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Full Length Research Paper

Prevention of acute ammonia toxicity in bluga *(Huso huso)*, using natural zeolite

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The research was accomplished in order to study the efficiency of natural zeolite to prevent acute toxicity of ammonia on *Huso huso.* The study was performed using Water static method for 96 h. Fish with average weight 46 ± 5 g and total length 22 ± 4 cm were exposed to four different concentrations (15, 30, 50, 75 mg L^{-1}) of ammonia salt. A group of fish was considered as control. Under stable condition, the lethal concentration of ionized ammonia was 50 mg L^{-1} during 96 h. In the lethal concentration of total ammonia, different amounts of 5, 10, 15 g L^{-1} granulated clinoptilolite zeolite were used. Results indicate significant differences between treatments and also with control ($p < 0.05$). With increasing clinoptilolite zeolite in each treatment, the survival rate of fish also increased significantly ($p < 0.05$). In lethal concentration of ammonia, the use of 15 g L^{-1} zeolite could prevent the mortality rate. Histopathological findings showed that major lesions were hemorrhage, hyperemia, hyperplasia, epithelial cells necrosis, degenerated tubules of kidney, expansion of Bowman's capsule and hepatocytes necrosis.

Key words: Ammonia, histopathological, lethal concentration, zeolilte, *Huso huso.*

INTRODUCTION

Ammonia is one of the most toxic metals to aquatic organism and ecosystems. Ammonia appears to have a direct effect on the growth of aquatic animals (Colt, 2006) and it causes a decreased growth, disease resistance (Lemarie et al., 2004) or even cause fish mortality in intensive culture (Wang and Walsh, 2000). However, the concentration of these elements above tolerable levels is a disturbance factor for species survival and ecosystem stability. The toxic effect of trace metals is influenced by environmental factors such as salinity, pH, water hardness and temperature (Lemus and Hung, 1999).

All fishes are sensitive to minor fluctuations of ammonia compounds. Zeolites are used as a natural material to remove ammonia. One of the best zeolites for ammonia removal is clinoptilolite (Bergero et al., 1994). Clinoptilolite has been found very effective in removing ammonia from water by means of its excellent ion exchange capacity since the seventies of the last century (Wang and Walsh, 2000). Recently, it has been used in detergents, aquaculture ponds and nuclear treatment, but it also has large potentials for other applications in liquid waste treatment (James et al., 2000). This research tried to determine lethal concentration (LC_{50}, 96 h) of ammonia on beluga sturgeon (*Huso huso*) (Linneaus, 1758) and

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survey the effects of clinoptilolite zeolite on the removal of ammonia compounds from water environment.

MATERIALS AND METHODS

Fish selection

This study was carried out in the laboratories of the Department of Natural Resources of Gonbad University. Fries of *H. huso* with an average weight and lenght 46 ± 5 g and 22 ± 4 cm, respectively were collected from Sijaval Fish Culture Center. The experiments were done during spring in 2010. After collection, fish were acclimated to laboratory conditions for one week. They were kept in glass fiber tanks filled with 300 L of fresh water (under constant aeration, fixed temperature = 26°C and pH = 8.2). The experiments were done by water static method for 96 h (Sprague, 1969). Air-stone has been used in treatment as an aerator.

Determination of LC_{50}

First, five flasks of 35 liters each were used with concentrations of 0, 15, 30, 50 and 75 mg L^{-1} of ammonia (prepared with NH_4Cl, Merck). A control without addition of ammonia in the water was also tested. Fifteen fish were placed in each flask with their respective duplicates for a 96 h exposition period. The amount of ammonium chloride salt to be added in each aquarium was calculated after the volume of each aquarium was accurately determined. Observations were made at intervals of 24, 48, 72 and 96 h, respectively. Fish were not fed during this period. Percent of mortality was registered every 24 h. Then, dead fish were collected.

Zeolite efficiency test

At first, a substantial amount of ammonia in the water was measured measured and then in each basin some ammonia salt equivalent to 50 mg L^{-1} was added according to preliminary test. Granulated zeolite at 3 treatments of 5, 10, 15 g L^{-1} with three replications for each treatment was used. Then at the end of the test, every 12 h, the amount of ammonia from the water basin was measured.

Behavioral and histopathological studies

The behavioral changes of the healthy fish and the fish exposed to various doses of ammonia were recorded. Samples were randomly taken from gill, kidney and liver of fish and histopathological sections were prepared.

Statistical analysis

Comparisons of mean values of mortality rate in zeolite treatments were performed using one-way analysis of variance (ANOVA) followed by the Duncan's test. In all cases, the significance level adopted was 95%.

RESULTS

After the 96 h exposure, there were 0.00, 26.66, 53.33, 100 and 100% mortality at the 0, 15, 30, 50, 75 mg L^{-1} concentrations of ammonia, respectively (Table 1). Control survival was 100% in the test.

Figure 1 shows the relation between the ammonia concentration and survival rate of *H. huso* after 96 h. The results obtained from 96 h toxicity experiments revealed that there was a significant difference between treatments with each other when ammonia concentration was increased in treatment ($p < 0.05$). The 96 h LC_{50} obtained were 29.23 mg L^{-1} by the use of computer program based on liner Regression (Figure 2). In lethal concentration of ammonia (50 mg L^{-1}) amount of 0, 5, 10 and 15 g L^{-1} granulated zeolite was used. With increasing zeolite in each treatment, the survival rate of fish also increased significantly ($p < 0.05$) (Table 2). Most absorption of ammonia was recorded in the first 12 h of testing (Figure 3). After the 96 h exposure, no mortality was observed in lethal concentration of ammonia using 15 g L^{-1} concentrations of zeolite (Table 2). Most absorption of ammonia was recorded in the first 12 h of testing by zeolite (Figure 3).The behavioral changes of *H. huso* exposed to various concentrations of ammonia are as follows: In control group, there were no behavioral changes and deaths observed throughout the experiment.

In experimental groups, there were vertical and downward swimming patterns and sudden movements. The motion of fish became extremely slow and they displayed behavioral anomalies such as capsizing in water and loss of balance. Finally, the fish sank down to the bottom and became motionless. Histopathological studies showed that the common lesions of fish exposed to ammonia lethal concentration were hyperplasia, edema, hyperemia, hemorrhage and expansion of secondary lamella, expansion of Bowman's capsule, hemorrhage, inflammatory cells infiltration and hepatocytes necrosis (Figures 4, 5, 6, 7 and 8).

DISCUSSION

The toxicity of ammonia is generally assumed to be due to the concentration of the unionized ammonia molecule (NH_3) because of its ability to move across cell membranes (Colt, 2006). In alkaline waters, the amount of H^+ available to react with NH_3^+ and produce NH_4^+ is low, and in this condition the NH_3 plasma-water gradient is reduced, decreasing NH_3 excretion and consequently accumulating in the plasma and tissues. A regular response was generally observed in the mortality rate which increases with increased concentration of ammonia. Ammonia appears to have a direct effect on the growth of aquatic animals and it can have a serious effect on the incidence of disease, especially under less optimum conditions of temperature and dissolved oxygen (Colt, 2006). However, the lethal concentration in varieties of fish is different. It depends on species, age

Table 1. The relation between the ammonia concentration and the mortality rate of *H. huso.*

Concentration (mg L^{-1})	Number of exposed fish	Number of dead fish	Percentage of dead fish	Number of life fish	Percentage of life fish
0	15	0	0	15	100
15	15	4	26.66	11	73.33
30	15	8	53.33	7	46.66
50	15	15	100	0	0
75	15	15	100	0	0

Table 2. The relation between the zeolite amount and the survival rate of *H. huso* in lethal concentration of ammonia.

Zeolite amount (g L^{-1})	Concentration of ammonia (mg L^{-1})	Number of exposed fish	Number of life fish	Percentage of life fish
0	50	15	0	0
5	50	15	5	33.33
10	50	15	11	73.33
15	50	15	15	100

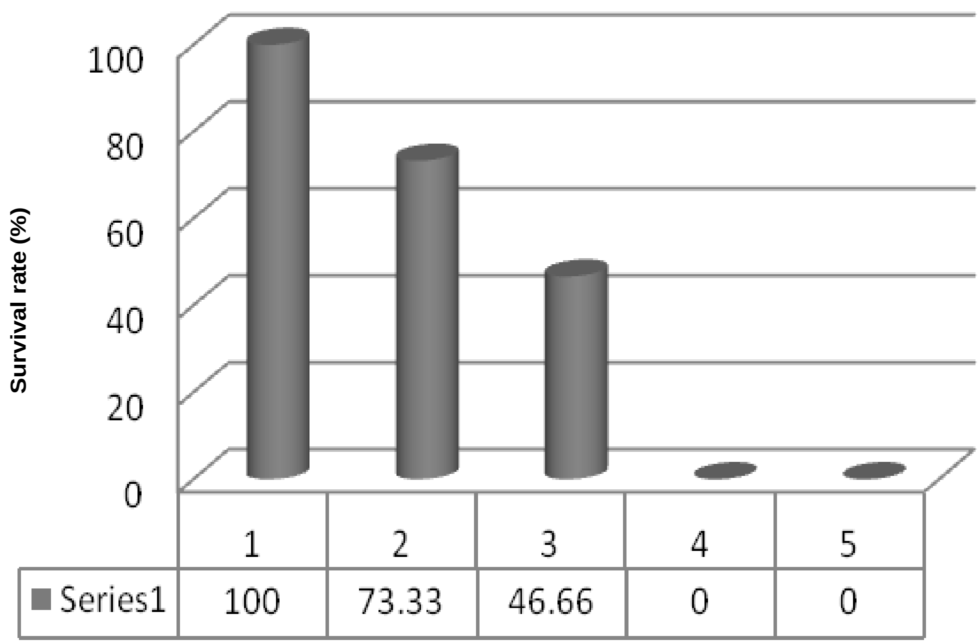

Figure 1. Histograms based on fish survival in different dosage of ammonia.

and environmental factors such as temperature, pH and hardness (Witeska and Jezierska, 2003; Giguere et al., 2004). Ololade and Oginni (2010) demonstrated that increase of toxicants concentration due to decrease in water pH and hardness caused significant direct relationship with 96 h, LC_{50} concentration of the fish.

Lloyed (1961) reported that in rainbow trout (*Oncorhynchus mukiss* Walbaum, 1792), the toxicity ratio of dissolved ammonia, zinc salt, lead and copper, as well as phenols began to increase markedly below 60% oxygen

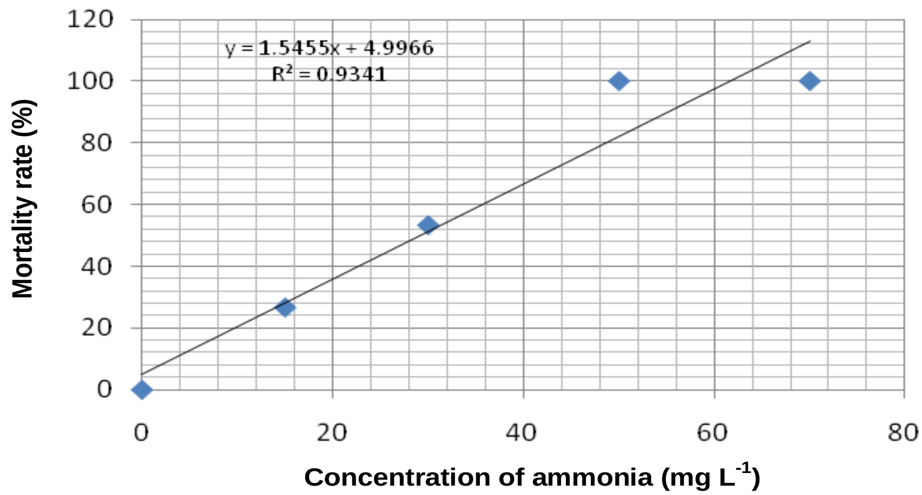

Figure 2. Linear regression based on fish mortality in different dosage of ammonia.

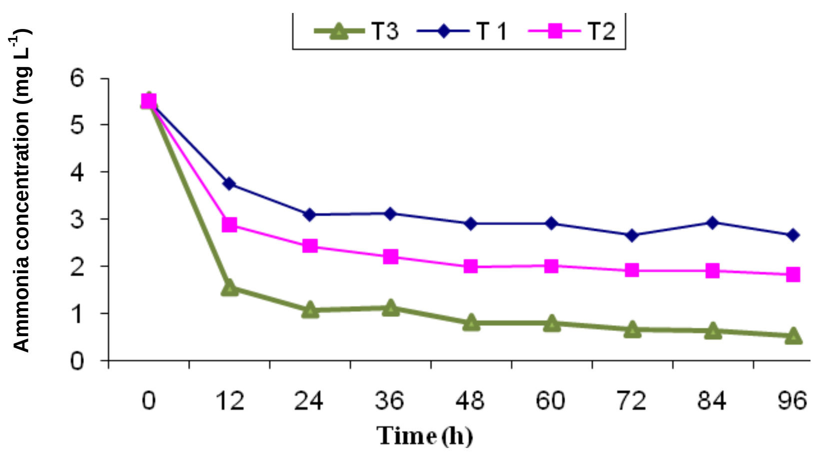

Figure 3. Decreasing the ammonia (NH_3) concentration during 96 h.

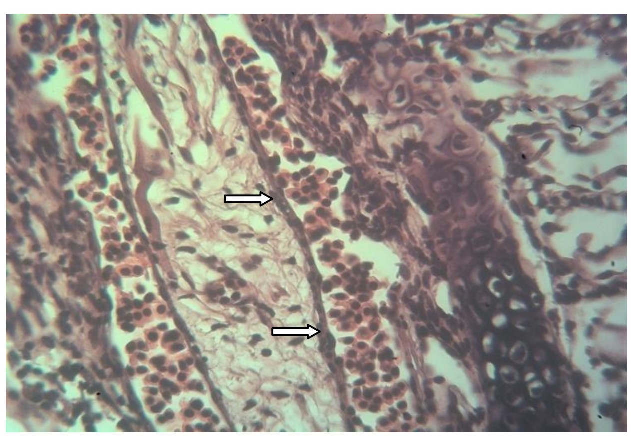

Figure 4. Gill histological section that exposure to lethal ammonia toxicity. Arrows show hyperemia (× 400).

Figure 5. Gill histological section that exposure to lethal ammonia toxicity. Arrows show edema (× 200).

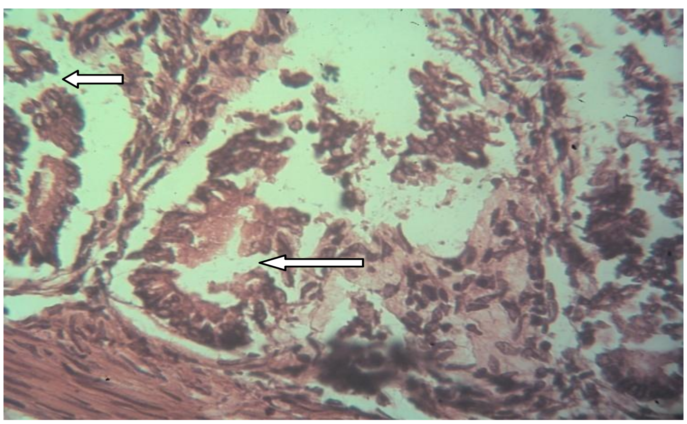

Figure 6. Kidney histological sections that exposure to lethal ammonia toxicity. Arrows show degenerated tubules of kidney (× 400).

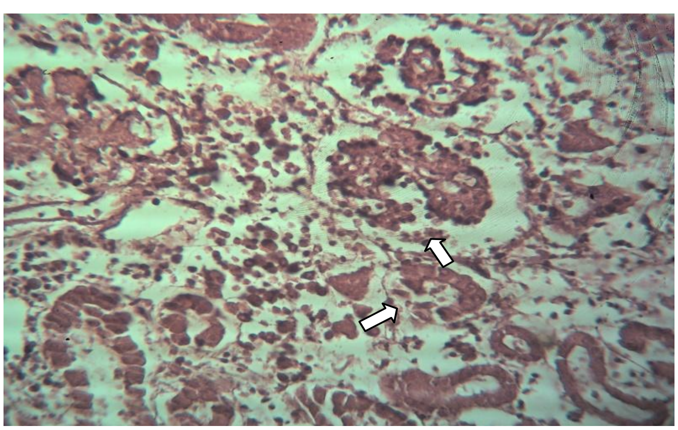

Figure 7. Kidney histological sections that exposure to lethal ammonia toxicity. Arrows show degenerated tubules of kidney and glumerol (× 400).

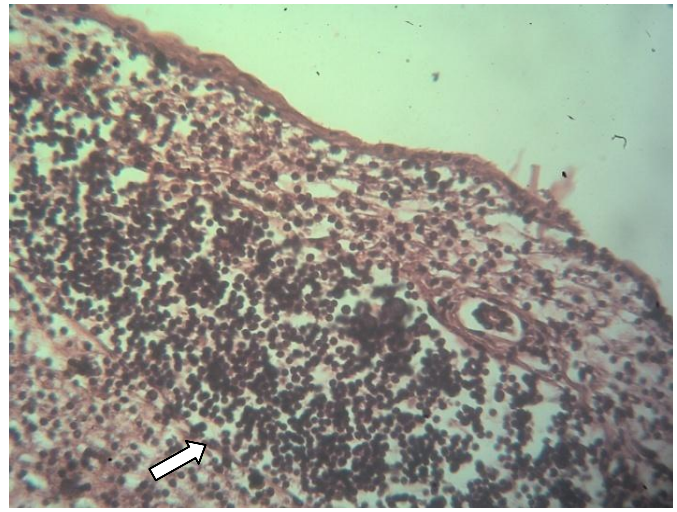

Figure 8. Liver histological sections that exposure to 30 mg/L of ammonia toxicity. Arrow shows inflammation (× 400).

saturation. However, in our research, the lethal concentration of ammonia was equal to 50 mg L^{-1}. Most mortality happened at earlier hours (Figure 3). Similar behavioral pattern has been reported with other metals such as ammonia on *O. mykiss* (Farhangi and Hajimoradloo, 2008), zinc on *Clarias gariepinus* Burchell, 1822 (Ololade and Oginni, 2010) and zinc on *Poecilia reticulata* Peters, 1859 (Gul et al., 2009). All these observations were more pronounced with increasing concentrations of toxicant. Consequently, the percentage and number of survivors decreased with increasing concentrations of toxicants in water. The differences between the mortality and survival rate in the control and experimental treatments were statistically significant ($p < 0.05$), particularly at higher concentrations. Similar reductions have been reported by Farhangi (2010) and Gul et al. (2009), when they exposed fish to toxicant metals under laboratory conditions. The primary test showed that there were no effect when 5 mg L^{-1} concentration of ammonia was used.

Different fish species have different sensibility to different concentrtion of the same metal. For example, Salmonids (that is, *Salmo salar* Linnaeus, 1758) are generally sensitive than the other fish species.

In the present research, the fish exposed to ammonia were observed to be highly irritable and displayed frenzied swimming when approached; their bodies were covered with thick mucus and finally died with mouths opened. These observations were similar to those of Olaifa et al. (2004) who worked with *Clarias gariepinus* (Burchell, 1822) on copper and Farhangi (2010) who worked with *Cyprinus carpio* (Linnaeus, 1758) on zinc.

Conclusion

The survival rate in beluga fish has increased by adding the amount of zeolites (Table 2). Applying zeolites was directly related to the removal of ammonia compounds in this study. So, the most efficient removal rate of ammonia by zeolite was achieved when granulated zeolite was applied at 15 g L^{-1} concentration. However during the trial, fish showed many severe reactions like disquiet and spasm. Tests revealed that zeolite quickly reduced $N\text{-}NH_4$ concentration after the first hour. The obtained results revealed that fish mortality is due to degenerated tubules and glumroles of kidney, necroisis of hepatocytes.

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