HISTOPATHOLOGICAL ALTERATIONS OF THE GILLS, LIVER AND KIDNEYS IN *ANABAS TESTUDINEUS* (BLOCH) FISH LIVING IN AN UNUSED LIGNITE MINE, LI DISTRICT, LAMPHUN PROVINCE, THAILAND

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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 the acidic water (pH 2-4) of an unused lignite mine in Li District, Lamphun Province, Thailand were examined and compared to those of farmed fish. Tissue abnormalities were found in all investigated organs. Deterioration and telangiectasia of gill filaments were 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 acidic water in this habitat causes severe damage to the internal organs of fish and consequently alter their physiological status. Since the water in this pond is utilized by local people, these findings highlight the need for adequate water treatment.

INTRODUCTION

The water reservoir at Ban Pu, Li District, Lamphun Province, Thailand is an abandoned lignite mine, whose water is highly acidic. However, surprisingly a population of climbing perch fish (*Anabas testudineus*) inhabit this water. 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). Acid water resulting from mining activity is highly toxic to most aquatic life. In this case, the acidity is not only harmful to aquatic life but to the food chain (Cotter and Brigden, 2006). 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 unused acid mines at Ban Pu, Li District, Lamphun Province, Thailand.

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MATERIAL AND METHODS

Adult *Anabas testudineus* fish (n = 10) were procured from an unused lignite mine (pH 2-4) at Ban Pu, Li District, Lamphun Province, Thailand. 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.

RESULTS

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). The pathological changes in the liver tissue of a fish inhabiting the unused lignite mine 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).

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. 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

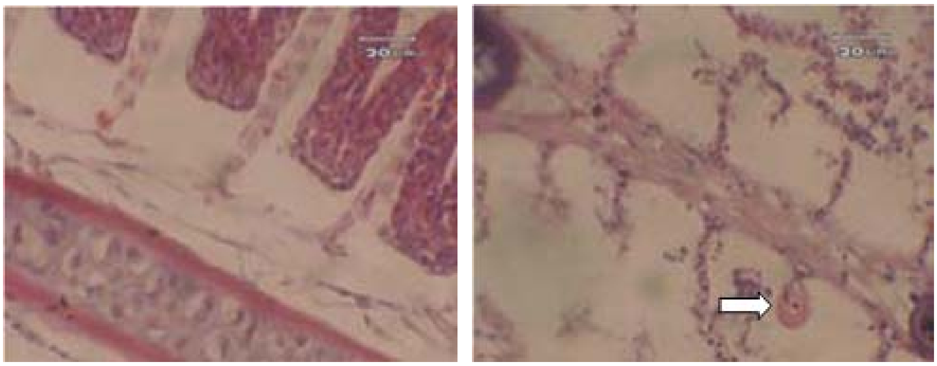

Fig 1–Photomicrograph of the gill of *Anabas testudineus* from farmed fish showing normal appearance of the gill lamellae (A) and of the *Anabas testudineus* at Ban Pu (B), showing desquamation of the epithelial lining and telangiectasia of the secondary lamellae (⇨).

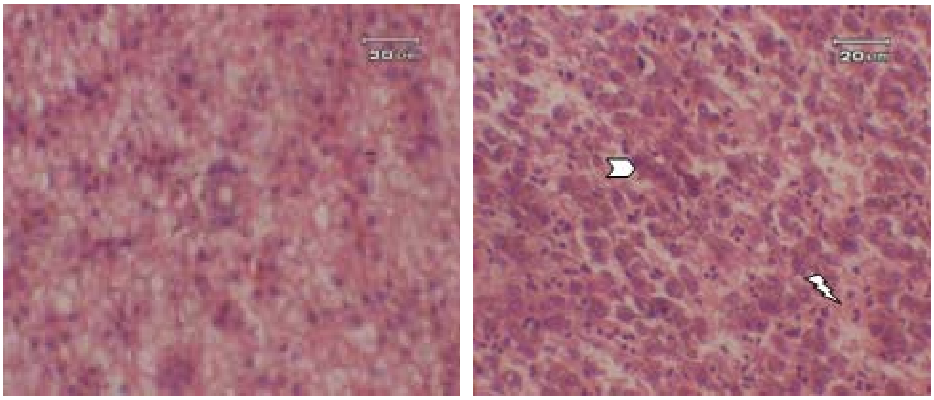

Fig 2–Photomicrograph of the liver of *Anabas testudineus* collected from farmed fish (A) and *Anabas testudineus* from Ban Pu (B) showing hemorrhagic liver tissue (⊳), blood congestion and necrotic cells (ϟ).

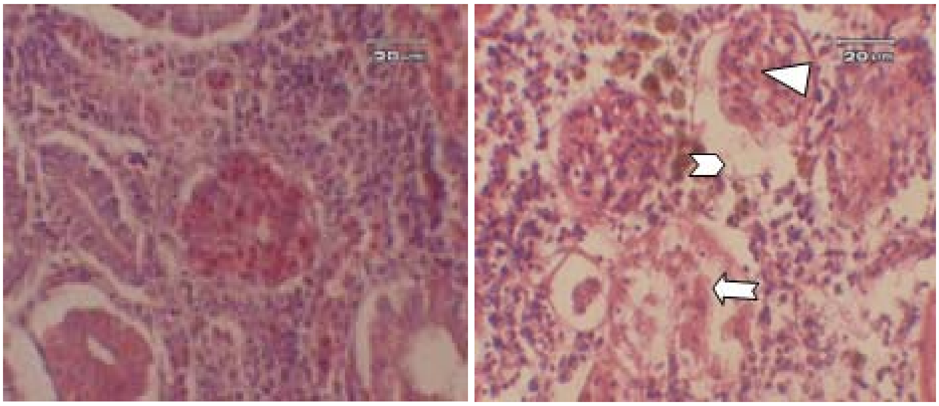

Fig 3–Photomicrograph of the kidney of *Anabas testudineus* from farmed fish (A) and *Anabas testudineus* at Ban Pu (B) showing detached epithelial cells of renal tubules (⇦) and glomerular contraction (◁) in the Bowman's capsule (⊳).

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 reabsorption 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 of extreme acidity (pH 2-4) resulting from mining activities. Complementary studies are needed for further evaluation of this problem.

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