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Research Article

Increasing of survival rate to *Acipenser persicus* by added Clinoptilolite zeolite in acute toxicity test of ammonia

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Abstract. The experiments were done to increase the survival rate to *Acipenser persicus* by added Clinoptilolite zeolite in lethal concentration of ammonia. Twelve fishes with average weight 26 ± 3 g were exposed to different concentrations (5, 15, 25, 35 mg L^{-1}) of total ammonia salt. A group of fish was considered as control. Under stable condition, the lethal concentration of total ammonia was 35 mg L^{-1} during 96 hours. In the lethal concentration of total ammonia different amount of 4, 8, 12 g L^{-1} Clinoptilolite zeolite were used. Results indicate significant differences between treatments with each other and also control ($p<0.05$). With increasing zeolite in each treatment, the survival rate of fish also increased significantly ($p<0.05$). By addition of 12g L^{-1} concentration of powdered zeolite, the mortality of fish could be prevented after 96 hours. Histopathological findings showed that major lesion were hemorrhage, hyperemia, hyperplasia and epithelial cells necrosis. Also observed was degenerated tubules of kidney, expansion of Bowman's capsule and hepatocytes necrosis by observations.

Key Words: acute toxicity, ammonia, *Acipenser persicus*, Clinoptilolite, lethal concentration.

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Introduction

The problem of water quality tends to be very important in the globalisation era, both for aquaculture and conservation (Colt 2006; Filimon *et al* 2009; Ozcan & Balik 2009; Telcean *et al* 2011). Fish production capacity can increase by identifying environmental factors and providing an appropriate environment for the fish (Imanpoor *et al* 2011). Heavy metals can negatively influence fish, in particular disrupting the endocrine system, and decreasing the quantity and quality of offspring (Popek *et al* 2009). Two of the principal methods to remove ammonia from water include nitrification and ion exchange (Emadi *et al* 2001). Materials such as oyster shell, rock, sand, activated carbon etc can be used as a substrate for the bacteria involved in nitrification. Ion exchange is a process in which ions from a synthetic or natural resin are exchanged with specific ions in wastewater. Some natural resins, such as zeolite, are used to remove ammonia (Bergero *et al* 1994), and one of the best zeolites is Clinoptilolite (Wang & Walsh 2000). Because of this, it can be the basis of its use in aquaculture.

Zeolites are aluminosilicate clay minerals that are mined from natural deposits or synthesized from other clay minerals. Zeolite crystals consist of a framework of SiO^{-4} tetrahedral into which Al^{+3} has been substituted for some of the Si^{+4} (Silapajarn *et al* 2006; Smical *et al* 2010; Smical 2011ab).

UIA (UnIonized Ammonia) in high levels showed different effects on variety of fishes. But until today, no studies respecting ammonia compounds toxicity have been achieved for beluga sturgeon *Acipenser persicus* Borodin, 1897. This research tried to determine lethal concentration (LC_{96}) of ammonia compounds on *A. persicus* and survey the effects of Clinoptilolite zeolite to removal of ammonia compounds from water environment.

Materials and Methods

The experiments were performed in Shahid Marjani Sturgeon Culturing Center, Iran. Temperature in all stages was maintained to 26 ^{O}C and pH was equivalent 8.2. The experiments were done by Water Static Method (Peyghan 1999) during 96 hours (4 days). Different amounts 0, 5, 15, 25, 35 mg L^{-1} of ammonia salt were used. In each basin, 12 fishes with average weight 26 ± 3 g and total length 12 ± 2 cm were placed. Treatments every 12 h were attended from behavior and mortality. Used ammonia was ammonium chloride (made by Merck Company, Germany). Total ammonia concentrations were measured with hack colorimeter DR/890 (made by USA). After the determination of the lethal concentration of ammonia (LC_{96}) to *A. persicus*, the main test to measure the efficiency of zeolite was assessed in removal of ammonia lethal concentration. The used zeolite was Clinoptilolite type with 90% purity which was prepared from Afrand Toska of Iranian Company. Hereby determination of the concentration and quantities of the used zeolite for absorbing of the lethal concentration of ammonia from the basin samples was the most important step of this research. Granulated

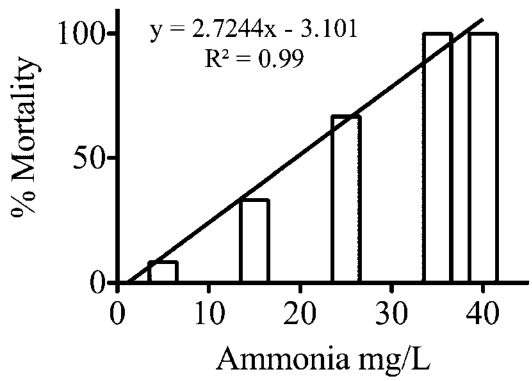

Figure 1. Fish mortality in different dosage of ammonia

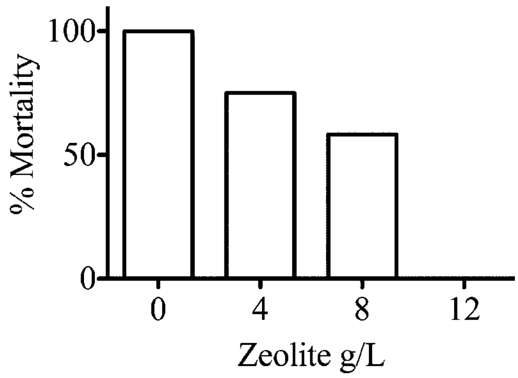

Figure 2. Reduction of mortality rate of the fishes in main test in different treatments.

zeolite at 3 treatments in 4, 8, 12 g L^{-1} with three replications for each treatment and a control treatment was used. In duration time of the experiments till to last test, at each 12 hours, the amount of ammonia in the water basin was measured and also all nominal behaviors of toxicity on fishes were observed. Airstone has been used in treatment as an aerator. Samples were taken from gill, kidney and liver of fish and histopathological sections were prepared. The results were compared by ANOVA (Analysis of Variance).

Results and Discussion

Based on the results in preliminary stage, highest mortality rates in doses of 35 mg L^{-1} were observed. Ionized ammonia lethal concentrations were equivalent to 35 mg L^{-1} in sturgeon fishes during 96 hours. High mortality rates were observed in the early hours. Mortality percentage of fish exposed to different concentration of ammonia (0, 5, 15, 25, 35 mg L^{-1}) after 96 hours is shown in Figure 1. All fish survived in control. Mortality percentage of fish was 0, 8.33, 33.33, 75 and 100%, respectively, in different concentration of ammonia. Results revealed mortality percentage of fish increase with increasing of ammonia concentration. The LC_{50} (median lethal concentration) was 18.65 mg L^{-1} by used of linear regression and relationship between mortality percentage and ammonia different concentrations (Figure 1).

At the beginning of the test, some behavioral symptoms such as gasping, swallowing water, the curvature of muscles, hit the basin sides, lack of balance and severe reaction to external factors were observed in fishes. At duration of the experiments the ammonia poisoned fish sat on the floor of the basins and finally died. Most absorption of ammonia in the first 12 hours after the start of testing was recorded. Eventually, the absorption rate of ammonia by zeolite treatments with increasing zeolites dramatically increased. Results indicate significant differences between treatments with each other and also control ($p<0.05$). The third treatment showed highest rate of absorption of ammonia than other treatments. With increasing zeolite in each treatment, the survival rate of fish also increased significantly ($p<0.05$). Application of 12 g L^{-1} granulated zeolite could be prevented the mortality after 96 hours (Figure 2). Results indicate that this ability to absorb ammonia by the zeolite after some time depends on the amount of zeolite that used for removal ammonia compounds and with decreasing ammonia, the zeolites reached the saturation point and not being able to continue the absorbing of this compounds.

The study of histological samples show that, the common lesions of fish gill exposed to ammonia lethal concentration were hyperplasia, edema, hyperemia, hemorrhage, expansion of secondary lamella, epithelial cells necrosis of gill and inflammation. In control group and zeolite without ammonia group less hyperplasia in tip of gill filaments and edema were observed too (Figure 3). The major lesions in kidney were such as expansion of Bowman's capsule, hemorrhage, hyperemia, degenerated tubules of kidney, epithelial cells necrosis of kidney and a lot of monocellular. In fishes kidney of control absorb any lesions (Figure 4). The lesions in liver were hyperemia, hemorrhage, inflammatory cells infiltration and hepatocytes necrosis (Figure 5). In the control samples there were no observed lesions. By adding of Clinoptilolite zeolite in lethal concentration of ammonia of water solution samples, less toxicity of ammonia and followed by less lesions of fishes have resulted.

The use of Clinoptilolite zeolite to increase survival rate to *A. persicus* was studied. Since, the ammonia toxicity is important for fish and shrimp that were reported by others (Frias Espericueta 1999; Mokarami & Emadi 2007; Farhangi & Hajimoradloo 2008; Peyghan 1999; Knoph 1996). Chen (1992) indicated that levels of $N\text{-}NH_3$ from 0.01 to 1.0 mg L^{-1} reduced growth of fresh water fish. He reported MATC (maximum acceptable toxicant concentration) for shrimp is below 5 mg L^{-1} of $N\text{-}NH_4$ or 0.35 mg L^{-1} of $N\text{-}NH_3$. Lloyd (1961) reported for the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) that below 60% oxygen saturation, the toxicity of dissolved ammonia, zinc salt, lead and copper, as well as phenols increase markedly. Wickens (1976) reported that above 0.1 mg L^{-1} of $N\text{-}NH_3$ growth of *Macrobranchium rosenbergii* (De Man, 1879) post larvae in salinity of 0.5 ppt (for 6 weeks of exposure) was significantly reduced to 60-70%. Muir (1982) reported effects of $N\text{-}NH_3$ (to 0.130 mg L^{-1}) on growth for rainbow trout. According to Knoph (1996) 48h-LC_{50} was 59.4 mg L^{-1} of $N\text{-}NH_4$ (0.34 mg L^{-1} of $N\text{-}NH_3$) for smolt salmons.

No acute ammonia toxicity data exist for *A. persicus*. In the case of ammonia, it acts as both a acute toxin and a chronic stressor. The unionized ammonia molecule (NH_3) is the toxic form because of its ability to move across cell membranes (Colt 2006).

Figure 3. Arrows show edema and hyperemia of fish gills exposed on lethal ammonia toxicity (*100).

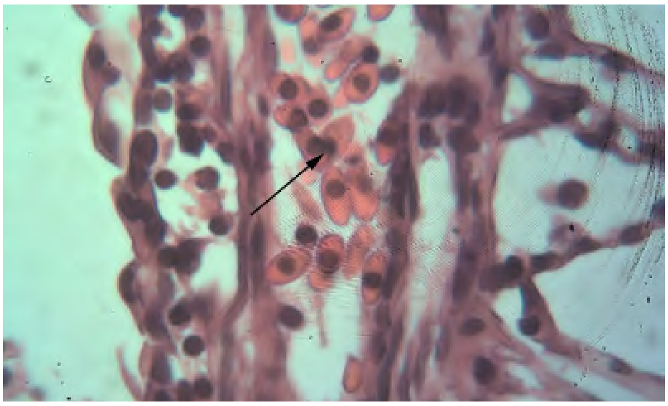

Figure 4. Arrow shows hemorrhage of the gill histological section exposed on lethal ammonia toxicity (* 100)

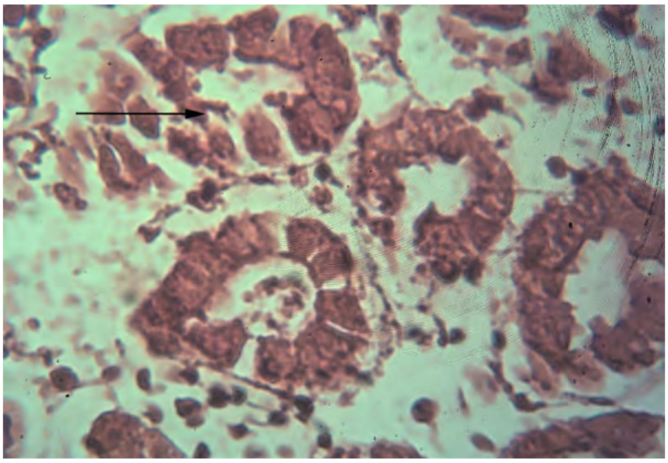

Figure 5. Arrow shows degenerated tubules of kidney histological section exposed to lethal ammonia toxicity (* 400).

In alkaline waters the amount NH_4^+ is low. In this situation, the gradient between plasma NH_3 and water is reduced, decreasing the excretion of NH_3 and hence it accumulates in the plasma and tissues. Most biological membranes are permeable to ammonia but relatively impermeable to ammonium ions. The specific biochemical mechanism of ammonia toxicity in fish is not fully explained, but it has been shown that ammonia intoxication impairs ATP production, induces a store depletion of polysaccharide and plasma ions, alters the neuronal synaptic transmission, and induces leucopenia, inflammation and degeneration of gills and kidneys. An ammonia molecule is too small to act as an antigen and cannot directly modify the immune system by acting in the usual way. 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). It was found that some teleost fish can employ ureogenesis when environmental conditions are not in favor of ammonia excretion, e.g. high alkalinity water, limited water sources or high environmental ammonia (Randall *et al* 1989; Iwata & Deguchi 1995). The lethal concentration in varieties of fishes is different and depends to species, age and environmental factors in water. Temperature, salinity, particulate matter, food availability, current speed and water depth have been examined in multiple- and single locality studies, and have been found exert varying degrees of influence upon their growth and condition (Celik *et al* 2009). In this study the lethal concentration of ammonia was equal to 35 mg L^{-1} NH_4^+. The most mortality has happened at earlier hours. Similar results were obtained by Mokarami & Emadi (2007) and Farhangi & Hajimoradloo (2008). The last authors also reported that 24h-LC_{50} was 15.77 mg L^{-1} of total ammonia for rainbow trout. The present experiments showed addition of NH_4Cl salt to trials as an N-NH_4 cause to appear some nominal signs on fish behavior. So, the experiments showed at high ammonia concentration signs had happened such as slowly gill ventilation, air gulping, bending muscles, increase of opercular and buccal movement and hyper excitability. Similar results were also obtained by Farhangi & Hajimoradloo (2008) and Peyghan (1999). In the fish culture systems, various factors exacerbate ammonia toxicity, such as CH_4, H_2S and low oxygen and high temperature. Various reasons are found for fish mortality as following:

- degenerated tubules of kidney and glumroles, they cause to disorganize kidney function (Peyghan 1999; Svoboda & Vykusova 1995);
- disorient of osmoregulation conclusion gill harm (Knoph 1996);
- declination of high and special of ammonia to brain, that it causes convulsion (Svoboda & Vykusova 1995);
- ability reduction of oxygen transfer by blood on one hand and increasing of plasma ammonia on the other hand (Mokarami & Emadi 2007; Knoph 1996). Similar results were obtained by the present experiments.

The survival rate in beluga fishes by adding the amount of zeolite has increased. Applying of zeolites was directly relative to removal of ammonia compounds in this study. The most efficient removal rate of ammonia by zeolite was achieved when granulated zeolite was applied at 12 g L^{-1} concentration. However, during the trial, fishes showed many severe reactions like disquiet and spasm. According to Peyghan (1999) findings, application of 10 g L^{-1} of zeolite concentration could prevent carp mortality at lethal concentration of total ammonia (150 mg L^{-1}) after 24 hours. Farhangi & Hajimoradloo (2008) reported that the use of 15 g L^{-1} powder zeolite can prevent mortality rate of rainbow trout fish in the lethal concentration of total ammonia. Chiayvareesajja & Boyd (1993) and Mokarami & Emadi (2007) demonstrated that finely zeolite is more effective than coarser zeolite for ammonia removal. Therefore zeolite powder was used in trials. Tests revealed that zeolite quickly reduced N-NH_4 concentration after the first hours. The concentration of N-NH_4 decreased drastically with increasing zeolite concentration. The experiments showed that N-NH_4 concentration decreased by 80-90%, however the complete removal of N-NH_4 was not achieved even when zeolite was applied at 12 g L^{-1} concentration. These findings are in agreement with studies of others (Chiayvareesajja & Boyd 1993; Mokarami & Emadi 2007; Peyghan 1999). Chiayvareesajja & Boyd (1993) showed that application 2 g L^{-1} of zeolite at 2 mg L^{-1} of N-NH_4 concentration could remove the N-NH_4 by 80-90%. Bergero (1994) revealed that in water containing 10 mg L^{-1} of N-NH_4 around 80% of ammonia could be removed by adding 100 g zeolite per 45 L water, so finally concentration was about 2.06 mg L^{-1} of N-NH_4. Although fish survived in these treatments, evidence of ammonia toxicity was present.

By using of zeolite in the main test, removing of ammonia molecules was faster by increasing of the zeolite concentration in different treatments until zeolite reached the saturation point. Hereby a particular amount of zeolite needed to be added as an active absorbance to remove the ammonia from the water solution.

Conclusions

We have considered that zeolites have a great potential to absorb the toxic compounds such as ammonia in water solution. During this research we have found that the normal amount of zeolite for absorbing the ammonia from the basin or fish ponds is about 2-5 g L^{-1}. This concentration of zeolite could decrease the toxicity of ammonia on the water solution and prevent the mortality of fish. The use of zeolites seems to be a promising matter in intensive aquaculture systems. Also it is mentionable that the main advantage to use the zeolites as absorbers is their recycling to back as fresh zeolites for multi applications.

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