

Hematological and Biochemical Changes Induced by Water Pollutants in Fishes Collected from Ramgarh Lake of Gorakhpur (U.P) India

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ABSTRACT- Ramgarh taal is an important water body of Gorakhpur, U.P, India, which received untreated effluents from different sources of Gorakhpur city. The aim of the present study was to evaluate the toxic impact of these effluents on water quality of water and its toxic effect on fish population presented in this lake with two sets of experiments (1) Fish caged in pollution free water i.e. control group and (2) Fish directly caught from different sites of water body. The effluents discharged in this water body caused significant alterations in pollution parameters of water and significantly altered the biochemical profile (total amino acid levels increased while total protein, Glycogen and nucleic acid levels decreased from the levels of control group) and hematological parameters (PLTs, WBCs, RBCs, HGB, HCT, MCV, and MCHC etc) decreased from the levels of control group. Seasonally variations in Physico-chemical parameters (Temperature, pH, DO, BOD and COD) of water body also measured. Which were much higher than the tolerance limit recommended by General standard by Central Pollution Control Board of India.

Key-words: Amino acid, Biochemical parameters, *Channa punctatus*, Hematological, Herbicides, Parameters, Pesticides, Physiological, Pollution

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INTRODUCTION

Environmental pollution is a worldwide problem because human activities are get polluted environment. Day by day Environmental contaminates are attributed to producing of different products, are release many toxic chemicals to capable of interacting aquatic or terrestrial ecosystem. Many types of toxic substance are lipophilic and weren't adversely affected by water ^[1] i.e. they are easily able to performed for penetrate cell membrane and frequently poses high bioaccumulation factors. The effluent discharge from industrial waste, agricultural waste, sewage, that is directly drainage into water bodies without treatment they constitute biohazards to man and other living organism such as aquatic animals because they contain toxic substances detrimental to serious effects on health ^[2,3].

Since much effluent discharged on ground or in water bodies and they are not treated properly results these effluents rapidly accumulated in the system of water bodies through food chain ^[4]. Fish are important members of aquatic ecosystem but now days they get more polluted because different effluent sources are directly drainage in to reservoir water and affect on aquatic flora and fauna as well as human health ^[5]. Many water bodies such as Lake, pond, river etc have different types of flora and fauna they cannot avoid exposure to these toxic effluents and chemical, which used in the form of fertilizer for well production of crops, that suspended or dissolved these water bodies thus that living organism move to favorable condition to avoid unfavorable condition ^[6]. Industrial waste is the most major types of effluent which polluted environmental system. The fresh water bodies are being affected by exclusive influence of human activities ^[7]. Thus it is truly stated that the Ramgarh Lake is more polluted because it receives huge amount of effluent sources from agricultural waste, house hold material, automobiles washing waste etc from different sites. The aim of this study was evaluated the effects of pollutants on flora and fauna of Ramgarh Lake and how much effects economically on fisheries as well as this data provide awareness baseline information for people

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settled around the lake and government to take action for treatment polluted water of Ramgarh Lake.

MATERIALS AND METHODS

Discription about study area and effluents discharged

The present study area was Gorakhpur situated in the east of Uttar Pradesh, India on National highway- 28 lies between Lattitude 26° 46' N and Longitude 83° 22' E, cover the geographical area of 3483.8 sq. Ramgarh Lake is a large natural lake situated southeast of Gorakhpur in eastern Uttar Pradesh. It lies within the floodplain of Rapti River and outflow into it through a drain called Gurrah Nalla. The lake had maximum water spread of about 723 hactares in 1970s but has now shrunk to 678 hactares. It maximum water depth has also decline from 4.5 m in 1990s to less than 3.5 m at present. Now-a-day Ramgarh Lake has more polluted because it receives huge amount of effluent discharge from different sites and its effects on flora and fauna of lake. These pretreated effluents from the remainder of the different sources are collected into a single drain and release into the Ramgarh Lake.

PHYSIOCHEMICAL STATUS

Collection and analysis of polluted water sample

Effluents and polluted water samples were collected from different sites of Ramgarh lake in glass stopper bottle at undisturbed stage from the three part of the each selected sites –

A- Entry point of effluent in the lake

B- 200 meters away from the entry point in downstream

C-200 meters away from the entry point in upstream in summer, rainy and winter season

Sampling was done from the ranging from 25 to 50 cm at various points. Care was taken to avoid any disturbance by loose sediments. The collected samples transported immediately to the laboratory and the physical and chemical characteristic of polluted water samples estimated (Table 1). The samples were collected analyzed for temperature, pH, dissolve oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD) values on the basis of APHA^[8] method.

BIOCHEMICAL STUDY

Collection of fish

10-15 healthy *C. punctatus* (commonly snake head) of an average total length of 170.57 cm and average weight of 1002.88 gm were brought to laboratory from Gorakhpur hatchery Chhapi, Gorakhpur (U.P.), India. They were maintained for acclimatization for two–three weeks in aerated aquarium of 45 liter capacity. These acclimatized fish were used for control group as well as brought polluted fish from selected site of Ramgarh lake and it directly used

for biochemical analysis i.e. uncontrolled group.

Commonly snake head (*C. punctatus*) is member of the Channidae family. It is identified by body elongated and cylindrical. Eyes are comparatively small and located anterior part of head and lower jaw slightly protruding, absent barbells.

Experimental design

Potential effect of effluents was studied in the form of biochemical parameters (protein, total free amino acid, glycogen and nucleic acid) in the set of two experiments:

- Fish caged in pollution free water i.e. control
- Fish directly caught from Ramgarh Lake.

Analysis of Biochemical parameters

After completion of experiment the caged fishes and captured fishes were brought to the laboratory and washed with water and killed by severe blow on head and operated muscles and liver tissue quickly dissected out in ice tray and used for biochemical analysis.

Protein

Protein levels were estimated according to the method of Lowery^[9] using standard solution of bovine serum albumin.10% TCA using for homogenates liver and muscle tissues.

Total Free Amino Acids

We were estimated that the total free amino acid on the basis of spice^[10] method. Homogenates 50 mg tissues were prepared in 95% ethanol, centrifuged at 6000 xg and used for amino acid estimation.

Glycogen

Glycogen was estimated by the anthrone method of Van Der Vies^[11]. In the present experiment, 50 mg of tissue were homogenates with 5 ml of TCA. The homogenate was filtered and 1.0 ml filtered was used for assay.

Nucleic Acid

Observation of nucleic acid (DNA and RNA) was performed, by methods of Schneider^[12]. Using diphenylamine and Orcinol reagents, respectively. Homogenates tissue was prepared in 5% TCA at 900 C, centrifuged at 5000 xg for 20 min and supernatant was prepared and used for estimation. Both DNA and RNA have been expressed as µg/mg tissue.

Hematological experiment

For the study of hematological, taken 2 healthy fish (each site) about 20 cm length and 200 g weight. Blood samples of approximately 4 ml were taken from each by puncturing caudal vein with sterile heparinized syringes and needles. Samples were taken into labeled Eppendorf tubes containing 0.5 mg ethylene diamine tetra acetic acid (EDTA) an anticoagulant; it was properly mixed and used for hematological analysis for all hematological parameters

such as red blood cell (RBC), count, the total white blood cell count (WBCs), hemoglobin percentage (Hb%), Erythrocytes sedimentation rate (ESR) and the packed cell volume (PCV), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC), in the natural product laboratory of D.D.U Gorakhpur University for hematological experiment by an Autoanalyser (Celltac α) NIHON KOHDEN.

RESULTS

The data represented were discussed on the basis of three seasons i.e. summer, rainy and winter. The results of physico-chemical parameters of water samples and analysis of hematological and biochemical parameters of fish caged in unpolluted water body and polluted fish directly caught from different sites of Ramgarh Lake are given in Table 1 & Table 3 respectively.

Physico-chemical parameters study

The results of physico-chemical analysis of effluent water samples from selected sites are given in Table 1. The data described on the basis of three seasons i.e. summer, rainy and winter. This study has been done during May 2015- December 2015.

Temperature of effluents water samples varied with different seasons. The highest temperature observed in summer season at site-1 which is 30.0 ± 0.03 and lowest temperature 15.6 ± 0.07 in winter season at site-2. The pH value measure of the acidity and alkalinity of water and is one of most stable measurements. Highest value of Ph observed in all three season at site-1 i.e. 7.6 ± 0.10 - 8.5 ± 0.06 . Dissolved oxygen (DO) is important for water bodies animal and during study of research work lowest DO in all sites with the comparison of central pollution control board of India. Measurement Biological oxygen demand (BOD) during all three season find that the highest value of BOD in site-1 and lowest in site-3 as well as the Chemical oxygen demand (COD) maximum in summer, rainy and winter at site-1 which is 1126.6 ± 1.46 , 1180 ± 2.886 and 1210 ± 2.856 respectively and minimum at site-3 which is 425 ± 2.145 , 291 ± 4.509 and 254 ± 1.527 of summer, rainy and winter respectively. But both BOD and COD has been highest value in all sites in all season as comparison to central pollution control board of India.

Hematological Observation

The effect of effluents on haematological parameters of *C. punctatus* is studied. Haematological parameters of polluted fish and those fish were caught from unpolluted area i.e control group showed significant ($p < 0.05$) difference. The effluents from different sites were highly toxic for flora and fauna. During hematological, it was observed that the highly decrease level of WBCs and RBCs from the level of control group (Table-2). Results concerning red blood cells counts exhibited a decrease in their number, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MHC), mean corpuscular volume (MCV) were significantly decrease than control groups. The level of PLT, WBCs, and RBCs of site-1 was lowest as comparison to site-2 and site-3. Thus results were obtained that site-1 was more polluted area as comparison to site-2 and site-3.

Bio-chemical Observation

The effluents discharged from site-1, 2, and 3 in Ramgarh Lake, its caused serious effects on flora and fauna. During biochemical experiment results observed that the significant alteration in protein, free amino acid, glycogen and nucleic acid in liver and muscles tissue of fish *C. punctatus*. Total protein was reduced to 27%, 30%, 34%, 35%, 42%, 34% of control, nucleic acid level such as DNA was reduced to 48%, 26%, 43%, 33%, 52%, 48% of control, similarly, RNA level was reduced to 40%, 45%, 48%, 35%, 40%, and 43% of control. Glycogen level was reduced to 64%, 48%, 67%, 44%, 755 and 78% of control, while total free amino acid was induced to 53%, 54.5%, 49.6%, 49.6%, 32.5% and 53.8% of control respectively in liver and muscle tissue in summer, rainy and winter season of site-1 (Table 3). Similarly trend of result were observed for site-2 (Table 3). The value of all biochemical parameters of site-3 was up to above 60-80% of control, its mean approximately same to control group, so it is clear that the fish collected from the site-3 was less polluted to effluents than site- 1 and site- 2.

Table 1: Physico-chemical characteristic of polluted water samples collected from different sites of Ramgarh lake in summer (May to June 2015), rainy (August to September 2015) and winter (November to December 2015)

Characteristic	Season	Site-1	Site-2	Site-3	General standard by central pollution control board of India
Temperature	Summer	30.0±0.03	29.9±0.09	29.9±0.012	Shall not exceed 5°C above the receiving water temperature
	Rainy	25.0±0.06	26.8±0.15	26.2±0.02	
	Winter	17.5±0.03	15.6±0.07	17.6±0.10	
pH	Summer	8.5±0.06	6.9±0.03	7.0±0.09	5.5-8.5 mg/L
	Rainy	8.0±0.06	7.0±0.03	8.0±0.06	
	Winter	7.6±0.10	7.4±0.07	7.0±0.13	
DO mg/L	Summer	7.3±0.02	8.5±0.01	10.5±0.02	30 mg/L
	Rainy	9.8±0.02	10.2±0.01	10.3±0.06	
	Winter	8.3±0.03	10.3±0.03	10.4±0.03	
BOD mg/L	Summer	473±1.722	277±2.314	76.8±0.983	30mg/L
	Rainy	482±1.453	288±2.887	77.5±0.345	
	Winter	501±1.145	235±1.577	72.1±1.201	
COD mg/L	Summer	1126.6±1.14	430.8±1.640	425±2.145	250 mg/L
	Rainy	6	681±7.264	291±4.509	
	Winter	1180±2.886	456±4.409	254±1.527	
		1210±2.856			

Table 2: Values of Hematological Parameters (mean±SE) of fish from different sites directly caught by from polluted site of Ramgarh Lake during May 2015 to December 2015

Items	Season	Fish reared in cage in unpolluted water body	Fish caught from polluted Ramgarh lake		
			Site-1	Site-2	Site-3
PLT(lac/mm ³)	Summer	188.7±0.88	20.7±0.64(11)	25.2±0.09(13)	32.2±0.12(17)
	Rainy	196.2±0.09	18.8±0.04(9)	25.1±0.07(13)	44.3±0.09(22)
	Winter	176.6±0.45	18.3±0.003(10)	20.4±0.15(11)	21.6±0.009(12)
WBC(10 ³ /mm ³)	Summer	5.6±0.06	0.3±0.06(5)	0.6±0.25(11)	0.4±0.01(17)
	Rainy	6.3±0.06	7.3±0.12(116)	18.4±0.09(292)	21.2±0.09(336)
	Winter	7.30±0.03	1.29±0.08(17)	1.36±0.16(19))	2.02±0.003(28)
RBC(10 ⁶ /mm ³)	Summer	4.5±0.02	0.45±0.01(10)	0.5±0.01(11)	1.3±0.01(29)
	Rainy	4.8±0.02	2.7±0.06(16)	1.2±0.01(25)	1.3±0.01(27)
	Winter	5.37±0.15	2.23±0.004(41)	2.32±0.003(43)	3.13±0.10(58)
HGB(g/dl)	Summer	10.2±0.12	1.670.04(16)	2.2±0.06(21)	2.3±0.06(22)
	Rainy	9.5±0.01	4.5±0.01(47)	5.7±0.01(60)	9.3±0.01(97)
	Winter	12.3±0.31	1.16±0.004(8)	1.21±0.006(10)	1.81±0.02(15)
HCT	Summer	351.7±0.88	5.5±0.01(1)	10.5±0.07(3)	15.6±0.12(4)
	Rainy	357.0±1.53	13.5±0.08(4)	28.3±0.09(8)	17.6±0.04(5)
	Winter	325.5±2.75	17.2±0.05(5)	17.01±0.19(5)	15.26±0.03(5)
MCV(µg)	Summer	54.7±0.88	12.2±0.06(22)	13.4±0.01(24)	15.5±0.02(28)
	Rainy	53.7±1.20	10.5±0.09(19)	11.3±0.09(21)	13.7±0.06(26)
	Winter	60.26±0.05	14.5±0.006(24)	13.35±0.005(22)	14.57±0.14(24)
MCH(pg)	Summer	47.6±0.16	13.5±0.06(28)	20.5±0.12(43)	33.4±0.01(70)
	Rainy	44.0±0.09	17.6±0.01(40)	35.2±0.12(80)	37.5±0.15(85)
	Winter	58.0±0.17	18.2±0.003(31)	22.21±0.005(38)	23.3±0.004(40)
MCHC(g/dl)	Summer	30.4±0.09	10.6±0.25(35)	14.3±0.06(47)	25.2±0.09(82)
	Rainy	39.3±0.07	33.2±0.12(84)	32.7±0.06(83)	36.4±0.37(92)
	Winter	28.8±0.28	20.84±0.09(72)	22.08±0.036(76)	21.78±0.04(76)

Values are mean ±SE of six Replicates. Values given in parenthesis are % level. Data were analyzed through student's t- test. Significant (p<0.05), when treated groups were compared with control

Table 3: Shows Biochemical parameters i.e Total protein (µg/mg), Total free amino acid (µg/mg), Glycogen (µg/mg), Nucleic acid (DNA and RNA) (µg/mg) in mean± SE values in different tissue of *Channa punctatus* fish May 2015 to December 2015

Parameters	Season	Tissue (50 mg)	Fish reared in unpolluted water body	Magnitude		
				Site-1	Site-2	Site-3
Protein (µg/mg)	Summer	Muscle	96.0±0.02	20.13±0.07(27)	30.96±0.005(32)	42.28±0.015(44)
		Liver	75.3±0.01	22.55±0.025(30)	25.06±0.009(33)	45.45±0.21(60)
	Rainy	Muscle	89.2±0.02	30.31±0.008(34)	31.22±0.03(35)	43.12±0.02(48)
		Liver	72.0±0.01	25.61±0.006(35)	24.86±0.004(34)	33.11±0.005(45)
	Winter	Muscle	60.2±0.004	25.45±0.02(42)	23.56±0.05(39)	27.76±0.03(46)
		Liver	64.5±0.002	22.14±0.05(34)	22.68±0.01(35)	38.51±0.02(60)
Amino acid (µg/mg)	Summer	Muscle	7.2±0.08	38.22±0.16(530)	34.08±0.09(413)	36.35±0.04(504)
		Liver	6.9±0.09	37.63±0.39(545)	26.23±0.07(380)	21.98±0.06(318)
	Rainy	Muscle	8.2±0.18	40.7±0.05(496)	35.9±0.33(437)	41.5±0.50(506)
		Liver	7.8±0.02	38.7±0.10(496)	35.3±0.03(455)	39.9±0.35(532)
	Winter	Muscle	6.3±0.01	20.5±0.07(325)	32.7±0.46(519)	34.5±0.006(547)
		Liver	6.5±0.02	28.5±0.002(538)	30.3±0.042(466)	27.2±0.009(418)
Glycogen (µg/mg)	Summer	Muscle	14.0±0.01	9.09±0.01(64)	9.37±0.08(67)	9.47±0.007(67)
		Liver	17.2±0.02	8.40±0.007(48)	9.47±0.006(55)	9.46±0.01(55)
	Rainy	Muscle	12.15±0.85	7.61±0.009(67)	8.93±0.16(73)	9.20±0.01(74)
		Liver	15.3±0.16	6.71±0.002(44)	8.70±0.002(56)	9.19±0.01(60)
	Winter	Muscle	10.6±0.009	8.03±0.008(75)	9.1±0.07(86)	9.3±0.04(93)
		Liver	10.5±0.002	8.2±0.08(78)	9.05±0.01(86)	9.3±0.01(88)
DNA (µg/mg)	Summer	Muscle	22.5±0.01	10.8±0.01(48)	12.0±0.002(53)	15.3±0.02(68)
		Liver	27.0±0.002	7.1±0.001(26)	12.5±0.01(46)	15.0±0.01(55)
	Rainy	Muscle	24.3±0.02	8.3±0.17(34)	13.5±0.01(55)	14.10.004(58)
		Liver	28.0±0.001	9.4±0.005(33)	10.2±0.15(36)	12.4±0.09(44)
	Winter	Muscle	25.6±0.003	13.3±0.02(52)	14.0±0.004(55)	15.3±0.02(60)
		Liver	25.8±0.009	12.3±0.001(48)	12.0±0.01(46)	10.5±0.002(47)
RNA (µg/mg)	Summer	Muscle	27.2±0.005	10.8±0.01(40)	11.2±0.05(41)	22.4±0.001(82)
		Liver	28.5±0.002	12.4±0.02(45)	16.3±0.006(57)	24.4±0.001(86)
	Rainy	Muscle	27.0±0.002	13.2±0.005(48)	14.8±0.02(55)	22.0±0.001(81)
		Liver	30.5±0.001	11.6±0.01(35)	15.3±0.02(50)	25.5±0.02(83)
	Winter	Muscle	26.7±0.004	10.8±0.01(40)	11.5±0.007(43)	20.5±0.005(77)
		Liver	28.5±0.002	12.3±0.001(43)	13.2±0.005(46)	15.05±0.01(53)

Values are mean ±SE of six Replicates. Values given in parenthesis are % level. Data were analyzed through student's t- test. Significant (p<0.05), when treated groups were compared with control

DISCUSSION

The water temperature is one of the most important physical characteristics of aquatic ecosystem, as it affects the organisms^[13]. Temperature of waste water is commonly high because of addition of warm water from industrial activities^[14]. pH of water is an important factor. The fluctuation of pH is linked with chemical changes, species composition and life processes^[15]. The desirable limit of pH recommended by Central pollution control board of India and WHO^[16]. Chemical oxygen demand (COD), represented the amount of oxygen required oxidizing all the organic matter biodegradable and non-biodegradable. The maximum permissible limit of COD recommended by Central pollution control board of India and WHO^[16]. Hematological studies help in understanding the relationship of blood characteristics of the habitat and adaptability of the species in the environment. Many factors such as environmental and physiological are known to influence fish hematology; these include stress due to capturing, transportation, sampling, age and sex^[17]. The decrease in the RBC to hemolytic crisis that results in severe anemia in fish exposed to copper pollutant^[18] or due to reduction of hemoglobin synthesis affected by pollutants metals. The decrease in both Hb and PCV in this work indicates that effluent exposed fish are anaemic. It was found that RBC, Hb and PCV decrease in *Channa punctatus* and *Clarias lazera* exposed to heavy^[19]. It was stated that this decrease resulted in macrocytic hypochromic anaemia from effluent stress^[20]. The mechanism by which resin acids cause hemolysis is not clear, but these compound are associated with reduction in cellular ATP and diminished oxygen consumption. Declines in Hb concentration caused by hemolysis usually result in jaundice and elevated concentration of bilirubin in plasma^[21]. It was assumed that variation in values of blood indices (MCV, MCH and MCHC) may be a defensive mechanism against effluents toxicity through stimulation of erythropoiesis^[22]. Polluted waste water samples caused significant ($p < 0.05$) decrease in level of protein, glycogen, nucleic acid and whereas enhancement in total free amino acid. The depletion of protein fraction may have due to their degradation and possible utilization of degraded products for metabolic purposes. Depletion in tissue proteins of fishes due to low rate of protein synthesis under metallic stress was also reported by several workers^[23,24]. Several authors found a decrease in protein content on contamination with industrial effluents^[25,26-30] and alteration in aquatic organism exposed to toxicants was measured^[31]. Increase in free amino acid level was the result of breakdown of protein for energy requirement and impaired incorporation of amino- acid in protein synthesis^[32]. It also attributed to lesser use of amino acid^[33] and their involvement in the maintenance of an acid-base balance^[34]. Decrease in the glycogen content in tissue as a consequence of toxic stress felt by the animals during the experiment. Inhibition in DNA Synthesis might affect both

protein as well as amino acid levels by decreasing the levels of RNA in protein synthesis machinery^[35]. On the basis of the present investigation it may be concluded that effluent from the different sites have the profound effect on the biochemical as well as hematological, with particular reference to energy metabolism in fresh water fish *C. punctatus*.

Above study that Ramgarh Lake is more polluted due to excess effluent discharge from site-1 as comparison to site-2 and site-3, this pollution has been adversely affected the aquatic fauna as well as community's people in surrounding areas; economical depends on this lake for fishing. The results obtained site-1 (Padleyganj) was more polluted than site-2 and site-3 during analysis of water quality of polluted water samples from selected sites, and observation of hematological and biochemical parameters, of those fish which directly caught from polluted sites and those reared in laboratory condition i.e. control. However the seriousness and importance of this problem has been realized recently and not much work has been done on the toxic effects of effluent discharged on the water quality of Ramgarh Lake and fishes. Hence, the waste material i.e. house hold materials, agricultural waste, automobiles servicing waste was made to access the toxic impact on physio-chemical (Ramgarh Lake water), biochemical and hematological parameters of fresh water fish *C. punctatus*. So it is believed that the data obtained from this study will provide baseline information for making effective fishery Conservation programme in this area.

CONCLUSIONS

It is clear from above study that Ramgarh Taal got polluted due to effluents discharged from different sources of Gorakhpur city and this pollution has adversely affected the aquatic fauna as well as communities in surrounding areas, economically depended on this water body for fishing and other purposes. However the seriousness of this problem has been realized recently but not much work had done on the toxic effects of effluents on the water quality and fish fauna present in Lake. Hence, an attempt was made to assess the toxic impact of effluents on water quality and biochemical and hematological parameters of fish directly caught from the different sites of water body. So it should be believed that the data obtained from this study will provide baseline information for making effective fishery conservation programme in this area.

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