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Research Article

Study on the Impact of Brick-kiln Effluent on Histomorphology of Gill and Hepatopancreas of *Channa punctata* Bloch. with Reference to Mobilization of Thyroid Hormones

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ABSTRACT

This study deals with the effect of sublethal exposure of brick kiln effluent on lipid and protein peroxides, Aspartate Transferase (AST), Alanine Transferase (ALT) and Alkaline Phosphatase (ALP) activities and mobilization of thyroid-stimulating and thyroid hormones in gill and hepatopancreas of Channa punctata Bloch., with supporting histochemical investigations in these tissues. Acclimatized adult fishes of either sexes of three groups were reared separately in three aquaria. Aquarium-I comprised the normal control group, and the other two aquariums, i.e. Aquarium-II and Aquarium-III, comprised experimental groups of fishes exposed to 20ppm/L and 30ppm/L of brick kiln effluents, respectively. Before these, the LC_{50} value of the Fish for brick kiln effluent was evaluated as 55±0.50 ppm/L for 28 days.

Increased lipid and protein peroxides, decreased transaminase activities with hypothyroidic conditions were observed both in gills and hepatopancreas on treatment.

Significant alternations of these biochemical parameters were recorded, with some drastic changes in the histo-architecture regarding treatment issues. Gills showed hypertrophy with fusion and curving of gill lamellae. Spiked secondary gill lamellae were also observed to have several degenerative features. Decreasing hepatocytes and vacuolation at several places were recorded in hepatopancreas with necrosis of the tissues and patchy degeneration.

Keywords: *Effluent, gill, hepatopancreas, hypothyroidic, histomorphology.*

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INTRODUCTION

Modernization and urbanisation walk hand in hand, which remains essential for developing countries like India. The rural society is replaced by buildings, monuments, etc., where bricks are used as the building blocks. The brick manufacturing plants are set up to accomplish the necessity of development. Due to its large population scale, India stands as the second largest producer of Bricks after China. Though there is development, but the harmful aspects of such plants cannot be over looked. Maximum brick kiln utilizes coal as the primary fuel, which, when burned, causes air pollution (Kamyotra, 2015). Several researchers studied the effect of brick kilns on air pollution, some of which are cited here.

Emissions of sulphur, nitrogen and carbon monoxide oxides were observed in 2008 by Joshi, S.K. and Dudani, I. at Kathmandu Valley brick kilns causing air pollution. These pollutants cause severe respiratory diseases like tonsillitis, acute pharyngitis, etc., and affect household health and nearby schools (Joshi & Dudani, 2008).

Respiratory discomfort for nasal congestion and burning of the eyes, loss of visibility, etc., for brick kilns, were reported by several workers in Kathmandu Valley in 2013 (Pariyar, Das & Ferdous, 2013).

The brick kilns of Panzan village of Budgam district were found to be the major sources of oxides of nitrogen and sulphur, which crossed the National Ambient Air Quality Standards (NAAQS), causing major health hazards (Skinder, Pandit, & Sheikh & Ganai, 2014).

Studies conducted in Pakistan in 2019 on the fuel used in brick kilns, i.e. coal or rubber, reveal severe health hazards. The emission of CO_2, CO and SO_2, and several carcinogenic dioxins has drastic effects on human health (Khan et al., 2019).

Unlike the air pollution, the dreadful effects of these brick kilns on the nearby aquatic bodies were not vividly studied. Very little attention is paid in this context, including some valuable research. Elevation in the total solids, suspended solids, calcium hardness, and total hardness of the river water was recorded in the studies conducted in 2008 under the supervision of Khan R. and Vyas, H. on Ksipra River, Ujjain (Khan & Vyas, 2008).

Studies conducted in 2015 on the brick kilns of the Bhaktapur area, Nepal, reveal that the brick kiln decreases soil fertility. The soil fertility deteriorates by heavy metal contamination of lead and chromium, hampering agricultural land (Bisht & Neupane, 2015).

The effect of the brick kiln on several vegetables, namely *Brassica oleracea L*., *Phaseolus vulgaris L*. and *Solanum melongena L*., was recorded at Panzan Valley (Jammu and Kashmir) in 2015. Hence the food value of several vegetables is also threatened (Skinder et al., 2015).

In 2015, Dey, S. and Dey, M. from Cachar district conducted several studies on the ill effects of brick kilns. They reported a severe deterioration in the water quality, causing hazards to the aquatic life's food chain and food web (Dey & Dey, 2015).

Studies on the brick kiln areas of Rajshahi and Gazipur districts, Bangladesh, in 2021 depict major air pollutions caused by SO_2, NO_x, etc., which exceeded the National Ambient Air Quality Standards (NAAQS). Heavy metals like lead (Pb) and Chromium (Cr) were observed from cultivable lands and surface waters nearby the brick kilns (Saha et al., 2021).

In this study, *Channa punctata* Bloch. was used as an experimental model. This very resistant variety of Fish is thoroughly distributed in ponds, pools, ditches, lakes, rivers, etc. These wetlands are also the homes of several vulnerable species, which may be affected by the kiln refusals. So, the harmful impacts upon the resistant model species imply the destruction of certain vulnerable ones.

In this study, Lipid Peroxide (LPO), Protein Peroxide (PPO), the mobilization of the thyroid hormones (T3, T4 and TSH) and the histopathology of gills and hepatopancreas were studied. The study's main objective was to evaluate the impacts of coal effluents on the

histomorphology of the *Channa punctata* Bloch fish species.

MATERIALS AND METHODS

The brick kiln disposals, i.e. burnt and partially burnt coals, were collected from neighbouring localities of the brick kilns at Geramari Pt-III (Lat. 26.101682^0, Long. 80.997406^0) and Dumardaha Pt-II (Lat. 26.090333^0, Long. 89.921255^0) of Dhubri District to make a standard brick kiln effluent for the experimental purpose. These were first sun-dried and then finely grinded using mixer-grinder. Then some experiments of effluent preparation were done by mixing this powder with various amounts of deionized water, kept for a week and then filtered separately. From these effluents, the g/ml (conc. 1000 ppm/L) concentration of effluent was used for the experimental purpose with required dilutions.

The fish model i.e. *Channa punctata* Bloch. of approximately 85 ± 5 grams weight and 16.5±5.7 cm length were collected from the local market and the water bodies near the brick kilns. The fish species were treated in 1.5% KMnO4 solution and further acclimatized for 1 week. The fish model was fed with commercial food marketed as "Dr. Fish" and manufactured by Sai Tirupathi Aqua PVT. LTD. It contains several ingredients like mini shellfish, baby shells, yeast, wheat flour, wheat germ, egg powder, soybean meal, Spirulina, the larva of a fly, vegetable powder, vitamins and several minerals having crude 38% protein, 3% crude fat, 8% crude fibre, 16% crude ash, 10% moisture 4.5% calcium, 1.5% lysine, 1.5% total phosphorus as per the packet information from the manufacturer.

LC_{50} value was determined in the fish model using the brick kiln effluent following OECD Guidelines (OECD, 2019) and was found to be 55±0.53 ppm/L. After estimating the LC_{50} value, the fishes were segregated using three aquaria viz. Aquarium-I contains the normal control group of fishes, and the further two aquaria, i.e. Aquarium-II and Aquarium-III, containing fishes exposed to two sub-lethal doses, i.e., 2ppm/L and 3ppm/L of prepared brick kiln effluents respectively. After a considerable period of 4 weeks (28 days), the Fish were sacrificed using Diethyl Ether anesthetization. The tissues of the study animal, the gills and hepatopancreas, were dissected and washed in normal saline and preserved in deep freeze in separate labelled Eppendorf tubes. After keeping parts for histological preparation, the measured amount of tissue samples was homogenised using a fixed amount of deionised water. After homogenization these were centrifugation at 5000 rpm and the supernatants were further collected for enzymatic and hormonal assays. The biochemical assays were performed within few hours from the extraction of the tissues.

The estimation of lipid and protein peroxide in the tissues viz. gills and hepatopancreas was done by the photometric evaluation of molar extinction co-efficient of thiobarbituric acid. (Ohkawa, & Ohishi & Yagi, 1979).

Aspartate Aminotransferase (AST) activities in the gills and hepatopancreas was studied by reagent kit based on UV-Kinetic Assay techniques (Bergmeyer, Scheibe, & Wahlefeld, 1978). The Alanine Transaminase activities (ALT) in the preferred organs were studied using an ALT reagent kit (IFCC/Kinetic) (Bergmeyer and Holder, 1980). The Alkaline Phosphatase (ALP) activities in gills and hepatopancreas were also estimated by utilizing the ALP reagent kit (GSCC/Kinetic) (Bretandiere et al., 1977).

The measurement of the thyroid and thyroid-stimulating hormones, namely T_3 (Chopra, 1977), T_4 (Chopra, & Solomon, & Ho, 1971) and TSH (Spencer et al., 1995) in the gills and hepatopancreas of the fish species using the Enzyme Immuno Assay Technique.

All the biochemical assays and the photometric analysis were programmed in a semi-automated biochemistry analyser (“Benesphera C-61” manufactured by Benesphera - Avantor Performance Materials India LTD.). The ELISA reader ("Benesphera E-21” manufactured by Benesphera - Avantor Performance Materials India LTD.) was aided for several readings in the ELISA well plates.

The machine was pre-programmed with beneficiary kit specifications and dilution factors. All reagents were procured from Benesphera - Avantor Performance Materials India LTD.

For histological studies, the preparation and processing of tissues, i.e. the gills and hepatopancreas, was done by Bernet Method (Bernet et al., 1999). After sacrifice of the animal, the tissues of both the normal control and treated fishes were collected and fixed in 8% formal-saline. For dehydration procedures, the tissues were passed in several grades of alcohol, and after clearing them in xylene, the tissues were embedded in paraffin. Using rotary microtome, sagittal sections of 5µ thickness was mounted on glass slides. Xylene was utilized for de-paraffinization and the tissue-sections were hydrated by passing through several grades of alcohol. After successful hydration, the tissues were stained in double stained with haematoxylin and eosin stains in between dehydration-rehydration-dehydration process. The stained sections were studied using the Almicro Trinocular Research Microscope, and the images were captured in a Nikon D5300 DSLR Camera Body using Olympus Microscope Adaptor. Required labelling of the images was done with the help of MS Paint.

RESULTS

Table-1: Lipid peroxide, protein peroxide, transaminase activities and amount of thyroid hormones in gills and hepatopancreas of experimental fishes

Study Parameters	Experimental Fish Groups		
	Group-I Normal-Control Group	Group-II Fishes exposed to 20ppm/L of brick kiln effluents	Group-III Fishes exposed to 30ppm/L of brick kiln effluents
Lipid Peroxide in Gills (nmol/mg)	204.45 ± 0.137	227.48 ± 0.227 + 11.264 % *	249.76 ± 0.094 + 22.162 % *
Lipid Peroxide (LPO) in Hepatopancreas (nmol/mg)	189.26 ± 0.163	217.35 ± 0.205 + 14.842 % *	224.93 ± 0.164 + 18.847 % *
Protein Peroxide (PPO) in Gill (nmol/mg of Protein)	8.464 ± 0.005	9.815 ± 0.005 + 15.962 % *	11.327 ± 0.006 + 33.826 % *
Protein Peroxide (PPO) in Hepatopancreas (nmol/mg of Protein)	11.425 ± 0.006	13.546 ± 0.007 + 18.565 % *	14.943 ± 0.007 + 30.792 % *
AST (GOT) activity in gills (IU/L)	373.68 ± 0.339	352.78 ± 0.263 -5.593% *	317.4 ± 0.351 -15.061% *
AST (GOT) activity in hepatopancreas (IU/L)	565.44 ± 0.452	522.14 ± 0.357 -7.658% *	475.66 ± 0.404 -15.878% *
ALT (GPT) activity in gill (IU/L)	187.16 ± 0.323	168.02 ± 0.180 -10.226% *	132.55 ± 0.218 -29.178% *
ALT (GPT) activity in Hepatopancreas (IU/L)	276.63 ± 0.361	258.44 ± 0.233 -6.575% *	219.22 ± 0.260 -20.753% *
ALP activities in Gill (IU/L)	534.56 ± 0.140	507.37 ± 0.137 - 5.086 %*	459.44 ± 0.208 - 14.052 % *
ALP activities in Hepatopancreas (IU/L)	727.19 ± 0.122	694.18 ± 0.087 - 4.539 % *	667.18 ± 0.260 - 8.252 % *
Amount of TSH in Gills (IU/mg)	0.237 ± 0.0006	0.256 ± 0.0009 +7.550 % *	0.269 ± 0.0006 +13.381 % *
Amount of TSH in Hepatopancreas (IU/mg)	0.256 ± 0.0001	0.275 ± 0.0007 +7.550 % *	0.304 ± 0.0011 +19.092 % *
Amount of T_3 in Gills (ng/mg)	0. 633 ± 0.0006	0. 633 ± 0.0006 - 18.945 % *	0.448 ± 0.0007 - 29.167 % *
Amount of T_3 in Hepatopancreas (ng/mg)	0.816 ± 0.0006	0.762 ± 0.0008 - 6.614 % *	0.625 ± 0.0007 - 23.457 % *
Amount of T_4 in Gills (ng/mg)	11.946 ± 0.006	8.341 ± 1.200 - 30.178 % *	8.541 ± 0.009 - 28.503 % *
Amount of T_4 in Hepatatopancreas (ng/mg)	13.185 ± 0.006	11.509 ± 0.065 - 12.711 % *	8.939 ± 0.006 - 32.203 % *
"*" indicates Significant at p<0.05, "+…%" and "-…%" indicate percent increase and percent decrease respectively.			

Histological Alternations in the Gills-

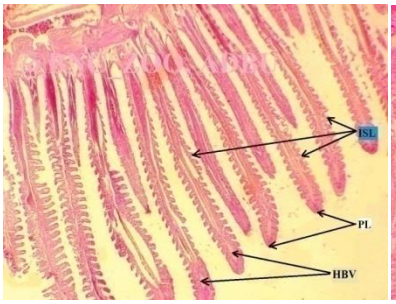

Fig. 1 Histomorphology of gill of Fish of Normal Control Group

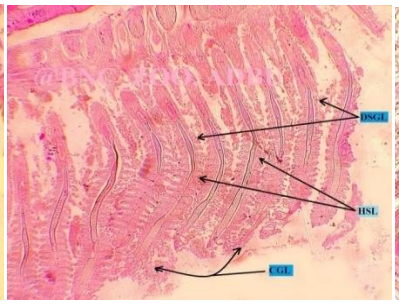

Fig. 2 Histomorphology of gill of fish exposed to 2ppm/L of brick kiln effluent

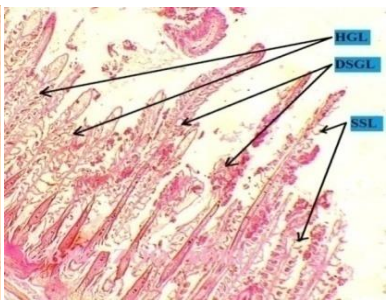

Fig. 3 Histomorphology of gill of Fish exposed to 3ppm/L of brick kiln effluent

PL = Primary Gill Lamellae, **ISL** = Intact Secondary Gill Lamellae, **HBV** = Healthy Blood Vessels, **DSGL** = Degenerated Secondary Gill Lamellae, **HSL** = Hypertrophy of Secondary Gill Lamellae, **CGL** = Curving of Gill Lamellae, **SSL**= Spiked Secondary gill Lamellae

Histological Alternations of Hepatopancreas-

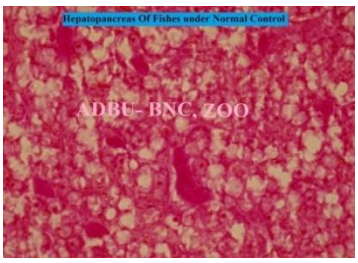

Fig. 4 Histomorphology of hepatopancreas of Fish of Normal Control Group

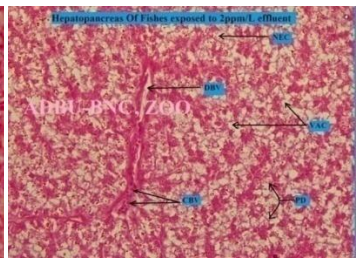

Fig. 5 Histomorphology of hepatopancreas of Fish exposed to 2ppm/L of effluent

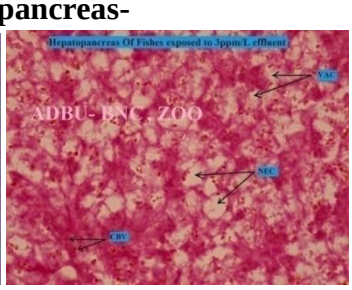

Fig. 6 Histomorphology of hepatopancreas of Fish exposed to 3 ppm/L of effluent

NEC = Necrosis, **DBV** = Dilated Blood Vessels, **VAC** = Vacuolation, **CBV** = Congestion of Blood Vessels, **PD** = Patchy Degeneration

DISCUSSION

Lipid peroxide in gills increased with deviations of +11.264% and + 22.162%, respectively, from normal control values on treatment with 2 ppm/L and 3 ppm/L of the brick kiln effluents. **I**n hepatopancreas, this was elevated by +14.842% and +18.847% on treatment with 2 ppm/L and 3 ppm/L of brick kiln effluents, respectively. Protein peroxide in the gills was observed to be increased by +15.962% and +33.826% on treatment with 2 ppm/L and 3 ppm/L of brick kiln effluent, respectively. In the hepatopancreas, this was increased by +18.565% and +30.792% on treatment with 2 ppm/L and 3 ppm/L of the brick kiln effluents, respectively. AST activities in gills were observed to be decreased by -5.593% and -15.061% on exposure to 2ppm/L and 3ppm/L of brick kiln effluent, respectively. In hepatopancreas, AST activities were observed to be declined by -7.658% and -15.878% on exposure to 2ppm/L and 3ppm/L of brick kiln effluent, respectively. ALT activities in the gills were noted to decline by -10.226% and -29.178% on exposure to 2 ppm/L and 3 ppm/L of effluents of brick kiln refusals, respectively. It was found to be decreased by -6.575% and -20.753% in hepatopancreas on exposure to 2 ppm/L and 3 ppm/L of effluents of brick kiln refusals, respectively. ALP activities in the gills were reduced by -5.086% and -14.052% on exposure of 2 ppm/L and 3 ppm/L of effluents from brick kiln refusals, respectively. In hepatopancreas, this was dropped by -4.539% and -8.252% on exposure of 2 ppm/L and 3 ppm/L of effluents from brick kiln refusals, respectively. The TSH level in the gills was elevated by +7.550% and +13.381% on exposure of 2 ppm/L and 3 ppm/L of effluents from brick kiln refusals, respectively. In hepatopancreas, this was increased by +7.550% and +19.092% on exposure of 2 ppm/L and 3 ppm/L of effluents from brick kiln refusals, respectively. The T_3 level in the gills was noted with a declination of -18.945% and -29.167% on treatment with 2 ppm/L and 3 ppm/L of brick kiln effluents; and in the gills its was marked with a reduction of -6.614% and -23.457% on treatment with 2 ppm/L and 3 ppm/L of brick kiln effluents respectively. The T_4 level in the gills was observed to be decreased by -30.178% and -28.503% on treatment with 2 ppm/L and 3 ppm/L of brick

kiln refusals, respectively. In the hepatopancreas, this was observed to be decreased by -12.711% and -32.203% on treatment with 2 ppm/L and 3 ppm/L of brick kiln refusals, respectively.

Curving of the gill lamellae and its fusion were observed in the fishes exposed to 2ppm/L of brick kiln effluents. Hypertrophy of the secondary gill lamellae also marked in several places. Degeneration of the secondary gill lamellae with spiked gill filaments and dilation of the blood vessels with the destruction of cartilages in the primary gill filaments were observed in fishes exposed to 3ppm/L of brick kiln effluents.

Lesions or atrophy of the hepatopancreas with progressive degeneration were observed in Fish exposed to 3ppm/L, with serious vacuolation, necrosis of the hepatocytes, patchy degenerations, and blood congestions at several places accompanied by some dilation in the blood vessels.

CONCLUSION

The elevation in lipid and protein peroxide activities clearly signifies the oxidative damages to the tissues of the treated fishes. Decreased activities of AST, ALT, and ALP in the tissues are due to huge-scale cell damage, for which these enzymes were insufficient for the transamination of essential amino acids to non-essential ones. Another important transaminase, the phenylalanine hydroxylase, has a key role in the transamination of tyrosine from phenylalanine (National Research Council, 2011; Udenfriend and Cooper, 1952). There is the highest possibility of loss of phenylalanine hydroxylase activities in the tissues of the treated fishes for the cell destruction for which deficiency of tyrosine content in the cellular pool. The hypothyroidic conditions observed in the tissues of the treated fishes are due to the less production of thyroid hormones, i.e., T_3 and T_4, as tyrosine is one of these precursors. (Hadley and Levine, 2009; Zhang et al., 2023). The thyroid hormones have a great role in promoting cell division. Their insufficiency results in insufficient nascent cell production in the healing of damaged tissues, causing ill growth and development and even death of the exposed fish.

The study thus conducted clearly reveals the fact that the brick kilns are not only the air polluting agents but also it causes several drastic impacts upon the aquatic system. The Fish *Channa punctata* Bloch is a resistant species, but its survival is also doubtful due to the worst impact of these kilns. So, if necessary measures are not taken by the Pollution Control Board, the Government, or certain NGOs, we may lose several vulnerable species from being extinct. So the study is conducted to draw the attention of these agencies and the laymen as well.

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Conflict of Interest:
There is no such evidence of conflict of interest.

Author Contribution:
All authors have participated in critically revising the entire manuscript and approving the final manuscript.

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