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IMPACT OF INDUSTRIAL WASTE WATER ON THE CHAMBAL RIVER AND BIOMARKER RESPONSES IN FISH DUE TO POLLUTION AT NAGDA, M.P.INDIA

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ABSTRACT
The impact of effluent from chemical and textile industries on the physico chemical characteristics of Chambal River was carried out for period of one year. Water sample were collected on quarterly basis beginning from the months of June to September, 2008 (rainy season) November, 2008-Febraury, 2009 (winter period) and March-may, 2009 (summer period) to reflect the seasonal factors. Three sampling stations were chosen along the river corresponding to the locations were notable industries discharges effluent into the river. The effluents discharged into stations 2 and 3 led to increase in pH, high BOD and COD values. 'T' test results revealed that turbidity, TDS, TSS, Electrical conductivity, DO, chloride, sulphate and hardness were significantly different among various stations sampled.

Antioxidant enzyme activities of fish (*M. tengara*) were determined to establish possible environmental impact of toxic effect on anthropogenic pollution on River Chambal. Fish were sampled in all seasons and from all sampling stations during 2008 and 2009. Blood was assayed for selected hematological parameters (haematocrit, hemoglobin, red blood cell counts, white blood cell counts, erythrocyte sedimentation rate, and total plasma protein and plasma glucose concentration). The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. Decrease in hemoglobin, haematocrit values, red blood cell counts, and total white blood cell counts were observed in winter and summer in both station 2 and 3. Plasma level of protein and glucose were also lower in the fish of station 2 and 3. The hematological indices MCHC, MCH and MCV were also lowered. In conclusion, the changes observed indicate that hematological parameters can be used as an indicator of toxicity related stress in fish. Fish from station 2 and 3 also revealed gradual decrease in albumin and INR. However high levels of ASP and ALT were observed in fish of station 2 and 3in all seasons. Histopathology of experimental fish revealed swelling and degeneration of hepatocytes, mild proliferation of bile ducts and hypertrophy of kupfer cells. The study underscores the need for immediate remediation programmers to control the poor water status of the sections of the river sampled.

KEY WORDS: biomarkers, Chambal River, *M. tengara,* physicochemical parameters, Transaminase enzymes

INTRODUCTION

Nagda is very close to tropic of cancer at 23'27N and 75'25 and 517 meters above MSL. Nagda is a city and municipality in Ujjain district in the Indian state of Madhya Pradesh River Chambal Originates from Barnagar (M.P) and joins River Yamuna after Udi at Jahika (U.P).From its origin onwards, tributaries, Khan, and Kshipra join river Chambal before Nagda, M.P. More than one lakh of residents in and around the Nagda area rely on water from Chambal River for public use, industrial supplies, power plant cooling and waste water treatment. The river receives water from different units of Grasim Industries and sewage from Nagda town. Waste after coming from the factory complex runs in a channel for about 3km and joins River Chambal near Juna Nagda.

Discharges of metal effluents into rivers may cause deleterious effects to the health (Tavares and Carvalho, 1992). Fish contaminants can reach man through the food chain (Pfeiffer, et al, 1985). Metals are released to the environment by both natural processes and anthropogenic sources, (Reddy and Baghel, 2010). Hematological indices are very important parameters for the evaluation of health and physiological status of an animal. Their changes depend on type of species, age, the cycle of the sexual maturity of spawners, and diseases (Vutukuru, 2005; Crook, 2006; Reddy *et al.*, 2010). Like in man, changes in the blood parameters of mice, which occur because of injuries or infections of some tissues or organs, can be used to determine and confirm the dysfunction or injuries of the organs or tissues.

The biochemical hub of the body, the liver, has a variety of transaminases to synthesize and breakdown amino acids and to interconvert energy storage molecules. The concentrations of these enzymes in serum are normally low. However, if liver is damaged the hepatocyte cell membrane becomes more permeable and some of these enzymes leak out into the blood stream. The two transaminases commonly measured are Alanine transaminase (ALT.E.C.2.6.1.2) and aspirate transaminase (AST; E.C.2.6.1.1) .In general, any damage to the liver will cause elevations in this transaminase (Raurich *et al.,* 2009).

The objective of the present study was to evaluate the toxicity of pollution on hematological indices and to measure the serum levels of ALT and AST in response to toxicity as potential biomarkers of hepatotoxicity. Therefore, the present study aimed to investigate the impact of industrial waste on hematological and biochemical indices of *Mystus tengara.*

MATERIAL AND METHODS

Water samples were collected on quarterly basis beginning from the month of June to September,2008(Manson), November-January, 2009 (winter) and March-May, 2009(summer) to reflect seasonal factors. Water samples from different areas were collected and designated as S1 to S3.

Description of Study stations

Station 1: This station is located at upstream at Methwasa village. Human activities are reduced here to bathing and fishing. This station was taken as the reference station (control) owing to the absence of discharge coming into the River from industries.

Station 2: This station is located near Mukteswar temple near Juna nagda.The discharges of industrial complex and domestic waste are drained into this station. It is poorly vegetated. It is about 2 km away from station 1.

Station 3: This station is located about 1 km far away from station 2. It is located between the village Juna nagda and Gidhgarh. It is also poorly vegetated.

Station 3: This station is located about 1 km far away from station 2. It is located between the village Juna nagda and Gidhgarh. It is also poorly vegetated.

Water quality analysis:

Sampling for water quality parameters was carried out in the five study stations on quarterly basis beginning from the month of June 2008-May 2009, covering Monsoon. Winter and summer seasons. Water from the selected sites were collected and taken in sterilized phosphate free pre cleaned polythene bottles. The samples after collection were immediately placed in dark boxes and processed within 6hrs of collection. The collected samples were analyzed for major physical and chemical water quality parameters like pH, electrical conductivity (EC), total dissolved solids(TDS), total suspended solids(TSS), total hardness(TH) dissolved oxygen(DO), Biochemical oxygen demand(BOD), chemical oxygen demand(COD).The procedure for analysis followed ' Standard methods of analysis of water and waste water(APHA).pH; the pH was measured directly either in the effluent channel or in the river using pH meter. Total hardness (TH): This was obtained by titrimetric methods (AOAC, 2002).Chloride (Cl): It was estimated according to methods given by AOAC (2002).

Histological Studies: This study was performed in winter only. The liver was fixed in formal saline at room temperature for 24 h before being dehydrated and embedded in paraffin wax (melting point 65oC). The tissues were sectioned at 5u and routinely stained with haematoxylin and eosin (H&E). Histological structure of the liver was evaluated under a light microscope (NIKON ECLIPSE E 400, USA) and photographed using digital camera attached to the microscope.

Hematological studies: Fish Mystus tengara of approximately same size and body weight were collected from different sampling stations and sacrificed. The blood was collected into sterilized tubes and transferred to tubes containing ethylenediamine tetra acetic acid -potassium (EDTA-K2) as an anticoagulant.

1. Erythrocytes (RBC) and WBC were counted immediately after blood collection in hemocytometer (Improved Neubauer. Weber scientific Ltd.) according to Wintrobe (1934).To measure hematocrit (Hct), ammonium heparinized hematocrit capillary tubes (Fisher scientific co.) were filled with blood and centrifuged for 5-min at 5000 x g in a micro capillary centrifuge (Haematokrit 24. Hettich). The percentage of hematocrit was determined by the use of a micro capillary reader. . Hemoglobin concentration was measured by the cyanmethaemoglobin method (Larsen and Snieszko, 1961) using a commercially available kit (Span, India). Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and Mean cell volume (MCV) were calculated using the formulae mentioned by Dacie and Lewis (2001).

Biochemical studies: Serum ALT and AST activity was assayed following the modified International Federation for Clinical Chemistry(IFCC) method laid down in monoenzyme kits (Siemens Diagnostics,Ltd, India). Total plasma protein and plasma glucose concentration were determined using commercial kits (Siemens Diagnostics, Ltd, India).

Coagulation test: The blood in the tube was maintained at room temperature for coagulation. (INR) The International Normalized Ratio (INR) was measured on the basis of blood clotting time.
Statistical analysis: Student's' test was applied to observe the significance of difference between control and experimental groups (Mahony and Michael 1986).

RESULTS

Water analysis: A summary of physico chemical parameters obtained in Chambal River for all the different stations are shown in Table.1. Results clearly indicate that the physico chemical parameters monitored in station 2 and 3 showed high levels of BOD, COD, TDS, TSS, EC and low DO in all seasons and exceeds the limits WHO standards. This must have been as a result of the nature of effluents discharged from the industries. However, pH and temperature did not show much variation in all sampling stations in all seasons.

Table-1: Annual Changes in physico chemical parameters of Chambal River in different segments and in different seasons of the year Season Rainy, Winter and Summer.

pH	7.2± 0.1	8.7± 0.1	8.9±** 0.1	7.1± 0.09	9.4±** 0.02	8.8± 0.9	6.8± 0.43	10.2± 0.7**	11.5± 0.64**
Temperature 'C	24.1± 0.1	24.0± 0.1	24.2± 0.09	22.4± 0.33	23.1± 0.44	22.8± 0.67	30.1± 0.23	31.0± 0.21	30.5± 0.54
TDS mg/L	32.13± 0.87	162.0± 3.3**	122.1± 9.9	110.10± 5.3	448.1± 6.3**	392.1± 3.2	92.1± 4.5	410.1± 12.1**	415.5± 13.4
TSS mg/L	44.1± 1.2	42.1± 5.3	44.0± 3.3NS	26.1± 2.2	148.1± 9.6**	140.2± 8.76	21.1± 7.6	128.0± 5.6**	126.0± 6.6
EC,umho/cm	110± 7.6	280± 9.7	350± 7.6**	85±2.3	180± 12.1	419± 13.2**	62± 2.3	310± 11.4	650± 23.6**
DO mg/L	7.2± 0.1	6.9± 0.02	4.8±** 0.09	7.5± 0.1	5.1± 0.8	3.9±** 0.07	6.9± 0.4	4.5±** 0.8	5.2± 4.3
BOD, mg/L	0.3± 0.01	24.2± 1.1	25.43± 1.1**	1.0± 0.01	54.4± 4.2**	51.2± 4.8	2.2± 0.09	45.5± 3.8**	46.8± 4.1
COD mg/L	0.8± 0.01	15.1± 1.1**	14.0± 0.9	12.0± 1.4	38.0± 2.6**	32.0± 1,8	10.0± 0.9	34.0± 2.1**	24.1± 1.8
Chlorides mg/L	1140± 25.1	1350± 43.1	1372± 38.7*	1170± 24.5	1322± 34.5**	2021± 75.2**	1210± 44.2	1344± 12.1	2685± 112.1*
Sulphate mg/L	220± 11.1	290± 15.4*	310± 18.4**	240± 18.7	700± 14.3**	690± 13.2	280± 9.9	1900± 44.7**	1780± 48.4
Total hardness	250± 12.1	400± 18.4**	390± 16.1	200± 9.67	1400± 34.3**	1380± 34.3	280± 8.11	1900± 46.7**	1780± 39.8

Histological Studies: Sections of Station 1 show the normal histological structure but Station 2 show mild swelling and degeneration of hepatocytes. At station 3, the micro sections of liver show degeneration of hepatocytes, hypertrophy of kupfercells and proliferation of bile ducts was observed.

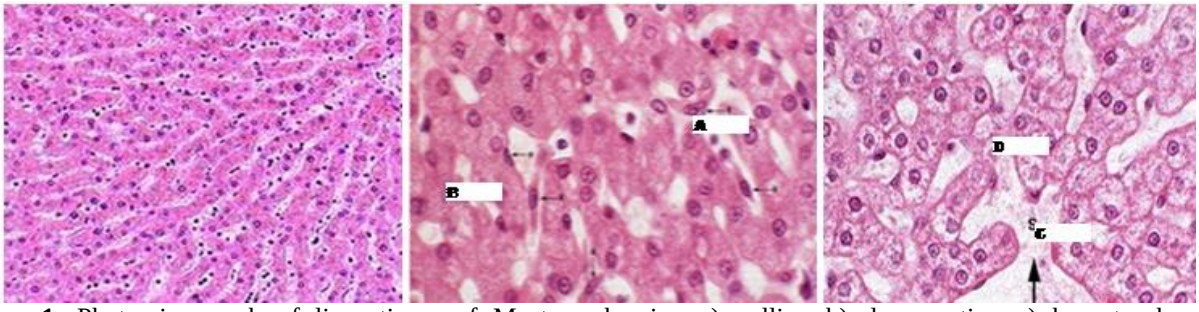

Figure-1. Photomicrograph of liver tissue of Mystus, showing a)swelling b) degeneration c) hypertrophy and d)necrosis and vacuolation in different stations of River Chambal.(400x).

Hematological studies: The main hematological alteration results includes significant decrease in haematocrit and hemoglobin concentration, and no significant decrease in red blood cell counts and erythrocyte sedimentation rates in the fish of Station 2 and 3 in all seasons. (Table-2). But a decrease in the total plasma protein was recorded in fish of Station 2 and 3 in all seasons. Plasma glucose levels decreased in the fish of both Station 2 and 3 in all seasons. The hematological indices of MCHC, MCH and MCV were also followed similar trend.

Results of the hemoglobin and haematocrit values in the fish of Station 2 and decreased significantly ($P < 0.01$) compared to those of the control. Though there was a decrease in red blood cell and erythrocyte sedimentation rate values of fish of both Station 2 and 3 , but the decrease was not much significant ($P > 0.05$) compared to the control fish. Similar trend was followed by WBC.

The hematological indices of mean corpuscular volume and mean corpuscular hemoglobin content (MCV and MCHC) were also decreased in fish of polluted zones in all seasons when compared with the control but were not statistically significant ($P > 0.05$). The mean corpuscular hemoglobin (MCH) decreased in the fish of both polluted stations but the decrease was only significant ($P < 0.05$) in Group II.

Table-2. Annual changes in some blood indices in *Mystus tengara* of Chambal River in different segments and different seasons of the year Season Rainy, Winter and Summer.

PARAMETER	S 1	S 2	S 3	S 1	S 2	S3	S1	S 2	S 3
Haematocrit %	38.4±0.94	26.8±0.79*	24.8±0.68**	39.1±.082	24.1±.011**	25.21±.072**	34.1±1.4	28.1±.081*	22.3±.068**
Hb.gm/100 ml	15.31±0.81	9.7±*0.7	8.34±0.69**	14.5±0.6	10.1±0.33**	9.3±0.28***	14.4±0.69	8.44±0.74**	8.90±0.5NS
RBC(million/ul)	2.63±0.1	2.41±0.06	2.24±0.08NS	3.62±0.22	1.24±0.08*	1.28±0.09**	2.59±0.06	2.29±0.06NS	2.12±0.07
WBC/10/L	4.7±0.2	5.43±0.1*	5.12±0.14*	4.4±0.11	5.12±0.31*	4.9±0.21NS	4.4±0.11	5.13±0.43*	5.11±0.21
MCHC (%)	37.28±5.63	31.97±4.35	34.63±4.03NS	34.2±2.34	30.91±3.3	32.11±3.2NS	34.1±4.1	30.1±2.12	29.11±3.4NS
MCV (ug)	240.18±20.12	232.4±24.04	204.8±20.28*	248.1±16.1	230 ±12.8	215.1±1.4**	238.1±10.1	209.1±12.1*	203.1±13.1*
MCH(g)	97.28±12.63	75.02±17.14*	68.3±5.99**	94.1±10.2	79.4±8.11NS	64.1±10.2**	92.1±13.1	70.11±8.7*	64.23±10.11*
Plasma protein gm/L	3.4±0.32	2.95±0.11*	1.89±0.37**	3.5±0.34	3.0±0.28NS	2.4±0.22*	3.40.22±	2.8±0.41*	2.9±0.44*
Plasma glucose mg/L	246.51±4.61	147.05±7.9**	162.11±7.41NS	282.1±6.54	200.11±8.1*	180.21±7.7**	244.1±6.72	188.11±0.43	190.1±6.71**

Biochemical studies

Alanine transaminase (ALT): The serum ALT and AST levels of *Mystus tengara* in control group were found to be normal in all seasons. But in the fish of Station 2 and 3 showed elevated levels of enzyme when compared to control (P> 0.01) in all seasons.

Total plasma protein and glucose: The protein and glucose values of fish of Station 2 and 3 were decreased (P <0.05) in all seasons.

Albumin: In contrast to the AST and ALT in fish of both polluted stations, the albumin content was significantly decreased in all seasons.

Coagulation test: The INR was normal in control fish. However it was enhanced in fish of both polluted stations in all seasons (P> 0.001).

Table-3. Annual Changes in bio chemical parameters in Mystus tengara of Chambal River in different segments and different seasons of the year Season Rainy, Winter and Summer.

Parameter	S 1	S 2	S3	S1	S2	S3	S1	S2	S3
ALT(IU/L)	60.01± 2.33	82.11± 3.11**	243± 7.2***	58.11± 3.1	98.22± 4.32**	238.22± 12.1***	64.11± 4.1	90.24± 3.6**	212.11± 9.98**
AST (IU/L)	40.7± 2.1	54.51± 3.4*	89.0± 5.4***	42.11± 1.9	62.78± 4.1*	94.11± 6.3***	46.7± 3.1	79.12± 6.1**	92.11± 7.6**
Albumin(g/dL)	4.11± 0.1	3.1± 0.12*	1.2± 0.06**	4.2± 0.5	2.8± 0.07*	1.8± 0.03**	3.9± 0.88	1.4±* 0.03	1.28± 0.02*
INR(Sec)	10.11± 1.1	44.5± 2.7**	75.1± 4.1***	9.2± 1.8	46.11± 4.6**	78.2± 5.8**	9.8± 1.9	46.22± 3.7*	58.2± 3.9**

*p> 0.05 **p> 0.01 *** p >0.001 NS= not significant

S 1 = Station 1. S2 = Station 2 S 3 = Station 3

DISCUSSION

The toxic effects of industrial effluent on the survival and biochemical profiles of Mystus tengara of Chambal River were critically examined and the corresponding results are compared and discussed.

In the present investigation, serum ALT and AST levels of Mystus tengara significantly increased exposed to when compared with the control in all seasons of the year. Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures to metals present in industrial effluent like may lead these metals to accumulate in the liver and cause pathological alterations (Braun beck, 1994). Moreover, cell injury of certain organs like liver leads to the release of tissue specific enzymes into the bloodstream (Burtis and Ashwood, 1996). Significant increase in transaminases (AST and ALT) activity in fish of both polluted zones could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver. Although it's precise biochemical functions in the Mystus are not fully understood, but contaminants present in water increased serum AST and ALT activities of reflecting a situation of tissue damage. Though not directly related to effluent toxicity, earlier studies also demonstrated an increased activity of ALT and AST may be due to leakage of hepatic cells which was also evidenced by our histopathologial studies (Reddy *et al.*, 2010b). Research indicates that ALT and AST can be used as biomarkers of cellular damage in blood plasma, protein degradation and liver damage (Markovich and James, 1999).

The major findings of this study are that heavy metals present in industrial effluent are more toxic and caused liver damage. The fish of both polluted stations showed significant increase in serum ALT and AST levels suggesting serious hepatic damage (Nimmi, 1990; Rajesh, 2009) .The present study provided new insights on the hepatotoxicity of industrial effluent, and indicated that ALT and AST activity can be used as a biomarker of hepatotoxicity. The liver is also responsible for the production of coagulation factors. The International Normalized Ratio (INR) measures the speed of a particular pathway of coagulation, comparing to normal. In the present investigation the INR was increased gradually in the fishes of both station 2 and 3 in all seasons of the year. It clearly indicates that due to the damage of liver in fish of polluted zones the synthesis of vitamin K- dependent coagulation factors have been impaired hence speed of clotting time was delayed.

CONCLUSIONS

The work presented here only threatened the chemical quality of the effluent, but it is equally important to extend the study to include a) measurement of a range of biological as well as physicochemical properties of soil which receive this polluted water for irrigation purposes b) identification and chemical analysis of plants grown on soils receiving this water and c) microbial analysis of soil (*Sail et a.l,* 2006).From the data obtained in this research must have been as a result of the nature of effluents discharged from the industries. Accordingly, water from these sampling stations is not free from the pollution and cannot be used for domestic purposes, drinking and even for agriculture.

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