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[Research]

Prevention of acute ammonia toxicity in beluga, *Huso huso*, using natural zeolite

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ABSTRACT

This study was accomplished to examine the efficiency of natural zeolite in preventing acute toxicity of total ammonia to *Huso huso*. The study was performed using Water Static Method in 96 hours. Fish averaged 46 ± 5 g in weight and 22 ± 4 cm in total length were exposed to four different concentrations (15, 30, 50, 75 mgL^{-1}) of ammonia and a group was considered as control. Under stable condition, the lethal concentration of ionized ammonia was 75 mgL^{-1} in 96 hours. In the lethal concentration of total ammonia, different amounts of 5, 10, 15 $g.L^{-1}$ granulated clinoptilolite zeolite were used. Results indicated significant differences between treatments and control ($p<0.05$). By 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 in gill filaments included hemorrhage, hyperemia, hyperplasia, epithelial cells necrosis. There were hemorrhage, hyperemia, degenerated tubules of kidney, expansion of Bowman's capsule in kidney and hepatocytes necrosis in liver.

Keywords: Ammonia, Histopathology, Lethal Concentration, Zeolilte, *Huso huso*

INTRODUCTION

The pollution of water and soil with metal cations has increased dramatically in the last 50 years as a consequence of the expedition of industrial activities. It can be dangerous to aquatic animals (Shemshadi *et al.*, 2012).

Ammonia is the principal nitrogenous waste product excreted by crustaceans and teleosts (Boyd & Tucker, 1998).It is one of the most toxic ions to aquatic organisms and ecosystems. all fishes are sensitive to minor fluctuations of ammonia compounds. Ammonia appears to have a direct effect on the growth of aquatic animals (Colt, 2006) and cause a decrease in growth, disease resistance (Lemarie´ *et al.*, 2004) or even kill the cultur fishes (Wang *et al.*, 2000). However, the concentration of these elements above tolerable levels is a disturbance factor for species survival and ecosystem stability. On the other hand, the toxic effect of trace ionsare influenced by environmental factors such as salinity, pH, water hardness and temperature (Lemus & Hung, 1999).

Thus, zeolite has been used as a natural material to remove ammonia. One of the best zeolites for ammonia removal is clinoptilolite (Bergero *et al.*, 1994). Zeolites are alumnosilicate clay minerals that are mined from natural deposits or synthesized from other clay minerals (Silapajarn *et al.*, 2006) that act as an ion-exchanging agents. In aquaculture practices, application of zeolite is suggested in ponds before stocking fry or during pond preparation (Briggs & Smith, 1996). Clinoptilolite has been found very effective in removing ammonia from water by means of its excellent ion exchange capacity since the seventies of last century (Wang *et al.*, 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). Sturgeons are the living representatives of an ancient and isolated

group of fish. they are important commercial fish and the Caspian Sea is the main habitat of the world largest population. *Huso huso* Linneaus, 1758 is a commercially important sturgeon spices which has become an endangered species. Probably, water quality changes in sturgeon culture is one of the main reason of mortality in larve and fingerling stage, this problem led us to conduct this study to determine lethal concentration (96 h LC_{50}) of ammonia in beluga sturgeon (*H. huso*), "the living representatives of an ancient and an endangered species" and to establish the proper amount of clinoptilolite zeolite for removal of ammonia compounds from water environment.

MATERIALS AND METHODS

Fish

This study was carried out in the Laboratory of Department of Natural Resources of Gonbad University (Gonbad, Iran). *H. Huso* fry averaged 46 ± 5 g in weight and 22 ± 4 cm in lenghth were collected from Sijaval Fish Culture Center, (Gonbad, Iran). The fish were acclimated to laboratory conditions for one week. They were kept in glass fiber tanks filled with 300 L fresh water (under constant aeration, temperature = 26 ± 2°C and pH = 8.2 ± 0.2). In the first series of experiments, 96 h LC_{50} value was determined following the static renewable bioassay method. (Sprague 1973). The experiments were done by Water Static Method during 96h in well aerated water.

Determination of LC_{50}

In the first series of experiments, 96 h LC_{50} value was determined following the static renewable bioassay method.

The experiments were conducted in five 35-lit flasks with concentrations of 0 (control), 15, 30, 50 and 75 mg.L-1 of total ammonia (prepared with NH_4Cl).

Forty five fish were placed in each treatment (15 in each repeat), with their respective duplicates, for a 96 hour exposure period. The amount of ammonium chloride in each aquarium was calculated after determining the exact volume of each aquarium. Observations were made 24, 48, 72 and 96 hours intervals. Fish were starved for 24 h prior to the experiment and throughout the bioassay study. The mortality rate recorded every 24 hours.

Zeolite efficiency test

In the second series of experiments, animals were exposed to different treatments for 4 days. The measured substantial amount of ammonia (75 $mg.L^{-1}$) was added to 3 treatments containing granulated zeolite (5, 10, 15 $g.L^{-1}$) with three replicates for each treatment. The amount of ammonia in each aquarium was measured every 12 hours.

Behavioral and Histopathological examination

The behavioral changes of the healthy fish and the fish exposed to various doses of ammonia wererecorded.25 specimens were randomly taken from gill, kidney and liver of fish exposed to lethal concentration of total ammonia and histopathological sections were prepared and stained in Hematoxilin and Eosin (H & E) according to available method (Bancroft et al. 1990).

Statistical Analysis

Comparisons of means ($p < 5\%$) were performed using split plot design and means comparison by the Duncan's test by SPSS.16. In all cases, the significance level adopted was 5%.

RESULTS

After the 96 hours exposure, there were 0, 28.88, 48.88, 97.77 and 100% mortality at the 0, 15, 30, 50, 75 $mg.L^{-1}$ concentrations of ammonia respectively (Table 1). Survival rate in control group was 100%. Table 1 shows the relation between the ammonia concentration and survival rate of *H. huso* after 96 hours. The results obtained from 96-hour toxicity experiments revealed that there was a significant difference between treatments by increasing the ammonia concentration ($p<0.05$) (Fig. 1). The 96-hour LC_{50} was 25.871 $mg.L^{-1}$ according to probity software (1.5) (Fig. 2). In the lethal concentration of ammonia (75 $mg.L^{-1}$), different amounts of granulated zeolite (0, 5, 10 and 15 $g.L^{-1}$) was used. By increasing zeolite in each treatment, the survival rate of fish was also increased significantly ($p<0.05$) (Fig. 3). Most absorption of ammonia was recorded in the first 12 hours of the experiment (Fig. 4). After the 96 hours exposure, no mortality was observed

in lethal concentration of ammonia, once 15g.L^{-1} zeolite was used in the trials (Table 2).

The behavioral changes in *H. huso* exposed to various concentrations of ammonia are as follow: In control group, there were no behavioral changes and deaths observed throughout the experiment. In experiment 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 in fish, exposed to lethal concentration of ammonia, showed: there was hyperplasia; edema, hyperemia, hemorrhageand expansion of secondary lamellain gills and hyperemia, hemorrhage and expansion of Bowman's capsule were showed in kidney. So, there were hemorrhage, inflammatory cells infiltration and hepatocytes necrosisin liver (Figs. 5 - 9). There was no lesion in control except some hyperplasia.

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

Concentration of total ammonia	N	Percent of mortality Subset for alpha = 0.05			
		1	2	3	4
0	3	.0000			
15	3		28.8833		
30	3			48.8867	
50	3				97.7767
75	3				1.0000E2
Sig.		1.000	1.000	1.000	.978

Means for groups in homogeneous subsets are displayed.

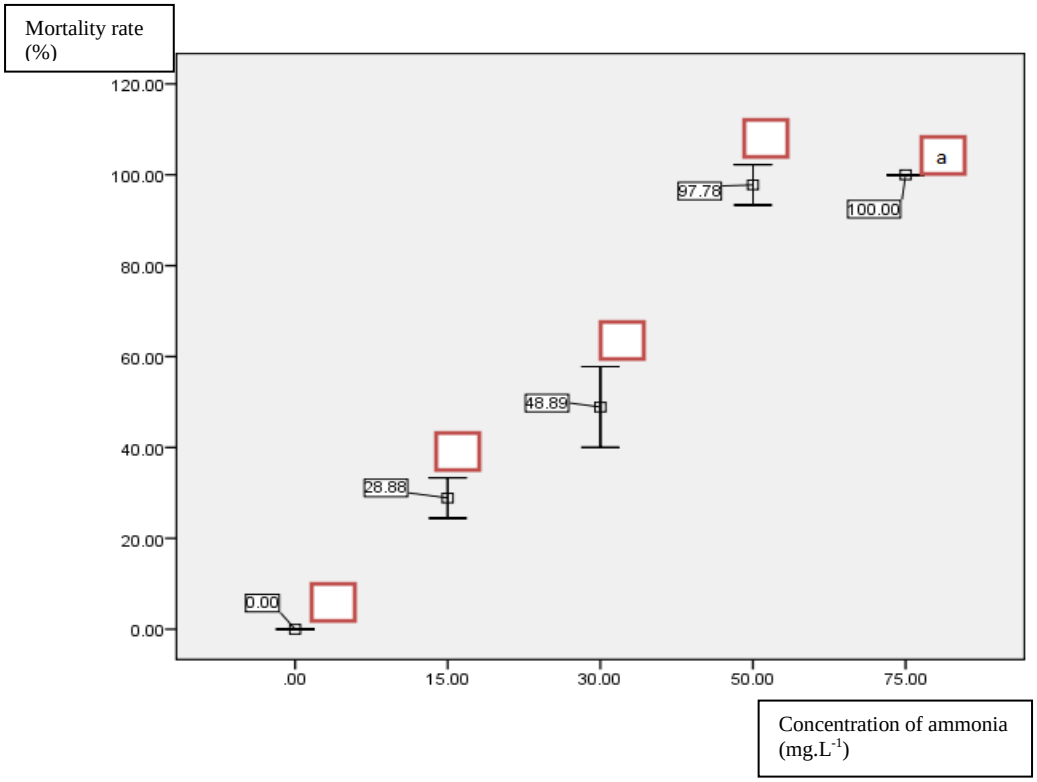

Fig 1. Fish mortality in different dosage of ammonia

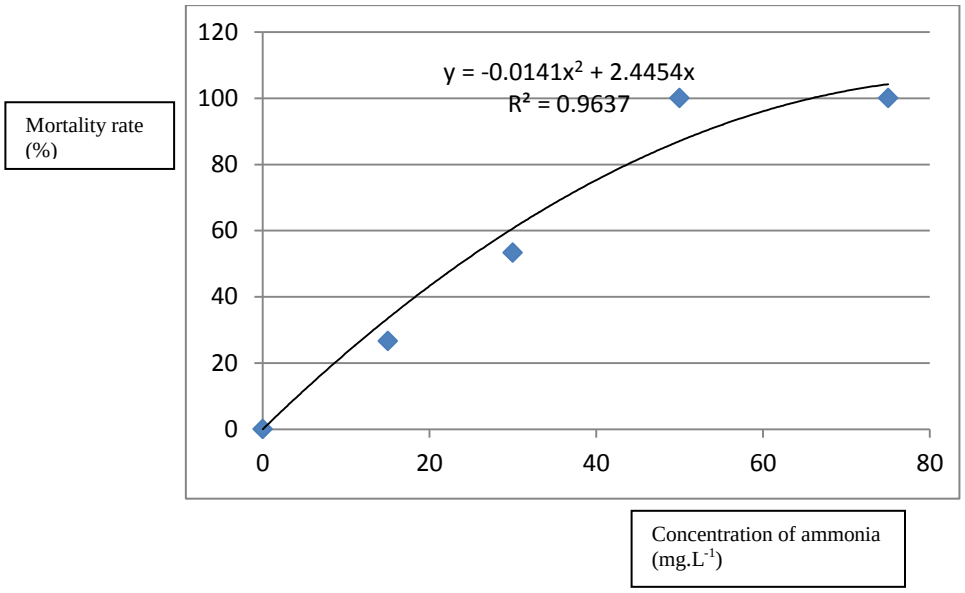

Fig. 2. Linear regression based on fish mortality in different dosage of ammonia

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

Concentration of zeolite	N	Percent of survival Subset for alpha = 0.05			
		1	2	3	4
0	3	.0000			
5	3		28.8867		
10	3			68.8833	
15	3				100
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

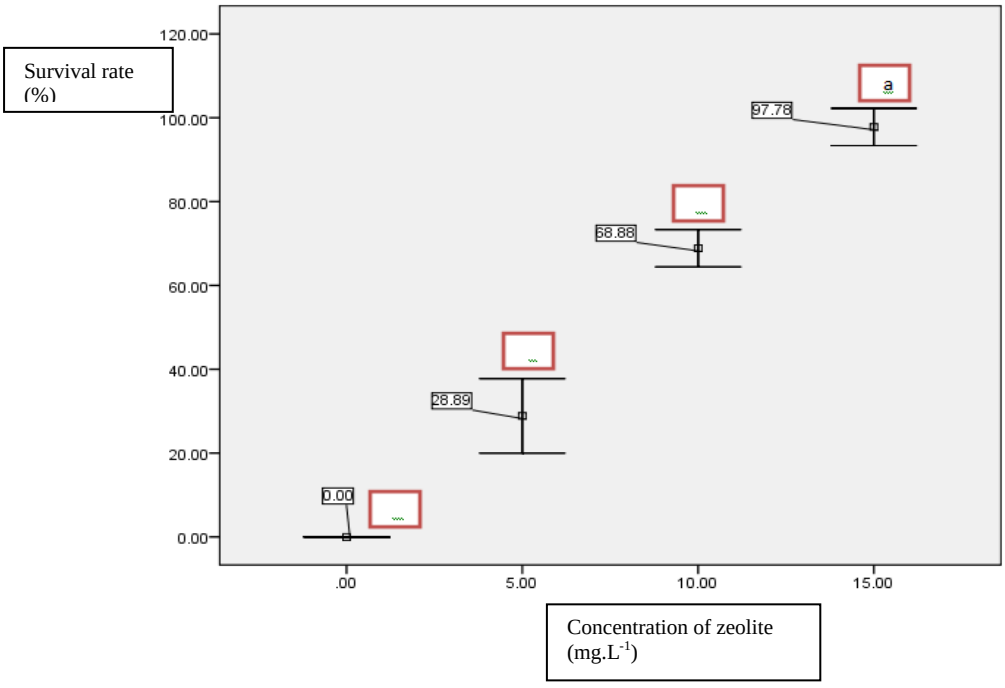

Fig 3. Survivalrate of the fish in different dosage of zeolite

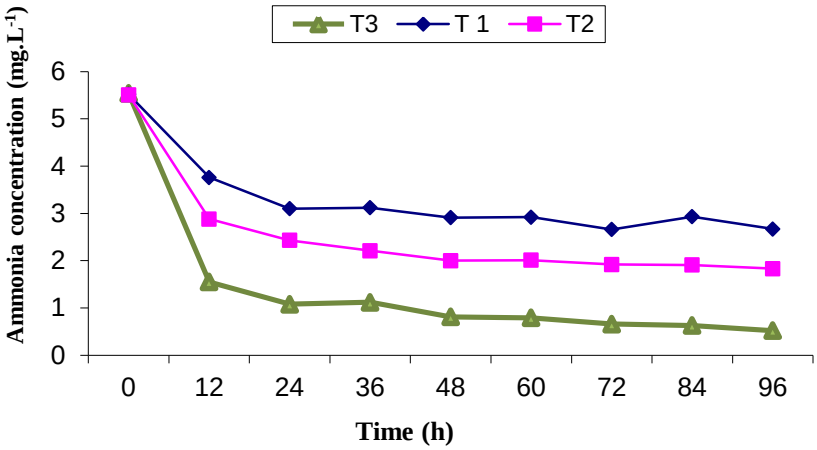

Fig. 4. Decreasing the ammonia (NH_3) concentration during 96 h

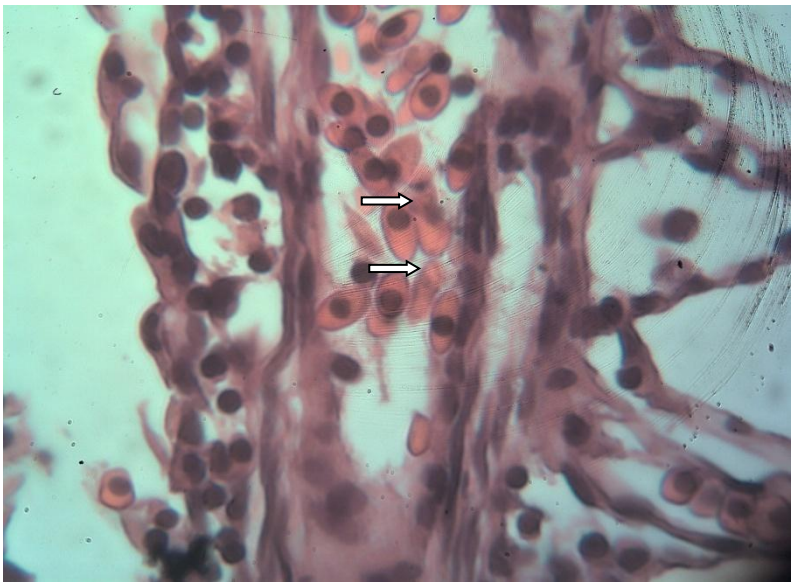

Fig. 5. Gill histological section exposed to lethal ammonia toxicity. Arrows show hyperemia (× 400, H & E)

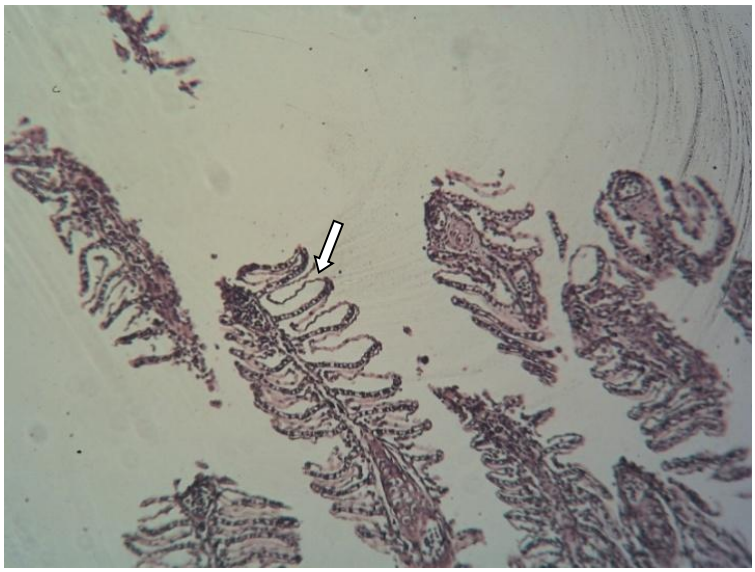

Fig. 6. Gill histological section exposed to lethal ammonia toxicity. Arrows show edema (×200, H & E)

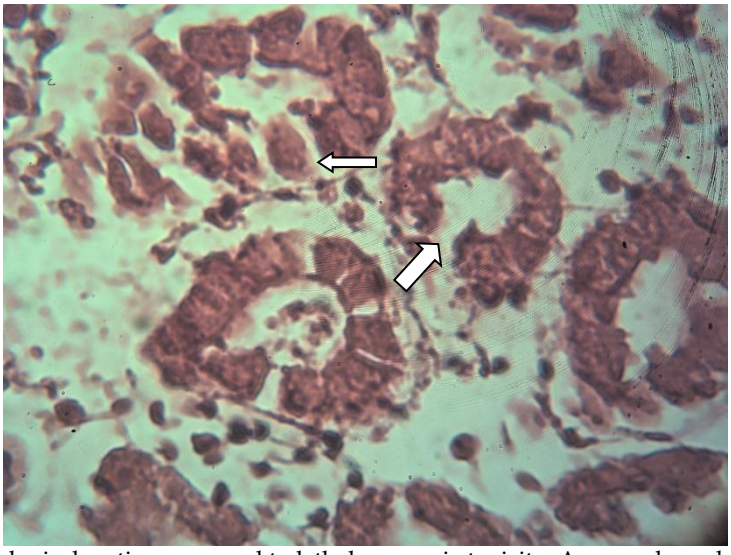

Fig. 7. Kidney histological sections exposed to lethal ammonia toxicity. Arrows show degenerated tubules in kidney (× 400, H & E)

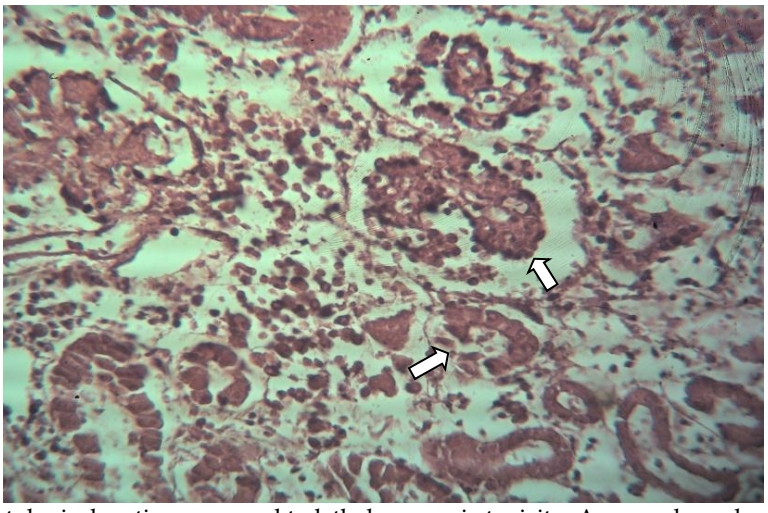

Fig. 8. Kidney histological sections exposed to lethal ammonia toxicity. Arrows show degenerated tubules of kidney and glumerol (× 400, H & E)

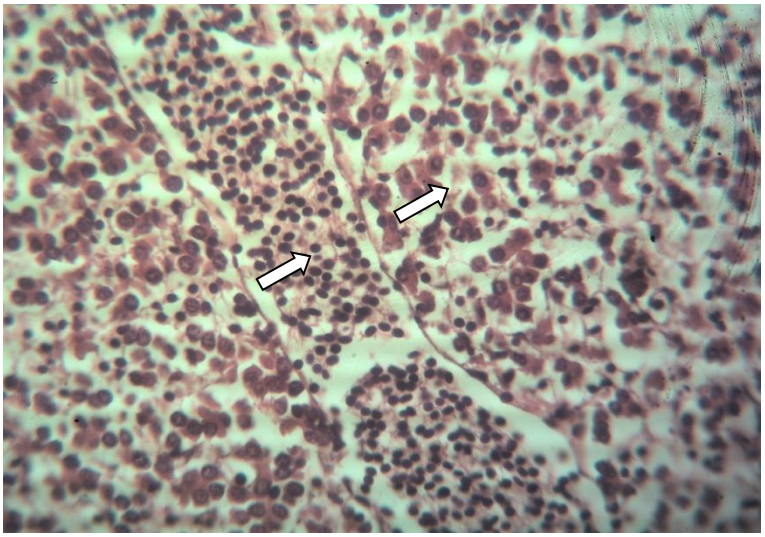

Fig. 9. Liver histological sections exposed to 30 mg.L^{-1} ammonia. Arrows show inflammation and necrosis (× 400, H & E)

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). Within the aquatic environment, at high concentrations, it exerts adverse effects by accruing structural damage, which affects the growth, development and survival of fish (Tuurala & Soivio, 1982). A regular response was increasing in mortality rate due to increased concentration of ammonia, it appears to have a direct effect on the growth of aquatic animals and 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 and environmental factors such as temperature, pH and hardness (Witeska & Jezierska, 2003; Giguere *et al*., 2004; Wong *et al.,* 1977; Lemus & Hung, 1999).

Lloyed (1961) reported for the rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) the toxicity ratio of dissolved ammonia, zinc salt, lead and copper, as well as, phenols begin to increase markedly below 60% oxygen saturation.In sturgeon, little information exists for ammonia toxicity. In the present study, the lethal concentration of ammonia was to 50 $mg.L^{-1}$ in *H. huso*. Most mortality happened at earlier hours. Consequently, the percentage and number of survivors decreased with increasing concentrations of ammonia in water.

Similar behavioral pattern has been reported about other ions such as ammonia on *Oncorhynchus mykiss* (Farhangi & Hajimoradloo 2008), Zinc on *Clarias gariepinus* Burchell, 1822 (Ololade & Oginni, 2010) and zinc on *Poecilia reticulata* Peters, 1859 (Gul *et al.,* 2009). All these observations were more pronounced with increasing concentrations of the toxicants. We observed significant difference in mortality rate between treatments and control particularly in higher concentrations of ammonia. Similar results have been reported by Farhangi and Hajimoradloo (2008); Gul *et al.,* (2009) and Farhangi (2010).

Our research showed that 5 $mg.L^{-1}$ concentration of ammonia did not affected the fish significantly ($p>0.05$).

Different fish species have different sensitivity to different concentrtion of the sameions. For example, Salmonids (i. e. *Salmo salar* Linnaeus, 1758) are more sensitive than the other fish species, due to their high oxygen need (Abdullah & Muhammad, 2006).

In the present study, the fish exposed to ammonia were observed to be highly irritable and displayed frenzied swimming when handled; 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 exposed *Clarias gariepinus* to copper and Farhangi (2010) who exposed *Cyprinus carpio* to zinc.The survival rate in beluga was increased by adding the amount of zeolites. The present experiments showed, that adding NH_4Cl salt to trials (>20 $mg.L^{-1}$ $N\text{-}NH_4$) cause slowly gill ventilation, air gulping, bending muscles, increasing opercular and buccal movement and hyper excitability in experimental fish. Similar results were also obtained by Knoph (1996) (0.34 $mg.L^{-1}$ $N\text{-}NH_3$ in smolts) and Peyghan, (1999) (150 $mg.L^{-1}$ $N\text{-}NH_4$ in common carp). Chiayvareesajja and Boyd (1993) showed that application of $2gL^{-1}$ zeolite for absorbtion of $2mg.L^{-1}$ $N\text{-}NH_4$ could remove $N\text{-}NH_4$ by 80-90%. Brgero (1994) revealed that in water containing $10mgL^{-1}$ $N\text{-}NH_4$, around 80% of ammonia could be removed by adding 100gr zeolite pre 45L water, so final concentration was about 2.06 $mg.L^{-1}$ $N\text{-}NH_4$. According to Peyghan (1999), application 10 $g.L^{-1}$ of zeolite could prevent carp mortality at lethal concentration of total ammonia (150 $mg.L^{-1}$) after 24 hours. James *et al.* (2000) reported, that application of 2, 4 and 8 $g.L^{-1}$ zeolite to Cu contaminated media, can completely remove Cu from water. In our attempt we observed, that application of 15 $g.L^{-1}$ zeolite, to ammonia contaminated media, remove ammonia from water completely and it quickly reduced $N\text{-}NH_4$ concentration after the first hours.

In the persent study, applying zeolite has directl relation to the removal of ammonia compounds. In aquaculture practices, application of zeolite is suggested in ponds before stocking fry or during pond

preparation (Briggs & Smith, 1996).When the concentration of ammonium ions exceeds the permissible limit in aquaculture ponds, it becomes toxic to fish life (Sampath *et al.* 1991; James *et al.* 1993) hence it is advisable to reduce the concentration below the permissible limit by application of zeolite. 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 hours. The obtained results revealedthat fish mortality is due to degenerated tubules and glumroles of kidney, necroisis of hepatocytes. In aquaculture practices, application of zeolite is suggested in ponds before stocking fry or during pond preparation (Briggs & Smith, 1996).

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جلوگیری از مسمومیت حاد با آمونیاک در ماهی بلوگا (*Huso huso*) با استفاده از زئولیت طبیعی

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چکیده

آزمایش ها به منظور بررسی کارآیی زئولیت طبیعی در جلوگیری از مسمومیت حاد با آمونیاک در فیل ماهی صورت گرفت. این آزمایش ها با استفاده از روش آب ساکن در مدت 96 اجرا شد. ماهیانی با وزن نسبی (5 ±46گرم) و طول کل (4±22سانتی متر) در معرض غلظتهای مختلف (15،30،50،75میلی گرم در لیتر) از آمونیاک قرار گرفتند. یک گروه بعنوان شاهد درنظر گرفته شد. تحت شرایط ثابت، غلظت کشنده آمونیاک کل درمدت 96ساعت معادل 75 میلی گرم در لیتر بدست آمد. در غظت کشنده آمونیاک کل، از مقادیر مختلف زئولیت کلینوپتیولیت (5،10،15 گرم درلیتر) استفاده شد. نتایج حاصل از آزمایش اختلاف معنی داری را در بین گروهها نشان داد($p<0.05$). با افزایش مقادیر زئولیت کلینوپتیلولیت نرخ بقا ماهیان بطور معنی داری افزایش یافت ($p<0.05$). استفاده از 15 گرم درلیتر زئولیت در غلظت کشنده آموناک کل توانست از تلفات ماهی جلوگیری کند. نتایج حاصل از آسیب شناسی نشان داد بیشترین ضایعات شامل خونریزی، پرخونی، هیپرپلازیا، نکروز سلولهای پوششی، تخریب مجاری کلیوی،اتساع کپسول بومن و نکروز سلولهای کبد بود. در گروه شاهد هیچگونه ضایعه ای مشاهده نشد.

* مولف مسئول