RESEARCH ARTICLE

Thermal power station effluent induced biochemical changes in the blood of freshwater fish, *Labeo rohita*

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ABSTRACT

The hazardous effect of heavy metal pollution from the water body on serum biochemistry of the freshwater fish, *Labeo rohita* (Rohu) was studied in this investigation. The aim of this study was to evaluate whether the waste effluent from thermal power station (TPS) were pose any hazardous changes in the blood biochemistry of common carp, *Labeo rohita* (rohu). The heavy metals like As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu were observed in water body adjacent to thermal power station in varying quantities that indicates the presence of heavy metal in water body. The biochemical changes in blood serum monitored through total protein, albumen, creatinine, total cholesterol and enzyme AST and ALT tests, were showed elevation in concentration which might be due to damage of the liver, kidney, and other tissues in the state of stress caused by exposure to metals.

Keywords: Heavy metals, water pollution, TPS, blood biochemistry, rohu.

INTRODUCTION

The main purpose of any industrial development is to provide an opportunity for better living and an employment to the people residing the area. Though industrial development produces more employment it is also responsible for the degradation of the environment by introducing various pollutants into the atmosphere which produces air, water and land pollution. Hence now there is need to protect the environment from these harmful effects at any possible limits. In recent years the energy demand has been increased so rapidly which is being largely met by using fossil fuel. The increasing demand for energy is the one of the challenges that faces the development of the country (Bashar, 2010). Thermal power plants are the main source of energy production in India where the energy is produced by using coal as a fossil fuel. Coal is largely composed of organic and some inorganic components such as including trace elements which have been cited as possible cause of health and environmental effects. Due to coal combustion a significant quantity and variety of trace elements are transformed into surrounding environment by various pathways.

In natural systems even a low concentration of heavy metals and trace elements can have beneficiary or harmful effect on aquatic biota. During recent years the environment is being contaminated with wide range of pollutants that includes heavy metals, trace metals, pesticides released from various domestic, industrial and other manmade activities, which are having harmful effect on ecological balance of the recipient environment.

Heavy metal contamination has been reported in aquatic organisms (Adham *et al*., 2002 and Olojo *et al*., 2005) and trace metal contaminations are important due to their potential toxicity for the environment and human beings (Gueu *et al*., 2007; Lee *et al*., 2007; Adams *et al*., 2008; Vinodhini and Narayanan, 2008). Heavy metals includes both essential and non essential elements that have a particular significance in ecotoxicology, as they are highly persistent and all have the potential to be toxic to living organisms (Storelli *et al*., 2005).

Major pollutants released by coal based power generation include sulphur, carbon and nitrogen compounds, heavy metals and fly ash. Coal operated thermal power plant can be a source of pollution, because ash derived from burning of coal containing heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) can contaminate water, presenting a potential hazard to the environment (Kanungo and Mahapatra, 2000).

Fly ash is a fine residue resulting from the burning of coal which is discharged into the surrounding environment either by dry or wet method. Chemically fly ash consists of Si, Al, Mg, Ca, K, Ti, and Fe in greater proportion with many trace elements such as V, Mn, Cr, Cu, Ni, As, Pb, Cd, and less quantity of various potential toxic elements. Chemical composition study of fly ash shows mostly the presence of four major elements Al, Si, Fe and Ca in the fly ash. Other metals such as K, Mg, Ba, Co, Cd, Zn, Mo, Pb etc. are present in traces. Though in the traces, compared to original coal, most of the elements are enriched in the fly ash, giving birth to the growing environmental concerns in the disposal and utilization in environment due to release of trace heavy metals.

According to Gupta *et al*. (2002) and Mehra *et al*. (1998) the major part of fly ash is disposed off in unmanaged landfills or lagoons which lead to environmental pollution through fly ash erosion and leachate generation along with metal contamination of surface and ground water resources and hencecan transfer these contaminants into the food chain.

Singh *et al*. (2004), Praharaj *et al*. (2002), Suresh *et al*. (1998) and Ramachandra *et al*. (2012) studied leaching of trace elements in coal ashes from Bokaro Thermal Power Station, Kharagpur, Vijayawada Thermal Power Station (VTPS), Andhra Pradesh and Yellur and surrounding villages closer to a thermal power plant in Udupi district, Karnataka State. They reported that nearly every naturally occurring element is likely to be present in coal and these get entertained in the resultant coal ash.

Chakraborty and Mukherjee (2009) studied the bioaccumulation of heavy metals like Fe, Zn, Cu, Mo, B, Si, Al, Cr, Pb, Cd, Hg and As in aquatic, terrestrial and algal species in the vicinity of thermal power station in fly ash contaminated areas in Uttar Pradesh. Studies of trace elements and the elements presents in fly ash are distributed into traction of the fly ashes based on volatilization temperature (Bahor *et al*., 1981).

Fish are located at the end of the aquatic food chain and are the inhabitants that cannot escape from the detrimental effects of these pollutants which may accumulate metals and pass them to human beings through food, causing acute and chronic diseases (Al – Yousuf *et al*., 2000; Vosyliene and Jankaite, 2006; and Farombi *et al*., 2007).

Biochemical biomarkers like glucose, total protein, creatinin, total cholesterol and enzymes like AST and ALT are frequently used as an indicator of the general state of health and early warning of stress in fish under stressful conditions (Barnhorn and Van-Vuren 2004; Abou El-Naga *et al*. (2005) and Osman *et al*. 2010). The pollutants interferes in the metabolic pathway and affects carboxyl, amino, sulphhydryl, phosphate and other groups of the molecules which result in the damage of enzyme systems by blocking active sites, immobilization of essential metabolites, modification of membrane structure and its permeability (Martinez-Porchas *et al*., 2011).

MATERIALS AND METHODS

A. Study site:

This study was conducted at pond in the vicinity of thermal power station (TPS) located at Koradi village of Dist. Nagpur during 2010 to 2012.

B. Collection of water sample for heavy metal analysis:

The water samples were collected from the pond of TPS for the heavy metal analysis and were further processed as, 5 ml of concentrated HNO_3 was added to a 50 ml of water sample to digest all the organic matter and to get the clear solution. The digested and cleared water samples were filtered using Whatman filter paper and made upto original 50 ml volume and injected into Inductively Coupled Plasma Atomic Emission Spectrometer (ICP - AES) for metal estimation.

C. Sampling and collection of the fishes:

The fishes, *Labeo rohita* (Rohu) were sampled with fishing net with the help of fishermen. These fishes were scrutinized. Below aged and diseased fishes were discarded and released into pond, only healthy and about 2 year old fished were kept for experimentation in container filled with pond water.

D. Collection of blood from fishes:

The blood sample was collected by puncturing the caudal fin with the help of syringe and needle.

E. Biochemical assays:

The biochemical tests for total protein, total cholesterol, creatinine, albumin and enzyme assays viz. AST and ALT were carried out by using standard techniques.

Each biochemical parameter was assessed in triplicate in both control and experimental fish. The blood samples from both control and experimental fish were centrifuged to separate the serum sample and the separated serum samples were then processed for biochemical and enzyme assays like liver and kidney function tests. For biomolecules, test like total proteins, total cholesterol, albumin and creatinine were carried out by using standard method for respective blood parameters.

i. **SGPT:** The activity of enzyme, Serum Glutamate Pyruvic Transaminase (SGPT) was observed by 2-4 DNPH method (Reitman and Frankle, 1957),
ii. **SGOT:** The activity of enzyme, Serum Glutamate Oxaloacetic Transaminase (SGOT) was observed by 2-4 DNPH method (Reitman and Frankle, 1957),
iii. **Total Protein:** Total Protein was estimated by Biuret method (Rosenthal *et al.,* 1956),
iv. **Albumin:** Albumin was estimated by Bromocresol green method (Spencer and Prince, 1977),

v. **Creatinine:** Creatinine was estimated by Alkaline picrate method (Rock *et al.,*1987),

vi. **Total Cholesterol:** Total Cholesterol was estimated by CHOD-PAP method (Allain *et al.,* 1974).

Statistical Analysis

All the results were subjected to statistical analysis to evaluate the authenticity of the results.

RESULTS

I. Heavy Metals in Pond Water

The concentration of heavy metals in pond water from TPS was shown in Table 01 shows presence of As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu in varying quantities. From the above data it is clear that the pond water is contaminated with heavy metals in different concentrations.

II. Biochemical Assays

During the present study various biochemical assays were performed to know the status of fish from the pond in the vicinity of TPS, where TPS effluent gets released. The findings of biochemical assays are indicated in the Table 02.

In this study significant differences were observed in biochemical parameters like total protein, total cholesterol, albumin, creatinine and serum enzyme (SGPT, SGOT) between control and exposed group except in globulin value which was found to be insignificant throughout the study period.

i. Serum SGPT and SGOT

The ALT and AST activity increased in TPS effluent exposed fish when compared with the control fish. The highest elevation in ALT activity was measured during May-Aug 2012 (90.84 ± 5.908 IU/L) and least during Jan. - Apr. 2011 (16.98 ± 2.661 IU/L), though the highest AST activity was measured in Sept. - Dec. 2010 (421.388 ± 23.174 IU/L) and least during May-Aug 2012 (174.842 ± 12.432 IU/L) in TPS effluent exposed fish.

ii. Total Protein

In the present study, total protein concentration in the serum of TPS effluent exposed fish was found to be increased as compared to control fish blood sample. It was observed to be higher during Sept. - Dec. 2011 (2.959 ± 0.175 g/dl) and lower in May – Aug. 2012 (2.250 ± 0.172 g/dl).

iii. Albumin

In the present study, it was observed that the serum albumin accounts more in the serum of exposed fish than the control. The elevation in serum albumin was observed in Sep. – Dec. 2011 (2.668 ± 0.301 g/dl) while it showed decline in Jan. – Apr. 2011 (1.950 ± 0.186 g/dl).

iv. Globulin

In the present study, the globulin concentration in the serum shows same trend as that of protein and albumin. The globulin concentration in the serum of effluent exposed fish was found to be highest during Jan. – Apr. 2011 (0.751 ± 0.304 g/dl) while it lowest during Sep. – Dec. 2010 (0.345 ± 0.166 g/dl).

v. Sreum Creatinine

In the present study, TPS effluent exposed fish showed increased serum creatinine than the control value. More serum creatinine was found in May – Aug. 2012 (0.785 ± 0.109 g/dl) and less in Jan. – Apr. 2011 (0.225 ± 0.050 g/dl).

vi. Total Cholesterol

In the present study, cholesterol level in the serum of TPS effluent exposed fish was generally found to be elevated as compared to that of the control value. Highest increase was observed during May-Aug 2011 (182.71 ± 4.612 mg/dl) while lowest values were observed during Jan. – Apr. 2012 (133.623 ± 38.813mg/dl).

Table 1: Shows concentration of heavy metals (in ppm) in water of TPS pond during the year September 2010 to August 2012. (all values are expressed in mean ± S.D.)

Metals	Sept.- Dec. 2010	Jan.-Apr. 2011	May-Aug. 2011	Sept.-Dec. 2011	Jan.- Apr. 2012	May - Aug. 2012
Al	0.42 ±0.042	0.357±0.102	0.364±0.125	0.346±0.059	0.309±0.039	0.304±0.014
As	0.215±0.021	0.166±0.017	0.171±0.042	0.185±0.017	0.146±0.040	0.125±0.022
Cd	0.096±0.010	0.238±0.052	0.095±0.006	0.085±0.013	0.090±0.009	0.089±0.008
Co	0.250±0.094	0.287±0.224	0.168±0.043	0.247±0.050	0.228±0.069	0.292±0.012
Cr	0.815±0.147	0.630±0.268	0.553±0.170	0.856±0.058	0.773±0.086	0.937±0.063
Cu	0.815±0.093	0.701±0.168	0.731±0.129	0.874±0.036	0.751±0.198	0.925±0.043
Fe	3.247±0.500	3.067±0.107	4.891±0.782	7.522±1.217	7.921±1.341	7.422±1.692
Mn	0.927±0.128	1.070±0.096	0.840±0.053	0.953±0.108	0.775±0.106	0.677±0.125
Ni	0.923±0.045	0.844±0.048	0.771±0.092	0.931±0.053	0.737±0.101	0.822±0.104
Pb	0.090±0.002	0.077±0.009	0.085±0.006	0.086±0.016	0.084±0.015	0.091±0.006
Zn	3.317±0.584	2.795±0.453	2.764±0.411	4.261±0.694	4.761±0.364	5.017±0.825

Table 2: Shows biochemical changes in serum of freshwater fish, *L. rohita* exposed to TPS effluent. (Values are expressed in Mean ± SD)

		SGPT	SGOT	TP	Albumin	Globulin	Creatinine	Cholesterol
Sept-Dec 2010	**C**	21.713 ± 1.216	336.466 ± 31.628	1.678 ± 0.678	1.211 ± 0.220	0.298 ± 0.146	0.193 ± 0.078	144.35 ± 10.453
	E	27.957 * ± 5.799	421.388 * ± 23.174	2.298 * ± 0.239	1.952 ± 0.238	0.345≠ ± 0.166	0.246≠ ± 0.058	150.565 ≠ ± 16.623
Jan-Apr 2011	**C**	15.4 ± 2.544	180.255 ± 12.264	1.558 ± 0.213	0.908 ± 0.059	0.65 ± 0.210	0.136 ± 0.027	132.16 ± 18.341
	E	16.98 * ± 2.661	231.87 * ± 21.187	2.701 * ± 0.171	1.950 ± 0.186	0.751 * ± 0.304	0.225 ± 0.050	142.226 ≠ ± 27.326
May-Aug 2011	**C**	22.915 ± 3.013	241.407 ± 16.75	2.15 ± 0.148	1.733 ± 0.221	0.228 ± 0.0624	0.208 ± 0.064	117.425 ± 8.602
	E	31.07 * ± 4.352	298.215 * ± 12.029	2.715 * ± 0.155	2.032 ± 0.114	0.633 * ± 0.253	0.42 ± 0.093	182.71 * ± 4.612
Sept-Dec 2011	**C**	25.018 ± 4.141	280.933 ± 13.835	4.365 ± 0.650	1.81 ± 0.176	0.34 ± 0.115	0.237 ± 0.0585	151.335 ± 11.551
	E	28.231 ≠ ± 1.728	331.065 * ± 9.431	2.959 * ± 0.175	2.668 ± 0.301	0.415 * ± 0.250	0.357 ≠ ± 0.046	173.458 * ± 13.876
Jan-Apr 2012	**C**	33.34 ± 5.601	183.757 ± 12.307	1.96 ± 0.196	1.65 ± 0.147	0.22 ± 0.077	0.191 ± 0.033	121.991 ± 9.239
	E	68.823 * ± 3.342	233.153 * ± 51.939	2.282 * ± 0.179	2.046 ± 0392	0.456 * ± 0.259	0.398 ± 0.084	133.623 ≠ ± 38.813
May-Aug 2012	**C**	34.068 ± 5.891	162.25 ± 7.404	1.9 ± 0.130	1.673 ± 0.217	0.31 ± 0.098	0.508 ± 0.179	136.971 ± 6.550
	E	90.84 * ± 5.908	174.842 * ± 12.423	2.250 * ± 0.172	2.623 ± 0.134	0.373 * ± 0.213	0.785 ≠ ± 0.109	164.476 * ± 23.435

SGPT = IU/L, SGOT= IU/L, Creatinine=mg/dl, TP (Total Protein)=g/dL, Albumin=g/dL, Globulin=g/dL, Cholesterol=mg/dl. C-Control, E-Experimental. Values represented by (*) significantly differ from control ($p<0.05$) and (≠) are non significant when compared with control.

DISCUSSION

The presence of heavy metals in any water body is important for carrying out various metabolic activities of living organisms but once they reach their maximum level, they serve as environmental pollutants that produce hazardous effects on the aquatic organisms.

In the present study, the water indicates presence of As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu in varying quantities when analyzed through ICP-AES. The heavy metal concentration values in water samples from TPS pond were found within permissible limit as per guidelines of APHA (2005) and USEPA (1986). Though the heavy metals concentrations were within permissible range they may cause threat to aquatic life in long run.

The present findings for heavy metal concentrations in water is supported by the observations of Thorat and Charde (2013) who had studied the physico-chemical properties of Kanhan river water receiving fly ash disposed waste water of Khaperkheda Thermal Power Station, Nagpur and had reported the concentrations of copper, cadmium, zinc, lead, mercury and arsenic metals were within normal range. Similar results were also reported by Junshum *et al.* (2007) when they studied water quality assessment in reservoirs and wastewater treatment system of the Mae Moh power plant, Thailand where the heavy metal concentration did not exceed both the surface water quality standards and the industrial effluent standards of Thailand.

The presence of pollutants in aquatic environment exerts its effect at cellular or molecular level which results in a significant change in biochemical responses and for monitoring of aquatic environment analysis of biochemical methods offer as an important biomarkers (Authman *et al.*, 2013b). So, analyses of serum constituents have been proved to be useful in the detection and diagnosis of metabolic disturbance and disease processes (Elghobashy *et al.*, 2001). Heavy metals are some of the most-active polluting substances as they can cause serious impairment to circulatory, metabolic, physiological, and even structural systems when high concentrations are present in aquatic ecosystems (Yang and Chen, 2003).

The kidney is one of the major target organs for environmental contaminants such as heavy metals, and they are important organs for metabolic waste excretion and heavy metal elimination in fish (Yang and Chen, 2003). Kidney function tests such as serum creatinine, uric acid and urea can be used as a rough index of the glomerular filtration rate where low values of creatinine, uric acid and urea have no significance but increasing values indicate the presence of disturbances in the kidney (Elghobashy *et al.*, 2001).

Serum enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) are considered to be important serum markers to investigate the health of animal species in concern. In addition, two major aminotransferases, AST and ALT, are the most significant enzymes involved in protein and amino acid metabolism (Folmar, 1993; Zikic *et al.*, 2001). Likewise, the other serum biomarkers such as glucose, triglyceride, total protein, and urea are commonly used to detect health of animals. Therefore, it was emphasized that measurement of serum biochemical parameters can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicants (Zikic *et al.*, 2001).During the present study, ALT and AST activity increased in TPS effluent exposed fish as compared with the control.

Transaminases like AST and ALT has a significant role in protein and amino acid metabolism and they may release into the blood following tissue damage and dysfunction. Singh and Reddy (1990), demonstrated that 0.25 mg Cu/L caused significant increases in serum AST and ALT activities in *Heteropneustes fossilis* with increases in exposure period. According to Oluah (1999) AST activity in *Clarias albopunctatus* increased significantly when exposed to 0.15 mg/L Zn and Hg for 21 days. Also Zikic *et al.* (2001) showed that plasma AST and ALT activities increased in Cd-exposed fish *Carassius auratus gibelio*. From the current study it was indicated that release of these transaminases into the blood circulation might occur due to damage of the liver, kidney, and other tissues in the state of stress caused by exposure to metals. It was suggested that serum enzymes such as ALP, AST, and ALT could be used as sensitive biomarkers in ecotoxicology to provide an early warning of potentially hazardous alterations in contaminated aquatic organisms (Levesque *et al.*, 2002; Vaglio and Landriscina, 1999, De La Torre *et al.*, 2000).

Cholesterol concentrations in the serum of TPS effluent exposed fish generally increased as compared to control. The present data is supported by other researchers showing increased serum cholesterol concentrations in metal-exposed fishes (Yang and Chen, 2003; Singh and Reddy, 1990; Canli, 1995). The cholesterol is an essential structural component of membranes and the precursor of all steroid hormones, its concentration may increase due to the liver and kidney failure causing the release of cholesterol into the blood. Exposure of fish to TPS effluent seems to elevate the level of serum cholesterol probably due to stress they caused by the toxicants. Heavy metals are known to have hazardous effects on cell structure, especially on the membranes. Therefore, increase in cholesterol may be a better indication of environmental stress.

In the present investigation, *Labeo rohita* collected from TPS pond showed an increase in serum creatinine. This may be attributed to the action of heavy metals and other pollutants on the glomerular filtration rate which causes pathological changes of the kidney (Oikari and Soivio, 1977). These recorded results are in agreement with that of Elghobashy *et al.* (2001) who observed increase in kidney functions in *Oreochromis niloticus* collected from the River Nile and some Egyptian lakes due to heavy metal pollution. Yang and Chen (2003) found significantly higher concentrations of creatinine in serum of intoxicated carp after 28 days of gallium (intermetallic elements) exposure. Zaki *et al.* (2009) observed a pronounced elevation of creatinine level in grey mullet after three weeks of exposure to 0.25 ppm of cadmium chloride and attributed it to kidney injury.

Hadi *et al.* (2009) reported that, creatinine and uric acid are biomarkers for muscle and purine metabolism, liver damage and kidney function. The rise in the creatinine of *Labeo rohita* collected from TPS pond may be attributed to heavy metals and other pollutants which affect the muscle metabolism. Hadi *et al.* (2009) reported that the increase of creatinine level might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism.

Serum albumin and globulin have been used as indicators of healthy status of the fish and considered as important indicators for the effect of pollutants in fish (Tayel *et al.*, 2007 and Mohammad *et al.*, 2013). Protein is also one of the important biochemical parameters which have been used to understand the general state of health and biological mechanism of metabolism under stress. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. So, due to this, proteins in liver degrade and the serum protein level increase.

The toxicity of these elements is due to their ability to cause, oxidative damage to living tissues. Toxic heavy metal can cause dermatological diseases, skin cancer and internal cancers (liver, kidney, lung and bladder), cardiovascular disease, diabetes, and anaemia, as well as reproductive, developmental, immunological and neurological affects in the human body. Prolonged exposure to water pollutants even in very low concentrations have been reported to induce morphological, histological and biochemical alterations in the tissues which may critically influence fish quality.

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