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Effect of Urea & DAP on Histological Parameters of *Anabus testudineus* (Kabai) Fish in rice field and ponds of Nabhata, Saharsa district

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ABSTRACT

The acidity of mine water generally makes it toxic to most organisms. The Gills, kidneys and livers of Anabas testudineus Bloch fish inhabiting paddy field in rainy season and ponds of Nabhata, Saharsa, Bihar, were examined and compared to those of farmed fish. Tissue abnormalities were found in all investigated organs. Deterioration of gill filaments was found. Liver tissue revealed hemorrhages, blood congestion and necrotic cells with mononuclear cell infiltration. In addition, hypertrophy of the epithelial cells of the renal tubules with reduced lumens, aneurisms of the renal tubules, and contractions of the glomeruli in the Bowman's capsule were observed. These histopathological findings suggest the some fertilisers like D.A.P and Urea in this habitat causes severe damage to the internal organs of fish and consequently alters their physiological status. Since the water in this pond is utilized by local people, these findings highlight the need for adequate water treatment.

1. INTRODUCTION

Paddy is cultivated in major portion of land in North Bihar where water is lodged about 3 to 4 months. The fish, *Anabas testudineus*, is the usual inhabitants of such water lodged areas. This fish have to face the unfavourable situation caused due to fertilizer toxicants of climbing perch fish (*Anabas testudineus*) inhabit this water. The climbing perch, *Anabas testudineus* (Bloch), also popularly known as *'Kabai'* is a well known air breathing edible fish, inhabiting fresh waters and brackish waters. The common name, climbing perch, originated from the Asian legend that Anabas climbs palm trees to suck juice. Probably the origin of this myth is that birds pick Anabas when they travel overland and place it on palm trees (Norman, 1975). Recently some related works have been initiated in our country as well (Saxena, 1973; Srivastava *et al*., 1977, Pandey and Shukla, 1980 and Shukla, 1982). Although, the extent of toxicity of fish by the toxicants is usually difficult to predict, yet toxicologist and environmental biologist have seriously felt the need of knowledge for the determination of an appropriate concentration which may be toxic to the fish in particular and in other animals in general. Because of direct and continuous contact with the environment, fish gills, organs for respiratory gas exchange, osmoregulation, excretion of nitrogenous waste products and acid-base regulation, are directly affected by contaminants. Histopathological features of the liver, ovaries, skeletal system and skin of organisms exposed to one or more toxins have been used as biomarkers (Hinton *et al*, 1985). Fish diseases and pathologies have also been used as indicators of environmental stress (Matthiessen *et al*, 1993). The ecological effects of aquatic pollutants are related to species distribution (Mosisch and Arthington, 2000). This study investigated the histological alterations caused by environmental contaminants in the gills, liver and kidneys of *A. testudineus* inhabiting Nabhata area,Saharsa district Bihar.

2. MATERIAL AND METHODS

The fish, *Anabas testudinues* (Bloch), belonging to the family Anabantidae of the order Perciformes, locally known as (kai) having the presence of suprabranchial accessory respiratory organs, were selected for the present study. Mostly *Anabas testudineus* locally pronunciated in Koshi zone as 'Kabai'. The fish were procured live from the local non-polluted water river of Koshi (Bihar) with the help of fishermen. They were brought to the laboratory in wide mouthed large earthen pots half filled with natural water and covered with mosquito net. Every effort was taken to give least stress to the fish during transportation. They were first thoroughly washed and then rinsed in 0.1% $KMnO_4$ solution to remove any sort of dermal infections. The fish were not supplied any food for the first three days of acclimation. They were adjusted to natural photoperiod and ambient temperature. Running tap water used in all the experiments and no aeration was done. They were fed with chopped goat liver every day and libitum at 11:00 A.M. sharp. The feeding was stopped 24 hr before the start of experiment (Static acute bioassay) and no food was supplied to them during the period of experimentation. However, the feeding schedule was strictly followed during chronic experiments to avoid starvational effects. The physic-chemical characteristic of water were analysed following the methods of APHA, AWWA and WPCF (1985). The pH was recorded by an electronic pH meter (Hana Hongkong). The fish were held in diluents water for at least one day before the start of a test. Exposure units were static and not aerated during the 96 hours test. The Urea and D.A.P. concentration were in logarithm series. The healthy and well-acclimatized fish were transferred one by one with the help of small hand net from the acclimatizing aquaria to the experimental ones. All the precautions as suggested by APHA, AWWA and WPCF (1985) were exposed to tap water simply. Assays were terminated and the results discarded if and when the control mortality exceeded 10% in addition, all promptness was taken to remove dead fish immediately upon discovery as

they might have depleted the dissolved oxygen of the solution and affected the tolerance of other fishes in paddy field. The liver, gills and kidneys were sectioned 1-2 mm, in thickness and fixed in 10% formalin and processed according to standard paraffin procedures. Five micrometer thick paraffin sections were placed on glass slides and stained with hematoxylin and eosin. Approximately 2-4 paraffin sections of each fish were histologically analyzed under a light microscope. Histopathological changes in these tissues were recorded and compared with controls under the guidance of a pathologist. The pathological changes in the gills of the fish from the unused lignite mine were observed. The most common gill changes were desquamation in secondary lamellae, lifting of the lamellar epithelium and telangiectasia (Fig 1b), but no gill abnormalities was observed in the gills of the control farmed fish (Fig 1a).

3. RESULT

The pathological changes in the liver tissue of a fish inhabiting in pond and paddy fields are shown in fig.2. Hemorrhage, blood congestion and necrotic cells were generally observed in the liver tissue. Mononuclear cell focal infiltration was observed (Fig 2b). There were no abnormalities found in the livers of the control fish (Fig 2a). The lesions in the kidneys of the fish exposed to acidic water in the unused lignite mine included degeneration of the epithelial cells of the renal tubules, degeneration of the glomeruli, hypertrophy of the epithelial cells of the renal tubules narrowing of the tubular lumen and glomerular contraction in the Bowman's capsule (Fig 3a). These were not observed in the kidneys of the farm fish (Fig 3b). Recently some related works have been initiated in our country as well (Saxena, 1973; Srivastava *et al*., 1977, Pandey and Shukla, 1980 and Shukla, 1982). Although, the extent of toxicity of fish by the toxicants is usually difficult to predict, yet toxicologist and environmental biologist have seriously felt the need of knowledge for the determination of an appropriate concentration which may be toxic to the fish in particular and in other animals in general. For such studies, several bioassay methods have been adopted in practice. Acute toxicity tests are the most accepted methods to determine the toxic level of a chemical on the test organism in a specific time. Death is an easily detectable and obviously most important adverse effect; as such the most common toxicity test is the acute mortality test. Based on this fact, several workers have determined toxic levels of various chemicals including fertilizers in the form of LC50 values (concentration lethal to 50% of the test organisms). LC50 values are quick and useful device for determining the toxicity of a particular pollutant to a particular species under specific physio-chemical condition (Shaw *et.al*; 1990 and Chaudhary, 1991).

4. DISCUSSION

Study of the gills in the control fish showed a typical structural organization of the lamella. Fish exposed to acid water had several histological alterations, namely desquamation of lamellar epithelium, fusion of the lamellae and lamellar aneurisms. In principle, as long as there is enough water in a rice field, it can serve as a fish culturing system. However, a rice field is by design intended for rice and therefore conditions are not always optimum for fish. At the most basic level is the fact that rice does not necessarily need standing water at all times to survive. Rice can be successfully grown in saturated soils with no standing water (Singh *et al*., 1980), and recent evidence on the system of rice intensification suggests that intermittent irrigation may increase rice yields. However, even with a continuously standing column of water, a flooded rice field is not necessarily an ideal place for growing fish. The water temperature can reach very high levels.

The gill abnormalities observed in this present study were similar to previous studies of low environmental pH on fish gill morphology, which showed separation of the epithelial layers of secondary gill lamellae, deformation of secondary lamellae and degeneration of chloride cells accompanied by hyperplasia of undifferentiated cells in the primary lamellae (Daye and Garside, 1976; Chevalier *et al*, 1985). Acidification of fresh water is usually associated with aluminum erosion from the substrate (Muniz and Leivestad, 1980). This metal has been reported to increase mucus secretion (Hendy and Eddy, 1989), which increases the blood-gas diffusion distance, resulting in a reduction in gas exchange (Ultsch and Gros, 1979). Heavy metal compounds associated with acidification have also been associated with a reduction of both carbonic anhydrase and Na+-K+ ATPase activities in salmonids, even at a relatively high pH (pH 5.0) (Staurnes *et al*, 1983). The acid stress also interferes with Na+/H+ exchange and Cl-/HCO3- exchange, thus, inhibiting Na+ and Cl- uptake (Saunders *et al*, 1983). Consequently, the fish dies of heart collapse because it cannot pump the more viscous blood due to sodium and chloride loss from the gills (Bulger *et al*, 2000). The lamellar fusions are defense mechanisms that reduce the branchial superficial area in contact with the external surroundings. These mechanisms also increase the diffusion barrier to the pollutant (Laurén and McDonald, 1985; Van Heerden *et al*, 2004). As in higher vertebrates, the kidneys of fish perform an important function relate to electrolyte and water balance and the maintenance of a stable internal environment. Following the exposure of fish to toxic agents, histological alterations have been found at the level of the tubular epithelium and glomeruli (Teh *et al*, 1997). Ortiz *et al* (2003) found kidneys of fish receive the largest proportion of post-branchial blood, and therefore renal lesions may be good indicators of environmental pollution. The lesions in the kidneys of *A. testudineus* exposed to acidic water in this study reveal the same patterns as previous studies. The kidney is a major site for toxic effects due to a wide variety of environmental pollutants (Foulkes and Hammond, 1975; Hook, 1980). Because of water re-absorption taking place in the distal tubules, relatively high concentrations of toxins may have an effect on renal cells. The renal contents may become acidified in some renal segments, which may provide an interaction with toxic substances. A higher level of H+ ions also affects kidney function, such as glomerular filtration and tubular

Reabsorption. Renal tubular cells contain a variety of transport enzyme systems, such as carbonic anhydrase, Na+-K+ATPase, Na+/ H+ exchange and Cl-/HCO3- exchange (Seldin and Giebisch, 1983). The acid water caused alterations of the liver parenchyma, such as vacuolization and necrosis. These alterations are often associated with a degenerative-necrotic condition (Myers *et al*, 1987). Several studies have shown a variety of changes in the liver of *Oreochromis niloticus* resulting from exposure to different toxic chemicals (Visoottiviseth *et al*, 1999; Figueiredo-Fernandes *et al*, 2006a, b). Several studies have reported that chronic accumulation of some heavy metals in fish livers causes hepatocyte lysis, cirrhosis and eventually death (Pourahamad and O'Brien, 2000; Varanka *et al*, 2001). Some heavy metals accumulate in the food chain, causing long term health effects to aquatic life and

eventually to humans (David, 2002; Cotter and Brigden, 2006). Since the water in this pond is utilized by local people, data from this study

highlights the need for water treatment. In the present study pathological changes of the gills, kidneys and liver were associate acidic water. Fish gills, kidneys and livers may serve as biomarkers for toxicity due to low environmental pH. The pathological changes observed in the gills, kidneys and Liver of *Anabas testudineus* fish in the present study were associated with water resulting from mining activities. Complementary studies are needed for further evaluation of this problem.

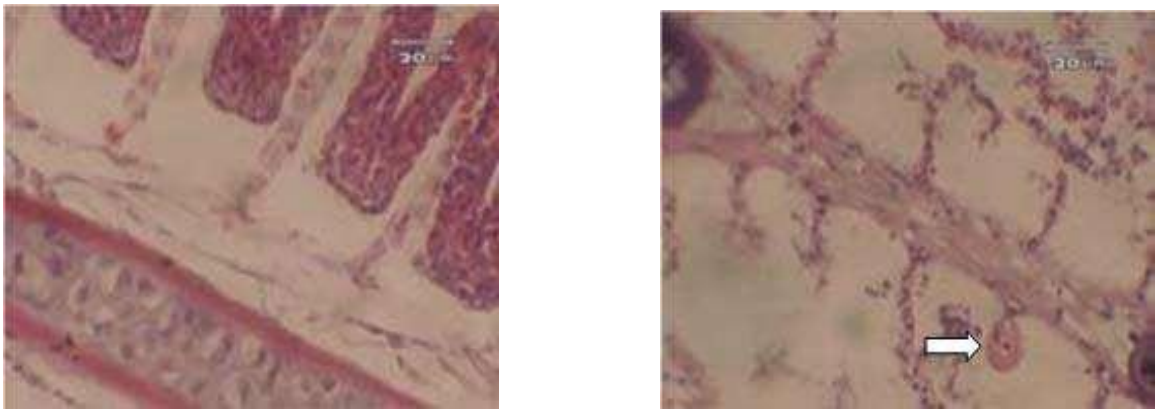

Fig 1–Photomicrograph of the gill of *Anabas testudineus* from farmed fish showing normal appearance of the gill lamellae (A) and of the *Anabas testudineus* at Ban Pu (B), showing desquamation of the epithelial lining and telangiectasia of the secondary lamellae (⇨).

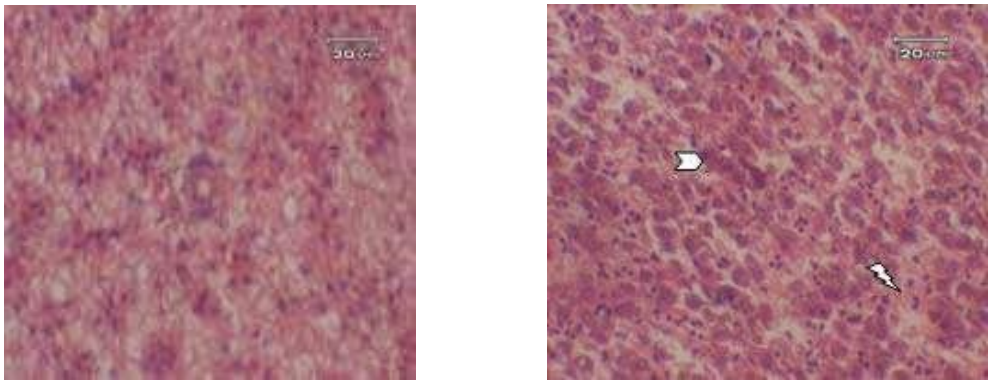

Fig 2–Photomicrograph of the liver of *Anabas testudineus* collected from farmed fish (A) and *Anabas testudineus* from Ban Pu (B) showing hemorrhagic liver tissue ( ), blood congestion and necrotic cells (⊃).

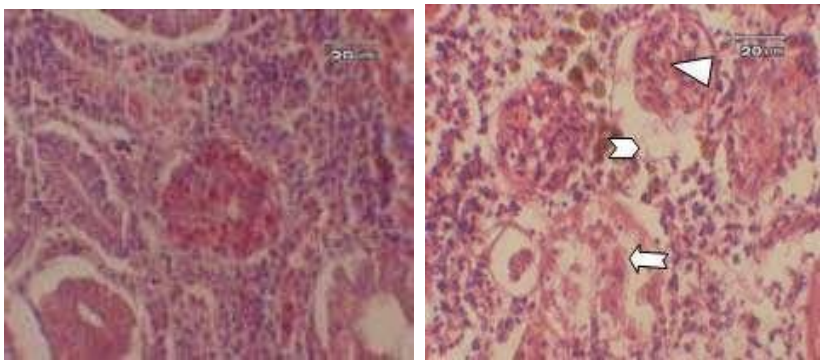

Fig 3–Photomicrograph of the kidney of *Anabas testudineus* from farmed fish (A) and *Anabas testudineus* at Ban Pu (B) showing detached epithelial cells of renal tubules (⇦) and glomerular contraction (◁) in the Bowman's capsule (⊃).

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