

## Impact of Pulp and Paper Mill Effluent on Water Quality of River Aami and its Effect on Aquatic Life (Fish)

Zuby Afroz and Ajay Singh

Department of Zoology, Natural Product Laboratory Deen Dayal Upadhyay  
Gorakhpur University Gorakhpur-273009 (U.P.) India

**Abstract:** River Aami is an important river of Gorakhpur region, U.P. India, which receives pulp and paper mill effluent of Rayana Paper Board Industries Ltd of Khalilabad, SantKabir Nagar, U.P. India. The aim of the present study was to evaluate the toxic impact of pulp and paper mill effluent on river Aami and its toxic effect on freshwater fish *Cyprinus carpio* with three sets of experiments: (1) fish caged in pollution free water (i.e control group) (2) fish caged in polluted river Aami (3) fish directly caught from River Aami. The pulp and paper mill effluent discharged in the river caused significant alteration in pollution parameters of river water and also significantly altered the biochemical profile (i.e. decreased the levels of total protein, Acetylcholinesterase activity and calcium concentration while increased the levels of blood glucose, activity of Transaminases and chloride concentration) of caged fish as well as fish caught from river Aami in comparison to control group. Seasonal variations in physico-chemical parameters (Temperature, pH, total solid, total suspended solid, BOD, COD) of river water were also measured which were found much higher than the tolerance limit recommended by WHO.

**Key words:** Pulp and Paper Mill Effluent • *Cyprinus carpio* • Acetylcholinesterase • Transaminases

### INTRODUCTION

Today most environmental problems are attributed to the production and release of toxic chemical capable of interacting with the environment and disrupting the ecosystem. Many of the toxic substances are lipophilic and weren't adversely affected by water [1] meaning they are easily able to penetrate cell membranes and frequently possess high bioaccumulation factors. Various devastating ecological effects and human disasters in the last 40 years have arisen majorly from industrial wastes causing environmental degradation [2, 3]. The discharges from these industries constitute biohazard to man and other living organisms in the environment because they contain toxic substances detrimental to health [4-6]. There has been an alarming and worrisome increase in organic pollutants [7]. Since many effluents are not treated properly, these products are discharged on the ground or in the water bodies and most of these discharges to water bodies accumulate in the system

through food chain [8]. The toxic chemicals produce stress on the aquatic organism like fish. Fish are important members of aquatic ecosystems and an important source of human food [9]. Fish in ponds, lakes and rivers cannot avoid exposure to these chemicals that suspended or dissolved in water, being less than land animals to move to favorable regions to avoid unfavorable condition [10].

The aquatic environment is severely affected by different types of chemicals which are toxic to the aquatic organisms. The paper industry has been one of the major sources of aquatic pollution in India. Papers and pulp effluent is serving threat to aquatic life because their effluent is one of the important reasons for the elimination of fish fauna from the rivers. Paper mill effluent consists variety of toxic components such as Chlorophenos, fatty acid and resin acid and these are the main acutely toxic and bioaccumulating compounds in bleached Kraft mill effluent (BKME) which might be responsible for causing metabolic impairment in the aquatic organisms which could even lead to their death [11]. The fresh water bodies

**Corresponding Author:** Ajay Singh, Department of Zoology Natural Product Laboratory Deen Dayal Upadhyay  
Gorakhpur University Gorakhpur-273009 (U.P.) India.  
Tel: +91-0551-2201171.

are being greatly affected by excessive influence of human activity [12]. Indian rivers act as temporary reservoirs for drainage of water and industrial effluent and often are highly contaminated with anthropogenic materials. This is true particularly for Aami River which receives bleached Kraft mill effluent from Rayana Paper Board Industries Ltd, Sant Kabir Nagar, Uttar Pradesh, India.

The aim of this study was to evaluate the toxic effect of pulp and paper mill effluent on water quality of river Aami using physico-chemical parameters and to study the alteration of biochemical profile of caged fish *Cyprinus carpio* placed in river Aami for three weeks and fish caught from river Aami in comparison to control group.

## MATERIALS AND METHODS

**Study Area and Paper Mill Description:** The present study area Gorakhpur is situated in the east of Uttar Pradesh, India, on National Highway-28 lies between Latitude 26° 46' N and Longitude 83° 22' E, covers the geographical area of 3483.8 Sq. km and The Sant Kabirnagar district lies between 26°, 47' and 26° 79' North Latitude and 83° 3' and 83° 3.45' East Longitude and bounded by Gorakhpur in the east, the study was carried at the Rayana Paper Board Industries Ltd, Khalilabad, Santkabir nagar (U. P.), which is one of the most rapidly developing and heavily polluted industrial belts of Gorakhpur region, Uttar Pradesh, India. In eastern Uttar Pradesh (India), river Aami is lifeline of Khalilabad, Santkabir nagar (U.P.) [11], River Aami originated from River Ghaghra and merges into the River Rapti, covering distance of about 250 km [13], This River receives bleached and unbleached effluents discharged from Rayana Paper Board Industries Ltd, Khalilabad, Santkabir nagar (U.P.). This water body is used for irrigation, fish catch to produce fish seed and fingerlings. Same water bodies are also utilized for disposal of the industrial wastes and sewage leading to the water pollution [11].

The Rayana Paper Board Industries Ltd, Khalilabad, SantKabir nagar (U. P.) is located adjacent to the Aami River, The mill uses Kraft and thermo mechanical pulping processes. The mill furnish is waste paper from soft and hard wood. The waste paper is chlorine bleached with calcium hydroxide or chlorine dioxide. This pretreated effluent and the effluent from the remainder of the mill operations are collected into a single drain and released into the Aami River. This has created health hazards not

only for local population but also resulted in disturbances of aquatic life of the Aami River which receive the effluent.

## Physico-chemical Study:

### Collection and Analysis of Effluent and Water Samples:

Effluent and water samples were collected from Rayana Pulp and paper mill and River Aami in glass stoppered bottles at undisturbed stage from the four selected sites, which are as follows:

**Site 1:** After the effluent treatment plant,

**Site 2:** Entry point of effluent in the river.

**Site 3:** 200 meters away from the entry point in downstream.

**Site 4:** 200 meters away from the entry point in upstream, in summer, rainy and winter season. Sampling was done from the depth 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 characteristics of Effluent and water samples estimated (Table 1).The samples collected were analyzed for temperature, pH, Total Solids (TS), Total Suspended Solids (TSS), Bio-chemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values. The procedure described in standard methods for the examination of sewage and industrial wastes APHA [14] has been adopted in the analytical techniques. The values obtained were compared with standards prescribed by WHO [15] and Central Pollution control board of India.

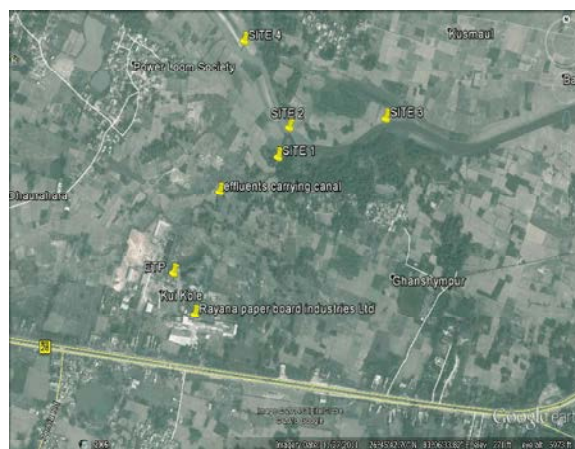


Fig 1: Location of working sites by google earth

**Biochemical Study:**

**Collection of Fish:** Ten healthy *Cyprinus carpio* (Common carp) of an average total length of  $15 \pm 0.57$  cm and average weight of  $100 \pm 2.88$  gm were brought to laboratory from Government hatchery Chhapia, Gorakhpur (U.P). They were maintained for acclimatization for two weeks in aerated aquarium of 40 liter capacity. They were fed properly through the holding period.

Common carp is one of the largest members of the Cyprinidae family. It is easily identified by two pairs of barbells on each side of the upper jaw. These bronze, brassy or yellow fish have serrated dorsal and anal fin spines. The common carp *Cyprinus carpio* is considered to be one of the chief edible fish in all regions [16].

**Experimental Design:** Potential effect of bleached effluents was studied in the form of Biochemical parameter (Blood Sugar, SGOT, SGPT, plasma Calcium & Chloride, Acetyl cholinesterase and Protein) in three sets of experiment:

- Fish caged in pollution free water i.e control
- Fish caged in polluted river Aami
- Fish directly caught from River Aami.

**Caging the Fish in Pollution Free Water and Polluted River Aami:** For caging two round net cages (made of 16mm mesh size nylon) were used. The nettings have no knots in order to prevent fish from being damaged by friction. Each Cage was provided with two stainless steel rings of 55cm in diameter. Sinkers, lines and floats were used to keep the cages in right position. Five fishes of uniform size and weight were caged in pollution free water body for three weeks and Five fishes of similar size and weight placed in cage were reared only in site 3 (i.e. 200 meters away from the entry point in downstream) for three weeks (March 2012-April, 2012) because fishes cannot survive at site-1 and site-2 due to heavy pollution.

**Fish Caught Directly from Polluted River Aami:** Three fishes of similar size and weight ( $195 \pm 28.86$  gm) were collected from site-3(200 meters away from the entry point in downstream) with cast net and brought to the Natural product laboratory of DDU Gorakhpur University for further process.

**Analysis of Biochemical Parameter:** 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

nervous tissues quickly dissected out in ice tray and used for biochemical and enzymatic analyses. Blood samples were collected from fish by cardiac puncture in EDTA containing Eppendorf tube (for plasma) and anticoagulant-free Eppendorf tube (for serum). Samples were centrifuged at 8000 rpm for 15 minutes and non-hemolyzed plasma and the supernatant serum was stored in the freezer at  $-20^{\circ}\text{C}$  and used for biochemical analysis.

**Plasma Calcium and Chloride Estimation:** Plasma Calcium and Chloride concentrations were determined by atomic absorption spectrophotometer.

**Glucose Estimation:** Serum Glucose concentration was measured by method of Siest *et al.* [17].

**Transaminases:** Activity of Serum transaminases (SGOT and SGPT) were measured by method of Reitman and Frankel [18] Homogenates (100mg/ml,w/v) were prepared in phosphate buffer for 5 minutes and centrifuged at 1000 g for 15 minutes.

**Acetylcholinesterase:** Acetylcholinesterase (AChE) activity was measured in the nervous tissue of fishes by the method of Ellman *et al.* [19]. Pooled nervous tissue (50 mg) dissected and homogenized in 1.0 ml of 0.1 M phosphate buffer pH 8.0 for 5 min. in an ice bath and centrifuged at 1000 g for 30 min at  $-4^{\circ}\text{C}$ . The Enzyme activity has been expressed in ( $\mu\text{m SH hydrolyzed/min/mg protein}$ ).

**Protein Estimation:** In Total protein estimation, homogenates (50 mg/ml, w/v) was prepared in 10% TCA. Total protein estimation, was made according to the method of Lowry *et al.* [20] from muscle tissues using bovine serum albumin as standard. Optical density was measured at 600 nm. Total protein content has been expressed as  $\mu\text{g/mg}$  of tissue.

**Statistical Analysis:** Each Experiment was replicated at least six times and the values have been expressed as  $\text{mean} \pm \text{SE}$  of six replicates. Student's 't' test and analysis of variance were applied to locate significant changes [21].

## RESULTS AND DISCUSSION

The results of physico-chemical analysis of effluent and water samples for different seasons are given in Table-1. The data presented are discussed on the basis of

Table 1: Physico-chemical characteristic of effluent and water sample collected from Rayana pulp and paper mill and different sites of river Aami in Summer(S) season (March 2012 to June 2012), Rainy (R) season (July 2012 to October 2012), Winter (W) season (November 2012 to February 2013).

Characteristics	Season	Magnitude				General standard by Central Pollution control board of India	WHO standard
		Site-1	Site-2	Site-3	Site-4		
Color	S R W	Dark brown	Dark brown	Brown	Yellowish	Disagreeable	Unobjectionable
Odour	S R W	pungent	pungent	pungent	Normal	Disagreeable	Unobjectionable
Temperature	S R W	32-35°C 28-31°C 22-25°C	30-33°C 28-31°C 20-22°C	28-30°C 25-28°C 18-20°C	28-30°C 25-28°C 17-20°C	Shall not exceed 5°C above the receiving water temperature	-
pH	S R W	8.89±0.0219 8.77±0.014 9.09±0.014	8.06±0.035 9.44±0.170 9.22±0.015	8.34±0.020 9.91±0.039 9.57±0.050	9.01±0.052 9.54±0.023 9.94±0.023	5.5-8.5	6.5-8.5
Total solid (mg/L)	S R W	4668±22.338 6566±109.138 4494±28.86	1803±18.559 2319±13.482 2858±30.11	1001±5.23 136±7.264 105±1.453	311±8.818 96±2.403 62±4.58	20-1000 mg/L	500-1500
Total suspended solid(mg/L)	S R W	1178±10.13 1946±22.113 980±3.929	758±14.449 1137±28.61 1104±10.535	291±4.509 111±1.201 78±0.577	197±9.597 75±1.157 48±0.577	100 mg/L	100 mg/L
COD(mg/L)	S R W	1263±19.220 1183±6.009 1210±2.886	681±7.264 313±1.855 456±4.409	533±4.409 280±2.886 428±3.511	425±5.131 254±1.527 322±5.364	250 mg/L	250 mg/L
BOD(mg/L)	S R W	598±4.371 482±1.453 503±1.154	288±1.855 73±0.8819 253±1.201	235±2.886 45±1.1547 213±0.577	199±2.081 39±1.453 77±1.201	30 mg/L	35 mg/L

Table 2: Level of bio-chemical parameters Random blood Glucose(mg/dl), SGOT and GPT (μmoles pyruvate/ mg protein/h), S.Calcium(mg/dl), S.Chloride(mEq/L), AChE( μm SH hydrolyzed/min/mg protein) and Protein (μg/mg) of caged *Cyprinus carpio* placed in unpolluted waterbody and polluted river Aami for three weeks and fish directly caught from polluted river Aami during March 2012-April 2012.

Items	Fish caged in unpolluted water body for three weeks	Fish caged in river Aami for three weeks	Fish caught from river Aami
Random blood Glucose(mg/dl)	60.0±6.50	65.0±3.75*	76.0±3.48*
GOT(μmoles pyruvate/ mg protein/h)	54.0±0.40	57.03±0.40*	62.20±0.52 *
GPT(μmoles pyruvate/ mg protein/h)	55.0±0.25	60.4±0.33*	66.28±0.40*
S.Calcium(mg/dl)	14.5±0.34	11.5±0.40*	8.4±0.34*
S.Chloride(mEq/L)	95.76±1.01	104.0±0.72	103.2±0.52*
AChE( μm SH hydrolyzed/min/mg protein)	0.066±0.0025(100)	0.038±0.0009*(57)	0.024±0.0004*(36)
Protein (μg/mg)	69.0±0.31	32.7±0.46*	30.3±0.47*

Values are mean ± SE of six replicates.

Data were analyzed through student't' test.

\*Significant at p<0.05

three seasons i.e. summer, winter and rainy. The result of analysis of biochemical parameters of fish caged in unpolluted water body, fish caged in polluted river Aami and fish directly caught from river Aami are given in Table-2. This study has been done during March 2012-April 2012.

Temperature of effluent and water sample varied with different seasons. The highest temperature was observed in summer season at Site-1(After ETP) which were 32-35°C and lowest temperature 17-20°C were

measured in Winter season at Site-4(200 meters away from the entry point in upstream). Among all the four sites lowest temperature were recorded in winter season and highest temperature were measured in summer season. The water temperature is one of the most important physical characteristics of aquatic ecosystem, as it affects the organisms [22]. Increase in temperature also increases the rate of microbial activity. Temperature increase may become barrier to fish migration and in this way seriously affect on reproduction of species. The major sources of

thermal pollution are industrial cooling systems working in a manufacturing plant or a power plant. Temperature of waste water is commonly high because of addition of warm water from industrial activities [23].

pH of water is an important environmental factor. The fluctuation of pH is linked with chemical changes, species composition and life processes [24]. It is a measure of the acidity or alkalinity of water and is one of the stable measurements. Higher values of pH were measured at site-4 in all three season which varied from 9.01-9.94. The desirable limit of pH recommended by Central pollution control board of India and WHO [15] for inland and irrigation water are 5.5-8.5 and 6.5-8.5 respectively. Aquatic organisms are sensitive to pH changes and biological treatment requires pH control or monitoring. Thus, pH is having primary importance in deciding the quality of waste water effluent. Waters with pH value of about 10 are exceptional and may reflect contamination by strong base such as NaOH and Ca (OH)<sub>2</sub> [25].

Total solids are the amount of solid present in dissolved and suspended form [11]. Maximum levels of total solids were measured at Site-1 ranged between 4494-6566 mg/l and minimum level of total solids at Site-4 varied between 62-311 mg/l throughout the study period. The desirable limit of Total solid recommended by Central pollution control board of India and WHO [15] are 20-1000 mg/l and 500-1500 mg/l respectively. Total suspended and dissolved solids affect organisms. They are products of run offs. They increase with increased rainfall and have adverse effects on dissolved oxygen [26].

Total suspended solid, the suspended matter consists of the particles of different types colloidal particles of various organic complexes [11]. The maximum levels of total suspended solids were found at Site-1 ranged between 980-1178 mg/l and minimum level was found at Site-4 varied between 48-197 mg/l. It was ranged between 758-1137 mg/l at Site-2 and at Site-3 it varied 78-291 mg/l for total suspended Solid.

COD (Chemical Oxygen Demand), represents the amount of Oxygen required oxidizing all of the organic matter both biodegradable and non-biodegradable. The maximum permissible limit of COD recommended by Central pollution control board of India and WHO [15] are same i.e. 250 mg/l while maximum values of COD was found much greater than standard values at Site-1 which varied between 1183-1263mg/l and minimum values were also greater than standard values which ranged between 254-425 mg/l at Site-4.

BOD (Biological Oxygen Demand) represents the amount of oxygen used by the microorganism to decompose the organic material. Maximum values of BOD were recorded during Rainy season at all the sampling sites. Maximum levels of BOD were measured at Site-1 ranged between 482-598 mg/l and minimum level of BOD occurred at Site-4 varied between 39-199 mg/l and these values are greater than the maximum permissible limit of BOD recommended by Central pollution control board of India and WHO which are 30 and 35 mg/l respectively (Table-1).

The level of bio-chemical parameters of caged fish placed in polluted river Aami and fish caught from the polluted river showed General stress syndrome Characterized by higher value of serum glucose level and transaminases (SGOT and SGPT).

There was hyperglycemic condition observed in the fish caught from river Aami and *Cyprinus carpio* caged in polluted river for three weeks. It was significantly higher ( $76 \pm 3.48$  &  $65.0 \pm 3.75$ ) in comparison to *Cyprinus carpio* caged in unpolluted water body ( $60.0 \pm 6.50$ ). The blood glucose levels have been used as an indicator of stress in fish. In early study hyperglycemic condition due to heavy metal stress related changes in fresh water fish *Catla catla* reported [27]. The hyperglycemic condition recorded on 7, 14 and 21 day in fresh water teleost *Oreochromis Mossambicus* due to stress induced by TIE(textile industrial effluent) [28]. The increase in blood sugar level in monogenean infected *Cyprinus carpio* recorded due to increase in the breakdown of liver glycogen or due to decreased synthesis of glycogen from glucose [29]. Hyperglycemic condition in naturally as well as experimentally stressed fishes may be due to impairment in the hormone level in blood involved in the carbohydrate metabolism [30-33]. Inhibition of cholinesterase in adrenal medulla which stimulates the breakdown of glycogen to glucose and increase in corticosteroid level which increases the blood glucose level in experimentally stressed fishes and ultimately causes hyperglycemia [34].

The activities of some enzymes like GOT (glutamic oxaloacetic transaminases) and GPT (glutamic-pyruvic transaminases) also indicates the impact of pollutant on fish. These enzymes are normally found within the cells of liver, kidney, heart and gills, but their increase in plasma indicates the tissue injury or organ dysfunction through its role in enzyme activation. The elevated levels of serum transaminases (SGOT and SGPT) are markers of liver dysfunctions that were observed in caged fish in polluted river and fish caught from polluted river, value

of SGOT significantly increased from  $54.0 \pm 0.40$  to  $57.03 \pm 0.40$  in fish caged for three weeks in Aami river and  $54.0 \pm 0.40$  to  $62.20 \pm 0.52$  in fish caught from same river, value of SGPT significantly increased from  $55.0 \pm 0.25$  to  $60.4 \pm 0.33$  in fish caged for three weeks in polluted river and  $55.0 \pm 0.25$  to  $66.28 \pm 0.40$  in fish caught from polluted river. This increased level of serum transaminases related to disruption of normal metabolism which is due to extensive alterations in the liver histology and indicates liver damage [29].

The activity of transaminases in fishes may be significantly changed under the influence of different toxic agents. It was reported that some metals, such as zinc, copper and cadmium significantly increase the activity of serum transaminases in some freshwater fishes [35, 36]. In a previous study in roach, *Rutilus rutilus*, alkaline phosphatase, serum GOT (glutamic oxaloacetic transaminases) and GPT (Glutamic-pyruvic transaminases) increased in fish exposed to mill effluent [37]. These enzymes are released into the plasma of fish only when tissues are damaged [35,38]. Transaminases play an important role in protein and amino acid metabolism. It was recorded that cadmium in plasma of goldfish significantly increased the activity of GOT and GPT and these results are in accordance with the results of previous investigators on freshwater fishes [39, 40]. Under the influence of different heavy metals or in the state of stress, damage of the liver, kidneys, heart and other tissues and organs may occur with concomitant liberation of transaminases into the circulation. If the water is polluted with different metals, such as zinc, copper and cadmium the activity of plasma transaminases in freshwater fishes are significantly altered [35, 41, 36].

Decreasing Calcium concentration and an increasing Chloride concentration pattern in the plasma has been observed in the caged common carp and fish caught from Aami river in comparison to fish caged in unpolluted river. The Calcium concentration was decreased significantly from  $14.5 \pm 0.34$  to  $11.5 \pm 0.40$  in Common carp reared in cage for three weeks in polluted river, from  $14.5 \pm 0.34$  to  $8.4 \pm 0.34$  in fish caught from polluted river Aami. Significant decrease was recorded in Calcium ion content of fresh water fish *Oreochromis Mossambicus* exposed to  $\text{CdCl}_2$  [42]. Calcium stimulates muscle contraction (i.e. promotes muscle tone and normal heart beat) and regulates the transmission of nerve impulses from one cell to another through its control over acetylcholine production.

We found increased chloride values in the common carp reared in cage for three weeks as well as fish caught from the contaminated river Aami in compared to fish from unpolluted water which is in agreement with the experiment of previous study [43, 37]. Chloride concentration was significantly increased from  $95.76 \pm 1.01$  to  $103.2 \pm 0.52$  in fish from Polluted Aami which was in agreement with previous study that fish from contaminated lake had higher values of plasma Chloride concentration in comparison to fish from uncontaminated lake [37]. Chloride concentration was non-significantly increased from  $95.76 \pm 1.01$  to  $104.0 \pm 0.72$  in caged fish. Oikari *et al.* [44] also reported that the concentration of plasma chloride in rainbow trout did not exhibit significant changes after 21 days after exposure to BKME. From previous study it was recorded that Calcium and Chloride concentration generally decreased in cyprinids following exposure to different stressors [45].

There was a significant decrease observed in Acetylcholinesterase activity in both group of fishes. It reduced significantly from  $0.066 \pm 0.0025$  to  $0.038 \pm 0.0009$  in fish caged in polluted Aami and decreased significantly from  $0.066 \pm 0.0025$  to  $0.024 \pm 0.0004$  in fish caught from Aami River (Table-2). Acetylcholine is the vital for the regulation of neurotransmitter in the sensory and neuromuscular system of fish [46]. Acetylcholinesterase has been one of the important biomarker to access the toxicity of different pollutants because it hydrolyzing the acetylcholine in to choline and acetic acid there by facilitating nerve impulse transmission from one cholinergic neurons to the next one [47]. Due to AchE inhibition there is marked increase in acetylcholine levels that causes continuous and excessive stimulation of the nervous system and this can lead to a tetany, paralysis and even death of the all organism [48].

Proteins occur in the body in the form of amino acids and other metabolites, which serve as building blocks of the body. Hence, protein content of the cell is considered to be an important tool for evaluation the physiological standards. Declinment in protein level was observed in caged fish and fish caught from the polluted river. Its values decreased significantly from  $69.0 \pm 0.31$  to  $32.7 \pm 0.46$  in fish caged in polluted Aami and  $69.0 \pm 0.31$  to  $30.3 \pm 0.47$  in fish caught from Aami River (Table-2). The reduction in protein content under effluent stress may be attributed to the utilization of amino acids in various catabolic reactions [49]. Several authors found a decrease in protein content on contamination with industrial effluents [44,50-54] and alterations in total proteins and their metabolites in aquatic organisms exposed to

toxicants was measured [55]. It was reported that the when fish were exposed to different effluent concentrations protein content of the gill, hepatopancreas and muscle decreased significantly [49]. The decline in protein content may be related to impaired food intake, the increases energy cost of homeostasis, tissue repair and detoxification mechanism during stress [56]. The food utilization decreases when the animals are under stress condition, which leads to the depletion of protein content in tissues. The reduction in protein content indicates that the tissue protein undergoes proteolysis, which results in the production of free amino acids and used in the TCA cycle for energy production during stress condition [49]. In previous study it was reported that Protein content of gonads and flesh of fish showed directly proportional reduction with effluent concentration compared to control [1]. In previous study, it was reported that sublethal doses of water sample collected from polluted site of river Aami caused significant alteration in the level of total protein in experimental fishes compared to control [57].

### CONCLUSION

In eastern Uttar Pradesh (India), river Aami is an important tributary of river Rapti, flowing through the Khalilabad city [11] and it is clear from above study that river Aami got polluted due to effluent discharge and this pollution has been adversely affected the aquatic fauna as well as communities people in surrounding areas, economically depend on this river for fishing and Agriculture purpose. However the seriousness and importance of this problem has been realized recently and not much work has been done on the toxic effect of Rayana Pulp and paper mill effluent on the water quality of river Aami and above fish. Hence, an attempt was made to assess the toxic impact of Rayana Pulp and paper mill effluent on the physico-chemical parameters of river Aami and biochemical parameters of *Cyprinus carpio*. So it is 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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