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STUDIES ON IMPACT OF HEAVY METAL CHROMIUM ON CATLA CATLA WITH REFERENCE TO BIOCHEMICAL PARAMETERS

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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.[1] Chromium and its derivatives such as sulphates, oxides, chlorides, nanoparticles etc have been found to have deleterious effect on neurology, ionoregulatory, physiology, biochemistry, metabolism and histological parameter in Catla catla fish.[2]

KEYWORDS: Glucose, glycogen, protein, lipid profile, ionoregulatory, nanoparticle.

INTRODUCTION
Chromium (Cr) is a naturally occurring element present in the earth's crust, with oxidation states (or valence states) ranging from chromium (II) to chromium (VI).[3] Chromium compounds are stable in the trivalent [Cr(III)] form and occur in nature in this state in ores, such as ferrochromite. The hexavalent [Cr(VI)] form is the second-most stable state.[4] Elemental chromium [Cr(0)] does not occur naturally. Chromium enters into various environmental matrices (air, water, and soil) from a wide variety of natural and anthropogenic sources with the largest release coming from industrial establishments. Industries with the largest contribution to chromium release include metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production. The increase in the environmental concentrations of chromium has been linked to air and wastewater release of chromium, mainly from metallurgical, refractory, and chemical industries. Chromium released into the environment from anthropogenic activity occurs mainly in the hexavalent form [Cr(VI)].[5] Hexavalent chromium [Cr(VI)] is a toxic industrial pollutant that is classified as human carcinogen by several regulatory and non-regulatory agencies.[5,6,7] The health hazard associated with exposure to chromium depends on its oxidation state, ranging from the low toxicity of the metal form to the high toxicity of the hexavalent form. All Cr(VI)-containing compounds were once thought to be man-made, with only Cr(III) naturally ubiquitous in air, water, soil and biological materials. Recently, however, naturally occurring Cr(VI) has been found in ground and surface waters at values exceeding the World Health Organization limit for drinking water of 50 µg of Cr(VI) per liter.[8] Chromium is widely used in numerous industrial processes and as a result, is a contaminant of many environmental systems.[9] Commercially chromium compounds are used in industrial welding, chrome plating, dyes and pigments, leather tanning and wood preservation. Chromium is also used as anticorrosive in cooking systems and boilers.[10, 11, 12]

The study was carried out to evaluate the toxicity in gill tissue of edible fish Catla catla using FTIR spectra. Fourier selfdeconvolution obtained by curve fitting was applied in the lipid (3000–2800 cm−1), carbohydrates

(1000–1100 cm−1) and in the amide region (1700–1600 cm−1). These spectral changes were used as biochemical parameters to assess the degree of toxicity. A disorder in lipid changes was measured by frequency shift and intensity changes in the CH2 asymmetric stretching band. This change in the fatty acid composition in fish could be used as biomarkers of toxic effect. Decreases in lactic acid (6–16%) clarify the lipid peroxidation which is the primary mechanism of toxicity. The deconvolution in the amide region shows peaks at 1621 cm−1, 1637 cm−1 due to β sheet; 1652 cm−1 and 1667 cm−1 due to α helix and 1683 cm−1 due to antiparallel β sheet. The results show a decrease by (3–7%) in α helix and increase by (13–40%) in β sheet structure. This shows β sheet formation of protein secondary structure due to toxicity. PCA plots indicate protein and lipids have strong positive loadings. The study shows the spectral variation is considered as an ideal biomarker with a high degree of accuracy of test organism to examine the toxicity of pollutants.[13]

MATERIALS AND METHODS

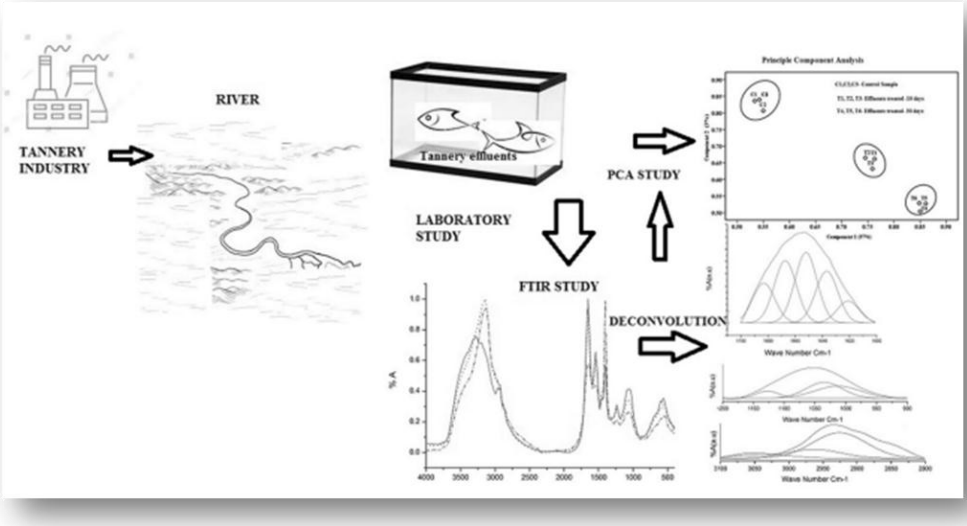
Catla catla were collected from the department of fisheries, Anantapur, Andhra Pradesh, and were immediately transported in big fish containers to the laboratory. Then they were released into large cement tanks contained of chlorinated tap water. The fish were fed with commercial fish pellets having around 40% protein content, and allowed to acclimatize for 15 days. Then the fish were isolated into batches having weight of 10 ±2 gms were maintained in static water without any flow.[14]

CARBOHYDRATE

LC50 of chromium chloride (trivalent chromium) to fish Catla catla was determined by "Probit method "of Finney. Based on the fact that the effect of a metal on fish becomes consistent within 96 hour of exposure. LC50 S/96 hours of trivalent and hexavalent chromium are considered as lethal concentrations. The LC 50/96 hours of hexavalent chromium to Catla catla is 100mg/L (Cr as 35.40mg/l). So, about 1/10th of the 96h LC50 lethal concentration was taken as sublethal concentration i.e., 59.68 mg/l, 100mg/l(Cr as35.40mg/lit) were the lethal concentrations, 5.96mg/l of trivalent chromium and 10mg/l(Cr as 3.54mg/lit) of hexavalent chromium respectively was taken as the sublethal concentration for further studies. The biochemical studies in this investigation were carried out on glucose levels in blood and glycogen levels in liver and muscle of the fish at 1,8,16, and 32 days on exposure to the sublethal concentrations of trivalent and hexavalent chromium. At the end of it, the healthy fishes were taken out and blood was collected from incision at the caudal vein region into the heparinized capillary tubes for studying blood glucose levels, and then the fishes were sacrificed, stunned to death and the required organs were dissected out from each animal using sterilized instruments. The organs are weighted accurately on an electrical semi-microbalance and transferred into ice jacketed micro beakers containing fish ringer solution. The fish ringer was prepared as per the composition given by Ekberg. The level of blood glucose was estimated by Mendel et al,(1954) method. Liver and muscle glycogen content was estimated by using the anthrone reagent method described by Caroll.[14]

PROTEIN: Protein profile of serum, liver and muscle

Total protein was determined according to the protocols of Bradford (1976)[16] as modified by Spector (1978)[18], taking BSA as a standard. Albumin was quantities using the diagnostic kit (Siemens Ltd., Gujarat, India). The intensity of colour 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.[1]



Graphical abstract of spectral profile index changes as biomarker of toxicity in Catla catla.

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. Total protein in the muscle fall in these parameters in fish Catla catla (Sobha et al., 2007). 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.[1]

Sample preparation: The gill tissues were lyophilised for 12 h to remove its water content completely. The samples were then ground with the help of an agate mortar and pestle to bring it in powdered form. Finely powdered tissues were mixed with pre-dried potassium bromide in a ratio of 1:100 respectively. It was subjected to a high pressure (3000 Psi) for 5 min in an evacuated die to produce a transparent sample pellet of 1 mm thickness and 13 mm diameter for use in FTIR spectrophotometer.[15]

Analysis: (i) FTIR- FT-IR spectra were recorded on NEXUS 470 spectrophotometer. A total of 256 scans were taken at a resolution of 4 cm−1 and averaged. A blank KBr disk was used as background. Pellets were scanned at room temperature (25 ± 1 °C) in the 4000–400 cm−1 spectral range. Background spectra were subtracted from the sample automatically. Each sample was scanned under the same conditions with three different pellets**. (ii) Statistical analysis:** The results were expressed as ± standard error of mean (SEM). Gill tissues of Catla catla tannery effluent treated group vs control group were analysed using the one-way ANOVA test using SPSS 16.0. P values of less than 0.05 were considered as statistically significant**. (iii) Principle component analysis (PCA):** The principle component analysis (PCA) was carried out using SPSS16.0 programming. It is used for data reduction from a larger sample. The PCA was used to our mean-centred, second derivative, and vector normalized spectral data. The results were displayed as score plots.[15]

LIPIDS: Serum total lipid was quantities using the diagnostic BQ Kit. Total cholesterol was estimated using the cholesterol dynamic extended stability (DES) diagnostic kit and HDL cholesterol using kit HDL-C. Triglyceride was determined by using the diagnostic kit. All these parameters were determined by a semiautomatic analyzer. Phospholipids were calculated by the method.[17]

Phospholipid= Cholesterol x 0:73 + 90.

Serum VLDL and LDL were calculated by the formula:

VLDL = Triglycerides/5

LDL = Total Cholesterol –(Triglycerides/ 5) – HDL

Statistical analysis: All values are given as mean ± SEM. Statistical differences among the means of reference and exposed were determined using Student's t-test.[1]

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.[25] Elevation in 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.[1] Total lipid was formed by the cholesterol, phospholipid, and triglyceride and so the elevation in its components leads to the increase in total lipid.[31]

RESULTS AND DISCUSSION

CARBOHYDRATE

The data on the levels of blood glucose (mg/100ml), liver and muscle glycogen (mg/gm wet wt), of the fish at 1,8, 16 and 32 days on exposure to sublethal concentrations of trivalent and hexavalent chromium, besides controls are presented in the following. According to the data the trivalent chromium exposed fish, the decrease and increase in blood glucose level and depletion and increase in liver and muscle glycogen content fish exposed to trivalent chromium indicate the metabolic imbalance and failure of metabolic haemostasis.[31] This could be due to the interaction of metal with neuroendocrinal coordinative centers which might have lead to the continuous breakdown of glycogen reserves in liver and muscle of fish by improper stimulation of enzyme machinery. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands fish exposed to toxicant through **glycolysis** or **Hexose Monophosphate pathway**.

Table 1: Blood glucose (mg/100ml) in Catla catla at different periods of exposure to sublethal to concentration of trivalent and hexavalents chromium. Each value is a mean of six replicants. Present change over the respective control is given in parentheses.[14]

S.D. ±: Standard Deviation; P: Level of Significance

ORGAN		EXPOSURE PERIOD IN DAYS								
			TRIVALENT CHROMIUM				HEXAVALENT CHROMIUM			
		CONTROL	1	8	16	32	1	8	16	32
BLOOD GLUCOSE mg/100ml	Mean S.D. %	92±4.20	72±3.82 (-21.73)	60±3.65 (-34.78)	84±6.29 (-8.69)	77±3.8 (-16.30)	52±4.4 (-43.47)	42±3.26 (-54.34)	81±3.05 (-11.95)	65±2.16 (-29.34)

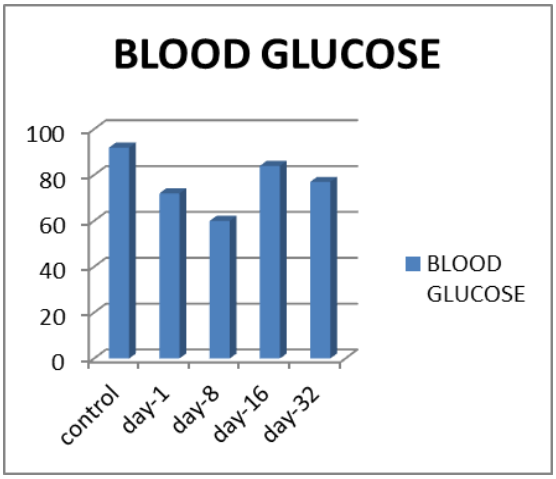
The differences between control and experimental are statiscally significant (P <0.005).

Table 2: Glycogen content (mg/gm wet wt.) of liver and muscle in Catla catla at different periods of exposure to sublethal concentration of trivalent and hexavalent chromium. Each value is a mean of six replicants. Percent change over the respective control is given in parentheses.[14]

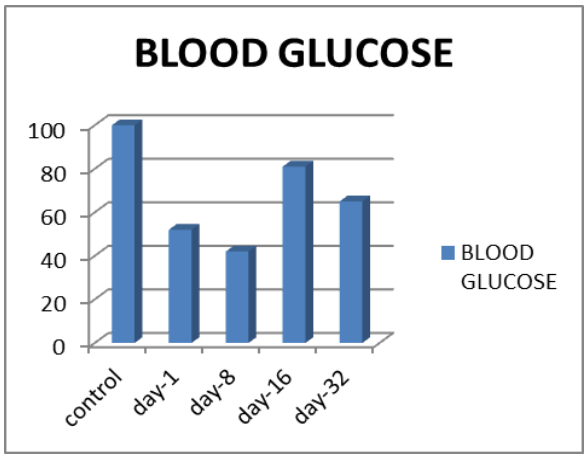
S.D. ±: Standard Deviation; P: Level of Significance

ORGAN		EXPOSURE PERIOD IN DAYS								
			TRIVALENT CHROMIUM				HEXAVALENT CHROMIUM			
		CONTROL	1	8	16	32	1	8	16	32
LIVER	Mean S.D. %	14.176 ±0.0342	7.126 ±0.0454 (-49.73)	10.260 ±0.0350 (-27.62)	12.671 ±0.0267 (-10.61)	16.546 ±0.0411 (-16.71)	11.645 ±0.0343 (-17.05)	10.188 ±0.0344 (-28.13)	9.248 ±0.0370 (-34.76)	8.128 ±0.0358 (-42.66)
MUSCEL	Mean S.D. %	1.981 ±0.0291	1.681 ±0.0334 (-15.14)	1.480 ±0.0342 (-25.29)	1.851 ±0.0291 (-6.56)	2.228 ±0.0371 (-12.46)	1.855 ±0.0281 (-6.36)	1.525 ±0.0386 (-23.01)	1.325 ±0.0386 (-33.11)	1.163 ±0.033 (-41.29)

The differences between control and experimental are statiscally significant (P <0.005).

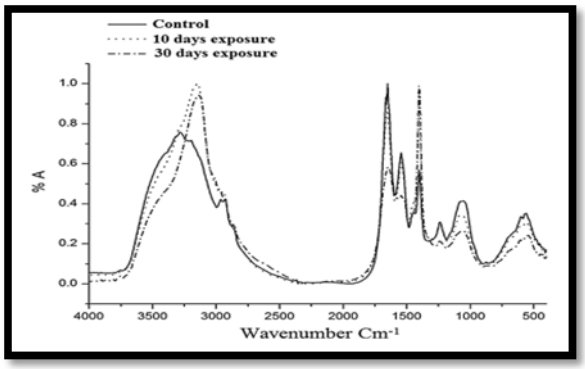


Blood glucose (mg/100ml) in trivalent Cr exposed fishes.



Blood glucose (mg/100ml) in hexavalent Cr exposed fishes.

PROTEIN



Control	10 days exposure	30 days exposure	Frequency assignment
3291(m)	3298(m)	3293(w)	Amide A: mainly N-H stretching of proteins
3089(w)	3081(w)	3079(w)	Amide B: N-H stretching of proteins.
1645(s)	1644(m)	1642(m)	Amide I:C=O stretching of proteins.
1536(s)	1527(vw)	1529(m)	Amide II:N-H bending and C-N stretching of proteins.
1398(vw)	1395(vw)	1379(vw)	COO^- symmetric stretch: fatty acids and amino acids

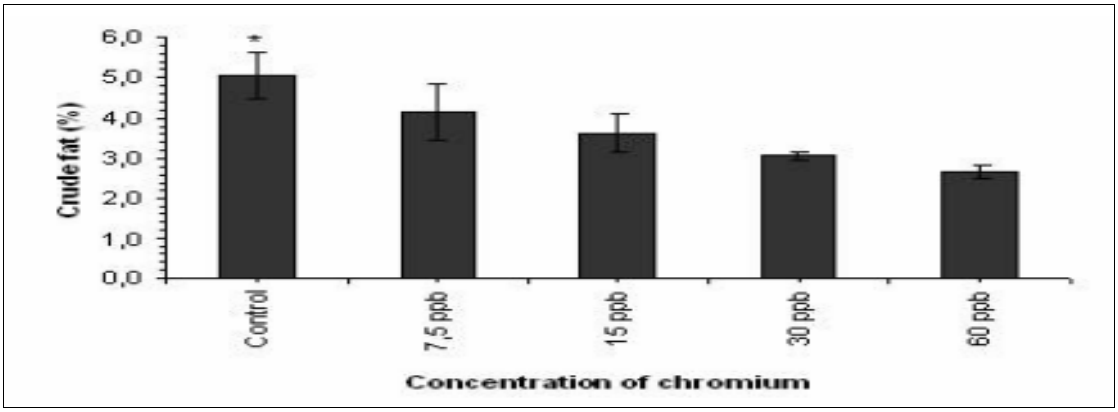
Average FTIR spectra of Catla catla showing control and tannery.

Tentative frequency assignment and their functional groups for the control effluents exposed to different days of exposure in the region of 4000-400 cm^{-1}. And tannery effluents treated gill tissues of Catla catla.

The average spectra of gill tissues of Catla catla of control and chronic exposures of tannery effluents for two different periods. The major and minor bands of the infrared spectra of the control groups, tannery effluents treated gill tissues were recorded in Table 1. As observed from Fig. 2 the bands centred at ~3291 cm−1 and 3089 cm−1 corresponds to amide A and amide B of proteins due to N–H/O–H modes of proteins. The bands rise at ~3013 cm−1 which indicates the presence of HC=CH group olefnic molecules. This band is used as a varying measure of degrees of unsaturation of phospholipids.[19] Lipids give rise to a number of absorption in FTIR spectra. The medium band which rises at ~2957 cm−1 is assigned CH3 asymmetric stretching. The peak at 2927 cm−1 and 2855 cm−1 can be assigned to asymmetric and symmetric stretching mode of CH2 modes.[20] This band is mainly screened for the lipids present in the biological system. Strong band ~1645 cm−1 is assigned to amide I and arises due to C=O of protein. Amide II bands appear ~1536 cm−1 due to N–H/C–N mode of vibration.[21,22] As observed from the Fig. 2 the intensity of amide bands decreases significantly due to the tannery effluents treated for both acute and chronic exposures. Medium intensity bands ~1392 cm−1 arise mainly from COO— symmetric stretching modes of fatty acids. The band is seen at ~1231 cm−1, and 1083 cm−1 were primarily assigned to the asymmetric and symmetric stretching modes of nucleic acids instead of phospholipids.[23] This band may overlap in the carbohydrates region, as revealed by the deconvolution.[24] There are several bands which appear in the 3000–2800 and 1800–1000 cm−1 region. These bands need extraordinary administration to information investigation since they comprise of several unresolved bands. We used Fourier self deconvolution techniques in the lipid regions (3000–2800 cm−1), carbohydrates region (1000–1100 cm−1) and amide region (1700–1600 cm−1).

LIPIDS

The nutritional benefits of fish are mainly due to the content of high quality protein and high content of the two kinds of omega-3 polyunsaturated fatty acids. Toxic heavy metals in fish can damage the positive effects of the omega-3 fatty acids present in fish their beneficial effects on heart disease risk.[26] Fatty acid composition and lipid metabolism affected depend on peroxidation in animals.[27] In the present study, ıt was showed that lipid levels decreased in muscle of fish exposed to Cr. Lipid peroxidation is the reaction of oxidative deterioration of membrane polyunsaturated fatty acids. The reduction of unsaturated fatty acids in muscle of fish in this study showed similarities that of Kawamoto et al.[28] The polyunsaturated fatty acids were significantly decreased after Cr+3 treatment compared to control group. This decrease might be due to induction of prostaglandin biosynthesis by Cr+3. This suggestion was confirmed by Choiet al.[29] and Figueired et al.[30]



CONCLUSION

The literature review concludes that the chromium derivatives such as ions, oxides, chlorides, nanoparticles pose a major impact on aquatic biota. Exposure of significant amount of chromium to wide array of fishes will involve a crucial and harmful impact are histopathology, DNA damage, behavioural changes such as swimming, cell disruption, metabolism, physiological changes etc. This can be concluded that with increasing heavy metals Cr will impose in the environment as well as biogeochemical cycle leading to toxicity in fishes.

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12. Spectral profle index changes as biomarker of toxicity in Catla catla (Hamilton, 1822) edible fsh studied using FTIR and principle component analysis M. Mohan Kumar1· S. Binu Kumari1· E. Kavitha2· B. Velmurugan3· S. Karthikeyan4 Received: 23 March 2020 / Accepted: 3 June 2020 / Published online: 16 June 2020 © Springer Nature Switzerland AG 2020.
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