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Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences (JABE)

A Peer Reviewed & Refereed, International Open Access Journal

www.jabe.in **Vol.1.Issue.2.2014 (Oct-Dec)** **E-ISSN:2394-2606**

BIOACCUMULATION OF COPPER IN *CIRRHINUS MRIGALA* (HAMILTON,1822) AND *CTENOPHARYNGODON IDELLA* (STEINDACHNER, 1866)

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Article History

Received on:17-10-2014

Revised on:29-11-2014

Accepted on:30-12-2014

ABSTRACT

Literature reveals that a considerable number of acute toxicity studies have been carried out with heavy metals in different species of fishes. However, only a few investigators attempted sublethal toxicity experiments with non-nutritive trace metals in culturable species of carps. In the present study, static bioassays were conducted on fingerlings of *Cirrhinus mrigala* (8±0.5 g) and *Ctenopharyngodon idella* (8.5±1 g), to study bioaccumulation of lead in six tissues, both edible (skin and muscle) and non-edible (gill, brain, liver and kidney), over a period of four weeks under sublethal conditions. Cupric chloride was used as the copper agent. Fish were exposed to 1/5 of LC 50 of copper calculated for *C. mrigala* (0.16 mg / l Cu) and that calculated for *C.idella* (0.53 mg / l Cu). The concentrations in the edible parts of both the species after 28 days of sublethal exposure, were well below the provisional tolerable daily intake of 7 ug/kg body wt. of lead per person, established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA),1982.

Cirrhinus mrigala, Ctenopharyngodon idella, copper, sublethal toxicity, bioaccumulation.

Introduction

The knowledge we have about uncontrolled use of chemicals is obtained from animal studies conducted under controlled condition on a short , intermediate , or long term basis. All of these studies are looking for dose–response relationships so that we may extrapolate them to the human situation, which is often uncontrollable [1]. Animal studies can tell us about no observable effect levels, which are used to derive acceptable daily intakes for environmental contaminants and other chemicals [2].

The impact of metals in aquatic environment has been widely investigated in studies examining accumulation in several species of fish [3,4,5,6,7,8,9]. Absorption of heavy metals can occur via two pathways, as discussed [10] and demonstrated in a comparative study [11]. The first is absorption from solution. Ion transfer through the gills serves as a good example. Metals may, however, also diffuse passively through skin and gills as a soluble complex down gradients created by adsorption at the surface. The second pathway is absorption from food or particles.

Copper is widely distributed in animal tissues, and is now regarded as a normal constituent of the human body, not as was formerly believed as a result of food and drinks contaminated with it [12]. Storage is mainly in the liver, the kidneys and the intestines. Metals like copper as part of a metabolic system, work as enzyme co-factors, and are essential elements for normal cellular functioning [13]. However, their higher quantities are toxic for the cell. On the other hand, copper may cause toxic effects even at low levels under

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www.jabe.in **Vol.1.Issue.2.2014 (Oct-Dec)** **E-ISSN:2394-2606**

certain conditions, thus implying a need for analytical monitoring of inhabitants like fish [14]. Copper sulphate is extensively used as an algicide in commercial fish culture ponds, and has also been used to control protozoan diseases in fishes. Culture operations in fish ponds extend for a period of 3-5 months. During this period, fingerlings are exposed to low levels of copper. Once the trace element is absorbed, it is transferred from the gills and intestine to the blood and distributed to other parts of the body [15], resulting in it's storage in both edible and non-edible organs.

In the present investigation, bioaccumulation of copper in six tissues, both edible (skin and muscle) and non-edible (gill, brain, liver and kidney), has been estimated over a period of 28 days under sublethal conditions.

Materials and methods

Male and female fingerlings of an endemic carp *Cirrhinus mrigala* (mrigal), and an exotic carp *Ctenopharyngodon idella* (grass carp) ranging in length from 3 ½ to 4'' and weight 8 ± 0.5g and 8.5 ± 1g respectively, were procured from a private fish farmer in Kaikaluru, Andhra Pradesh. They were acclimated at a temperature of 28 ± 2 ^{0}C and fed with rice bran and oil-cake.

The sub-lethal toxicity experiment was conducted with lead in both male and female fingerlings of *C. mrigala* (measuring 3 1/2 - 4" and weighing 8 ± 0.5 g) and *C. idella* (measuring 3 1/2 - 4" and weighing 8.5 ± 1 g) after acclimation for one week. Feeding was terminated 24 hrs. prior to the experiment .

Groups of 25 fish were exposed to 1/5 of LC 50 of copper calculated for *C. mrigala* (0.16 mg / l Cu) and that calculated for *C. idellus* (0.53 mg / l Cu) in 500 litre fibre-glass tanks, not exceeding 1 g fish /l, using static test method.

Aeration was avoided, as it might alter the results of the tests. Fish were maintained at 29 ± 2 0 C and fed on a weekly basis, once in the morning and once in the evening, for four weeks during the 28-day long experiment. Control fish were maintained under the same conditions, in water devoid of copper detectable.

Prior to exposing the fish to sublethal concentration, skin, muscle, gill, brain, liver and kidneys of control groups of both species were sampled. Every 7th, 14th, 21st and 28th day, 25 fish (belonging to both species) were sampled from the copper exposure groups, dissected, and skin, muscle, gill, brain, liver and kidneys neatly separated. 1 gm. weight of each tissue was weighed into 25 ml. conical flasks, and digested overnight with 7 ml. of pure nitric acid (AR grade, specific gravity : 1.42, Qualigens, India) and 3 ml. of hydrogen peroxide.

The tissues were analysed for copper concentration following the method of AOAC official method 999.10 (AOAC 2000) [16]. Samples were digested in Teflon containers using a microwave digester (LEM MARS 240/50 Niulab, Hyderabad, India). Tissues were homogenized, 3.0 g. of wet tissue was weighed into 100 ml. Teflon vials and digested overnight with 7 ml. of pure nitric acid (AR grade, specific gravity:1.42, Qualigens, India) and 3.0 ml. of hydrogen peroxide. The microwave parameters were 700 W power for 1 hr., with 40 minute heating time and 20 minute ventilation time. The digested contents were transferred to acid washed polypropylene bottles and made up to 25 ml. with double distilled water and subjected to lead content analysis by Atomic Absorption Spectrophotometer (Spectra AA 220, Varian, Australia). Statistical analysis was performed by two way ANOVA procedure of MINITAB, to determine any significant difference in lead accumulation level in the chosen tissues of *C.mrigala* and *C.idella*.

Results & Discussion

The amount of copper in the skin, muscle, gill, brain, liver and kidney of the control group of *C.mrigala* are 0.01, 0.02, 0.02, 0.007, 0.02 and 0.01 ug/g wet wt.

After 7, 14, 21 and 28 days of exposure to sublethal concentration (1/5 of LC 50) of copper (0.16 ppm.), the amount of copper in skin of *C.mrigala* recorded values 0.01, 0.02, 0.03, and 0.05 ug/g wet wt., in muscle, 0.02, 0.03, 0.03 and 0.04 ug/g wet wt., in gill, 0.03, 0.04, 0.06 and 0.09 ug/g wet wt., in brain, 0.01,

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0.01, 0.02 and 0.02 ug/g wet wt., in liver, 0.02, 0.03, 0.05 and 0.07 ug/g wet wt., and in kidney, 0.03, 0.03, 0.04 and 0.05 ug/g wet wt. respectively.

Analysis of variance for bioconcentration of copper by control (Group A) and copper treated (Group B) of *C.mrigala* is shown in Table 1. There is significant difference between the A Group (control) and B Group (copper treated) at 5% level of significance. Further, there is a significant mean difference in micrograms among the tissues at 5% level as per the significant p-values of the F-test mentioned above.

Table 1. Analysis of variance for bioconcentration of copper by Groups A (Control) & B (Copper treated) of *C.mrigala*

Source	DF	SS	MS	F	P
Group	1	0.0132	0.0132	14.179	0.004
Tissues	10	0.0093	0.0009	4.997	0.001
Error	24	0.0045	0.0002		
Total	35	0.0270			

The amount of copper in the skin, muscle, gill, brain, liver and kidney of the control group of *C.idella* are 0.09, 0.02, 0.03, 0.008, 0.07 and 0.04 ug/g wet wt. respectively.

After 7, 14, 21 and 28 days of exposure to sublethal concentration (1/5 of LC 50) of copper (0.53 ppm.), the amount of copper in skin of *C.idella* recorded values 0.14, 0.20, 0.38 and 0.39 ug/g wet wt., in muscle, 0.02, 0.03, 0.03 and 0.04 ug/g wet wt., in gill, 0.14, 0.22, 0.31 and 0.39 ug/g wet wt., in brain, 0.14, 0.14, 0.15 and 0.16 ug/g wet wt., in liver, 0.12, 0.21, 0.29 and 0.38 ug/g wet wt. and in kidney, 0.10, 0.13, 0.18 and 0.23 ug/g wet wt., respectively.

Analysis of variance for bioconcentration of copper by control (Group A) and copper treated (Group B) of *C.idella* is shown in Table 2. From the results it is concluded that there is a significant difference between the A Group (control) and B Group (copper treated) at 5% level of significance. Further, there is a significant mean difference in micrograms among the tissues at 5% level as per the significant p-values of the F-test mentioned above.

Table 2. Analysis of Variance for bioaccumulation of copper by Groups A (control) & B (copper treated) of *C.idella*

Source	DF	SS	MS	F	P
Group	1	0.4436	0.4436	13.175	0.005
Tissues	10	0.3367	0.0337	252.337	0.000
Error	24	0.0032	0.0001		
Total	35	0.7834			

Among the different tissues, brain recorded the lowest amount of copper in the control groups of both the species. It may be explained by the physico-chemical nature of the concerned heavy metals, which dictate their penetration across the blood-brain barrier and other barriers [16]. Skin recorded the highest level of copper in the control group of *C. idella*, and gill liver and muscle recorded maximum levels of copper in the control group of *C. mrigala*. Species-specific membrane permeability might be the reason behind the difference in concentration. Lakshmanan et al. [17] postulated that accumulation of a metal in different species is the function of their respective membrane permeability and enzyme system, which is highly species-specific, and because of this fact, different metals accumulated in different orders in different fish samples.

Comparison of bioconcentration of copper by different tissues of control (A Group) and copper treated (B Group) *C.mrigala* and *C.idella* is shown in Figures 1 and 2.

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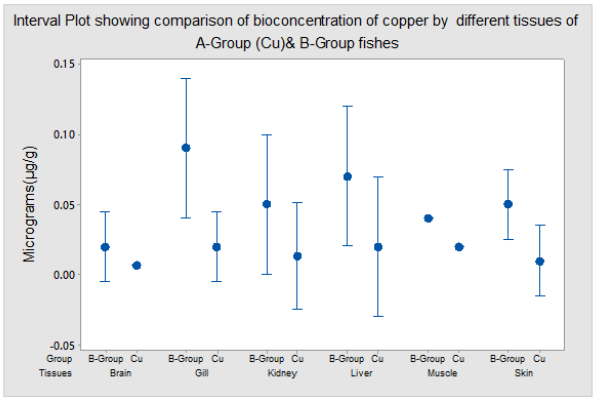

Figure 1.Comparison of bioconcentration of copper by different tissues of the control (A Group) and copper treated (B Group) *C.mrigala*

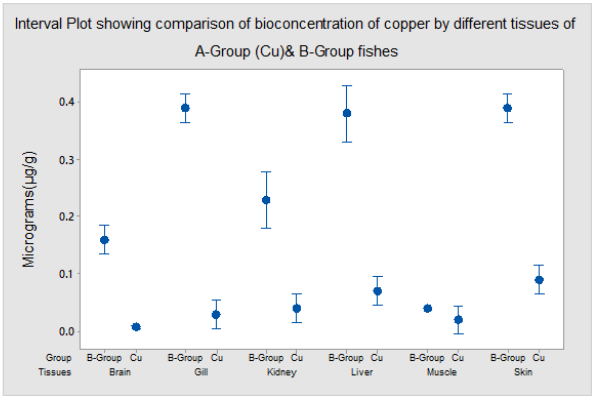

Figure 2. Comparison of bioconcentration of copper by different tissues of the control (A Group) and copper treated (B Group) *C.idella*

In copper treatment groups of both species, the bioaccumulation of copper increased with increasing exposure period.

The data indicated the following rank order of copper uptake and concentration (from highest to lowest values of copper at the end of the exposure period) in *C. mrigala* – gill > liver > kidney and skin > muscle > brain.

The higher metal concentration in gills might be directly due to the respiratory mechanisms of fishes. Similar observation was noted by Playle [18] who reported that during the respiratory process, the constant exposure of gills to the ambient water, and the consecutive filtering action for oxygen intake might enhance the metal concentration in gill tissues. It was also reported that, in general, the metal concentrations are lowest in muscle. Target organs, such as the liver and gills, are metabolically active tissues and accumulate heavy metals in higher levels, as was observed in experimental [19,20 *a*, 21] and field studies [22,23,24,25]. It is also evident that lead uptake and accumulation in the gills is more, because of it's high affinity with ion transport activity of various mineral ions [26,20 b,27].

The rank order of copper uptake and concentration (from highest to lowest values of copper at the end of the exposure period) in *C. idella* is as follows – skin > gill > kidney > liver > brain > muscle.

Metal accumulation in tissues of aquatic animals is dependent upon exposure concentration and period, as well as some other factors such as salinity, temperature, interacting agents and metabolic activity of the tissue in concern. Similarly, it is also known that the metal accumulation in the tissues of fish is dependent upon the uptake, storage and elimination [26,23].

A large number of biological variables play a significant role with regard to metal accumulation. These include interspecies variations [28,29] orientation to the sediment and behaviour [30,31], as well as life stages present [2]. Accumulation levels vary considerably among metals and species [32].

The amount of a metal accumulated is influenced by various environmental, biological and genetic factors, leading to differences in metal accumulation between different individuals, species, age, tissues, seasons and sites [33,34].

It is well understood that metal ions taken up by a fish through any route are not totally accumulated because fish can regulate metal concentrations to a certain extent, after which accumulation occurs. Therefore, the ability of each tissue to either regulate or accumulate metal ions can be directly related to the

total amount of metal uptake in that specific tissue. This metal regulation is due to the induction of low molecular weight metal-binding proteins, such as metallothioneins, which are closely related to heavy metal exposure and metals taken up from the environment can be detoxified by binding on these proteins [25,35].

The accumulation of metals by fish depends on the location, feeding behaviour, trophic level, age, size, duration of exposure to metals and homeostatic regulation activities of fish [36]. Kargin [37] has listed multiple factors that influence metal accumulation in fish such as season, physical and chemical properties of water. Age factor or maturity of fish may influence the accumulation of heavy metals [38]. Growth rate is important to stabilize the accumulation of metals.

Copper is essential for animals and plants, as it takes part in enzyme formation and participates in respiratory processes, with accumulation levels varying widely among aquatic organisms. Variations in copper concentration are related to levels of tolerance and toxicity symptom outbreaks, depending on species and period of passive accumulation [39]. This metal accumulates by several means, depending on environmental conditions and habits of species [40,41].

Copper is an essential micronutrient naturally occurring in unpolluted freshwaters, in concentrations ranging from 0.2 to 30 µg l-1 [42]. Contamination of aquatic systems, from mining, agricultural and [24,42]. As a consequence, concentrations of copper ranging from 50 to >560 ug l-1 have been reported in polluted areas all over the world [43,44, 45].

Conclusion

In the present study, the copper concentrations in different tissues of *C. mrigala* and *C. idellus* were negligible though detectable, in the edible (muscle) part of the fish after 28 days of sublethal exposure. These levels were much below the provisional tolerable daily intake of 500 ug/kg. body wt. of copper per person, established by the FAO/WHO Joint Expert Committee on Food Additives [45].

Acknowledgements

I am thankful to the University Grants Commission for selecting me under the Faculty Development Programme (FDP) during the XI plan, and extending financial support to pursue my Ph.D.

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