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ORIGINAL ARTICLE

Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish *Channa punctatus* inhabiting river polluted by Thermal Power Plant effluent



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Abstract Qualitative and quantitative assessment of heavy metals in the Thermal Power Plant effluent was performed to study the impact of their toxic effects on various biomarkers (carbohydrate, protein and lipid profiles). Heavy metals present in the water were in the order Fe > Cu > Zn > Mn > Ni > Co > Cr. Fe and Ni exceeded and Cr was equal to the USA standards set by UNEPGEMS. Glycogen in liver ($p < 0.001$) and muscle ($p < 0.01$) depleted significantly. Insignificant ($p < 0.05$) decline in blood glucose (−21.0%) and significant ($p < 0.05$) elevation in both total protein and globulin in serum, liver and muscle was noted. Albumin decreased significantly ($p < 0.01$) in serum but showed significant ($p < 0.05$) increase in liver and muscle. Thus A:G ratio fell in serum and rose in liver and muscle. Similarly lipid profile also gets altered where significant elevation in serum total lipid ($p < 0.01$), total cholesterol ($p < 0.01$), phospholipid ($p < 0.05$), triglycerides ($p < 0.001$), LDL ($p < 0.01$) was observed but significant ($p < 0.05$) decline in VLDL was recorded. These biomarkers suggested that fish become hypoglycemic, hyperlipidemic and hypercholesterolemic. Heavy metals also provoked immune response as evident from the rise in globulin. In conclusion the Thermal Power Plant wastewater containing heavy metals induced stress, making fish weak and vulnerable to diseases.

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1. Introduction

Rapid industrialization results in the production of large amounts of waste which is usually discharged into the nearby water bodies (Javed and Javed, 2013). Despite appropriate legislation the contamination of the aquatic ecosystem is on rise consequently the inhabiting flora and fauna will become the victim of anthropogenic pollution. The river at Kasimpur (27.218°N and 79.378°E), district Aligarh, India is a good example of such

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a situation. It is located in the vicinity of Harduaganj Thermal Power Station (HTPS) which has a total capacity of power generation of 700 MW and uses sulfur rich bituminous coal as fuel at the rate of 11,65,069 tonnes/annum and spills out great amount of wastewater which is characterized by high metal contents and ash (Javed and Usmani, 2012b). This wastewater is discharged into the river via iron pipes. Fish *Channa punctatus* is used as bioindicator of the river since it is the prevalent species and also serves as delicacy for locals. Heavy metals accumulate in tissues of the fish and in turn affects its physiology (Sia Su et al., 2013). Several studies have reported alterations in blood glucose and tissue glycogen levels, protein and lipid profile of fishes (Palanisamy et al., 2011; Yousafzai and Shakoori, 2011; Yacoub and Gad, 2012; Cogun and Şahin, 2013; Javed and Usmani, 2011, 2013c). Since these act as good biomarkers of fish health therefore their assessment is necessary in order to check the influence of heavy metal under field conditions. Xenobiotics (heavy metals) are known to induce oxidative stress and/or carcinogenesis/mutagenesis by mediating free radicals/reactive oxygen species (Tabrez and Ahmad, 2011a,b; Siddiqui et al., 2011). Few studies have been conducted, reporting bioaccumulation and concluding that toxic effects of heavy metals occur when accumulation was beyond the limits (Javed and Usmani, 2013c; Zutshi et al., 2010). The aim of the present study was qualitative and quantitative assessment of heavy metals in the effluent and their effect on the biomolecules like carbohydrate, protein and lipid profile of different tissues of fish *C. punctatus*. Since alteration in metabolism points out the fact that bioaccumulation occurred beyond limits.

2. Material and methods

2.1. Sample collection

Water was collected in pre-cleaned and acidified bottles and preserved by acidifying with 6 N HNO₃ for the estimation of heavy metals.

Dissolved oxygen (D.O), total solids (T.S), total dissolved solids (T.D.S) and total suspended solids (T.S.S) were determined using standard methods (APHA, 2005). The temperature and pH were recorded on the spot using thermometer (Deluxe, 6) and pH indicators (S.D. Fine Chemicals).

Live samples of *C. punctatus* ($n = 26$) were captured, sacrificed and then utilized for the analysis of blood glucose, tissue glycogen, protein and lipid profile. Reference *C. punctatus* ($n = 10$) was also collected from nearby water body which was utilized for culture practices.

2.2. Estimation of heavy metals in water samples

Heavy metals (Cr, Mn, Fe, Co, Ni, Cu and Zn) were estimated in river water using an Atomic Absorption Spectrophotometer (Perkin Elmer, Analyst A 800) as per the standard protocols of APHA (2005).

2.3. Biochemical assays

2.3.1. Blood glucose estimation

Prior to sacrifice of fishes blood was collected through cardiac puncture and was placed into the vials. Blood samples taken were centrifuged at 3500 rpm for 10 min to obtain serum.

The glucose levels in the serum were analyzed using the diagnostic kit Eco-Pak glucose (Accurex Biomedical Pvt. Ltd., India). The glucose levels in samples were measured spectrophotometrically (UV-VIS Systronics, 118) against blank at 505 nm.

2.3.2. Liver and muscle glycogen estimation

Fishes were dissected to remove the liver and muscle tissue to estimate the glycogen level. The glycogen levels in liver and muscle were measured by Anthrone reagent according to the protocols of Carrol et al. (1956).

2.3.3. Protein profile of serum, liver and muscle

Total protein was determined according to the protocols of Bradford (1976) as modified by Spector (1978), taking BSA as a standard. Albumin was quantitated using the diagnostic kit (Siemens Ltd., Gujarat, India). The intensity of color developed was measured by a spectrophotometer (UV-VIS Systronics, 118) at 595 and 628 nm for total protein and albumin, respectively. Globulin was calculated after subtracting the albumin content from the total protein. Albumin to Globulin (A:G) ratio was also calculated.

2.3.4. Serum lipid profile

Serum total lipid was quantitated using the diagnostic BQ Kit (3830 Valley Centre Dr., San Diego, CA). Total cholesterol was estimated using the cholesterol dynamic extended stability (DES) diagnostic kit (Transasia Bio-Medicals, India) and HDL cholesterol using kit HDL-C (Siemens Ltd., Gujarat, India). Triglyceride was determined by using the diagnostic kit (Siemens Ltd., Gujarat, India). All these parameters were determined by a semiautomatic analyzer (Erba Chem-5 Plus V₂). Phospholipids were calculated by the method of Covaci et al. (2006).

$$\text{Phospholipid} = \text{Cholesterol} \times 0.73 + 90$$

According to Friedewald's equation (1972) serum VLDL and LDL were calculated by the formula:

$$\text{VLDL} = \text{Triglycerides}/5$$

$$\text{LDL} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL}$$

2.4. Statistical analysis

All values are given as mean \pm SEM. Statistical differences among the means of reference and exposed were determined using Student's *t*-test.

3. Results and discussion

3.1. Physicochemical parameters and heavy metals in canal water

Physicochemical characteristics and heavy metal load of river water is given in Table 1. The estimated heavy metals were recorded in the order Fe > Cu > Zn > Mn > Ni > Co > Cr. Physicochemical analysis of water has long been employed to assess its quality. Temperature, pH, DO, TSS and TDS are often used as measures of water quality because any change in them reflects the pollution status of natural

Table 1 Physicochemical characteristics and total heavy metal load of canal water.

Physicochemical characteristics	Present study	WHO (guidelines) (mg L ⁻¹)	USA (standards) (mg L ⁻¹)
Temperature	27.6 ± 0.36°C	–	–
pH	6.9 ± 0.29	–	–
D.O	6.9 ± 0.0 mg L ⁻¹	–	–
T.S	652.0 ± 0.70 mg L ⁻¹	–	–
T.D.S	407.0 ± 0.06 mg L ⁻¹	–	–
T.S.S	245.0 ± 0.50 mg L ⁻¹	–	–
Cu	0.86 ± 0.007 mg L ⁻¹	2	1.3
Ni	0.12 ± 0.014 mg L ⁻¹	0.02	–
Fe	8.71 ± 0.84 mg L ⁻¹	–	0.3
Co	0.11 ± 0.014 mg L ⁻¹	–	–
Mn	0.21 ± 0.071 mg L ⁻¹	0.5	0.05
Cr	0.10 ± 0.014 mg L ⁻¹	0.05	0.1
Zn	0.3 ± 0.021 mg L ⁻¹	3	5

All values are given as mean ± SEM.

Values of heavy metal content in the present study are given as mean of triplicates ($n = 4 \times 3$), samples collected from 4 different zones of canal. Standard guidelines adapted for Water Quality for Ecosystem and Human Health, 2006 (prepared and published by the United Nations Environment Programme Global Environment Monitoring System (GEMS)/Water Programme).

Blank cells indicate that no, citable information was available.

waters. They were suitable for sustenance of fishes however Fe and Ni content exceeded the recommended guidelines set by the United Nations Environment Programme Global Environment Monitoring System (UNEPGEMS, 2006). Fe concentration was much high as compared to other metals which may be due to its high solubility in water and also the pipes used for discharge of effluents are made of Fe. Ni was also beyond the limits which may be due to its use in nickel plating on the machines, metal pipes etc. From other regions of Aligarh as well heavy metals are reported in water bodies beyond maximum permissible limits. Javed and Usmani (2012a, 2013a) reported that the water of sewage fed aquaculture pond at Panethi, Aligarh which is used as a source of commercial food fish also contains heavy metals in the order Fe > Mn > Zn > Co > Ni > Cu = Cr. In another study the sugar mill effluent dominated the river at village Satha, Aligarh also reported to contain heavy metals in the order Ni > Cr > Cu > Co (Javed and Usmani, 2013b). Tabrez and Ahmad (2009) reported that several folds higher values of these metals in the wastewater of lock factories, Aligarh and they were in the order Cu > Zn > Fe > Cr > Ni > Mn. River Manyame in Zimbabwe is subjected to large amounts of mining, metallurgical, agricultural runoff, and manufacturing industrial wastewaters which were reported to contain heavy metals in the order Zn > Fe > Cu > Cr > Ni (Nhiwatiwa et al., 2011). But the metal content in these waters are lower than the present study river water except in the wastewater of lock factories and River Manyame. In the present study possible reason for high levels of heavy metals could be attributed to the fact that this power station may not have efficient wastewater treatment plant due to which these metals are present beyond the maximum permissible limits. Although these metals are essential in traces but their excess becomes toxic and gets accumulated in tissues of fish *C. punctatus* due to which fish comes under stress.

3.2. Effect of heavy metals on glucose/glycogen of *C. punctatus*

Glucose and glycogen levels of tissues of *C. punctatus* are shown in Table 2. In the present study non-significant fall

(21.0%) was depicted in the blood glucose levels of the fish. Similarly glycogen reserves of the liver depleted significantly ($p < 0.001$). Moreover muscle glycogen level also declined significantly ($p < 0.01$) when compared to the reference. Other workers also observed that serum glucose first increased and then decreased upon chronic exposure until depleted (David et al., 2005; Zutshi et al., 2010). However in *Mastacembelus armatus* collected from same site elevation in serum glucose was observed, may be it was under acute stress (Javed and Usmani, 2013c). Depletion of the glycogen content in the liver and muscle was also observed by other workers in fish *Mystus cavasius* exposed to electroplating industrial effluent (Palanisamy et al., 2011), *C. punctatus* exposed to distillery effluent (Maruthi and Subba Rao, 2000). Srivastava and Srivastava (2008) reported that glycogen reserves consistently decreased from 8.18 to 5.3 mg g⁻¹ in *C. punctatus* when exposed to sublethal concentrations of ZnSO₄. Since carbohydrates serve as the instant energy source during stress so during acute condition blood glucose level increases due to glycogenolysis but reduction can be correlated to utilization of stored glycogen to meet the energy demand or chronic exposure. In liver, glycogen mobilized to glucose whereas in muscle glycogen/glucose served as readily available source of energy, thus hypoglycemia was observed.

3.3. Effect of heavy metals on protein profile of *C. punctatus*

Impact of heavy metals has also been observed on the protein profile of different tissues of *C. punctatus* and is shown in Table 3. Within the body albumin and globulin makes most of the proteins and alteration in the quantities of these proteins occur due to which the A:G ratio gets disturbed when intoxicated with xenobiotics/heavy metals. In the present study significant increase was observed in the serum total protein. Albumin level fall significantly ($p < 0.01$) and globulin showed significant ($p < 0.05$) rise over reference. Thus A:G ratio decline significantly ($p < 0.01$) as compared to the reference. Gopal et al. (1997) observed increase in serum total protein and globulin but decline in albumin in *Cyprinus carpio* when intoxicated with Cu and Ni. Increase in total protein, albumin

Table 2 Impact of Heavy metal concentrations on glucose and glycogen content of *C. punctatus*.

Tissues	Reference Glucose/Glycogen content	Exposed	Percent change over control (%)
Blood (glucose)	1.71 ± 0.02	1.34 ± 0.10	-21.0
Liver (glycogen)	3.90 ± 0.02	1.52 ± 0.01 ^{***}	-61.0
Muscle (glycogen)	1.19 ± 0.01	0.63 ± 0.02 ^{**}	-47.0

Values are mean ± S.D ($n = 8$).

Blood glucose is given in mg %.

Glycogen values are given in mg/g.

Student's *t*-test was used to test the significance.

+/- indicates increase or decrease over reference.

^{***} Significant at $p < 0.001$.

^{**} Significant at $p < 0.01$.

Table 3 Protein profile of reference and exposed *C. punctatus*.

Tissues	Total protein	Albumin	Globulin	A:G
Reference serum (mg/ml)	1.58 ± 0.005	1.12 ± 0.01	0.464 ± 0.004	2.43 ± 0.001
Exposed serum (mg/ml)	1.62 ± 0.00 [*]	0.175 ± 0.001 ^{**}	1.443 ± 0.03 [*]	0.121 ± 0.003 ^{**}
Reference liver (mg/g wet weight)	1.17 ± 0.03	0.313 ± 0.003	0.86 ± 0.033	0.36 ± 0.016
Exposed liver (mg/g wet weight)	13.04 ± 0.66 [*]	6.9 ± 0.10 ^{**}	6.14 ± 0.56	1.13 ± 0.085
Reference muscle (mg/g wet weight)	0.93 ± 0.07	0.172 ± 0.001	0.76 ± 0.07	0.24 ± 0.005
Exposed muscle (mg/g wet weight)	14.35 ± 0.35 [*]	3.35 ± 0.05 ^{**}	11.00 ± 0.30 [*]	1.69 ± 1.39

All values are expressed as mean ± SEM, ($n = 7$).

^{**} $p < 0.01$.

^{*} $p < 0.05$.

and globulin was observed in liver and muscle in the present study. Corroborating results of total protein in the muscle was also reported in *M. cavasius* (Palanisamy et al., 2011). In the muscle of *Oreochromis niloticus* the increase in total protein was reported (El-Serafy et al., 2013). Other workers however reported fall in these parameters in fish *Catla catla* (Sobha et al., 2007) and *O. niloticus* (Yacoub and Gad, 2012). A:G ratio is an index used to track changes in the composition of serum or plasma (Mazeoud et al., 1977) and its normal value lies between 0.8 and 2.0, reported for mammalian models. In the present study the ratio of A:G was less than 0.8 in serum and muscle of exposed fish. However in liver of exposed fish the A:G ratio was 1.13 which was higher than 0.8 but below 2.0. Since albumin is entirely produced by the liver so an increase in albumin and total protein in the present study could be attributed toward the protein synthesis for utilization to meet high energy demand. The low albumin level than globulin in all the cases including the present case (excluding liver) could be ascribed as the utilization of albumin to meet the immediate energy demand hence rapid synthesis takes place in the liver, and higher globulin levels to meet the immunotoxic challenges. It is also said that the fishes which have low levels of globulins were less likely to survive in polluted waters because it impart immune resistance. Moreover the increased protein levels in the liver could also be due to the effort of liver to repair their damaged tissues as a result low levels were reported in serum despite synthesis. Therefore estimation of total protein alone to check health status of fish would be misleading. After all necrosis of hepatic tissue was more likely the cause of the disturbance of protein constituents in Thermal Power Plant effluent exposed fish *C. punctatus*.

3.4. Effect of heavy metals on lipid profile of *C. punctatus*

Serum lipid profile of both the samples of *C. punctatus* is shown in Table 4. In the exposed fish lipid fraction occurs in the order total lipid > phospholipid > total cholesterol > HDL > LDL > triglycerides > VLDL however in reference fish the trend was total lipid > phospholipid > total cholesterol > triglyceride > LDL > HDL > VLDL. Alterations observed in profile were significant increase in the total lipid ($p < 0.01$), cholesterol ($p < 0.01$), phospholipids ($p < 0.05$), HDL and LDL ($p < 0.01$) levels when compared to reference. Other workers also recorded significant elevations in these parameters (Vinodhini and Narayanan, 2008; Zutshi et al., 2010; Shalaby, 2001; Hanan et al., 2013). Elevation in

Table 4 Serum lipid profile of reference and exposed *C. punctatus*.

Profile	Reference <i>C. punctatus</i>	Exposed <i>C. punctatus</i>
Total lipid	490.01 ± 0.51	534.43 ± 0.064 ^{**}
Total cholesterol	149.41 ± 0.45	230.05 ± 0.049 ^{**}
Phospholipid	199.06 ± 0.32	257.93 ± 0.036 [*]
Triglycerides	142.60 ± 0.23	46.45 ± 0.150 ^{***}
HDL	35.55 ± 3.00	133.65 ± 0.08
LDL	80.98 ± 0.86	87.11 ± 0.170 ^{**}
VLDL	27.8 ± 0.76	9.29 ± 0.15 [*]

All values are given in mg/dl and as mean ± SEM, ($n = 7$).

^{***} Statistically significant at $p < 0.001$.

^{**} $p < 0.01$.

^{*} $p < 0.05$.

these parameters particularly cholesterol is ascribed due to the mobilization of lipid either through oxidation or a process of gradual instauration of lipid molecules from the synthesis site for subsequent utilization. Since after carbohydrate in general and glycogen in particular, it's the lipid molecules which are utilized to overcome the stress. Total lipid was formed by the cholesterol, phospholipid, and triglyceride and so the elevation in its components leads to the increase in total lipid. However the triglyceride fraction showed significant ($p < 0.01$) decline in the present case. Corroborating values of triglycerides were reported in *Heteropneustes fossilis* when exposed to As and zeolite in a laboratory based study (Balasubramaniam and Anil, 2013). Similarly the VLDL fraction decreased significantly ($p < 0.05$) since its concentration depends on the triglyceride fraction. Triglyceride-rich lipoproteins (VLDL) are believed to be the components of the innate, non adaptive immune defense system thus their decline leads to immune suppression. Elevation in lipid profile is either due to disturbance in the metabolism of lipids or may be due to impaired clearance from plasma which favors liver dysfunction. As a result hyperlipidemia, hypercholesterolemia, premature atherosclerosis and excessive deposition of fat take place. Furthermore lipid, particularly phospholipid is the important structural component of cell membrane, subsequent alteration in lipid profile will lead to changes/damages in the membranes. Consequently the intracellular and membrane bound enzymes are released into the circulation. Very limited work had carried out both in field and laboratory based studies in fish pertaining effect of heavy metals on lipid profile.

These biomolecules particularly proteins and polyunsaturated fats (PUFA) are also prone to oxidative damage caused due to these transition metal ions. The elevation or decline in these parameters could be due to carbonylation or peroxidation by free radicals/ROS generated as a result of metal toxicity (Tabrez et al., 2011). The free radicals/ROS either induce or suppress the antioxidant defense system leading to the imbalance between the two, and consequently cause oxidative stress (Sheriff et al., 2014).

The effluents released from the power plant contain heavy metals consequently in water Fe and Ni were beyond the limits and Cr was equal to the USA standards whereas Mn was higher than the USA standards set for ecosystem and human health. Hence Cr and Mn can also be a threat to the life of living organisms. These heavy metals besides affecting the quality of water also influence major protein food source in the form of fish. These metals may accumulate in fish tissues due to which fish comes under stress which is confirmed by studying alterations in major biomolecules of fish. Therefore *C. punctatus* can suitably be used as bioindicator for monitoring the water quality of river. Furthermore these heavy metals cause elevation or decline in carbohydrate, protein and lipid profile, which are served as suitable biomarkers of fish health. No doubt power plants are necessary for development but on the other hand they have important impacts on aquatic habitats, their diversity and livelihood of humans over long periods if nothing is done toward pollution mitigation.

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