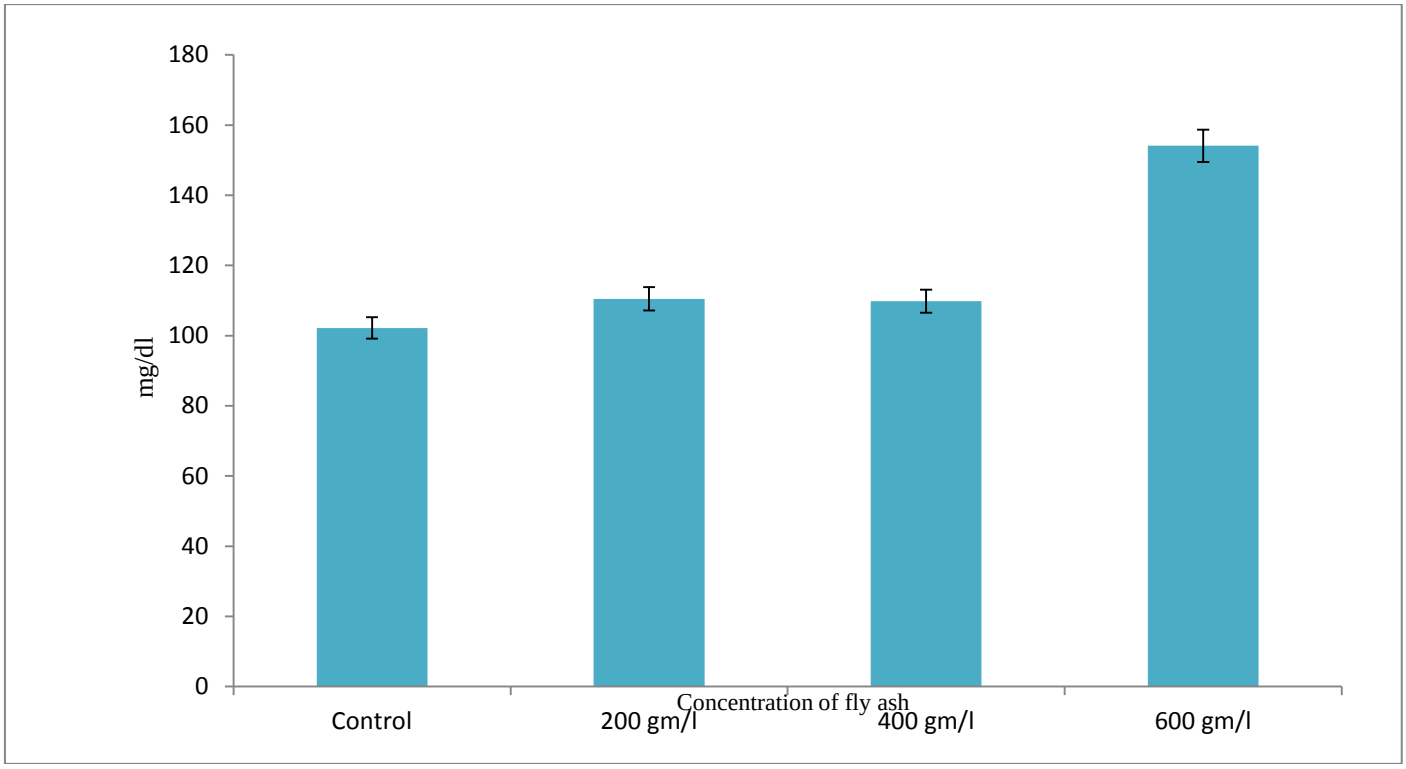

Figure-4: Changes in triglycerides (mg/dl) of fish *Heteropneustes fossilis* (Bloch) on exposure to fly ash.

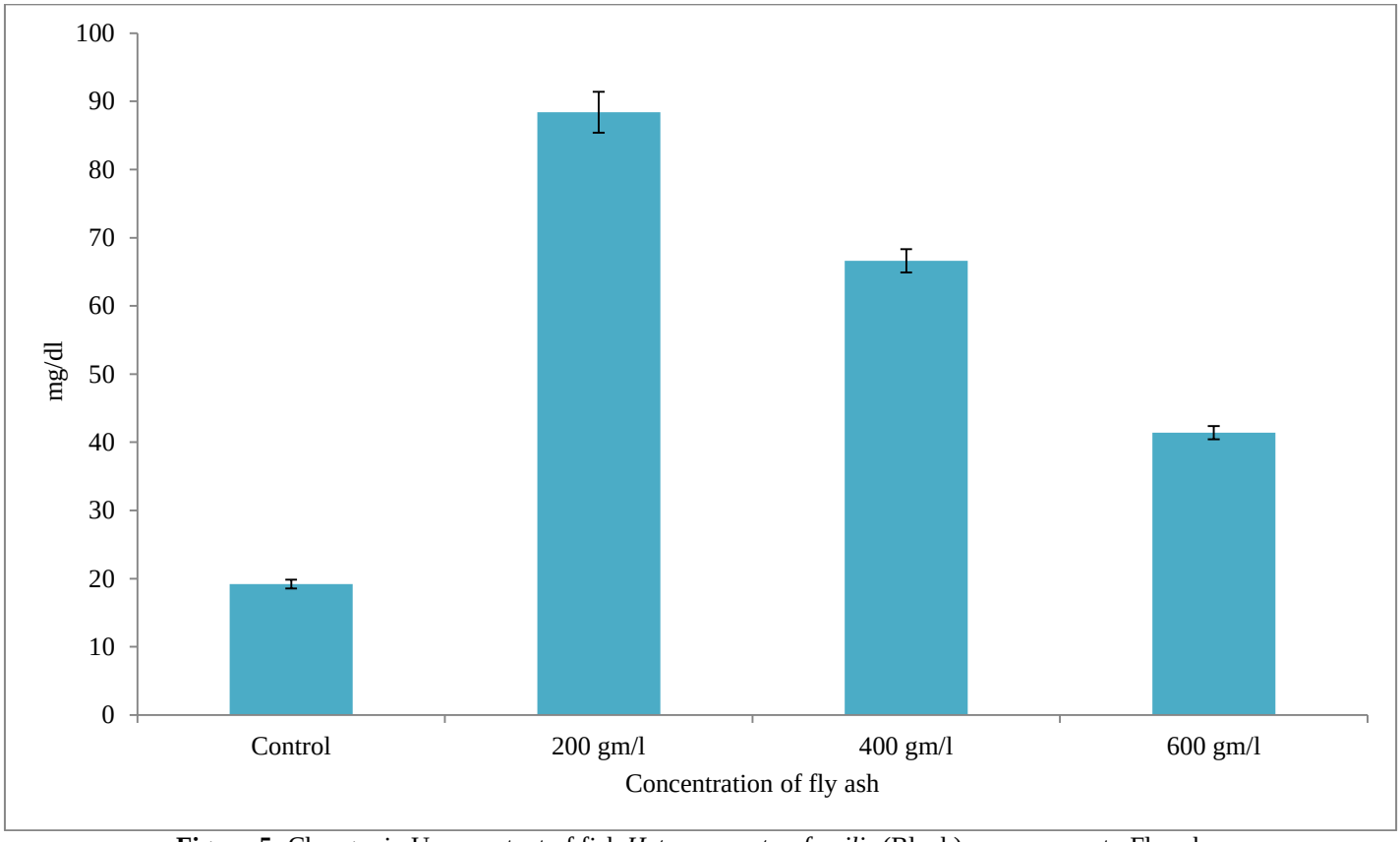

Figure-5: Changes in Urea content of fish *Heteropneustes fossilis* (Bloch) on exposure to Fly ash.

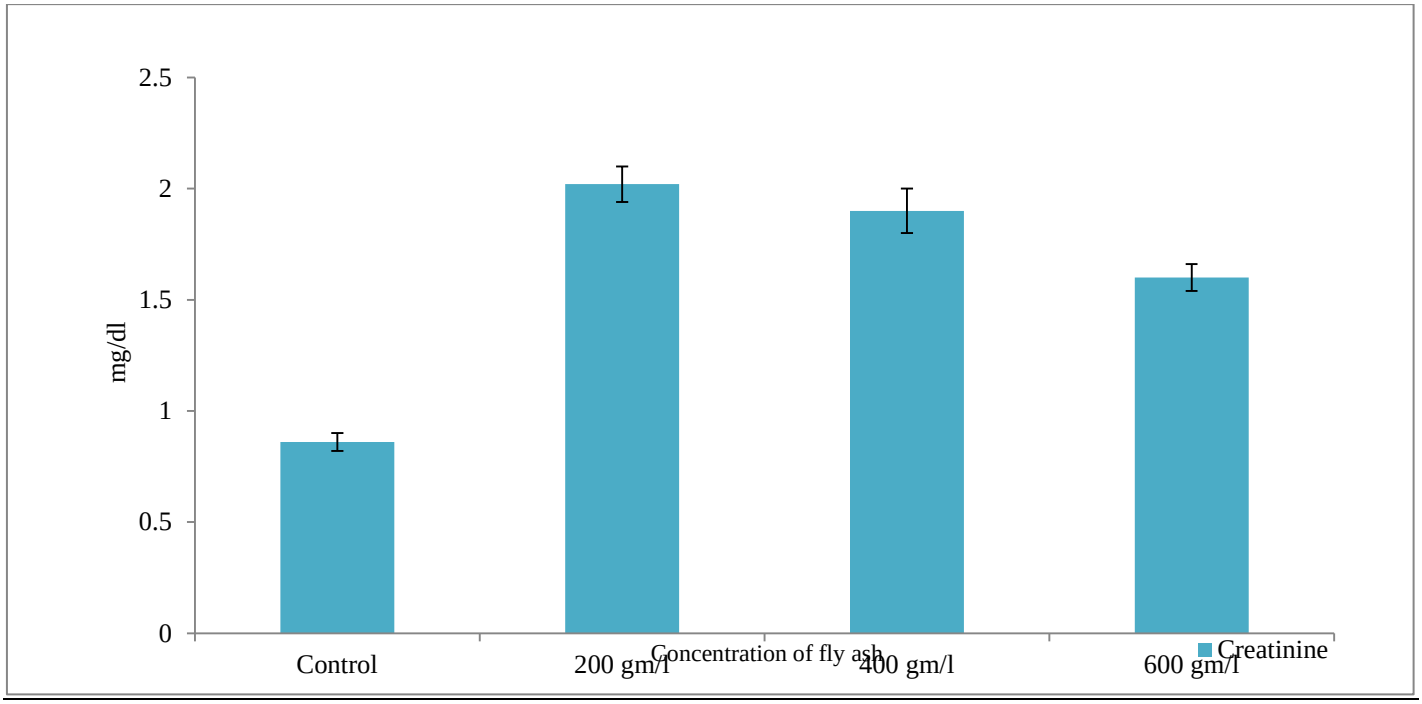

Figure-6: Changes in Creatinine (mg/dl) of fish *Heteropneustes fossilis* (Bloch) on exposure to Fly ash.

Conclusion

Study of plasma biochemistry of fish indicates the physiological condition of fish on a given environment. In this piece of work it was found that there was alteration in the biochemical parameter of blood in fish when exposed to fly ash. Results clearly indicates stress due to fly ash toxicity with impact on kidney functioning. Further research is required to know the safe value of fly ash in the environment to the fish.

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