Impact of brick-kiln effluent on transamination of tyrosine and mobilization of thyroid hormones in gills and hepatopancreas of *Channa punctata* (Bloch, 1793) with reference to histo-architecture of the tissues

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Abstract
Dhubri district of Western Assam, India comprises of large number of brick-kilns yielding huge amount of ashes of burnt or partially burnt coal, supposed to be the major soil and water pollutants of the locality. This study dealt with the histological investigations of the gills and hepatopancreas of *Channa punctata* Bloch collected from some brick kiln-adjacent wetlands of Dumardaha Pt. II and Geramari Pt. III areas of Dhubri District with supporting observation of lipid and protein peroxidation, mobilization of thyroid and thyroid stimulating hormones, phenylalanine-tyrosine ratio in the tissues and serum ammonia-urea ratio. Studies carried out both in freshly collected fishes, acclimatized fishes and experimental fishes treated with two sub-lethal concentrations of an effluent prepared using brick-kiln refusals collected from the studied areas.
During acclimatization process of about first five weeks mortality was observed in some of the collected fishes and rest survived thereafter. Histopathological lesions were marked both in gills and hepatopancreas of freshly collected fishes, were not seen after eight weeks of survival. Similar lesions were again observed in fishes exposed to brick-kiln effluent with varied degree. Rate of lipid and protein peroxidation were observed more in both gills and hepatopancreas of freshly collected fishes and effluent-treated fishes with hypothyroidic conditions and big phenylalanine-tyrosine ratios in comparison to fishes of eight weeks survivability. These indicate healing of tissues at about eight weeks. Reobserving of similar histopathological lesions in the effluent-treated fishes revealed that brick-kiln refusals are toxic to fishes.

Key-words: Brick kiln, transamination, peroxidation, hypothyroidic, histopathology, fish

Introduction-
Bricks serve as essential building materials for various construction projects, contributing to urbanisation and modernization in developing countries like India. To meet the growing demand for bricks, numerous brick kilns have been established across the country, making India second largest brick producer after China (Kamyotra, 2015). However, while focusing on modernization, the significant impacts of brick kilns cannot be overlooked. Coal is burnt as the fuel for the manufacture of bricks which is the main source of air pollution. Although several studies have investigated the air and soil pollution caused by the brick kilns, there is a significant gap in research regarding their impact of water pollution.
It was investigated in 2008 that brick kilns surrounding the bank of Ksipra River in Ujjain caused an augmentation in the concentration of total solids, suspended solids which increased the total hardness and calcium hardness of the river water (Khan & Vyas, 2008). Studies conducted in 2008 at Kathmandu Valley revealed that oxides of sulphur, nitrogen and carbon monoxide was released from the brick kilns leading to air pollution. The health condition of the residents was greatly challenged since these air pollutants caused severe respiratory disorders like tonsilitis, acute pharyngitis etc (Joshi & Dudani, 2008). In 2013, it was reported from Nalin Chouk, Bhaktapur district that the air pollution due to brick kilns caused severe respiratory diseases as well as burning of eyes and loss of visibility in the people residing nearby the brick kilns (Pariyar et al., 2013). At Panzan village (Jammu and Kashmir), several air pollutants including oxides of nitrogen and sulphur and particulate air pollutants crossed the National Ambient Air Quality Standards (NAAQS) causing severe health hazards were reported in 2014 (Skinder et al., 2014).
In 2015, it was reported that the fertility of the soil depends upon the distance from the brick kilns. And the fertility of the agricultural lands decreased due to the accumulation of heavy metals like chromium and lead (Bisht & Neupane, 2015). In 2015, it was reported from Panzan Valley (Jammu and Kashmir) that brick kiln refusals led to the deterioration in the food value of vegetables namely *Solanum melongena L*., *Phaseolus vulgaris L*. and *Brassica oleracea L*. (Skinder et al., 2015)
From the investigations conducted at the Cachar district in 2015, it was noticed that the brick kilns caused deterioration of water quality affecting the food web in the nearby aquatic bodies (Dey & Dey, 2015)

In 2019, studies revealed that coal or rubber used as fuel in brick kilns led to the high emissions of CO_2, CO and SO_2 and several carcinogenic dioxins causing some toxic effects. (Khan et al., 2019). *Channa punctata* (Bloch, 1793), a very hardy species of fish, was selected as the model organism for the study for its distribution in the study area. The wetlands neighbouring the brick kilns are the habitat for not only the selected hardy fish model, but also the home for several vulnerable aquatic species. Anomalies in the activities of the certain enzymes and hormonal markers in *Channa punctata* (Bloch, 1793), can depict the drastic impact upon the survivability of the species and other vulnerable species present in aquatic system as well.

On studying the previously conducted investigations, the current study aimed in evaluating the following objectives in the model organism *Channa punctata* (Bloch, 1793)-

1. To study the total protein content in the gills and hepatopancreas of the fishes.
2. To analyse the oxidative stress on gills and hepatopancreas by investigating the peroxidation (lipid and protein peroxides).
3. To study the transamination of phenylalanine (Phe) to tyrosine (Tyr) by studying the activity of the transamination enzyme phenylalanine hydroxylase (PAH) and the quantification of amino acids i.e. phenylalanine and tyrosine in gills, hepatopancreas and serum.
4. To study the mobilization of thyroid stimulating hormone (TSH) and the activity of thyroid hormones i.e. triiodothyronine (T_3) and thyroxin (T_4) in the gills and hepatopancreas as well as the serum.
5. To investigate the impact upon the nitrogenous wastes namely ammonia and urea.

The main objective of the study was to evaluate the impacts of brick kiln effluents on the biochemical marker enzymes with reference to histo-pathology of the gills and hepatopancreas of the fishes so that some vulnerable organisms can be saved from extinction.

Materials and methods-

Burnt or partially burnt coal ashes (brick kiln refusals) from two selected brick kiln sites at Dumardaha Pt. II and Geramari Pt. III areas in Dhubri district were collected. Brick kiln effluents were prepared by mixing measured amount of refusals to fixed amount of deionised water in a concentration of 10 ppm (stock).

The selected resistant fish species, *Channa punctata* Bloch. which weighs 87 ± 5 grams and 17.2 ± 4.2 centimetres approximately were collected by netting from the wetlands neighbouring the brick kilns. The fishes were treated for any minor injuries with 1.5 % of potassium permanganate ($KMnO_4$) solution for 4 hours (Floyd & Klinger, 2002). A fish food containing several ingredients like vitamins, several minerals, wheat flour, wheat germ, soyabean meal, Spirullina larva, mini shellfish, baby shells, yeast, larvae of fly, vegetable powder, marketed as “Dr. Fish” was utilized during the entire study.

After $KMnO_4$ treatment, fishes were euthanised by utilizing diethyl ether anesthetization to dissect out the gills and hepatopancreas of the fishes. The respective tissues were washed in normal saline and bulk amount of tissue was homogenised using deionised water for enzymatic and hormonal assays within 2 hours of tissue extraction. The homogenised tissue sample was centrifuged at 5000 rpm and the supernatant was collected for biochemical assays and kept in deep freeze. Small portions of the tissues were washed in Phosphate Buffer Saline (PBS) and further homogenized in PBS to estimate the PAH activity, were also kept in deep freeze till assays. Some amount of tissue samples of 5×5×5 cubic millimetre thickness was further preserved in separately labelled eppendrof tubes containing 10 per cent neutral buffered formalin (NBF) for histo- pathological analysis.

Some fishes were further acclimatized for 5 weeks (35 days) and LC_{50} study was conducted further. the LC_{50} value was found to be 5.5 ± 0.11 ppm (OECD, 2019) with the prepared brick kiln effluent. On determination of LC_{50}, fishes were randomly differentiated into 3 different aquaria each containing 60 litres water volume. The fishes in “Aquarium-I” were acclimatized fishes which were supposed to be normal control and the other two aquaria “Aquariua-II & III” containing experimentally treated fishes with two sub-lethal concentrations of effluent i.e. 2 ppm and 3 ppm respectively. After 4 weeks (28 days) of exposure, the gills and hepatopancreas of the fishes were dissected out and preserved for further biochemical and histological analysis.

For estimation of PAH, Phe, Tyr, TSH, T_3, T_4 and urea content in the serum, blood was collected from the the caudal vein. Collected blood samples was further centrifuged at 5000 rpm for 10 minutes and the supernatant was collected for estimation of TSH, T_3, T_4 and urea content in the serum.

The estimation of ammonia in the serum of the fishes were conducted by puncturing the caudal vein of the fishes. The blood was collected in heparinised glass capillaries which was sealed immediately using petroleum jelly to prevent any loss of ammonia from the blood and the assay was performed immediately after serum collection.

Amounts of total protein in the gills and hepatopancreas of freshly collected, acclimatized (normal control) and the experimentally treated fishes by estimated by the method of Lowry et. al., 1951.

The assays for lipid and protein peroxide were conducted in the gills and hepatopancreas of both the freshly collected, acclimatized (normal control) and the experimentally treated fishes were done by photometric estimations of molar extinction co-efficient of thiobarbituric acid (Ohkawa et al., 1979).

Assays for Phenylalanine Hydroxylase (PAH) activity were conducted in the gills and hepatopancreas as well as the serum of the freshly collected, acclimatized (normal control) and the experimentally treated fishes by the Sandwich ELISA reagent assay kit developed by Assay Genie (Kohl and Ascoli, 2017).
Estimation of phenylalanine and tyrosine activity were conducted in the gills and hepatopancreas as well as the serum of the freshly collected, acclimatized (normal control) and the experimentally treated fishes by thin layer chromatography and spectrophotometry of separated spots (Culley, W. J., 1969).
The estimation of TSH in the gills and hepatopancreas as well as the serum of the freshly collected, acclimatized (normal control) and the experimentally treated fishes was conducted with the aid of TSH microwell ELISA kit following the Quantitative Enzyme Immuno Assay (EIA) principle (Beck-Pacozz and Persani, 1994), (Caldwell et. al., 1985), (Fisher, 1996).
The estimation of Triiodothyronine (T_3) was conducted in the gills and hepatopancreas as well as the serum of the freshly collected, acclimatized (normal control) and the experimentally treated fishes with the utilization of T_3 microwell ELISA kit following the Competitive Enzyme Immuno Assay (EIA) principle (Young, Pestaner and Gibberman, 1975), (Braverman, 1996), (Braverman and Utigen, 1996), (Chopra, 1977).
The estimation of Thyroxine (T_4) was conducted in the gills and hepatopancreas as well as the serum of the freshly collected, acclimatized (normal control) and the experimentally treated fishes with the aid of T_4 microwell ELISA kit following the Competitive Enzyme Immuno Assay (EIA) principle (Chopra et al., 1971), (Charkes, 1996).
The estimation of ammonia in the serum of the of the freshly collected, acclimatized (normal control) and the experimentally treated fishes was conducted by Megazyme's ammonia assay kit (Bergmeyer and Beutler, 1990).
The determination of urea in the serum of the freshly collected, acclimatized (normal control) and the experimentally treated fishes was determined by Modified Berthelot method utilizing the urea kit (Fawcett and Scott, 1960).

The estimation of biochemical and hormonal assays was photometrically estimated by a pre-programmed biochemistry analyser "Benesphera C-61" with beneficiary kit specifications and dilution factors was used for photometric analysis of enzymatic assays.
The histo-architectural analysis of the tissues preserved in neutral buffered formalin was conducted by the principles of Godkar and Godkar (2008). Dehydration of tissues were conducted by passing the tissues at different grades of alcohol and further sectioned using a rotary microtome of 5 µ thickness which were double stained using haematoxylin and eosin stain. The stained sections were viewed under Almicro Trinocular Research Microscope and the images of the tissues were captured using Nikon D5300 DSLR camera body using Olympus Microscope Adaptor.

Results-

Table no. 1- Showing Total protein content, lipid peroxide, protein peroxide, phenylalanine hydroxylase activity, phenylalanine and tyrosine contents in the gills, hepatopancreas and serum of freshly collected, acclimatized (normal-control) and the experimentally treated fishes

Sl. No.	Studied Parameters	Tissues sample studied	Freshly collected fishes	Acclimatized fishes	Fishes treated in 2 ppm of effluent	Fishes treated in 3 ppm of effluent
1.	**Total Protein Concentration (mg/ml)**	Gills	10.848 ± 0.008 - 10.575%	12.131 ± 0.007	10.765 ± 0.008 - 11.257%	9.250 ± 0.008 - 23.668%
		Hepato-pancreas	15.134 ± 0.013 - 7.355%	16.335 ± 0.010	14.231 ± 0.009 - 12.883%	11.756 ± 0.008 - 28.034%
2.	**Lipid peroxide activity (n mol/ml)**	Gills	225.729 ± 0.071 + 8.833%	207.408 ± 0.076	227.358 ± 0.050 + 9.619	249.43 ± 0.081 + 20.261%
		Hepato-pancreas	210.654 ± 0.091 + 10.011%	191.454 ± 0.033	210.467 ± 0.060 + 9.931%	224.764 ± 0.086 + 17.398%
3.	**Protein peroxide activity (n mol/ml)**	Gills	9.766 ± 0.010 + 15.662%	8.443 ± 0.008	9.831 ± 0.009 + 16.439%	11.345 ± 0.008 + 34.364%
		Hepato-pancreas	13.528 ± 0.001 + 18.336%	11.432 ± 0.008	13.546 ± 0.006 + 18.486%	14.944 ± 0.007 + 30.716%
4.	**Phenylalanine Hydroxylase (pg/ml)**	Gills	84.653 ± 0.017 - 21.209%	107.44 ± 0.017	89.916 ± 0.010 - 16.311%	82.423 ± 0.013 -23.285%
		Hepato-pancreas	126.325 ± 0.010 -12.480%	144.339 ± 0.009	129.336 ± 0.012 -10.394%	97.51 ± 0.009 -32.444%

		Serum	78.437 ± 0.010 -12.299%	89.437 ± 0.008	79.632 ± 0.008 -10.963%	62.764 ± 0.008 -29.823%
5.	**Phenylalanine (µg/ml)**	Gills	2.339 ± 0.009 + 103.445%	1.150 ± 0.006	2.434 ± 0.010 + 111.727%	3.075 ± 0.009 + 167.525%
		Hepato-pancreas	2.640 ± 0.006 + 28.693%	2.052 ± 0.005	2.678 ± 0.009 + 30.545%	3.656 ± 0.006 + 78.199%
		Serum	2.054 ± 0.006 + 9.221%	1.881 ± 0.009	2.351 ± 0.007 + 25.015%	2.567 ± 0.006 + 36.522%
6.	**Tyrosine (µg/ml)**	Gills	1.849 ± 0.007 - 17.730%	2.248 ± 0.008	1.755 ± 0.008 - 21.931%	1.079 ± 0.007 - 51.996%
		Hepato-pancreas	2.057 ± 0.008 - 16.300%	2.457 ± 0.006	1.850 ± 0.007 - 24.704%	1.460 ± 0.008 - 40.597%
		Serum	1.645 ± 0.008 - 11.256%	1.854 ± 0.007	1.560 ± 0.009 - 15.868%	1.234 ± 0.010 - 33.472%
"*" indicates Significant at p<0.05, "+…%" and "-…%" indicate percent increase and percent decrease respectively.						

Table no. 2- Showing TSH, T_3, T_4, Ammonia and Urea contents in the gills, hepatopancreas and blood serum of freshly collected, acclimatized (normal-control) and the experimentally treated fishes

Sl. No.	**Studied Parameters**	**Tissues sample studied**	**Freshly collected fishes**	**Acclimatized fishes**	**Fishes treated in 2 ppm of effluent**	**Fishes treated in 3 ppm of effluent**
7.	**TSH (µIU/ml)**	Gills	0.271 ± 0.0014 + 14.903%	0.236 ± 0.0009	0.256 ± 0.0007 + 8.298%	0.269 ± 0.0007 + 14.098%
		Hepato-pancreas	0.281 ± 0.0008 + 9.879 %	0.256 ± 0.0007	0.275 ± 0.0010 + 7.302%	0.305 ± 0.0007 + 18.977%
		Serum	6.792 ± 0.0010 + 40.760%	4.825 ± 0.0009	6.840 ± 0.0006 + 41.757%	8.354 ± 0.0008 + 73.129%
8.	**T_3 (µg/dl)**	Gills	0.528 ± 0.0010 -16.672%	0.634 ± 0.0009	0.515 ± 0.0014 - 18.817%	0.448 ± 0.0006 - 29.401%
		Hepato-pancreas	0.748 ± 0.0010 - 8.337%	0.816 ± 0.0014	0.768 ± 0.0014 - 5.787%	0.626 ± 0.0009 - 23.308%
		Serum	6.449 ± 0.0008 - 11.081%	7.252 ± 0.0011	6.735 ± 0.0009 - 7.129%	5.626 ± 0.0009 - 22.426%
10.	**T_4 (µg/dl)**	Gills	10.145 ± 0.0007 - 15.072%	11.945 ± 0.0008	10.142 ± 0.0011 - 15.010%	8.542 ± 0.0008 - 28.493%
		Hepato-pancreas	11.416 ± 0.0009 - 13.416%	13.185 ± 0.0009	11.509 ± 0.0010 - 12.709%	8.939 ± 0.0012 - 32.202%
		Serum	5.693 ± 0.0006 - 11.406%	6.426 ± 0.0009	5.896 ± 0.0008 - 8.245%	4.923 ± 0.0010 - 23.386%
11.	**Ammonia (gm/L)**	Serum	58.447 ± 0.007 + 38.742%	42.126 ± 0.010	73.276 ± 0.010 + 73.944%	97.442 ± 0.008 +131.311%
12.	**Urea (mg/dl)**	Serum	29.648 ± 0.008 + 79.325%	16.534 ± 0.009	38.540 ± 0.008 + 133.113%	47.944 ± 0.007 + 189.992%
"*" indicates Significant at p<0.05, "+…%" and "-…%" indicate percent increase and percent decrease respectively.						

Histoarchitectural examinations-
Gills-

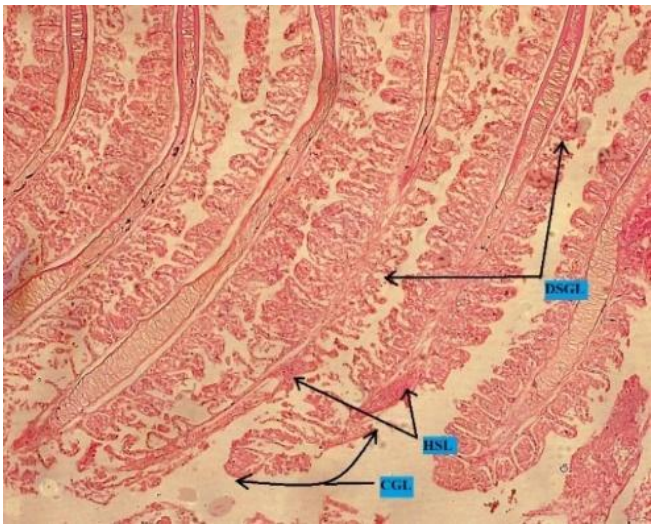

Figure 1- Histoarchitecture of gills of freshly collected fish

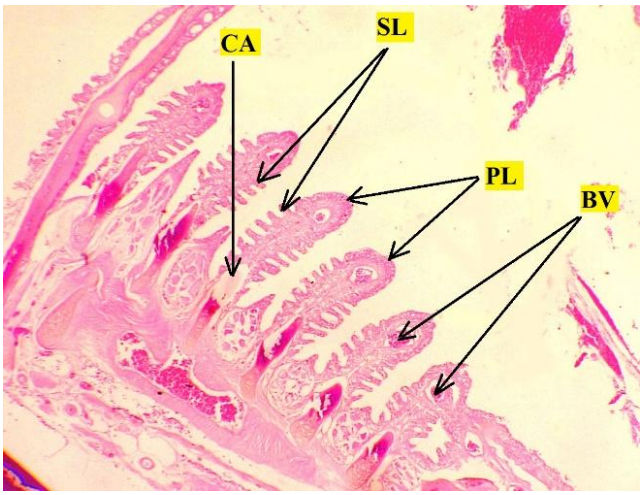

Figure 2- Histoarchitecture of gills of acclimatized fish

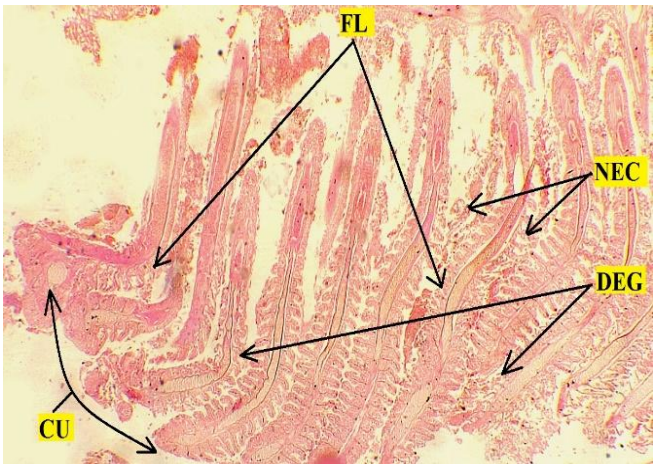

Figure 3- Histoarchitecture of gill of fish exposed to 2 ppm of effluent

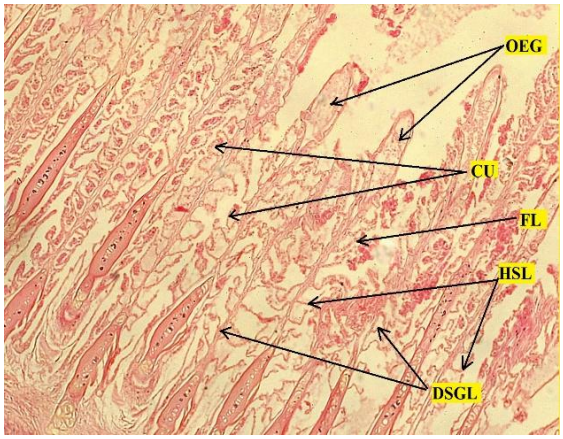

Figure 4- Histoarchitecture of gills of fish exposed to 3 ppm of effluent

(CGL- Curved gill lamellae HSL- Hypertrophy of gill lamellae, DSGL- Degeneration of secondary gill lamellae, CA- cartilage, SL- Secondary gill lamellae, PL- Primary gill lamellae, BV- Blood vessels, CU- Curving of gill lamellae, FL- Fusion of gill lamellae, NEC-Necrosis of gill epithelium, DEG- Degeneration of gill lamellae, OEG- Oedematous changes in gills)

Hepatopancreas –

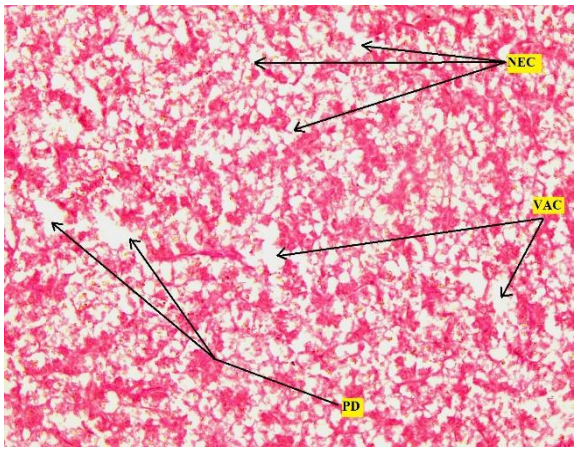

Figure 5- Histoarchitecture of hepatopancreas in freshly collected fish

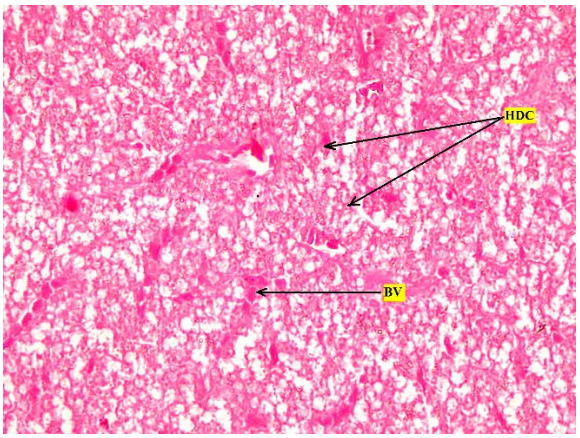

Figure 6- Histoarchitecture of hepatopancreas in acclimatized fish

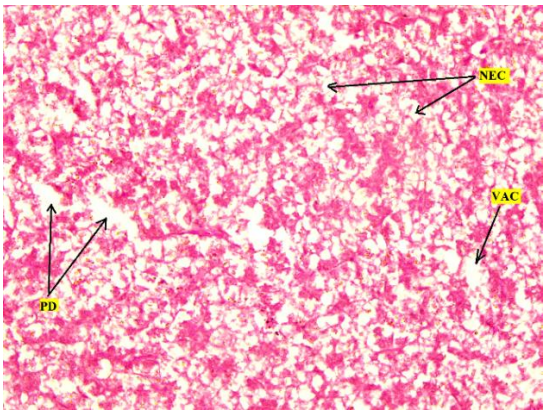

Figure 7- Histoarchitecture of hepatopancreas in fish exposed to 2 ppm of effluent

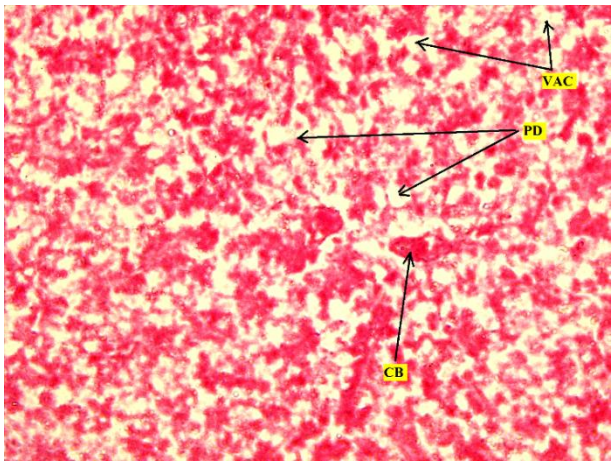

Figure 4- Histoarchitecture of hepatopancreas in fish exposed to 3 ppm of effluent

Where, HDC= High density of cells, BV= Blood vessels, NEC= Necrosis, VAC= Vacuolation, PD= Patchy degeneration, CB= Congestions of blood vessels.

Discussions-

The total protein content in the gills of the freshly collected fishes were found declined by – 10.575% in comparison to the acclimatized fishes (normal control) and when these acclimatized fishes were treated with 2 ppm and 3 ppm of effluent, it also decreased by – 11.257% and – 23.668% respectively. The total protein content in the hepatopancreas of the freshly collected fishes was found decreased by – 7.355% in comparison to the acclimatized fishes and on further treatment with 2 ppm and 3 ppm of effluent, it further decreased by -12.883% and – 28.034% respectively.

The lipid peroxide (LPO) in the gills of freshly collected fishes was found elevated by + 8.833% from the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, the it increased by + 9.619% and + 20.261% respectively. The LPO in the hepatopancreas was estimated to be augmented by + 10.011% in comparison to the acclimatized fishes and when these acclimatized fishes were treated with 2 ppm and 3 ppm of effluent, it was found elevated by + 9.931% and 17.398% respectively.

The Protein peroxide (PPO) in the gills of freshly collected fishes was found increased by + 15.662% from the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, it increased by + 16.439% and + 34.364% respectively. The PPO in the hepatopancreas was estimated augmented by + 18.336% in comparison to the acclimatized fishes and when these acclimatized fishes were treated with 2 ppm and 3 ppm of effluent, it was found increased by + 18.486% and +30.716% respectively.

The Phenylalanine Hydroxylase (PAH) activity in the gills of the freshly collected fishes was found declined by – 21.209% in comparison to the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, it decreased by – 16.311% and -23.285% respectively. In the hepatopancreas of the freshly collected fishes, PAH activity decreased by – 12.480% and on treatment with 2 ppm and 3 ppm of effluent, it declined by – 10.394% and – 32.444% respectively. The PAH activity in the serum of the freshly collected fishes was found declined by – 12.299% in comparison to the acclimatized fishes and it was again decreased by – 10.963% and – 29.823% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.

The Phenylalanine (Phe) content in the gills of the freshly collected fishes was found augmented by +103.445% in comparison to the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, it elevated by +111.727% and +167.525% respectively. In the hepatopancreas of the freshly collected fishes, the Phe content elevated by +28.693% and on treatment with 2 ppm and 3 ppm of effluent, it increased by + 30.545% and + 78.199% respectively. The Phe

content in the serum of the freshly collected fishes was found augmented by + 9.221% in comparison to the acclimatized fishes and it again elevated by + 25.015% and + 36.522% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.
The Tyrosine (Tyr) content in the gills of the freshly collected fishes was found declined by – 17.730% in comparison to the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, it decreased by –21.931% and – 51.996% respectively. In the hepatopancreas of the freshly collected fishes, the Tyr content decreased by – 16.300% and on treatment with 2 ppm and 3 ppm of effluent, it declined by – 24.704% and – 40.597% respectively. The Tyr content in the serum of the freshly collected fishes was found declined by – 11.256% in comparison to the acclimatized fishes and it again decreased by – 15.868% and – 33.472% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.
The Thyroid stimulating hormone (TSH) content in the gills of the freshly collected fishes was found augmented by +114.903% in comparison to the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, it elevated by +8.298% and +14.098% respectively. In the hepatopancreas of the freshly collected fishes, the TSH content elevated by + 9.879% and on treatment with 2 ppm and 3 ppm of effluent, it increased by + 7.302% and + 18.977% respectively. The TSH content in the serum of the freshly collected fishes was found augmented by + 40.760% in comparison to the acclimatized fishes and it again elevated by + 41.757% and + 73.129% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.
The Triiodothyronine (T_3) content in the gills of the freshly collected fishes was found declined by – 16.672% in comparison to the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, it decreased by – 18.817% and – 29.401% respectively. In the hepatopancreas of the freshly collected fishes, the T_3 content decreased by – 8.337% and on treatment with 2 ppm and 3 ppm of effluent, it declined by – 5.787% and – 23.308% respectively. The T_3 content in the serum of the freshly collected fishes was found declined by – 11.081% in comparison to the acclimatized fishes and it again decreased by – 7.129% and – 22.426% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.
The Thyroxine (T_4) content in the gills of the freshly collected fishes was found declined by – 15.072% in comparison to the acclimatized fishes and on treatment with 2 ppm and 3 ppm of effluent, it decreased by –15.010% and – 28.493% respectively. In the hepatopancreas of the freshly collected fishes, the T_4 content decreased by – 13.416% and on treatment with 2 ppm and 3 ppm of effluent, it declined by – 12.709% and – 32.202% respectively. The T_4 content in the serum of the freshly collected fishes was found declined by – 11.406% in comparison to the acclimatized fishes and it again decreased by – 8.245% and – 23.386% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.
The ammonia concentration in the serum of the freshly collected fishes was found augmented by + 38.742% in comparison to the acclimatized fishes and it was again elevated by + 73.944% and + 131.311% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.
The urea content in the serum of the freshly collected fishes was found augmented by + 79.352% in comparison to the acclimatized fishes and it was again elevated by + 133.113% and + 189.992% when the acclimatized fishes were treated with 2 ppm and 3 ppm of effluent respectively.

Histoarchitectural studies-

Gills-

The histoarchitectural studies of the gills (Figure 1, 2, 3 and 4) clearly portrayed the impacts upon the organ on exposure to effluents. When the fishes were freshly collected, severe changes were noticed in the gills including curving of gill lamellae and degeneration of secondary gill lamellae. But after acclimatization of the fishes the organ of the fish healed to some extent and therefore, they are considered as the normal control fishes. When these acclimatized fishes were further treated with 2 ppm and 3 ppm of effluents, curving of gill lamellae, fusion of the gill epithelium leading to the degradation and necrosis of gill epithelial cells was observed in the organ which depicts drastic impacts upon the organ. The degree of degradation is much higher in the fishes exposed to higher concentration of effluents i.e. 3 ppm of effluent.

Hepatopancreas-

The histoarchitectural analysis of the hepatopancreas also depicted the stress upon the fishes due to the brick kiln effluent (Figure 5, 6, 7 and 8). When the fishes were freshly collected, the hepatopancreas of the fishes were observed with several harmful aspects like decrease in the cell density and patchy degeneration of the tissue which healed to some extent when the fishes were acclimatized with increase in the density of the cells. On treatment of these acclimatized fishes, which were considered to be normal control, with 2 ppm and 3 ppm of effluent, necrosis and vacuolation of the cells leading to the decrease in the density of the cells, clearly displayed the damages caused to the organ. The respective tissue of fishes exposed to 3 ppm of effluent were much degenerated than the fishes exposed to 2 ppm of effluent.

Conclusion-

Bricks plays a vital role for certain construction purposes and with the increase in population, the manufacture and utilization of bricks has also increased. The harmful aspects caused by these brick kilns upon the environment cannot be

ignored. From the study it can be concluded that the brick kiln refusals have worst impact on the study fish i.e. *Channa punctata* (Bloch, 1793) causing tissue damage by increased lipid and protein peroxidations for which transaminase enzymes including phenylalanine hydroxylase released from tissues to blood, for which transamination process from the essential amino acid phenylalanine to non-essential tyrosine goes down with several folds leading to insufficiency of tyrosine for the synthesis of thyroid hormones (T_3 and T_4) in the thyroid gland and their mobilizations to tissues. This hypothyroidic condition hampers in maintaining BMR and also on cell division process essential for growth and healing of damaged tissues which lead to mortality of the fishes. As gills are the chief excretory organs of the fishes excreting ammonia, on damage of gills due to toxicity hampers in the excretory process for which high concentration of ammonia in fish body leading to another kind of toxicity. For management of this high concentration of ammonia the fishes change their excretory process from ammonotelism to ureotelism to some extent. Toxicity of enhanced ammonia level also cause retardation in growth, metabolism and even death of the effluent exposed fishes.

If such drastic impacts were observed in a resistant fish species leading to its mortality so the survivability of several vulnerable species becomes doubtful. In such a small area like Dhubri district numerous brick kiln sites are noticed and these establishments not only affects the air and soil but also has drastic impacts upon the aquatic life too which was observed from the study. So, these brick kilns are threat to the conservation of several species in the wild. A section of people depends upon the fishes caught in the wetlands nearby the brick kilns as source of food and earnings so the food value and the economy of the fisherman is also being threatened. From the very study, the Pollution Control Boards as well as certain Governmental and Non-Governmental Organisations were made aware of the dreadful impacts of these brick kilns and the layman as well.

Conflict of Interest-

There is no such conflict of interest.

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