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The Influence of Calcium and Sodium on Aluminum Toxicity in Nile Tilapia (*Oreochromis niloticus)*

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Abstract: Aluminum (Al) is one of the most important factors in the toxicity of acidified waters to freshwater fish species. Two experiments were conducted to evaluate the influence of water strength on the toxicity of aluminum in fish by comparing the mortality of Nile tilapia (*Oreochromis niloticus)* exposed to aluminum with addition of Ca^{++} or Na^{+}. A preliminary bioassay determined the 96h LC_{50} of aluminum nitrate to be to 580µgl^{-1}. In first experiment, Nile tilapia were exposed to this concentration with combination of five different amounts of calcium giving water ionic strength ranging from 5.3 to 7.1 (10^{-4}M) . In the second experiment fish were also exposed to the LC_{50} of aluminum but with five different amounts of sodium giving different water ionic strength as in the first experiment. The addition of Ca^{++} and Na^{+} was calculated in order to obtain an identical increase in water ionic strength in both experiments. Fish mortality decreased systematically with the addition of either base cations, Ca^{++} or Na^{+}. Furthermore, increasing ionic strength with Na^{+} was a larger extent than the corresponding increase in ionic strength by the addition of Ca^{++}. The interaction between aqueous aluminum and gill surface was also discussed. It has been demonstrated that gills of tilapia exposed to Al-alone showed a severe fusion of lamellae and filaments and abnormally large amount of mucus. In contrast, theses structural lesions were reduced to some extent by adding Ca^{++} or Na^{+}.

Key words: Nile tilapia, calcium, sodium, aluminum, toxicity, Egypt

INTRODUCTION

In aquatic ecosystems, the levels of toxic metals have increased either directly, as a result of atmospheric deposition and waste-water discharge and runoff (e.g., Pb, Hg, Cu and Zn), or indirectly, through increased solubilisation and mobilization from sediments owing to acidification (e.g., Fe and Al). Mobilization of aluminum is one of the most important consequences of the acidification of soil water systems from the edaphic to aquatic environment (Rosseland and Staurnes, 1994). Aqueous aluminum is recognized by many authors as the principal toxicant killing freshwater fish in acidified waters (Allian and Wilson, 1999; Guibaud and Gauthier, 2003). Aluminum primary leaches out from soils with low pH-buffering capacity i.e., low dissolution rates of base cations. Such acid sensitive catchments are characterized by slowly weatherable soils and rocks, often with only a thin soil cover. Within these catchments, the soil water and runoff concentration of terrestrial derived base cations, like Ca^{++} and Na^{+}, are normally low. Furthermore, acidification is responsible for the depletion of base cations from the soil complex and runoff (Palmer and Driscall, 2002). Accordingly, it has repeatedly been observed that negative impacts of acidification on freshwater fish are most severe in low conductivity waters (Rosseland and Henriksen, 1990).

The ionic strength is important for the Al-toxicity as the interaction between aqueous aluminum and the gill surface is central for the mechanism of acute Al-toxicity in fish (Poléo, 1995; Bjerkenes *et al*., 2003; Alstad, *et al*., 2005).

Ionic binding is probably of major importance for the interaction between aluminum and the gill surface this is because cations may compete for the negatively charged sites on the gill surface and also increased ionic strength will reduce the rate of ionic binding between opposite charged molecules (Geromyszkelkowska and Szubartowska, 1999). The ionic strength (I) is defined as: $I = 0.5 \sum c_i z_i^2$.
(c^i) is the molar concentration and (z_i) the charge of an ion (i) and the sum is taken over all ions in the solution.

Increase ionic strength may reduce Al-toxicity both by reducing the ability of aluminum to bind to the gills by ionic bonds, or by competition between base cations and positively charged aluminum for negatively

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charged sites on the gill surface (Andrèn *et al*., 2006). Ca^{++} is the predominant cation in most natural waters, and the concentration of Ca^{++} is positively correlated with ionic strength. It is well documented that high concentrations of Ca^{++} can reduce Al-toxicity in fish (Playle *et al*., 1989). This multigating effect has most often been attributed to Ca^{++} through its effect on fish gill permeability. Ca^{++} is important in stabilizing biological membranes, maintaining the integrity of cell to cell junctions, and controlling ion and water permeability across epithelial tissues (McDonal, 1983) It is possible that the mitigating effects of Ca^{++}, and base cations in general can, to some extent, be explained by the water ionic strength (Kroglund and Finstand, 2003).

According to this, it has been indicated that not only calcium, but also high water sodium concentration may reduce Al-toxicity in fish (Brown, 1981; Dietrich *et al*., 1989).

In the present study we investigated the possible effect of ionic strength on the toxicity of aluminum. We tested the prediction that an equal increase in ionic strength, by the addition of Ca^{++} and Na^{+} respectively, will reduce the Al-toxicity to the same extent. Also, because of the main target of the metals is the gill, which is the main site of several functions, gas exchange, acid-base regulation and nitrogenous excretion, the influence of aluminum on the gill were investigated.

MATERIAL AND METHODS

Nile tilapia (*Oreochromis niloticus*), 49 ± 5 gm, were obtained from a local hatchery. The experimental fish were brought into the department, where they were kept and acclimatized for two weeks prior to the experiments. Two bioassays were conducted to facilitate evaluation about calcium and sodium effects on acute aluminum toxicity. The first bioassay determined the amount of aluminum nitrate needed to affect 96h LC_{50} at ionic strength of 5.3 to 7.1 (10^{-4}M). The second bioassay was conducted to evaluate the mortality response of Nile tilapia exposed to potentially toxic concentration of aluminum nitrate in waters with different ionic strengths. Two experiments were conducted: one varied calcium and the other varied sodium. Each combination of calcium or sodium with water ionic strength was replicated in four aerated aquaria.

Experiment 1 involved exposing fish to the LC_{50} (580µgl^{-1}) of aluminum nitrate in environment with five different concentrations of calcium giving water ionic strength ranging from 5.3 to 7.1 (10^{-4}M). Experiment 2 involved exposing fish also to 580 µgl^{-1} aluminum nitrate but in environment with five different concentrations of sodium giving water ionic strength identical to experiment 1 (Table 1).

Table 1: Water quality data (pH and ionic strength) for Nile tilapia exposed to (LC_{50}) 580 µgl^{-1} Aluminum nitrate using different amounts (1,2,3,4 and 5) of Ca and Na.

Exposure		pH	ionic strength
Al-only		5.66±0.08	4.55±0.05
Al + Ca	1	5.64±0.09	5.38±0.07
	2	5.66±0.07	5.88±0.06
	3	5.64±00.6	6.13±0.09
	4	5.70±0.08	6.63±0.06
	5	5.67±0.05	7.13±0.08
Al + Na	1	5.64±0.09	5.38±0.06
	2	5.66±0.07	5.88±0.07
	3	5.64±00.6	6.13±0.03
	4	5.70±0.08	6.63±0.05
	5	5.67±0.05	7.13±0.04

Histological studies: Gills were removed and fixed for two days in buffered formalin (10%) and routinely embedded in paraffin wax for light microscopy examination. Sections were cut at 5µm and stained in with haematoxylin and eosin (H&E).

The Al-stock solution was prepared by dissolving $Al(NO_3)$ $9H_2O$ in distilled water. The pH of this stock solution was kept low (pH 2.0) in order to ensure that the total amount of Al was present as Al^{3+} before it was added to the water. The pH (2.0) of the Al-stock solution was rapidly raised to 5.5, as this solution was mixed with distal water (pH6.8). The experiment which received only untreated water acted as control. Water temperature varied between 18 ± 4 ºC during the experimental period.

Water pH was measured using a Radiometer PHM-80 with a Radiometer GK-2401C combined glass-electrode. The conductivity was measured using a Radiometer CDM-80.

Experimental fish were randomly distributed through aquaria of 100L. here were 25 fish in each of aquarium and 3 tanks for each treatment. In the control, however, we used 12 fish. Each exposure aquarium was examined for dead fish at least once a day. Fish were judged to be dead when opercular movements had ceased and no swimming response could be elicited by stimulation of the caudal peduncle. Water temperature, conductivity and pH were measured daily throughout the experiments.

Statistical Analysis:

The results from each treatment were compared with control results by using Student's t-test. A value of<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

While no mortality was observed in the control fish, mortality was recorded in all Al- exposures. 580µg l^{-1} concentration of Aluminum nitrate was required to affect a 96-h LC_{50} for Nile tilapia placed in water at pH 5.5 and temperature of about 21.5ºC. There were significant differences in mortality between the Al-only exposures and the Al-exposures with base cations added (Fig. 1) Mortality was lowest in the Al+Na-exposure. Also in the Al+Ca-exposure mortality was significantly lower than the corresponding Al-only exposure, but significantly higher than Al+Na-exposure (P<0.01) (Fig. 1).

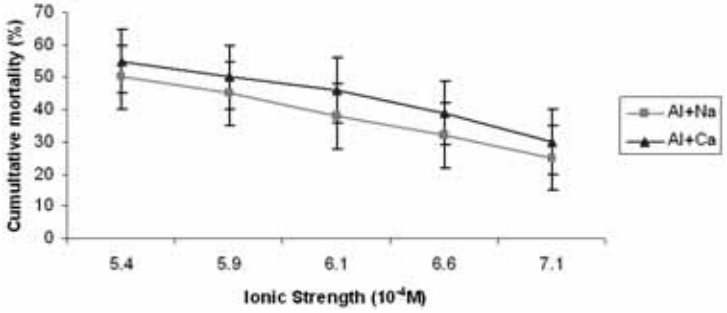

Fig. 1: Cumulative mortality of Nile tilapia exposed to 250µl^{-1} (LC_{50}) aluminum nitrate at varing ionic strengths.

Our results confirm previous observations that the acute toxicity of aluminum is reduced with the addition of base cations to Al-rich water. In addition to this, we observed that an equal increase in ionic strength due to the addition of Ca^{++} and Na^{+}, respectively, did not reduce the Al-toxicity to the same extent. Sodium seems to be the more effective cation in reducing Al-toxicity.

In this study we observed that both the addition of Ca^{++} and Na^{+} had an effect on the Al-toxicity in tilapia. This agrees well with the literatures. It has been accepted for a long time that aqueous Ca^{++} mitigates Al-toxicity in fish (Brown 1983; Playle and Wood. 1989). It has also been demonstrated that high concentrations of Na^{+} in the water reduces Al-toxicity (Brown 1981; Dietrich *et al*., 1989). The The effect of aqueous Ca^{++} has been attributed to its stabilizing effect on the gill epithelium (McDonald, 1983). Increasing the concentration of aqueous Na^{+} might reduce passive efflux and facilitate compensatory Na^{+}. Poléo and Bjerkely (1997) found that salmon exposed to aluminum did not suffer from ion regulatory disturbances despite enhanced mortality (75% after 68h). Andrèn *et al*. (2006) also demonstrated that Al-toxicity in rainbow trout at relatively high pH was predominately due to hypoxia rather than ion regulatory disturbances. Therefore, in our study we might assume that, at least to some extent, the tilapia was suffering from hypoxia. So, it is reasonable to attribute the mitigating effects of Ca^{++} and Na^{+} to their contribution to water ionic strength, reducing the possibility for aluminum to interact with gill surface.

As already mentioned, our experiments demonstrate that increasing Ca^{++} or Na^{+} concentrations reduces the toxic effects of aluminum. The highest effect was obtained by the addition of Na^{+}, the reason for this difference could be that Ca^{++} and Na^{+} do not influence Al-chemistry in the same manner. Na^{+} is more effective in ameliorating the Al-toxicity than Ca^{++}, because on equivalent basis, about a 1.5 times higher amount of Na^{+} is needed to obtain the same ionic strength increase as by Ca^{++}. Thus, the possibility of a Na^{+} ion to be present near the surface of the fish gill should be higher in comparison to Ca^{++}. On the other hand, Ca^{++} is a divalent ion and should therefore have better qualitative properties in competition for cation exchanges sites than monovalent Na^{+}, like substituting aluminum present on negatively charged sites at the gill surface. However, Ca^{++} as a divalent ion will have a thicker hydration shell than Na^{+}, which may prevent Ca^{++} to come close to the gill surface as Na^{+}.

The interaction between aqueous aluminum and gill surface seems to be of major importance for the acute toxicity of aluminum in fish (Exley *et al*., 2002; Poléo & Bjerkey, 2000). In the present study, it has been investigated that gills of tilapia exposed to Al-alone showed a severe fusion of lamellae and filaments and abnormally large amount of mucus (Fig. 2A). In contrast, theses structural lesions were reduced to some extent by adding Ca^{++} or Na^{+} with aluminum (Fig. 2, B and C).

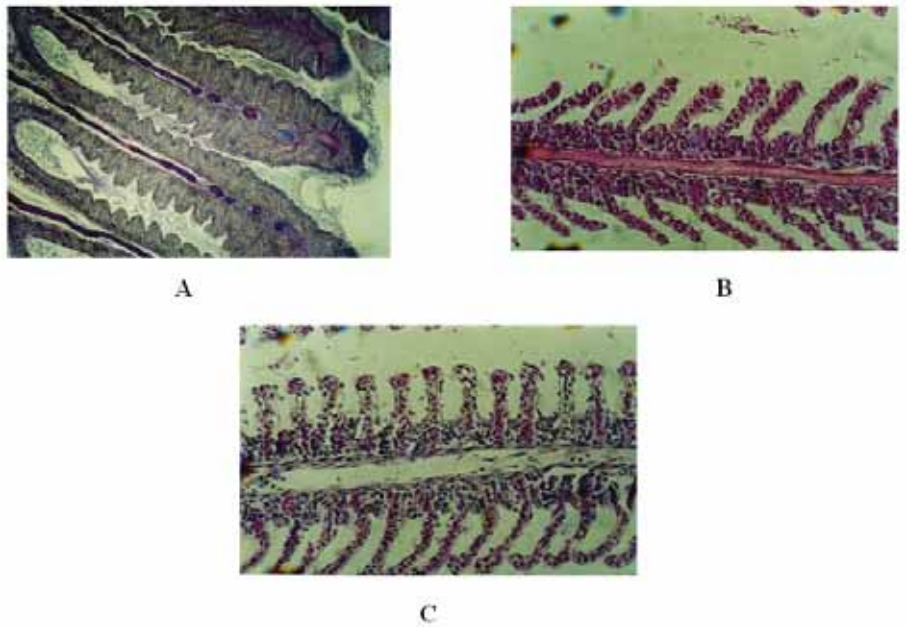

Fig. 2: Histological appearance of gills of Nile tilapia exposed to aluminum. A: aluminum only, B: aluminum + calcium and C: aluminum + sodium. (H & E) X 400.

Intensive mucus production and large non-tissue spaces have been reported in the gills of fish exposed to high concentration of various metals (Muniz and Walloe 1990). Müeller *et al*. (1991) concluded that as a result of lamellar fusion, the lamellar surface area may be reduced by much as 75%. This would impair ion uptake and oxygen delivery to the tissues. Enlargement of non-tissue space could result in inadequate gas exchange, and, consequently in a reduced diffusion capacity.

It is generally accepted that the main target of aluminum is the gills of fish. Death of animals is due mostly to impaired gill functions such as respiration and ion exchange. Intensive production of mucus caused by Aluminum in this *study* suggests that high mortality should attribute to respiratory failure than to impaired ion regulation between neighboring lamellae and intensive mucus was produced. Al-accumulation and hypoxia seem to be very important for the effects of aluminum (Poléo, 1995; Polléo & Bjerkely, 2000). Ionic strength should therefore play a key role for the Al-toxicity in fish because it is essential for the chemistry on all aquatic surfaces, including the fish gill.

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