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Histopathological evaluation of environmental gill disease (EGD) in the cultured rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

Gills are the important and sensitive organs of fish which are exposed to many environmental factors. This study was conducted to investigate the histopathological alterations of the gills of the rainbow trout cultured in one the farms of Chaharmahal and Bakhtiary in Iran. In summer of 2011, massive mortality (over 70 percent) was occurred in these fishes. With the primary examination of affected fishes, the gill samples were collected and placed in formalin 10% then sent for histopathological tests. It was distinguished that the impact of influent water polluted by rural waste water having high pH and ammonia led to gill alterations. Several histological changes were observed in the gills that included proliferative of mucosal cells, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae, detachment of epithelium layer in primary lamella, lamellar fusion, edema in secondary lamellae, dilation and congestion in blood vessels of gill filaments. These histopathological findings suggest that influent water in considered farm with high ammonia and pH causes severe damage to the respiratory organs and consequently impact on the physiological status and incidence of mortality in fishes.

Key words: EGD, Rainbow trout, Histopathology

INTRODUCTION

Gill is one the most important organ in the fish body, so that it has very closely contact with outside of the body which results in increase the contact with pathogens and environmental pollutants. The gills, which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants and toxins[1,2].

Ammonia is the main final product of nitrogen metabolism in freshwater fish. A very small part of metabolic products is represented by urea. The balance between production and excretion of ammonia may be disturbed by various endogenous and exogenous factors [3,4]. Pollution of the aquatic environment is a serious growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment led to various deleterious effects on the aquatic organisms [5]. Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly via the food chain [6].Pathologic changes have been reported in the gills of many fish as a result of exposure to different toxicants [2,7,8]. Histopathological

alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism[9]. Ammonia is a naturally occurring product of biological metabolism, but high concentrations are often associated with human sources such as sewage treatment plants, agricultural and feedlot runoff, coal coking and gasification plants, and fertilizer manufacturing plants [10].
The aim of this study was to investigate the impact of the environmental conditions of Sabzekooh stream on the gill histological structures of the rainbow trout that cultured in south area of Chaharmahal-va-Bakhtiary province in Iran.

MATERIALS AND METHODS

Study Area: The present study was conducted in the farm that was located at south area of Chaharmahal-va-Bakhtiary province in Iran. The annual production of fish is 20 ton and supplied by Sabzekooh stream that sometimes polluted with rural waste water.

Water Analysis: Some chemical parameters of influent water were measured and listed in table1.

Sampling: Fish samples were collected during summer 2011, measuring about 10-12 cm in total length and 5-7g in weight. After showing clinical signs and recording of them, parts of gills were carefully removed and prepared for histological studies.

***Histological investigations*:** Specimens from gills were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 4-6µm then stained with haematoxylin and eosin and examined microscopically [11].

RESULTS AND DISCUSSION

Mortality was occurred in fishes with 3-5 gram in weight and after five days reached to 70 percent of total biomass. Sign of affected fishes include weakness, lethargy, anorexia, movement and crowding near the pool inlet. In the many of fishes raised opercle, exophthalmia and darkening of skin were seen. Also in the clinical signs of diseased fish the accumulation of mucus in the gill opening, diffusion of gill filament, anemia and corrosion in top of filament were observed (fig1).Sometimes fishes appear the irritation, agitation and jump out of the water.

The histological alterations found in the gills of the fish included proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae(fig 2), dilation of the marginal channel, severe degenerative and necrotic changes in gill filaments and secondary lamellae(fig3), curling of secondary lamellae and mucous cells proliferation. Edematous changes, lifting of the lamellar epithelium, were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Also, dilation and congestion in blood vessels of gill filaments were observed.

Gill disease has been responsible for large scale mass mortalities as well as poor growth and performance in farmed salmon and has been a serious financial burden for some sectors of the industry. The lamellar fusions are defense mechanisms that reduce the bronchial superficial area in contact with the external surroundings. These mechanisms also increase the diffusion barrier to the pollutant [12]. The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants. Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since; in general, these results in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants [1]. Epithelial lifting and lamellar fusion in rainbow trout (*Oncorhynchus mykiss*) exposed to petroleum residues were observed [13].

Table 1-Some chemical parameters of influent water

Parameter	COD(mg/l)	BOD5(mg/l)	NO2(mg/l)	NO3(mg/l)	NH3(mg/l)	pH
Value	8.6	1	0.054	9.4	0.1	8.50

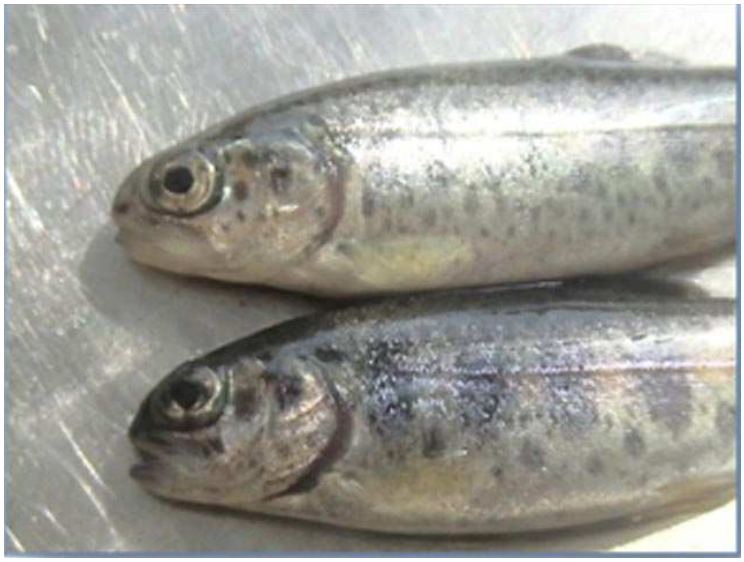

Fig 1: Raised opercle, exophthalmia, darkening of the skin and swollen gills are visible in the affected fishes

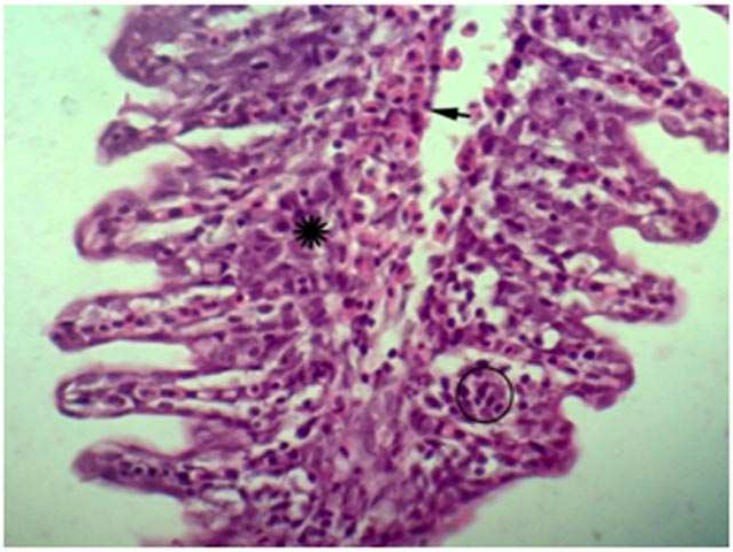

Fig. 2: Gills of fish showing the proliferation in the epithelium of gill filaments and secondary lamellae, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae , proliferation of mucous cells (⋆),aggregations of inflammatory cells in gill filaments(O), dilation and congestion in blood vessels of gill filaments(arrow) (X400)

Similar alterations in the gills have also been reported in the fishes exposed to metals [14] and organic contaminants [15]. Such alterations are non-specific and may be induced by different types of contaminant [16]. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake [1].

Results of the present study revealed the fishes manifest histopathological changes in gills during summer season. The pH of the water was 8.5 and Ammonia level was 0.1 mg/l. these parameters are dependent to each other and increases of one leads to change in another one. Increase in two parameters result in sever alteration in some organs specially gills. The results of other authors confirmed the present findings. The toxic nature of ammonia affects the gill lamellae by bulging the tip of gill, degeneration of gill filaments, necrosis of gill epithelium cells, changes of disruption, vacuolization with dense fluid under the layer, mucous coating on the surface of gill lamellae [17].The physic-chemical analysis of water quality revealed that influent water used in the desired Farm is placed in the class

C. These waters in the classification are moderately polluted and unsuitable for culture of salmoninds [18]. It was shown that Exposure silver catfish, *Rhamdia quelen* to waterborne ammonia increased total ammonia levels in both tissues and also induced gill epithelium damages such as lamellar fusion and edema as compared with controls at different pH. Gill histology may be used as early indicators of ammonia toxicity in silver catfish [19]. pH is a measure of the acidity of water, and is important in understanding the chemical balance of the water. PH is a strong determinant of the solubility and availability of both nutrients and pollutants. Most natural water bodies will have pHs close to 7, depending on the local geochemistry. The histological changes observed in the gills in the present study indicate that the fish were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress. Such information confirms that histopathological alterations are good biomarkers for field assessment. It must be emphasized that histopathology is able to evaluate the early effects and the responses to acute exposure to chemical stressors. In conclusion the present study showed that histopathology is a useful biomarker for environmental contamination.

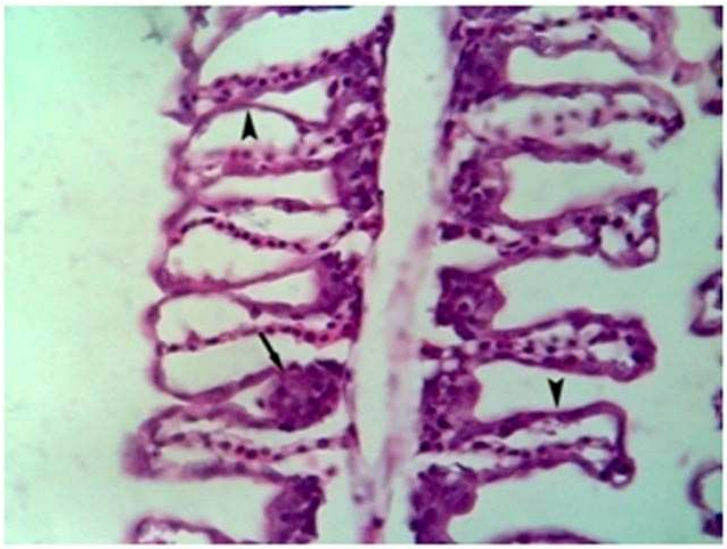

Fig. 3: Gills of fish showing the proliferation in the epithelium of secondary lamellae (arrow) detachment of epithelium layer in the secondary lamella (arrowheads), degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae, curling of secondary lamellae, and atrophy of secondary lamellae (X400)

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