The effect of Calcium Pre-Exposure on the Acute Copper Toxicity to Juvenile Nile Tilapia, *Oreochromis niloticus*

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ABSTRACT

The toxicity of copper to Nile tilapia, *Oreochromis niloticus* was evaluated by determining the 96-hr LC_{50}. Two experiments were done to investigate the effects of pre-exposure of Nile tilapia to 4 different Ca-salts and concentrations on the acute copper toxicity in indoor laboratory. Expt. (1) was carried out to determine the 96-hr LC_{50} of Cu^{2+} in copper sulfate to fish pre-exposed to 4 different Ca-salts (Ca-oxide, Ca-carbonate, Ca-chloride and Ca-sulfate). Expt. (2) was carried out to determine the optimum Ca^{2+} concentration, to minimize the Cu^{2+} toxicity. Comparing the copper toxicity to the Nile tilapia, pre-exposed to 100 mg Ca^{2+}/L as Ca-oxide, Ca-carbonate, Ca-chloride and Ca-sulfate, the 96-hr LC_{50} of Cu^{2+} was 14.27, 11.19, 10.18 and 9.29 mg Cu^{2+}/L, respectively, while the 96-hr LC_{50} for control was 5.03 mg Cu^{2+}/L.

Expt.(1), showed that the highest copper LC_{50} was obtained in case of Ca-oxide pre-exposure (14.27 mg Cu^{2+}/L). The 96-hr LC_{50} of Cu^{2+} to fish exposed to 0, 50, 100 and 200 mg Ca^{2+}/L was 5.03, 13.23, 14.27 and 13.09 mg Cu^{2+}/L, respectively. The 96-hr LC_{50} values were slightly increased with increasing the calcium concentration ($P>0.05$), and the optimum one was obtained at 50-100 mg Ca^{2+}/L.

Keywords: Calcium, copper, liming agents, Nile tilapia, toxicity.

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INTRODUCTION

Copper sulfate is often used as an algaecide in commercial and recreational fishponds to control the growth of phytoplankton and filamentous algae. It is also used to control certain fish diseases *(1, 2)*. The concentrations of copper sulfate used for phytoplankton control are seldom directly toxic to fish, but do kill large numbers of invertebrate food organisms such as rotifers, cladocerans and copepods *(1)*. However, above a specific concentration, copper is toxic to fish including such cultured species as salmonids, cyprinids and catfish *(3)*. As a result, the treatment recommendation for the use of copper sulfate for finfish is 0.5-1 mg/L *(1, 4)*.

Nile tilapia is a native fish species of Egypt that grows faster in warm months, and has become more popular allover the world because it can be cultured in a variety of aquaculture systems and are favorable food fish *(5)*. However, Nile tilapia is omnivorous fish and could consume detritus, phytoplankton and zooplankton *(6, 7, 8)*. Thus, fish which graze the plankton organisms is liable to the accumulation of copper in their tissues, which may reach the toxic concentration.

Copper toxicity is known to be regulated by alkalinity, hardness and pH of water *(9)*. Therefore, recommendations for the safe use of copper sulfate have been based on hardness *(10, 11)*, total alkalinity *(1, 12, 11)*, and pH of the water *(9)*. The high concentrations of calcium, a major component of hardness, are also thought to limit copper toxicity by protecting the ion-regulating mechanisms at the gills, from the disruptive effects of copper *(13)*. One means to increase the uptake of calcium by aquatic organisms is to increase the level of environmental calcium through the application of liming agents. The total plasma calcium level of the common carp (40-50 mg/l) is raised in tap water (0.15 mM/L Ca^{2+}) and remains within 3 mmol/L, while fish kept in high calcium freshwater shows larger hypercalcemic responses *(14)*. The calcium supplied through liming reduce the uptake of heavy metals *(15, 16)*.

The objective of the present study was to determine the effect of pre-exposure to calcium ions on copper toxicity in juvenile Nile tilapia, *Oreochromis niloticus.*

MATERIALS AND METHODS

Experimental procedures

Healthy fish of Nile tilapia, *Oreochromis niloticus* (L.) were collected from fish hatchery of the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia. Fish weighing 2-2.5 g were acclimatized to the laboratory conditions in indoor tanks for 2 weeks. The fish were distributed randomly in glass aquaria of 150-liter capacity at a rate of 100 fish per aquarium. Each aquarium was supplied with compressed air via air-stones from air pumps. Well-aerated water was provided from a storage fiberglass tank. During the experimental period, fish were fed frequently a diet which contained 35% crude protein to satiation twice daily. The excreta was removed by siphoning half of the aquarium water and replacing it by an equal volume of water containing the same calcium or copper concentrations.

Analysis of the physico-chemical parameters of water

Water samples were collected daily, at 15 cm depth from each aquarium. The dissolved oxygen and temperature were measured on site with a YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). The dissolved oxygen concentrations ranged from 6.6 to 7.5 mg/L and were above 75% of saturation in each aquarium. The ambient water temperature was approximately stable for the experimental duration and ranged from 26 to 28°C. The pH and free ammonia were measured using Hach kits (Hach Co., Loveland, Colorado, USA). pH ranged from 8.5 to 9.0, and free ammonia concentration was less than the critical level (<0.1 mg/L) in all the water treatments. The total alkalinity and total hardness were measured by titration *(18)*. The total alkalinity and total hardness ranged from 160 to 200 mg/L as $CaCO_3$ and from 120 to 150 mg/L as $CaCO_3$, respectively.

Two experiments were carried out to investigate the effect of pre-exposure of Nile tilapia to 4 calcium salts (calcium oxide, calcium carbonate, calcium chloride and calcium sulfate) on the acute copper toxicity in indoor laboratory. The dose of Ca-salts and copper sulfate was calculated, in our experiments, according to the molecular weights of Ca and Cu, respectively.

The first experiment was carried out on 500 fish to determine the 96-hr LC_{50} of Cu^{2+} to fish previously exposed to 4 different calcium salts. The fish were divided into 5 equal groups. Gp. (1) was the control; non-exposed to Ca-salts. Gps (2-5) were exposed to 100 mg Ca^{2+}/L of calcium oxide, calcium carbonate, calcium chloride and calcium sulfate, respectively for 4 days.

After exposure to Ca-salts, fish of each group were transferred to Ca-salt free aquarium for the determination of the 96-hr LC_{50} *(17)* of Cu^{2+} as copper sulfate. For determination of the Cu^{2+} toxicity, fish of each group were randomly divided into 10 equal sub-groups. Each sub-group was exposed to different concentrations of Cu^{2+}.

From the 1st experiment it was concluded that the optimum Ca-salt which minimized Cu^{2+} toxicity was Ca-oxide. The second experiment was carried out to determine the optimum Ca concentration (as Ca-oxide), which could be used to minimize the Cu^{2+} toxicity. However, fish were classified into 4 equal groups. Gp. (1) was kept as control, while gps. (2-4) were exposed to 50, 100 and 200 mg Ca^{2+}/L for 4 days. After pre-exposure to different concentrations, the Cu^{2+} 96-hr LC_{50} of each group was determined *(17)* in order to select the optimum Ca^{2+} concentration which could be used to minimize Cu^{2+} toxicity.

Statistical analysis

The obtained data were subjected to one-way ANOVA and the differences, among the means, were done at the 5% probability level using Duncan's new multiple range test. Correlation analyses were performed by fitting the data into a linear curve, selecting the model giving the best fit. The software SPSS, version 10 (SPSS, Richmond, USA) was used *(19)*.

RESULTS

The toxicity of copper to Nile tilapia was carried out by determining the 96-hr LC_{50}, which indicates the toxic concentration at which 50% of fish die. Comparing the copper toxicity to Nile tilapia pre-exposed to 100 mg Ca^{2+}/L of different calcium salts (calcium oxide, calcium carbonate, calcium chloride and calcium sulfate), our results showed that (Fig. 1) the 96-hr LC_{50} of Cu^{2+} in copper sulfate to fish pre-exposed to calcium oxide, calcium carbonate, calcium chloride and calcium sulfate was 14.27, 11.19, 10.18 and 9.29 mg Cu^{2+}/l, respectively, while, the 96-hr LC_{50} for the non-pre-exposed control group was 5.03 mg Cu^{2+}/l.

The pre-exposure of the fish to 100 mgCa^{2+}/L resulted in the lowest toxicity of Cu^{2+}. The data in Fig. (2) showed that the 96-hr LC_{50} of Cu^{2+} to fish pre-exposed to 0, 50, 100 and 200 mg Ca^{2+}/L was 5.03, 13.23, 14.27 and 13.09 mg Cu^{2+}/L, respectively. The optimum 96-hr LC_{50} of Cu^{2+} was observed at level of 50 mg Ca^{2+}/L with insignificant differences with calcium treatments ($P<0.05$). The least 96-hr LC_{50} was obtained in the control group (5.03 mg Cu^{2+}/L; $P<0.05$).

Our results (Fig 3) showed that the Nile tilapia could tolerate Cu^{2+} toxicity up to 20 mg/L when they were pre-exposed to Ca-oxide and Ca-chloride. Fish survival rate was declined by increasing the Cu^{2+} concentration ($r^2 = 0.9454$ and 0.8673 for Ca-oxide and Ca-chloride, respectively). On the other hand, the fish pre-exposed to Ca-carbonate and Ca-sulfate showed the least survival rate ($P<0.05$). However, all the Ca-salts supported the fish survival rate, with different percentages, up to 40 mg Cu/L after which no fish survived except in case of calcium oxide that supported the fish survival rate up to 70 mg Cu/L (Fig. 3).

The optimum survival rate (Expt. 1) was obtained with fish pre-exposed to Ca-oxide. Expt. (2) showed that the fish which were pre-exposed to different doses of Ca-oxide equivalent to 0 (control), 50, 100 or 200 mg Ca^{2+}/L revealed that all doses of Ca-oxide supported the fish survival against copper toxicity better than the control ($P<0.05$; Fig 4). This support reached up to 20 mg Cu^{2+}/L except at 50 mg Ca^{2+}/L where fish could tolerate up to 30 mg Cu^{2+}/L after which the fish survival rate decreased with increasing the copper toxicity up to 60 mg Cu^{2+}/L. In control fish, no survival threshold was observed where copper toxicity inversely affected fish survival up to 40 mg Cu/L after which no fish survival was observed.

DISCUSSION

Fish are naturally exposed to a variety of metals including both essential and non-essential elements. Copper is one of the essential metals that after absorption from gills and intestine is transported by metallothionein into blood circulation. After exposure of rainbow trout (*Oncorhynchus mykiss*) to copper for 4 hr, gill damage was recorded ***(20)***. Similar findings were recorded in fish exposed to sublethal concentration of copper for 96 ***hr (21).*** Disruption of the gill function by exposure to copper was found on several occasions ***(22, 23)***. This disruption leads to changes in the diffusion distance across the gill epithelium (24), which might impede gas change, leading to tissue hypoxia ***(20).***

Water chemistry especially pH, alkalinity and hardness could affect the heavy metal toxicity. In this study herein, the total hardness and total alkalinity increased significantly after the application of Ca-salts to aquaria water where the total alkalinity and total hardness ranged from 160 to 200 mg/L as $CaCO_3$ and from 120 to 150 mg/L as $CaCO_3$, respectively. These increases in both factors supports more fish survival. In this regard, **Miller and Mackay *(25)*** found that the incipient LC_{50} of copper for juvenile rainbow trout (*Salmo gairdneri*) increased when hardness was increased from 12 to 100 mg/L and alkalinity was held at 10-50 mg/L. Also, **Wurts and Perschbacher *(3)*** studied the LC_{50} of copper to channel catfish (*Ictalurus punctatus*), and found that the mortality rate was decreased when calcium hardness level increased from 20 to 250 mg/L, when bicarbonate alkalinity was held at 75 mg/L.

Copper, as a divalent cation, would have chemical activity and ionic form similar to the calcium ion (and possibly magnesium). So, in soft water, copper may compete with calcium for the active sites in the gills, and calcium failed to significantly reduce copper toxicity. Liming did not only supply calcium for uptake; but increased the levels of environmental calcium that reduces the uptake of heavy metals *(15, 16)*. When cadmium (Cd) concentrations are already present, the protective effect of liming was reduced, as Cd competes with calcium uptake *(26)*.

It is supposed that if the solubility of Ca-salts are the same, the release of calcium to the rearing water would be the same, however, the effect on the copper-toxicity-reduction would be the same. So, the LC_{50} herein differed as Ca-salts differed because the solubility of each Ca-salt differed depending on the pH of the rearing water. The pre-exposure of fish to Ca-salts increased the fish survival more than the control fish group. So, the pre-exposure to liming agents in heavy metal-free water would seem to be necessary to prevent the adverse effects associated with future exposure to heavy metals. The pre-exposure of Nile tilapia to calcium could bind the active sites on the gills, and the post-exposure to copper ion does not find free sites to bind, so, copper toxicity is significantly reduced. This hypothesis could explain the result encountered in this study, where the pre-exposure of Nile tilapia to calcium, irrespective of the type of salts increases the survival rate more than the control (non pre-exposed to calcium). In this concern, **Kaviraj and Dutta** *(27)* found that the pre-exposure to liming agents might be effective in reducing the acute toxicity of Cd to carp. Their study has shown that the LC_{50} value of Cd to common carp (*Cyprinus carpio*) is 165 mg/L, while the toxicity is reduced and the LC_{50} value of Cd increased to 235 mg/L when the fish were pre-exposed to 100 mg/L quick lime. Moreover, the uptake and distribution of Cd in fish was reduced after the fish were acclimated to selected calcium concentrations *(28)*.

There was no significant difference in the LC_{50} of fish before exposure to different Ca^{2+} concentrations over 50 mg Ca^{2+}/L. This result may be due to binding of the free active sites on the fish gills by the released Ca^{2+}, however, the excess Ca^{2+} cation remained free. The number of free active sites, on the fish gills, may depend on fish species, age and size. It has been theorized that the calcium-activated proteins control the passive and energy dependent process regulating ion metabolism at the gills *(29, 30)*. It is likely that copper competes directly with calcium for the same binding sites on ion regulating proteins. Therefore, high concentrations of calcium would keep the binding sites maximally saturated preventing copper from binding and interfering with the normal protein functions (i.e. ion metabolism).

It could be concluded that the pre-exposure of fish to 50-100 mg Ca^{2+}/L could support the fish survival in the polluted water. Further work is needed to investigate the changes in the biochemical aspects and the growth performance of fish, previously exposed to calcium before copper toxicity.

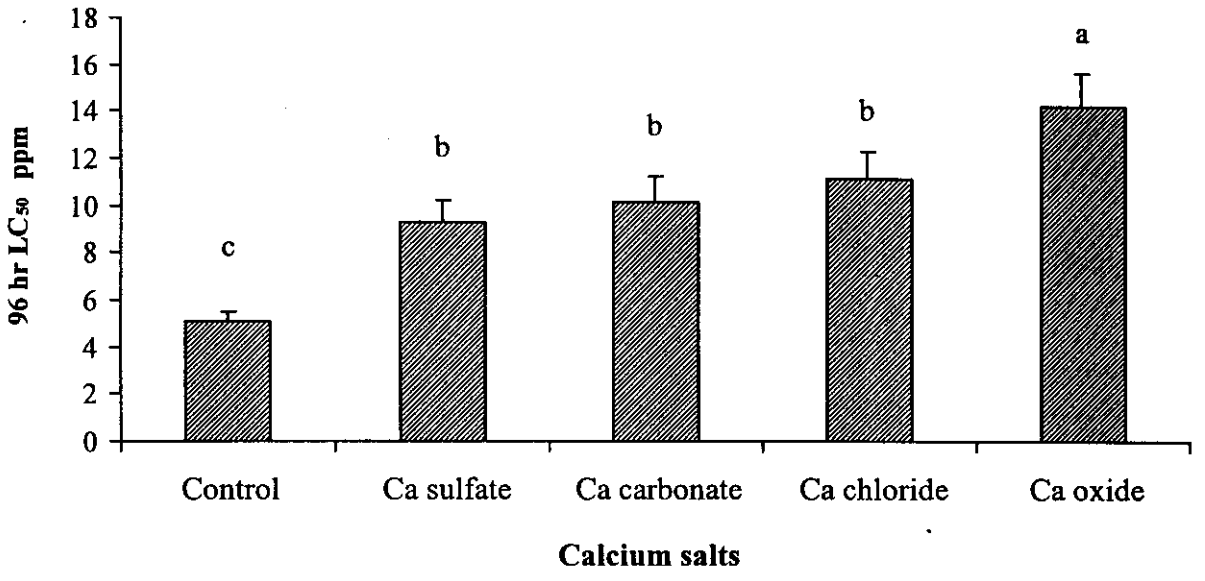

Fig. 1: The 96-hr LC_{50} of Cu^{2+} (ppm) to Nile tilapia pre-exposed for 4 days to different calcium salts. Bars assigned with the same letter are not significantly differed at $P<0.05$.

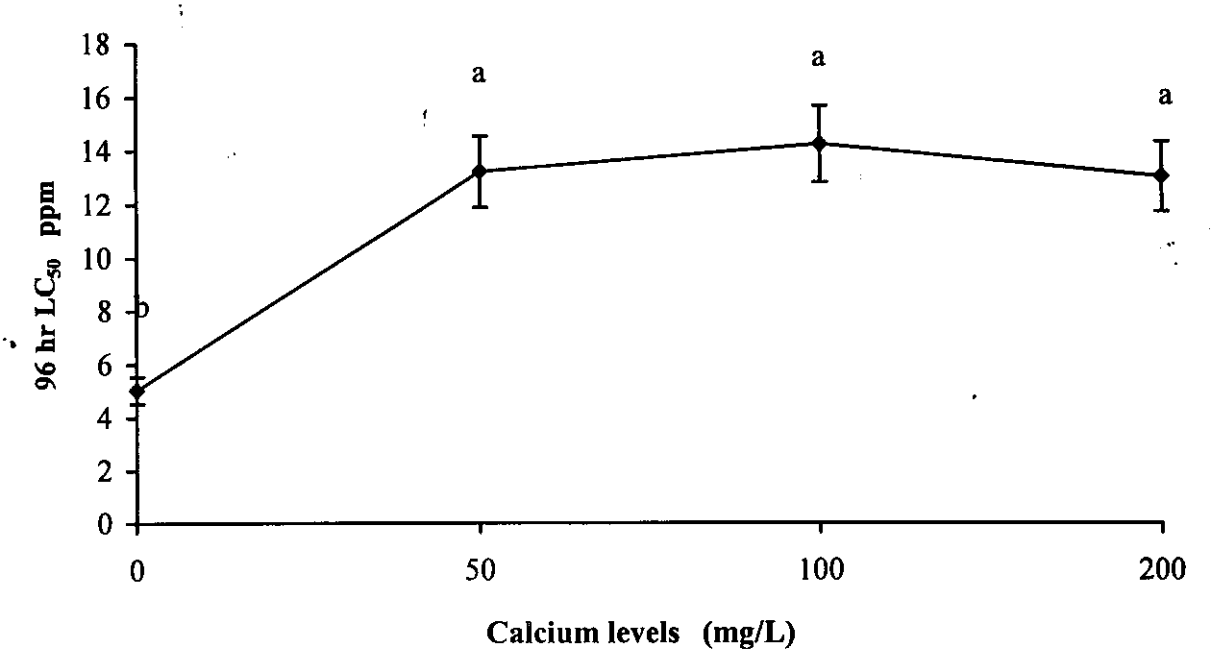

Fig. 2: The 96-hr LC_{50} of Cu^{2+} (ppm) to Nile tilapia pre-exposed for 4 days to different doses of calcium oxide. Points assigned with the same letter are not significantly differed at $P<0.05$.

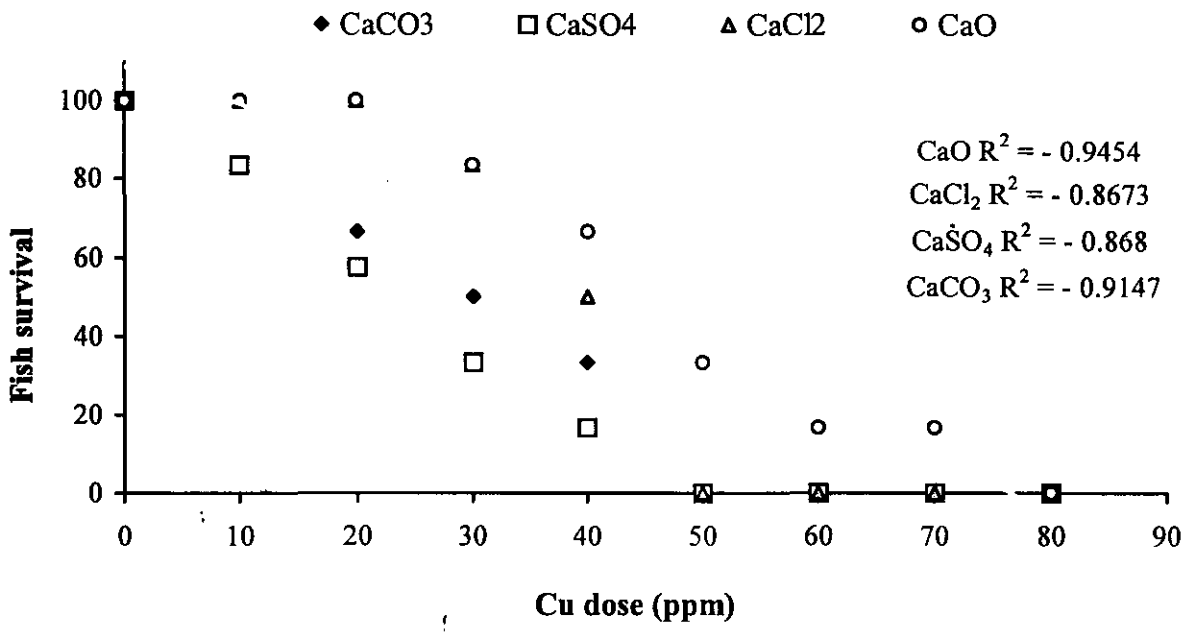

Fig. 3: Fish survival (%) of Nile tilapia pre-exposed for 4 days to different Ca salts and exposed to different doses of copper for 96 hr.

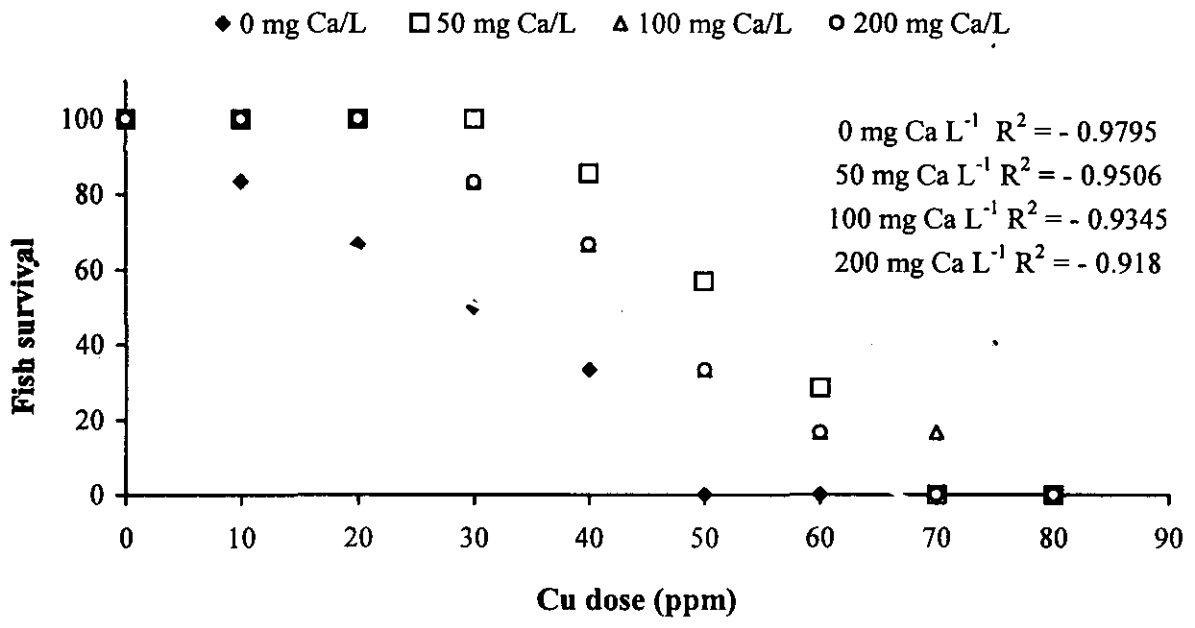

Fig. 4: Fish survival (%) of Nile tilapia pre-exposed to different CaO concentrations and exposed to different doses of copper for 96 hr.

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الملخص العربى

تأثير تعرض زريعة البلطي النيلي للكالسيوم على سمية النحاس

محسن عبد التواب – ممدوح عبد العزيز علي موسى

قسم بحوث بيئة وبيولوجى الأسماك - المعمل المركزي لبحوث الثروة السمكية

بالعباسة - أبو حماد – شرقية

أجريت هذه الدراسة لمعرفة أفضل أملاح الكالسيوم في رفع مقاومة أسماك البلطي النيلي لسمية النحاس حيث تم تعرض أسماك البلطى النيلى لأحد أملاح الكالسيوم وهى أكسيد الكالسيوم و كلوريد الكالسيوم وكبريتات الكالسيوم وكربونات الكالسيوم لمدة ٤ أيام عند تركيز ١٠٠ مجم كالسيوم فى اللتر ثم تعرض هذه الأسماك لجرعات مختلفة من النحاس. تم تعيين الجرعة النصف مميتة من النحاس بالنسبة للسمكة محل الدراسة وقد وجد أن الجرعة النصف مميتة من النحاس فى الأسماك التى لم تتعرض لأي من أملاح الكالسيوم هي ٥,٠٣ مجم نحاس لكل لتر والتى ارتفعت في الأسماك التي سبق تعرضها لأى من أملاح الكالسيوم المذكورة سابقا غير أن أفضلها كان أكسيد الكالسيوم (١٤,٢٧ مجم نحاس لكل لتر). بعد ذلك تم تعريض مجموعة أخرى من الأسماك محل الدراسة لتركيزات مختلفة من أكسيد الكالسيوم تكافئ ٠ ، ٥٠ ، ١٠٠ ، ٢٠٠ مجم كالسيوم لكل لتر لمدة ٤ أيام ثم تعرضت هذه الأسماك لجرعات مختلفة من النحاس وتم تعيين الجرعة النصف مميتة من النحاس. وقد وجد أن أفضل هذه التركيزات هو التركيزات ٥٠-١٠٠ مجم كالسيوم لكل لتر حيث كانت الجرعة النصف مميتة للأسماك التي سبق تعرضها لهذه الجرعة هي ١٣,٢٣ مجم/لتر. ونستنتج من ذلك أن تعرض الأسماك لأكسيد الكالسيوم (٥٠-١٠٠ مجم كالسيوم لكل لتر) لمدة ٤ أيام يقلل من أثر سمية النحاس عليها.