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# Accumulation of Copper in Liver, Gill and Muscle Tissues of *Anguilla anguilla* (Linnaeus, 1758)

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**Abstract:** Accumulation of copper in liver, gill and muscle tissues of *Anguilla anguilla* was studied after exposing the animals to 1.00, 2.00 and 4.00 ppm Cu over 96 h period. The levels of metals in the studied tissues were determined using atomic absorption spectrophotometric techniques. All the copper concentrations tested increased the tissue levels of the metal significantly in liver and gill tissues compared to control fish while metal accumulation in muscle tissue was statistically significant only at 4.00 ppm Cu. Accumulation of the metal in gill tissue showed no difference between the concentrations tested despite the differences observed in liver and muscle tissues. Accumulation of the metal was highest in liver tissue and lowest in muscle tissue.

**Key words:** Fish, *Anguilla anguilla*, heavy metal, copper, accumulation, tissue

## INTRODUCTION

Heavy metals enter aquatic environments by a number of natural sources such as erosion, weathering and volcanic activities. Their levels in these environments however, increased drastically due to mainly anthropogenic sources such as urban and industrial inputs use of excess amounts of fertilizers and pesticides and over irrigation (Fergusson, 1990; Gregory *et al*., 2002).

Excess levels of heavy metals in these environments while resulting in accumulation and metabolic, physiologic and pathologic disturbances in aquatic organisms (Friedman and Gesek, 1994; Yamano *et al*., 1998; Novelli *et al*., 1999) their transference to higher levels of food chain in concentrated amounts results in significant health problems and even death in receiving organisms (Hilmy *et al*., 1985; Tort and Torres, 1988).

Copper is needed in trace amounts for carrying out vital functions in animal organisms (Cousins, 1985). It is used in electric industry, alloys, chemical catalyzers, dyes and algaecides as a raw material (Torres *et al*., 1987). Discharge of copper from these sources without any treatment increases its concentration in aquatic environments and hence shows its toxic action to water organisms. Studies carried out with various fish species showed that excess copper causes structural deformations in gill tissue and in vertebrate (Stagg and Shuttleworth, 1982; Lauran and McDonald, 1985) accumulation in metabolically active tissues such as liver, kidney gill and spleen (Handy *et al*., 2002) and changes in biochemical and hematological parameters (Arslan *et al*., 2006; Ciftci *et al*., 2008).

Although, heavy metals are absorbed through gills and/or gastrointestinal track by fish organisms, independent of the uptake way they are primarily accumulated in metabolically active tissues such as liver and kidneys (Erdem, 1990; Heath, 1991). Gills are indirect contact with the outer environment hence with the pollutants (Hodson, 1988). Liver acts in the transformation of basic nutrients and in detoxification and storage of toxic materials (Cicik, 2003). Muscle however, is not an active tissue in accumulating metals but it is important to determine the levels of metals in this tissue since it forms the main consumable part of the fish.

*Anguilla anguilla* is consumed as a protein source and shows a wide distribution in fresh and salt waters. Since their freshwater habitats are mostly under the influence of rural, industrial and agricultural activities, it is important to study metal accumulation in their tissues which reflects the pollution status of the environment.

Determination of heavy metal accumulation in tissues of edible species is also important as far as human health is concerned, therefore present study was undertaken to determine the levels of copper in liver, gill and muscle tissues of *A. anguilla* after exposing the animals to 1.00, 2.00 and 4.00 ppm concentrations of the metal over 96 h.

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## MATERIALS AND METHODS

*A. anguilla* was obtained from the drainage channels of Goksu Delta, a conservation area in Silifke, Mersin. Since metal accumulation depends on metabolic activity, length and weight of fish similar size of fish, 37.57±3.18 cm in length and 72.11±3.27 g in weight were used in experiments. Experiments were carried out under controlled laboratory conditions having a temperature of 24±1°C and illuminated for 12 h.

Fish were placed in 8 glass aquaria, 40×100×40 cm in size and were adapted to laboratory conditions for 30 days. The same sizes of 4 glass aquaria were used in the experiments. The first three aquaria were filled with 120 L of 1.00, 2.00 and 4.00 ppm Cu solutions and the fourth one was filled with the same amount of tap water and used as control. Experiments were run in triplicate each containing 2 fish and hence 24 fish were used in total.

Some physical and chemical parameters of the experimental media were as follows; Temperature: 22±1°C; pH: 8.03±0.05; Dissolved oxygen: 7.39±0.52 ppm $O_2$; Total hardness: 267.4±4.13 ppm $CaCO_3$; Total alkalinity: 394±0.59 ppm $CaCO_3$.

Experimental tanks were aerated using a central aeration system and water in experimental and control tanks were replaced once in 2 days to avoid changes in concentration due to adsorption, precipitation and evaporation. Fish were fed once a day with *Lumbricus terrestris* (Ciftci *et al*., 2008) at amounts of 2% of the total biomass.

Fish were removed from the aquaria at the end of the experimental period, they were decapitated and their liver, gill and muscle tissues were dissected.

Dissected tissue samples dried at 105°C for 72 h and their dry weights were recorded. They were transferred to glass tubes and 2:1 v/v nitric acid ($HNO_3$, 65%, 1.40, Merck) and perchloric acid ($HClO_4$, 60%, 1.53, Merck) mixture was added. Tissues were digested at 120°C for 8 h. They were then transferred to polyethylene tubes and their volumes were made up to 5 mL with distilled water. Copper levels in the samples were measured using an atomic absorption spectrophotometer.

Statistical analyses of the data were carried out using variance analysis and Student Newman Keul's procedure (SNK) (Sokal and Rohf, 1969).

## RESULTS AND DISCUSSION

No mortality was observed in any copper concentration during the 96 h of exposure period. Some behavioral changes were observed such as coordination disturbances in swimming activities, rejection of food uptake and swimming towards the water surface at the beginning of exposure. The mean levels of copper accumulation in liver, gill and muscle tissues of *A. anguilla* exposed to 1.00, 2.00 and 4.00 ppm concentration of the metal over 96 h are shown in Table 1 together with the statistical evaluation of the data.

Table 1: Copper accumulation in tissues of *A. anguilla* exposed to various concentrations of the metal over 96 h (μgCu $g^{-1}$ dry wt)

| | Liver | | Gill | | Muscle | |
|---|---|---|---|---|---|---|
| Factors | X±Sx | * | X±Sx | * | X±Sx | * |
| Control | 30.30±2.72 | as | 3.32±0.21 | bs | 0.87±0.01 | bs |
| 1.00 ppm | 95.54±1.72 | at | 22.31±0.43 | bt | 0.95±0.11 | cs |
| 2.00 ppm | 142.17±9.39 | ax | 21.07±0.83 | bt | 0.95±0.09 | cs |
| 4.00 ppm | 122.43±1.54 | ay | 20.68±2.08 | bt | 1.74±0.17 | ct |

*= SNK; Letters a, b, c and s, t, x, y show differences among the tissues and concentrations, respectively. Data shown with different letters are significant at the $p<0.05$ level; X±Sx = Mean±Standard error

Copper accumulation significantly increased in liver and gill tissues at all the concentrations tested whereas accumulation was only significant at 4.0 ppm Cu in the muscle tissue compared with the control fish ($p<0.05$). Tissue accumulation of copper differed significantly between the concentrations tested except the gill tissue ($p<0.05$). The following relationship was found among the tissues in accumulating copper; Liver>Gill>Muscle.

The effect of heavy metals on mortality in fish was shown to depend on external concentration and exposure period (Abel and Papoutsouglou, 1986). Exposure to sublethal concentrations of copper over 30 days did not cause mortality in *A. anguilla* (Ciftci *et al*., 2008), *A. rostrata* (Gill *et al*., 1993) and in *Tilapia nilotica* and *Cyprinus carpio* (Kargin and Erdem, 1992), prolonged exposures however, did result in mortality. No mortality was observed in *A. anguilla* exposed to 1.00, 2.00 and 4.00 ppm copper over 96 h which may be due to various adaptation mechanisms such as the increase in the synthesis of glutation and metallothioneins and/or slowing down metabolism or the tested concentrations of copper was in the tolerance limits of this species at the period tested (Saglamtimur *et al*., 2003).

Fish show some behavioral changes together with metabolic and physiological changes when exposed to heavy metals as in the present study. Studies carried out with various fish species revealed that reduction in swimming performance, rejection of food and increased operculum movements are observed in copper exposed fish (Ansari, 1984; Venkataramana and Radhakrishnaiah, 2001; Ali *et al*., 2003).

Copper accumulation in fish depends upon concentration of the external medium exposure period and the tissue in concern. It was shown that accumulation of heavy metals accumulation increase with increasing

exposure periods to sublethal concentrations (Kargin and Erdem, 1992; Cicik *et al*., 2004) and that accumulation is generally higher in liver and lower in muscle tissues (Erdem, 1990; Cinier *et al*., 1999; Daglish and Nowak, 2002; Cicik, 2003).

## CONCLUSION

Liver and gill tissue accumulation of copper in *A. anguilla* exposed to copper over 96 h showed an increase compared with control fish whereas the muscle accumulation only increased at 4.00 ppm Cu. The difference among the tissues in accumulating copper was probably be due to differences in the metabolic activity of the tissues and capacity to syntheses of metal binding proteins.

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