Biological Sciences

Pak J Sci Ind Res 2000 43 (6) 359-362

The Uptake and Loss of Absorbed Dissolved Copper by *Clarias angullaris* Fingerlings

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(Received 19 March 1998; accepted 17 April 2000)

The uptake and loss of dissolved copper in media by the African mud fish *Clarias angullaris* was studied in water of 25mg l^{-1} hardness expressed as $CaCO_3$ of pH 6.8 at 27±2°C in a static test system. The uptake experiments involved exposure of fish to 0.5, 0.75 and 1.0 mg Cu^{+2} l^{-1} test solutions from which the fish absorbed and retained 0.9912, 1.3010 mg and 1.4036 mg Cu^{+2} g^{-1} wet fish wt. respectively, after 11 days. In the loss experiments, the fish were exposed to 0.5, 0.75 and 1.0 mg Cu^{+2} l^{-1} for 7 days and the surviving fish were allowed to recover in copper-free-water for over 20 days. Approximately 50% of the absorbed copper was shed from all fish within 2 days. After 20 days the fish held 0.1019, 0.1296 and 0.1010 mg Cu^{+2} g^{-1} wet wt from previous exposures to 0.5, 0.75 and 1.0 mg Cu^{+2} l^{-1} respectively.

Key words: Dissolved copper, Uptake and loss; *Clarias angullaris.*

Introduction

Heavy metals in solution have been found to be absorbed and retained by fish (Coombs 1974). Bryan (1976) reported that such absorption is probably due to passive diffusion down a gradient between the mucus covering, the entire body surface and the gills on one hand and the internal organs on the other.

The uptake of copper may be reduced initially because several fish species have the ability to adapt to potentially toxic copper levels at least on temporary basis. According to Lauren and McDonald (1978) a&b) the ability of fish to adapt depends on changes in both sodium ion (Na^+) transport and permeability of the whole body. The two authors have shown that the rainbow trout sequestered copper in a sulfhydryl-rich and soluble protein tentatively identifie as metallothionein present in both the gills and liver. There is the assumption that the quantity of metallothionein in the gill and liver increases with exposure to copper. It may be inferred that the assumed increase in metallothiona in quantity in response to the influx of copper may be the factor responsible for the ability of the fish to adapt on temporary basis.

In the above context Brungs *et al* (1973) exposed the brown bullhead up to 20 months to copper level of up to 104mg l^{-1} in water 202mg l^{-1} expressed as $CaCO_3$. They found that copper was not accumulated in the operculum or red blood cells but the gills and liver levels increased with exposure greater than 27-53µg l^{-1} and kidney level increased at 104µg Cu^{+2} l^{-1}. Benoit (1975) reported that with increasing exposure time and copper concentration the bluegill accumulated more copper.

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Copper may be used in fish culture although it is toxic to fish (Oronsaye and Ogbebo 1995). This study examines the uptake, retention and loss of the metal from fish previously exposed to the metal.

Materials and Methods

Fingerlings of the experimental fish *Clarias angullaris* were obtained and transported to the laboratory in river water contained in 60 l capacity plastic containers. The fish were held in the laboratory for three weeks in aerated laboratory water having a pH of 6.5 and total hardness of 25mg l^{-1} as $CaCO_3$ at 27±2°C to acclimatize to the diluted water. During the holding period the fish were fed a daily ration of *Lumbricus* sp. For the uptake experiments the fish in batches of 25 were exposed to 0.5, 0.75 and 1.0mg Cu^{+2} l^{-1} in 24 litres of tapwater in glass aquaria in three separate experiments. To avoid the uptake of copper by the gut the fish were not fed during exposure to the metal. The length of exposure ranged between one to tweleve days.

In the experiments on the loss of copper after previous exposure to the metal new batches of fish were exposed to 0.5, 0.75 and 2.0mg Cu^{+2} l^{-1}. Exposure was for seven days and surviving fish were allowed to recover in fresh uncontaminated water for periods ranging from one to thirty days. Only the fish which recovered were fed a daily ration of live earthworms (*Lumbricus sp.*); all experiments were carried out using the static system and a control experiment was run in each series of experiments.

The experimental tanks and the 50cm^3 pyrex beakers used for "wet digestion" of fish were washed with detergent, soaked overnight in 5% nitric acid and rinsed with distilled water

(Leonard 1971; Oronsaye 1987). The experimental fingerlings alongwith their controls were removed at daily intervals and their copper content was measured after they were allowed to swim in uncontaminated water for five minutes to remove copper adhering to their body surface. The fish were then pithed and dried between a filter paper. One fingerling was placed in each beaker. The wet weight and dry weight (after drying in an oven at 80±5°C to constant weight) were recorded. Each dried fish was milled separately and stored in polythene bags in a dessicator. The fish were not ashed.

Perchloric acid, nitric and concentrated sulphuric acids (Analar Grades in ratio of 1:5:1) were added to one gram of each milled specimen in conical flasks (Oguzie 1996). The conical flask and contents were transferred to a hot plate at 85±2°C in a fume chamber and allowed to boil. Digestion lasted about 12 h. When fats appeared in solution as indicated by colour change to dark brown, more nitric acid was added. Complete digestion was shown by white crystalline substance obtained after white fumes of perchloric acid ceased. About 5ml of distilled water was added to each completely digested specimen and brought to boiling for five minutes on the hot plate before being made to 25ml and allowed to cool and settle.

The amount of copper in each sample was measured by a Varian Techtron Spectr. AA-10, an Atomic Absorption Spectrophotometer calibrated previously using standard copper solution. The levels of copper found in fish tissue are shown in Fig 1-4 in which one fish sample represents one point.

Results and Discussion

Uptake of copper. The fish that died in experiments were not analysed for copper residue in their tissue. The stickleback *Gasterosteus aculeatus* (Pascoe and Mattey 1977; Oronsaye 1987) has been shown to accumulate heavy metal after death. Thus analysis which could result in very high metal concentration in tissue were avoided.

The mean concentration of copper in whole body preparations of 10 unexposed *C. angullaris* was 0.036 mg Cu^{+2} g^{-1} wet weight in the range of 0.02 to 0.051 mg Cu^{+2} g^{-1} wet weight. Copper is an essential element and has a biochemical role as an enzyme activator in animal tissue (Ray and Jerome 1987). Copper is a major constituent of heamocyanin in the blood pigment of invertebrates some of which serve as fish food. The recorded body load of 0.036mg Cu^{+2} g^{-1} wet weight of fish in the present study can be assumed to be normal mean value. The value can be compared to the range 0.010 to 0.070mg Cu^{+2} kg^{-1} wt for the gills and 0.02 to 0.123 mg Cu^{+2} kg^{-1} wt for the muscles of *Tilapia zilli* whereas 0.230 mg Cu^{+2} kg^{-1} was recorded for the muscles of *Clarias gariepinus*. These fish were analysed by Oguzie (1996) from water containing enhanced levels of the heavy metals.

Fish exposed to 0.5mg Cu^{+2} l^{-1} for one day increased their body load from a mean of 0.036mg Cu^{+2} l^{-1} to 0.4432mg Cu^{+2} g^{-1} wet wt which is about 12.3 times normal mean value. After three days of exposure the absorbed metal was reduced to 0.3500mg Cu^{+2} g^{-1} or 9.7 times normal mean value (Fig 1). The copper residue in the body increased steadily after the 4th day. At the end of experiment. after 11 days the fish surviving in the 0.5mg Cu^{+2} l^{-1} had 0.9912 mg Cu^{+2} l^{-1} wet fish wt. This represents about 28 times normal mean value. As for the fish exposed to 0.75mg Cu^{+2} l^{-1} the body residue was 0.4621mg Cu^{+2} g^{-1} wt after one day. As it is shown in Fig 1 the absorbed metal was reduced after two days to 0.3104mg Cu^{+2} g^{-1} wet wt.

The reduction in the concentration of absorbed heavy metal may be explained by the fact that because of homeostatic mechanism and because *C.angullaris* is euryhaline the fish adapts to sudden influx of ions. A similar observation has been reported for *G.aculeatus* exposed to zinc and cadmium (Matthiessen and Brafield 1977; Oronsaye 1987) and the brown bullhead exposed to copper (Brungs *et al* 1973).

With increasing exposure period the metal residue in the fish exposed to 0.75mg Cu^{+2} l^{-1} increased. At the end of exposure for 11 days the fish had 1.3010mg Cu^{+2} g^{-1} wet wt which is an increase of 32.5 times normal value.

The fish exposed to 1.0mg Cu^{+2} l^{-1} absorbed and retained the metal for 6 days before the metal was reduced from 1.0002 to 0.811mg Cu^{+2} g^{-1} fish wt. . Thereafter. the amount of copper retained by the fish also increased with increasing exposure period (Fig 1). This observation is in accordance with the results of previous studies on the accumulation of copper by

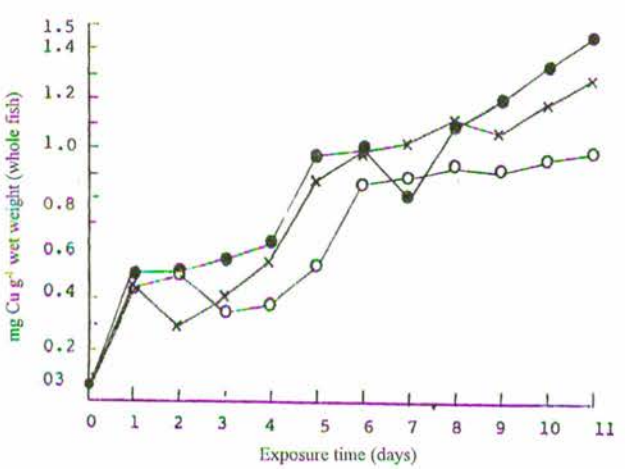

Fig 1. Uptake of copper by fish when exposed to 0.5 mg (o), 0.75 mg (x) and 1.0 mg $Cu^{2+}l^{-1}$(●).

fish (Brungs *et al* 1973; Benoit 1975). Brungs *et al* (1973) reported that absorbed copper levels in the gills and liver of the brown bullhead increased with exposure to concentrations greater than 27-53µg Cu^{+2} l^{-1}. Benoit (1975) exposed the bluegill to copper solutions in Lake Superior water and reported that fish showed increased copper levels in the gill at a concentration above 40µg l^{-1} while those in the liver and kidney increased at a concentration above 162µg l^{-1}. The present study using *C.angullaris* confirms an increased copper residue in fish with increasing exposure period.

Loss of absorbed copper. When the fish exposed to 0.5mg Cu^{+2} l^{-1} for 7 days were allowed to recover in copper-free water about 50% of the absorbed copper was lost from the fish after 2 days (Fig 2). The fish previously exposed to 0.75 and 1.0mg Cu^{+2} l^{-1} when allowed to recover, shed about 50% of the absorbed copper in less than 2 days (Fig 3 - 4). The rate of loss of copper from the fish previously exposed to 0.5mg Cu^{+2} l^{-1} was slow when compared to those having been exposed to 0.75 and 1.0mg Cu^{+2} l^{-1}. Thereafter in all recovery experiments, the rate of efflux of copper from fish slowed down. The results may be explained like this. Copper is an essential element required for the normal life processes of fish. In this context Krezeski *et al* (1968) demonstrated the presence of large amount of a 10.000-Da-metal binding protein containing copper in 11 species of freshwater fish in which copper is sequestered. The absorbed and retained copper is probably held by this protein and the initially effluxed metal may be the metal that was unbound. Hence the initial rate of efflux was fast when the fish were allowed to recover in copper-free water. As it is shown in Fig 2 and 4 it appears that on extending

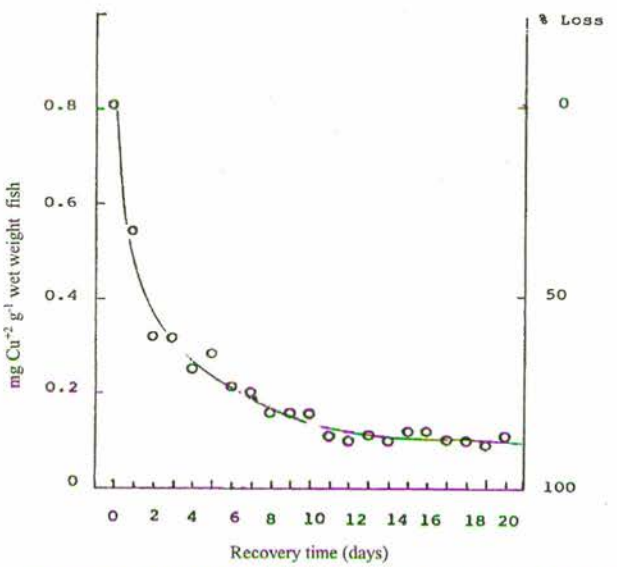

Fig 2. Loss of copper from tissue of fish while in copper-free water after previous exposure to 0.5 mg $Cu^{2+}l^{-1}$.

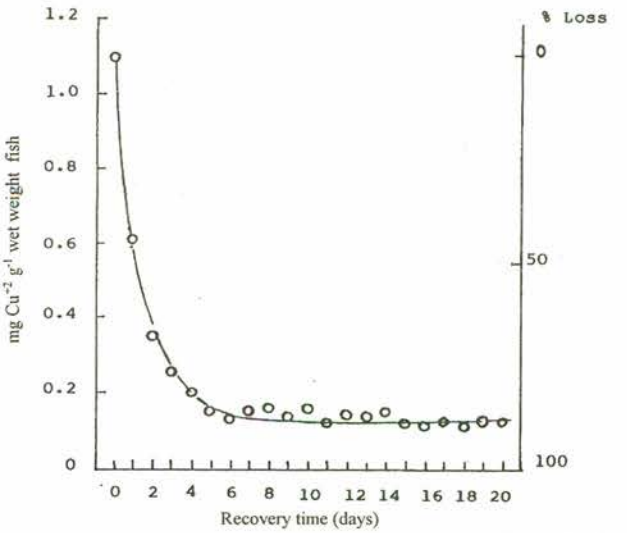

Fig 3. Loss of copper from tissue of fish while in copper-free water after previous exposure to 0.75 mg $Cu^{2+}l^{-1}$ for seven days.

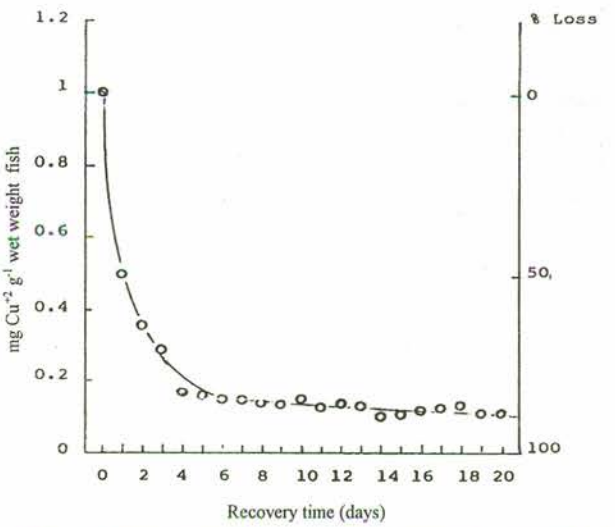

Fig 4. Loss of copper from tissue of fish while in copper-free water after previous exposure to 1.0 mg $Cu^{2+}l^{-1}$ for seven days.

the recovery period beyond 20 days all copper may not be lost from the fish for the rate of efflux on the absorbed metal slows down to almost zero. It can be imagined that the bound metal needed for the normal biochemical processes will not be released and could conceivably enhance the metal load of the fish.

Conclusion

The study has revealed that when copper containing compounds are been applied to live fish, the metal is absorbed and retained. Also when the source of copper is removed there is a corresponding loss of the metal from the fish. The efflux is not total because some of the absorbed metal is retained by the fish probably for its normal bio-chemical processes.

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