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Organochlorine pesticide residues in some selected wetland sediments and occurrence of hepatic neoplasm in air breathing fish from North Bihar, India

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Abstract : Concentration of organochlorine pesticides (OCPs) were determined in liver sample of air breathing fish mangur (*Clarias batrachus*) and soil from few selected wetlands of North Bihar particularly Supaul and Saharsa district. Soil and fish were collected from the various test zones for the assessment of organocholrine pesticide residues. Various organochlorine group of pesticide incurred were αHCH, βHCH γHCH, Endosulfan DDT and DDE etc. HCHs, Aldrin and Endosulfan were the dominant contaminants suggesting their current use in neighbouring agricultural areas. Fishes liver and soil sample from the reference site showed almost negligible percentage of these organochlorine groups of pesticides. Simultaneous screenings of fish liver were made to see the risk of occurrence of pre neoplastic and neoplastic liver lesion based upon histopathological findings. The histo-architecture of liver of *C. batrachus* from the test zone (B) had a number of necrotic changes and enlarged perisinusoidal areas, increased eosinophilic inclusion, pyknotic and heterochromatized nuclei. Vacuolation refers to the Initiation of pre-neoplastic changes occurring in liver. Vacuolar degeneration, Karyomegely, fibrosis of central vein, focal vacuolation and multi-focal hemosidorosis and occurrence of apopotic bodies from site A. Pronounced feature of ductular metaplasia of hepatocyte leading to neocholangiolar structure from site C.

Key Words: Orgalochlorine pesticides, Fish, Liver cell.

INTRODUCTION

The fish as a bioindicator species plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to change in the aquatic environment (Mayer *et al*, 1991; Moor 1991).[1,2] The sudden death of fish indicates heavy pollution which can be measured in terms of biochemical, physiological or histological response (Sounders 1969, Dutta, *et al.* 2003)[3,4]. In aquatic environment pesticide undergo a biotic degradation by hydrolysis, and enter in aquatic organism directly through gill or epithelial tissues. The harmful chemicals accumulate in specific organs and then get biomagnified. Fishes take up most of the xenobiotics from the surrounding water by passive diffusion through gills or gastro-intestinal tract. After uptake the chemical are transmitted and deposited in the fatty portion of the tissues (Kumari *et al* 2001)[5]. Liver is the target organ, which not only resists the deleterious effects of pesticides but also detoxifies it. Entering to an organism xenobiotic bind to specific cellular structure called receptor that is localized on the cell surface or inside the cell either in its cytoplasm or on cell organelles (Yamaguchi *et al.* 2003)[6]. Many reports are available for the study of pesticide residues in wetland (Kumari *et al.*2000)[5] and its impact on various organs Muir (2003)[7],Carla (2004)[8],Couch(1993)[9], Rawat *et al.*(2002)[10] but a

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systematic approach regarding prevalence of organochlorine pesticides in soil & fish liver sample from wetlands of North Bihar are meager. Thus present investigation a few wetland test zones of North Bihar have been selected to estimate organochlorine pesticides residues in soil and liver of fish and occurrence of neoplasm.

MATERIALS AND METHODS

Clarias batrachus were captured by local fisherman from the three test zone wetlands in around Saharsa and Supaul District of Bihar viz – Chitragupta Mandir Chaur – (A), Gramharnia Chaur (B) and Hardi chaur (C) and screened to find out the neoplastic and pre-neoplastic liver lesions. Approximately 200 fish adult/ female were collected and screened (Table – I) and collected from these wetlands. Unhealthy fishes were dissected on spot and liver tissues were dissected out for the estimation of pesticide residue and histopathological analysis. Among the fish haul, healthy *Clarias batrachus* of 14-27" length and 50-110 gm±10 gm wt were brought to the laboratory and after disinfections with 0.1% $KMNO_4$ solution they were kept at room temperature in large plastic pool for acclimatization. They were fed with pelleted food made up of wheat flour and egg with a pinch of starch as binder @ 4-5% of their body weight. They were also fed with chopped goat liver on every 3rd day to fulfill their dietary requirement.

Quantification of pesticide residues in soil and fish liver

Soil sediments were collected at the four edges of each wetlands/chaur using a spade. The entire samples were sealed in polythene bags, storing at 0°C and transported to the laboratory within two days. Freeze dried sample were then passed through 1.0 nm sieve to separate sample and other debris. After crushing with anhydrous sodium sulphate powdered soil sample were further processed in n-Hexane along with processed liver sample send to IITR, Lucknow for estimation of organochlorine pesticide residues (Text graph- I & II).

Histopathological analysis

The liver tissue of fishes from reference site and those of various test zones were fixed for light and electron microscope. For light microscope, tissues were fixed in neutral formalin, and processed as per routine method in the laboratory. Photographs were taken on Trinocular Microscope (Labomed CXR III) fitted with Olympus digital 14 megapixel camera. Light photomicrogrph of liver cell, is depicted in Plate-I.

RESULTS AND DISCUSSION

The liver of normal fish from reference site has continuous mass of hepatic cells with cord like formation. The cells were large with more or less centrally placed nucleus and homogenous cytoplasm. Clear division of hepatic cells into lobules has not been observed in most of the hepatic cells. Hepatocytes were intact with dense cytoplasm. Architecture of hepatic artery was very distinct. Sinusoidal spaces were well organized and opened into central vein (Fig-1)

Few or no lesion was observed in the tissue of fish taken from reference site and site A except some vacuolar degeneration, Karyomegely, fibrosis of central vein, focal vacuolation and multi-focal hemosidorosis. (Fig.-2).

The histoarchitecture of liver of *C. batrachu.s* from the test zone (B) wetlands had a number of necrotic changes and enlarged perisinusoidal areas, increased eosinophilic inclusion, pyknotic and heterochromatized nuclei, and extensive vacuolation (refers to the Initiation of pre-neoplastic changes) in liver (Fig- 3).

Few histological examination of liver tissue from test zone C also revealed re-organization of liver tissue, characteristic of micro and macro-nodular cirrhosis. Pronounced feature of ductular metaplasia of hepatocyte leading to neocholangiolar structure. Hepatocyte tending to form rosette with the bile canaliculi located in the center (Fig.-4).

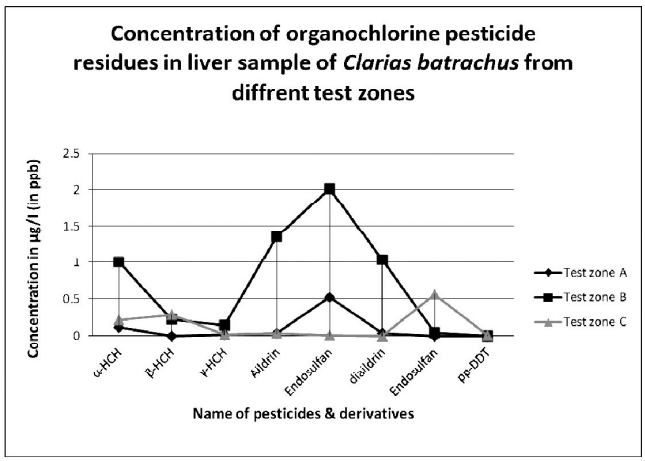
A comparative analysis of pesticides in soil and fish liver shows accumulation of various organochlorine pesticides viz. αHCH βHCH γHCH , aldrin, endosulphan, DDE, DDT etc. the presence of αHCH in soil varies from1.117±0.002µg/l to 0.147±0.017µg/l. Aldrin was ranging between 0.04±0.042 µg/l to 0.341±0.005µg/l. Estimation of organochlorine pesticide residue in fish liver collected from different test zone showed the variation in the accumulation of αHCH βHCH γHCH , aldrin and DDT whereas fishes collected from reference site showed almost negligible percentage of organochlorine pesecticides. Presence of pesticides in fish liver reveals

the persistent use of pesticides which has also been reported by Halden (1965)[11]. Mass mortality and behavior of brook and atlantic salmon on stream polluted by agricultural pest (Saunders, 1969)[3]. Pesticidal poisoning in fish is considered to be very serious, as fish forms a major food resources for mankind affecting the consumers health (Dubois,1971)[12] and may also adversely affect the yield of yeast. Presence of pesticide residues in liver tissues in the present investigation can be correlated with the reports of (Yamaguchi,2003)[6], in the muscles of fish from Upper Thames river. Muir *et al* (2003)[7] have also observed DDT, HCH & PCBs in fishes from Barents sea Canadian Arctic. Occurrence of neoplasm and other degenerative changes in the present investigation, have also well documented about the pathogenesis of liver lesion with anthropogenically introduced contaminants Robert *et al* (1991)[13]. Mayers *et al* (1992)[1] have shown that hepatic neoplasm as biomarkers of contaminant exposure in fish. They have also measured fluorescent aromatic compounds in Bile and Polychlorinated biphenyl PCBs. Carla M. *et al* (2004)[8] have examined fish liver for toxicopathic lesion and analyzed for selected chlorinated hydrocarbon such as PCBs, DDTs,and di-aldrin.George *et al* (1996)[14] have shown Rainbow trout liver as an alternative model for environment carcinogenesis research. Parallal diagnosis of cell and tissue pathologies in *c. batrachus* liver showed that lysosomal per turbation sensitivity reflected onset of progression of liver injury comprising focal to extensive necrosis and fibrosis, as indicated by highly significant correlation between the breakdown of lysosomal stability and degree of liver lesion. Injury of lysosomal membrane by lypopholic toxic compound may lead to leakage of the hydrolytic lysosomal enzyme causing disturbance of cell function, resulting in degeneration and possibly neoplasm (Moore 1985). Further (Moore *et al* 2007)[15] very well illustrated about the hepatocellular neoplasm in adult winter flounder from Boston Harbour, as in the present investigation Mark *et al* (2007)[16] have studied the progression of hepatic neoplasia in medaka exposed to diethylnitrosamine. Couch (1993)[17] have very well compared about neoplastic hepatocyte with normal one under Light and Electron microscopy. He has also well documented the hepatocellular carcinomas in teleost fish. Moore et al (1991)[2] in their studies have used the cellular marker of pollutant exposure and liver damage in fish. Donald *et al* (1984)[18] have shown the effect of chemical pollutants poses stress in bottom dwelling fish and these are more prone to liver neoplasm and other diseases. Angela *et al* (1992)[19] have shown the histochemical and cytochemical indices of toxic injury in the liver of dab *Limanda limanda*. In all the wetland test zones, test zone B seems to be the most toxic followed by test zone C and test zone A (Reference site) as shown in the text graph (I, II). Organochlorine pesticide accumulation in soil and fish liver and occurrence of liver anomalies itself confirm it. It may be concluded that the bottom dwelling air breathing *Clarias batrachus* from these wetlands is being worst sufferer by the accumulation of organochlorine pesticides and other pollutants. Fishes are affected with a variety of multiple, co-occurring hepatic lesion including unique degenerative condition, putatively pre-neoplastic foci of cellular alteration and neoplasms. These lesions represent morphologically identifiable step forming sequence of progression ultimately leading to the development of neoplasm (confirmed by laboratory experiment). This progression sequence paralleled the pattern identified in experimental model of chemically induced hepatocarcinogen in fish. Since very little or no work has been carried out previously in this region the present data could serve as base line data, although only three test zones were selected. However, the data obtained could not fully represent the environmental condition on wetlands and fishes in the North Bihar. More detailed investigations in terms of sampling sites and fishes are therefore necessary.

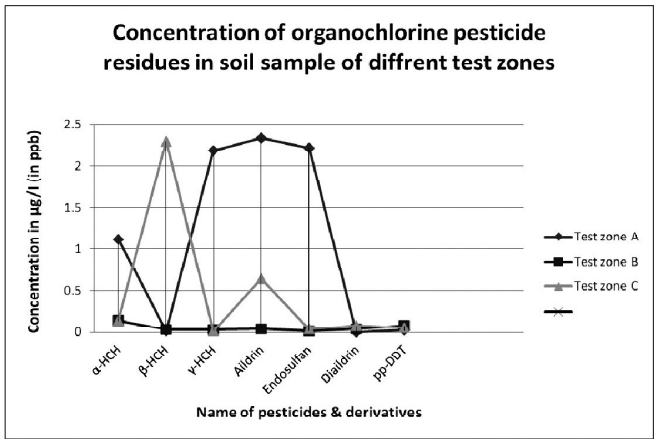
Table – I: Resume of the test fish *Clarias batrachus* surveyed from different test zones after external and internal screening during 2011-2012

Type of the ova	Test Zone A	Test Zone B	Test Zone C
Skin			
Skin lesion	5%	10%	1%
Skin tumor	-	2%	1%
Ulcerative spot	-	15%	50%
Degeneration	5%	10%	10%
Liver			
Necrosis	-	2%	2%
Loss of cell limit	5%	15%	5%
Cytoplasm density	-	10%	5%
Melanomacroph10%	10%	10%	5%
↑ in bile duct	-	5%	2%
Ovary			
Ovarian tumor	-	-	-
Testis			
Tumor	-	-	-

(-) represent absence of lesion for the approx. 200 individual studied.



Text Graph- I



Text Graph-II

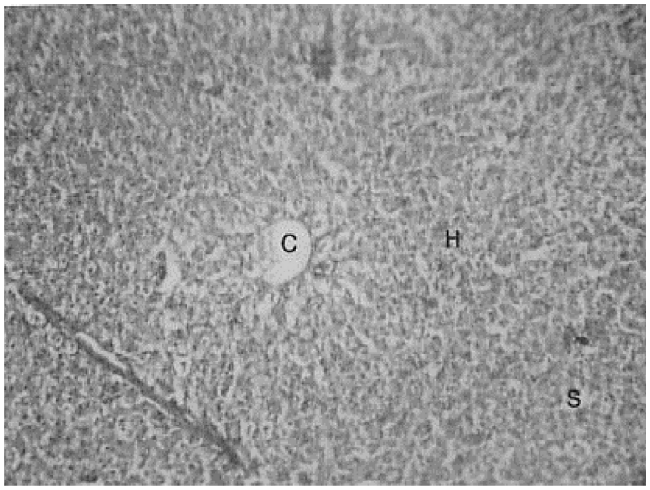


Fig-1

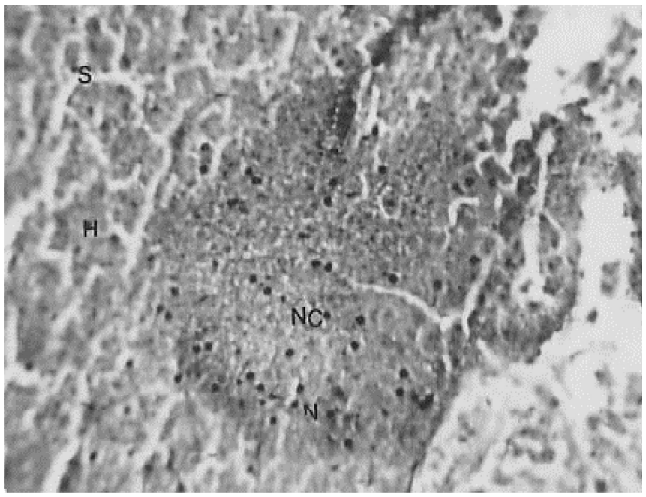


Fig-2

Fig-3

Fig-4

Photo micrograph of *Clarias batrachus* liver. fig-1 showing normal liver parenchyma with distinct central vain and hepatic cords. Fig-2 Fish liver from test zone A with degenerated parenchyma at portal tried fig- 3 Fish liver from test zone B showing nodular cirrhosisFig.4.Fish liver from Test zone C ,bile duct leading to neocholangiolar structure.c X 200.

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