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# ACCUMULATION AND HISTOPATHOLOGICAL EFFECTS OF ARSENIC IN TISSUES OF SHINGI FISH (STINGING CATFISH) *HETEROPNEUSTES FOSSILIS* (BLOCH, 1794)

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## Abstract

A 60-day experiment was conducted to compare the accumulation and toxicological effects of arsenic in muscle, intestine and liver of shingi fish, *H. fossilis* (Bloch) after exposure to two concentrations (7.0 and 20.0 ppm) of arsenic trioxide. The highest/maximum level of accumulation of arsenic was observed in the liver whereas the lowest level of arsenic was found to accumulate in the muscle tissues at the end of exposure period. It is apparent from the study that the damage of the liver of test fish due to 15 days exposed period was less compared to the damage caused by 60 days exposure periods. The intensity of histological alterations was observed to increase gradually with the arsenic concentration and the exposure time.

Key words: Arsenic, *Heteropneustes fossilis*, Accumulation, Tissues, Histopathology

## Introduction

Study of toxicology pertaining to aquatic animals has become important in water pollution studies. Heavy metal contaminants in aquatic ecosystems pose a serious environmental hazard because of their persistence and toxicity. Among the heavy metal pollutants, arsenic (As) receives a special attention due to its potential health hazard to aquatic fauna and human life in particular. The recent research has suggested that As acts as an endocrine disruptor at extremely low concentrations (Stoica *et al.* 2000). The presence of As in industrial wastes and its high toxicity along with considerable bioaccumulation in freshwater fishes make it a toxicant that should be given due consideration in aquatic toxicology. The term bioaccumulation refers to the wastes which have been reconcentrated in organisms often having undergone initial dilution in environment producing toxic effects in fishes (Dallinger *et al.* 1987). Availability of heavy metals in the aquatic ecosystem and its impact on the flora and fauna had been reported by many investigators (Nayak 1999 and Shrinivas and Balaparameswara 1999).

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The accumulation of heavy metals in different tissues of fish may cause various physiological defects and mortality (Torres *et al.* 1987). Heavy metals accumulated in the tissues of aquatic animals may become toxic when accumulation reaches a substantially high level (Kalay and Canli 2000). The pattern of accumulation of metals in animals differs from metal to metal and organ to organ during their functional status. Most of the investigations pertaining to heavy metals contaminants in aquatic systems are dealt either with toxicity or with accumulation (Rushforth *et al.* 1981 and Khadiga *et al.* 2002). Heavy metals have been shown to be concentrated in the liver of various fishes (Sorensen 1991 and Rao *et al.* 1998). The highest concentrations of As was recorded in the liver, while the lowest one was in the muscle. Mormede and Davies (2001) suggested that the liver was the target organ, showing the detoxification and accumulation role of the liver. Muscle is generally considered to have a weak accumulating potential (Erdoğrul and Erbilir 2007, Uysal *et al.* 2009 and Bervoets and Blust 2003). Histological changes associated with heavy metals in fish have been studied by many authors (Thophon *et al.* 2003, Mohamed and Gad 2005, Athikesavan *et al.* 2006, Giari *et al.* 2007, Jiraungkoorskul *et al.* 2007 and Van Dyk *et al.* 2007). Hence, the present study was aimed to investigate the accumulation of As in *Heteropneustes fossilis* (Bloch) and the associated histopathological changes in three organs (muscle, liver and intestine) at laboratory condition.

**Materials and Methods**

Special care was taken to make sure that the fish were approximately of similar size and weight. Fishes with almost similar length (8.15±0.51 cm) and weight (6.25±0.75 g) were collected from local market and were acclimatized under laboratory conditions (29.0±1 $^0$C). Fishes were transported to the laboratory in large buckets with proper covering and frequent agitation. On arrival at the laboratory, these were immediately released into three big tanks containing tap water and then maintained there for about 6-7 days in a static condition. Fishes were fed on artificial feed twice daily. Any debris or unwanted particles were removed from the tank after feeding. The water medium was changed at 24 hours interval to remove the metabolic-pollutants. Air compressor with air stones was used for oxygenation of water. The water quality parameters of the acclimation tank were studied at times. However, after acclimation, only healthy fishes were used for experiment. Arsenic trioxide ($As_2O_3$) was collected from the BDH laboratory (England) in original package form. By mixing with tap water two different concentrations of $As_2O_3$ was used as stock solution. The fishes were exposed to two concentrations for a period of 1, 15 and 60 days in glass aquaria containing 10-20 L water. Tap water stored in the tank for two months confirming the settlement of iron, were used for the experiment. The water was aerated for one day before starting the experiment. Stone aerators connected to a compressed air supply were used to maintain an adequate level of dissolved oxygen in each aquarium. The liver, intestine and muscle tissues of control and treated fishes were isolated and dried in an oven at 105 $^0$C for 24 hours. The known amounts of dried tissues

were digested with nitric acid and perchloric acid. After the accomplishment of complete digestion, the digested samples were made-up to 25 ml with metal free double distilled water and arsenic measurements were made using atomic absorption spectrophotometer (Begum *et al.* 2005). Values were expressed as µg/g, dry weight.

At the end of the exposure period, muscle, intestine and liver were collected from the *H. fossilis* and preserved in small plastic vials with 10% buffered neutral formalin (Begum *et al.* 1996). The number at section of samples was prepared using a microtome, stained and studied under a photomicroscope (Olympus, CH4O, Japan). Photomicrographs were taken after the examination of histological condition of each of the tissue slides.

**Results and Discussion**

The physico-chemical properties (temperature, dissolved oxygen, pH, carbon dioxide, alkalinity, total hardness and ammonia concentration) of the tap water were monitored during the acclimation period and trial with fishes exposed to $As_2O_3$ (Table 1). It is evident from the data that (Table 1), the water quality parameters did not fluctuate greatly among the different treatment aquariums as well as between different experimental trials. Moreover, the water quality was always within the normal ranges.

Table 1. Physico-chemical parameters of aquarium water with different treatments of arsenic (As).

| Concentration of arsenic (ppm) | Temperature ($^0$C) | Dissolved $O_2$ (mg/l) | $P^H$ | $CO_2$ (mg/l) | Alkalinity (mg/l) | Hardness (as $CaCO_3$) mg/l | Ammonia mg/l |
|---|---|---|---|---|---|---|---|
| BDL (control) | 29 (27.5-30.0) | 6.2 (6.1-6.3) | 7.1 (7.1-7.3) | 19.1 (18.2-20.0) | 129.8 (125.1-134.5) | 226 (224-229) | 0.27 (0.24-0.30) |
| 7 | | 5.7 (5.4-6.1) | 7.32 (7.5-7.6) | 20.9 (19.8-22.0) | 130 (129-131) | 225 (220-230) | 0.18 (0.10-0.26) |
| 20 | | 5.3 (5.2-5.4) | 7.26 (7.2-7.31) | 20.2 (18.0-22.4) | 129 (128-130) | 213 (212-214) | 0.15 (0.089-0.21) |

BDL- Below detection level

The levels of arsenic (As) accumulation in the liver, intestine and muscle tissues of *H. fossilis,* during exposed to control, 7 and 20 ppm of concentration for 1, 15 and 60 days are presented in Table 2. In the present investigation, the highest level of As accumulation (10.01 ± 0.55) and (16.26 ± 0.34) was found in the liver and lowest level (3.24 ± 0.25) and (6.55 ± 0.10) in muscle to 15 and 60 days of exposure periods, respectively. Similar pattern of accumulation of As in the liver tissues of *Mugil cephalus* has been reported by Maher *et al.* (1999) and found significantly higher than in any other tissues. Pazhanisamy *et al.* (2007) investigated the accumulation of As in *Labeo rohita*

after exposed in two sub lethal concentration of As trioxide. They found that the maximum level of accumulation of As was in the liver whereas, the lowest level in the muscle tissue at the end of 28 days of exposure. Similarly, the distribution and accumulation pattern of heavy metals in the liver of various teleosts fishes have been studied by Noel-Lambot *et al.* (1978) and Thiruvalluvan *et al.* (1997). In the present study, the rate of accumulation was found to increase gradually with the As concentration and the exposure time. The findings are identical with the report of Karuppasamy (1999) while, described the bioaccumulation as dose and time dependent in phenyl mercuric acetate exposed fish *Channa punctatus*.

Table 2. Accumulation of arsenic (µg/g, dry weight) in tissues of *H. fossilis* exposed to different concentration and exposure periods (days).

| Tissues | Concentration | Exposure period in days | | |
|---|---|---|---|---|
| | | 1 | 15 | 60 |
| Muscle | 0.28 (Control) | 0.53 ± 0.17 | 1.38 ± 0.12 | 2.16 ± 0.11 |
| | 7.0 | 1.99 ± 0.90 | 3.24 ± 0.25 | 6.55 ± 0.10 |
| | 20.0 | | | |
| Intestine | 2.43 (Control) | 2.10 ± 0.17 | 3.81 ± 0.29 | 8.50 ± 0.21 |
| | 7.0 | 3.47 ± 0.14 | 5.67 ± 0.27 | 11.10 ± 0.23 |
| | 20.0 | | | |
| Liver | 3.39 (Control) | 5.38 ± 0.33 | 6.62 ± 0.46 | 7.49 ± 1.11 |
| | 7.0 | 8.09 ± 0.67 | 10.01 ± 0.55 | 16.26 ± 0.34 |
| | 20.0 | | | |

Then, with the rate of accumulation of As and days of exposure, the histopathological study also showed drastic changes in muscle, intestine and liver tissues of the exposed fish. These findings suggested that the observed changes were undoubtedly as a result of various toxicological impact of As exposure. Even, these changes were directly related with the concentration of 7 and 20 ppm of As along the 1, 15 and 60 days of exposure (Plate 1a). However, at 7 ppm exposed to As, the muscle tissue exhibited dystrophic changes with marked thickening and separation of muscle bundles after 15 days of exposure (Plate 1b), but, such a change after 60 days of exposure, the vacuolar degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis were observed (Plate 1c). Again, at 20 ppm of As concentration, the muscle tissues exhibited dystrophic changes with marked thickening and vacuolar degeneration of muscle bundles along with severe intramuscular edema after 15 days of exposure (Plate 1d) while, atrophy and edema of muscle bundles as well as splitting of muscle fibers were seen in 60 days of As exposure of fish (Plate 1e). It is true that like gills, muscle tissue also come into close contact with pollutants dissolved in water. Therefore, the muscles of *H. fossilis* showed degeneration in muscle bundles accompanied with focal areas of necrosis as well as atrophy and vacuolar degeneration. Similar findings had been observed in Nilotica fish by Mohamed (2008).

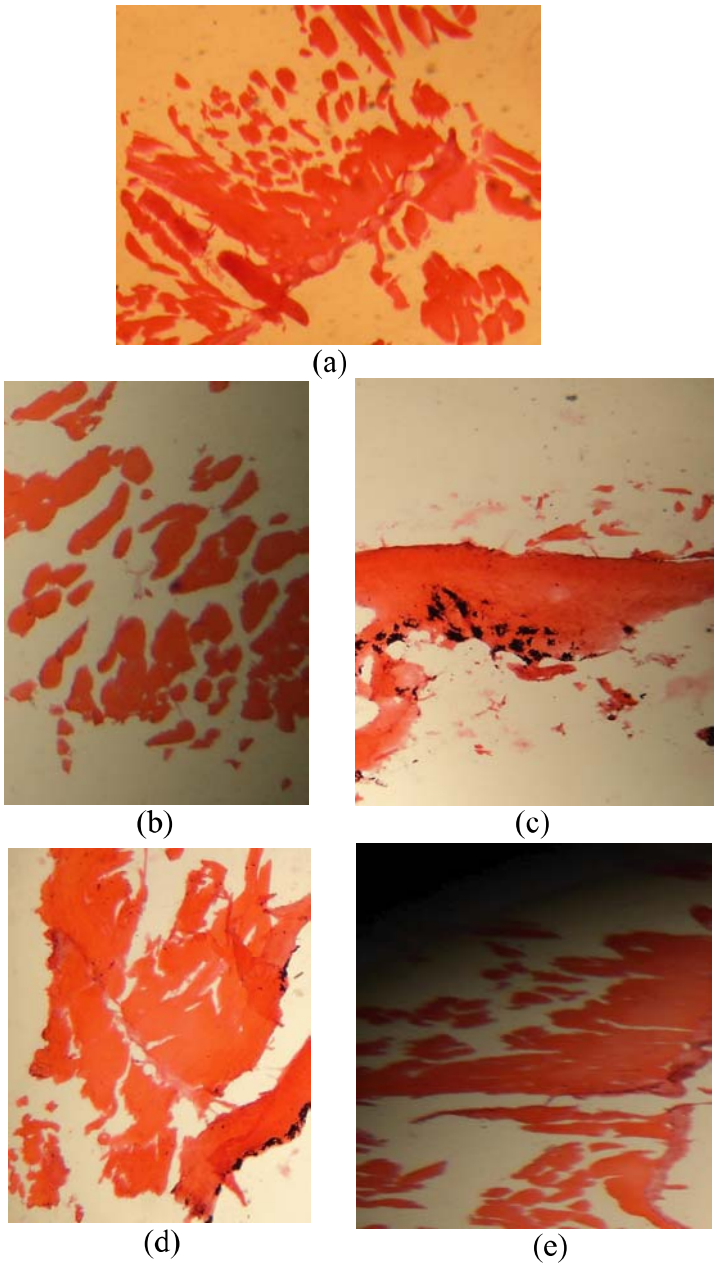



Plate 1. (a) Muscles of fish showing the normal, (b) separation in muscle bundles, (c) focal area of necrosis, (d) intramuscular edema and (e) splitting of muscle fibers and atrophy of muscle bundles.

In the case of intestine (Plate 2a), the histopathological changes so obtained in 7 ppm of As concentration and 15 days of exposed included partial intactness of serosa but, more or less organized mucosa and disorganized villi (Plate 2b). The same organ, even at same concentration after 60 days of exposure exhibited partially damage of muscles, but disorganized, slightly swollen and shorten of villi (Plate 2c). This in 20 ppm concentration of As and after 15 days of exposure showed damaged serosa disorganized and consequent fussion of mucosa, degeneration and edema between the intestinal submucosa and lamina propria (Plate 2d). Further, these damages were characterized by the increases in number of goblet (mucosal) cells, width of the lamina propria and degeneration of villi after 60 days of As exposure (Plate 2e). The findings suggest that however, uptake of As and other metals occur mainly through gills but may also occur *via* intestinal epithelium. Therefore, the histopathological alterations so far observed in the intestine tissues of studied fish may be a result of uptake of toxic As. The present results are in agreement with those observed by many investigators about the effects of metals on fish intestine (Giari *et al.* 2007 and Hanna *et al.* 2005).

Observations on the fish liver (Plate 3a) revealed that in 7 ppm of As concentration, and 15 days of exposure primary degeneration occurred in the hepatocytes (Plate 3b), which at the end of 60 days showed further changes like focal areas of necrosis, haemorrhage and haemolysis between the hepatocytes (Plate 3c). On the other hand, fish exposed to As concentration of 20 ppm, the hepatocytes became more irregular and lose their polygonal shape, areas of hepatocytes with eosinophilic cytoplasm (Plate 3d). Moreover, haemosiderin was seen around central veins and hepatoportal blood vessels. In some cases, dilation and intravascular haemolysis in hepatoportal blood vessels were noticed at 60 days of exposure (Plate 3e). Sorensen (1991) stated that the liver is a critical target organ for As toxicity in fish due to the role it plays in metabolism and detoxification. Such a statement is supported by the results of our present study, as livers of shingi fish exposed to different concentrations of As showed significant changes in architectural and structural arrangements, as well as areas of inflammation and focal necrosis. Similar alterations have been observed in fish liver being exposed to As in both the laboratory and field conditions (Gilderhus 1966 and Joshi and Sahu 2007).

Thus, comparing the entire findings it is clear that the highest/maximum level of As accumulation obtained in the liver and intestine, whereas, the lowest in the muscle tissues at the end of exposure periods. At the same time, it indicates about the intensity of histological alterations are also dose (As concentration) and time dependent. In conjugation of these two facts, the As trioxide affects severely on the tissues of internal organs of *H. fossilis* leading to life threat and poor reproductive performance. Moreover, the present study suggests further detailed investigation on the possible As pollution sources as well as the rate of accumulation in different fish tissues and possible histological changes.

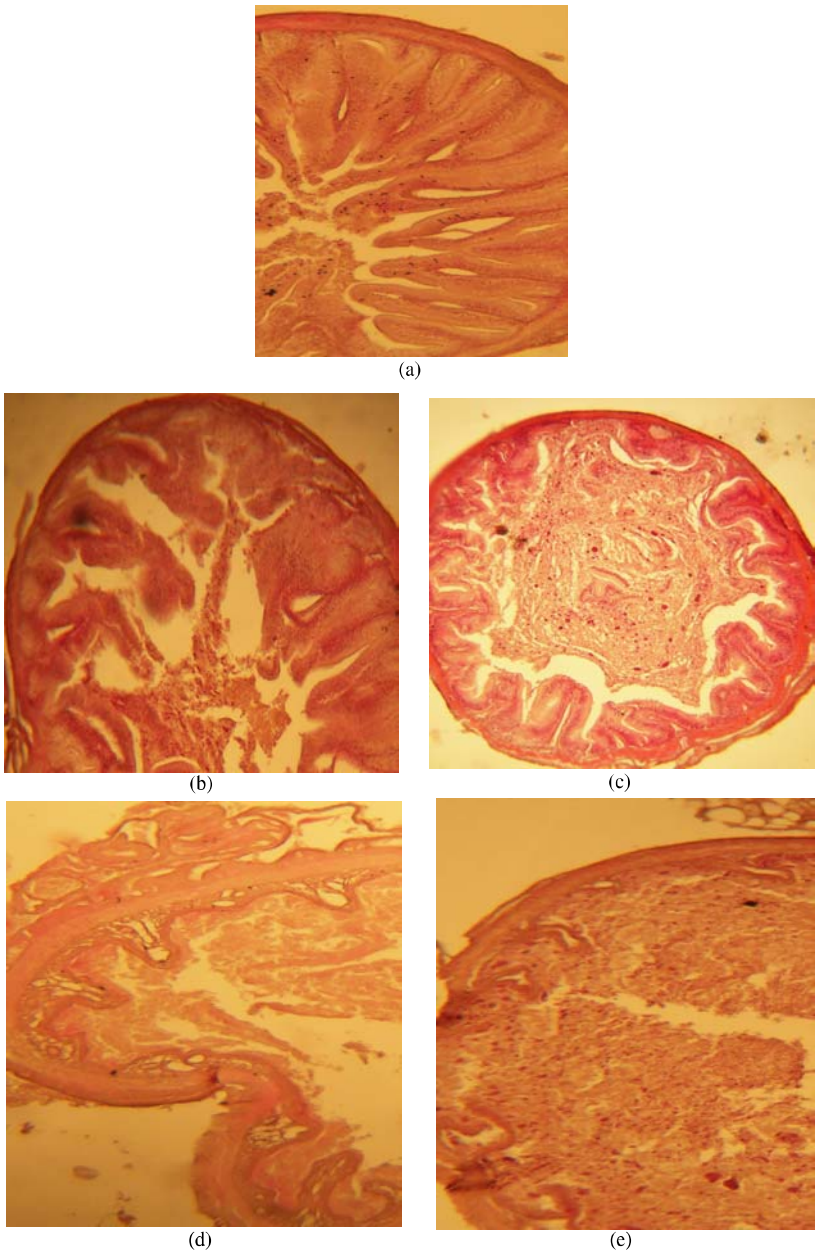
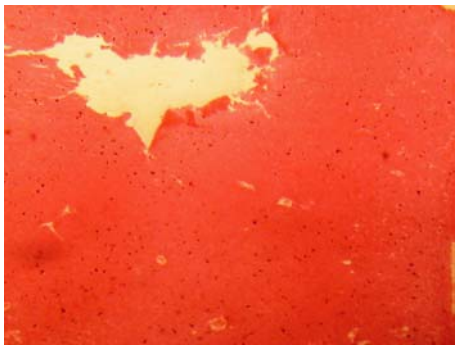
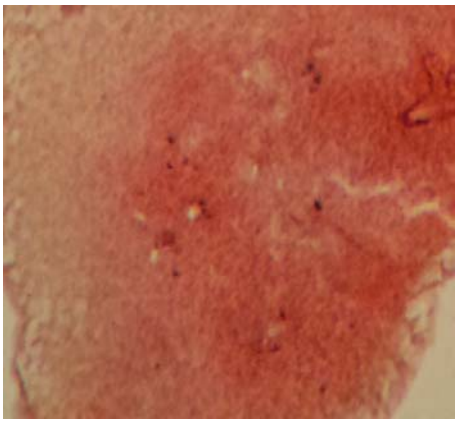


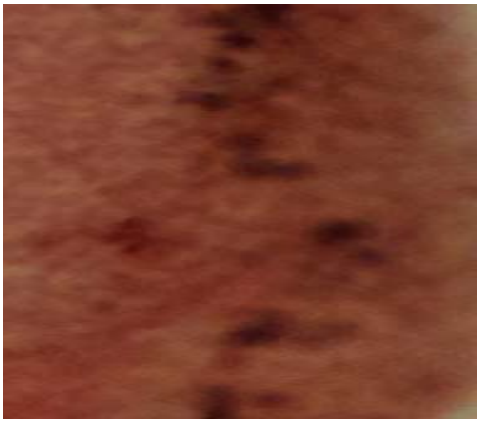
Plate 2. (a) Intestine of fish showing the normal, (b) serosa showed partial intacness and disorganized villi, (c) severe damage in muscularis and villi slightly swollen and shortened, (d) degeneration and edema between the intestinal submucosa and lamina propria and (e) increase in the width of lamina propria and villi degenerated.



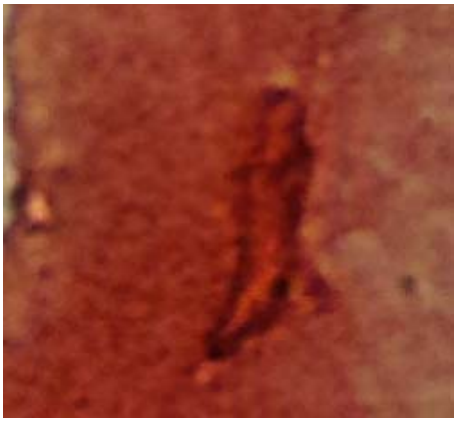
(a)



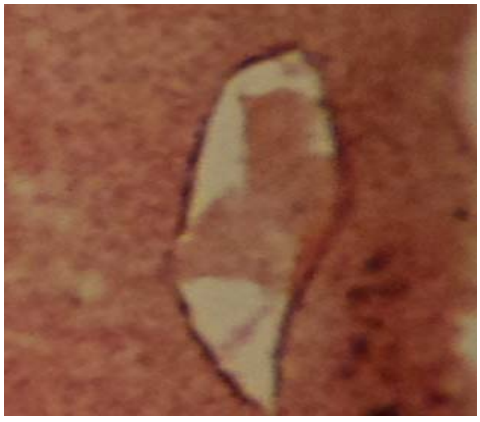
(b)



(c)



(d)



(e)

Plate 3. (a) Liver of fish showing the normal, (b) vacuolar degeneration, (c) focal areas of necrosis, (d) haemorrhage and haemolysis between the hepatocytes, intravascular haemolysis in blood vessels and (e) intravascular haemolysis in hepatoportal blood vessels.

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