ACUTE TOXICITY STUDIES ON INDIAN FLYING BARB, *Esomus danricus* (HAMILTON-BUCHANAN), IN RELATION TO EXPOSURE OF HEAVY METALS, CADMIUM AND COPPER

Suchismita Das*¹ and Abhik Gupta ²

1. Department of Life science, Assam University, Silchar (INDIA)
2. Department of Ecology and Environmental Science, Assam University, Silchar (INDIA)

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ABSTRACT

Aquatic organisms are good indicators of environmental pollution because they concentrate pollutants in their tissues directly from water and sediments, and through them, it is also possible to assess the bioaccumulation of pollutants in the food chain. Only biological monitoring can tell us what toxic materials are doing to organisms. The susceptibility of fish to a particular xenobiotic is a very important factor for lethal concentration, LC50 values (concentration that kills 50% of test population). The fish that is highly susceptible to toxicity of one chemical may be less or non-susceptible to the toxicity of another at same concentration of that chemical. The purpose of acute toxicity test is to assess various abnormalities caused due to administration of a chemical to animals and to determine the order of lethality of the chemical. In aquatic toxicology, acute lethal toxicity tests with fish or invertebrates are usually intended to assess the numerical value of toxicity, to compare potencies of toxicants and to assess the effects of environmental variables on toxicity. Fishes are sensitive to external perturbations in aquatic medium. Therefore, it has become imperative to determine whether a potential toxicant is harmful to fish and if so, to find out the relationship between toxicant concentration and its effects on fish. *Esomus danricus* (Hamilton-Buchanan) is endemic to North India and found in shallow water bodies adjoining paddy and tea cultivation. In the present study, behavioural changes and acute toxicities for two heavy metals Cadmium and Copper were determined. Acute toxicity (LC50) on *E. danricus* for 24, 48, 72 and 96hrs for Cd were 19254µg/l, 12773.6µg/l, 8432 µg/l and 6363 µg/l respectively. The LC50 values for 24, 48, 72 and 96hrs for Cu were and 1.02µg/l, 0.25µg/l, 0.07µg/l and 0.01µg/l respectively. Copper showed toxic effects at much lower dose than Cadmium. Several behavioural manifestations were also noted for exposure durations.

**Key Words**: LC50, Toxicity, Cadmium, Copper, *E. danricus*

* Author for correspondence
INTRODUCTION

Freshwater is vital to life yet its environment is frequently being treated with greatest carelessness in terms of pollution. Freshwaters are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity always associated with the danger of accidental discharges or criminal negligence. Massive deforestation and industrialization are consequently destroying natural water by adding effluents and wastes into it. Of particular cause of concern are occurrence of heavy metals\(^1\) which are rapidly discharged into water bodies as wastes and agricultural run-off. Heavy metals are trace metals with a density at least five times that of water, they cannot be metabolized by the body and hence they are bio-accumulative and inhibit biological processes. Some heavy metals such as Co, Cr, Fe, Mn, Mo, Ni, Se, Sn, V and Zn are essential for growth, As and Ba may have possible beneficial uses. To be able to normally develop and function, an organism needs these minerals. While Bi, Cd, Hg, Pb and Ti appear to have no apparent metabolic function and so are termed non-essential elements. A deficit or an excess of one or more microelements may disturb the organism's internal equilibrium. Heavy metals may affect organisms directly by accumulating in their body or indirectly by transferring to next trophic level\(^2\). They cause serious impairments in metabolic, physiological and structural system when present in high concentrations\(^3\), influence behaviour by impairing mental and neurological function, neurotransmitter production and utilization, and altering numerous metabolic body processes.

Objectives

Toxicity tests have been proven to be powerful tools and were recommended along with other methodologies to obtain information on the ecological impact of contaminants\(^4\). Knowledge of acute toxicity of a xenobiotic often can be very helpful in predicting and preventing acute damage to aquatic life in receiving waters as well as in regulating toxic waste discharges. Indian flying barb, *Esomus danricus* is an active teleost species belonging to subfamily Rasborinae and hails from fast flowing and shallow streams of North East India. In Barak Valley, tea and paddy cultivation form the major agricultural activities. Needless to say, the shallow water bodies adjoining tea and paddy cultivation practically serve as sink for pesticides, chemicals, fertilizers and some domestic discharges affecting biota in general and fishes in particular. It would thus be very interesting to note the acute toxicity and behavioral responses of selected heavy metals, Cadmium and Copper on Indian flying barb found in such waters.

MATERIAL AND METHODS

*E. danricus* of almost equal length (~47 mm) and weight (~0.838g) were obtained from freshwater ponds Assam University campus, Barak valley, South Assam, India, and kept in 100 litre glass tank, fed with fish food and acclimatized in laboratory conditions seven days prior to experimentations. Temperature, pH and dissolved oxygen under laboratory condition were 29°C, 6.8 and 5.5 mg/l respectively. Actual concentrations of two heavy metals Cadmium and Copper were taken from their respective compounds (CdCl\(_2\) \(\cdot\) H\(_2\)O and CuCl\(_2\) \(\cdot\) 2H\(_2\)O respectively manufactured by Marc, Germany). Stock solutions were prepared
using distilled water. Serial dilutions were prepared from stock solution as per dilution techniques\(^5\-^7\). For toxicity tests, ten fish each were kept in three litres capacity glass aquariums containing various concentrations of test chemicals and acute toxicities were monitored at 24, 48, 72 and 96-h durations. Controls were also simultaneously maintained. The corresponding LC50 values were estimated by Probit analysis\(^8\). Observed quantal response was death manifested in cessation of gill and operculum movements of test fish.

**RESULTS AND DISCUSSION**

Acute toxicity (LC50) on *E. danricus* for 24, 48, 72 and 96 hrs for Cd were 19254µg/l, 12773.6µg/l, 8432 µg/l and 6363 µg/l respectively. The LC50 values for 24, 48, 72 and 96hrs for Cu were 1.02µg/l, 0.25µg/l, 0.07µg/l and 0.01µg/l respectively. Copper showed toxic effects at much lower dose than Cadmium. The behaviour of the fish in both the control and test solution were noted every 24 h up to 96 h. The fish showed a marked change in their behaviour when exposed to different concentrations of test solution. The fish exposed to Cu appeared irritable and showed frenzied swimming activity when the bowls were approached. Their bodies were covered with thick mucus. Behavioural manifestations of acute toxicity such as grouping, loss of equilibrium, erratic swimming, surfacing and darting movements were observed. For Cd, the major clinical symptoms such as inappetance and ataxia appeared after 2-3 days exposure. At higher concentration of the Cd, the exposed fish showed erratic movements. The other signs of toxicity such as loss of equilibrium, gradual onset of inactivity, erratic swimming with irregular collision to the inner glass walls of the aquarium along with circular movements were observed.

**Table 1 : LC 50 values(95% Lower and Upper CI) of Cd and Cu on *Esomus danricus***

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>LC50 OF Cd (µg/l)(95% Lower and Upper CI)</th>
<th>LC50 OF Cu (µg/l) (95% Lower and Upper CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>19254.0±4049* (12629.8, 30829.1)</td>
<td>1.02±0.45* (0.439, 2.67)</td>
</tr>
<tr>
<td>48 hrs</td>
<td>12773.6±2796 (8182.5, 20475.5)</td>
<td>0.25±0.11 (0.103, 0.643)</td>
</tr>
<tr>
<td>72 hrs</td>
<td>8432.0±1911 (5299.1, 13594)</td>
<td>0.07±0.04 (0.029, 0.221)</td>
</tr>
<tr>
<td>96 hrs</td>
<td>6363.0±1240 (3990.7, 9675)</td>
<td>0.01±0.008 (0.005, 0.046)</td>
</tr>
</tbody>
</table>

*Mean±SE
The exposed fish shed fins and huge destruction of dermal mucous layer was marked. Scar and ulceration of the gill lamellae was observed in the Cd exposed fish. At higher exposure periods, profuse bleeding of gills was observed. At later exposure periods, fish became over excited and periodic outburst of erratic swimming was noted, ultimately the fish died of suffocation. The exposed fish showed high levels of sensitivity. Any types of external stimuli like a flash of light, movements near aquarium and sound, the sluggish fish instantly starts vigorous movement and many a times collide with aquarium walls. After a few moments of such activity, the fish becomes senseless and ventilation rate almost stopped. The reaction and survival of aquatic animals depend not only on the biological state of the animals and physico-chemical characteristics of the water but also on the type of toxicant and the duration of exposure to it. Other fish characteristics such as, body size, feeding habits and sexes can also be considered as variable for LC50 of metals for different species of fish. Cadmium (Cd) is a nonessential element that causes severe toxic effects in aquatic organisms in very low concentrations. Cd is an anthropogenic contaminant considered in the priority pollutant lists. About three-quarters of Cd are used in batteries, predominantly in rechargeable nickel-cadmium batteries. Most of the remaining quarter is used mainly for pigments, coatings and plating, and as stabilizers for plastics. Concentrations of cadmium associated with background fresh water systems are estimated to range between 0.05-0.2 μg/l. While Cd is released through natural processes; anthropogenic cadmium emissions have greatly increased its presence in the environment. In aquatic systems, Cd quickly partitions to sediment, but is readily remobilized through a variety of chemical and biological processes. Toxicity of Cd to aquatic organisms varies with the type and life stage of organisms, hardness, temp-erature and presence of other toxicants and the duration of exposure. Heavy metals like Cd have been reported to exert a wide range of effects on fish such as disturbances in osmo regulation, respiration, gill and tissue damage, reduced energetic resources and poor performance. Juvenile
trout (*Oncorhynchus mykiss*) have higher 48 h LC50. Acute toxicity of Hg, Zn, and Cd in rainbow trout showed that fish was more susceptible to Hg, followed by that of Zn and Cd. In water hardness of 100 mg/l Ca, carp fry and fingerling (*Cyprinus carpio*) have Cd 96 h LC50 of 4.3 and 17.1 mg/l, respectively. In chinook salmon, LC50 values for 96 hour exposures ranged from 1.1-3.5 μg Cd/l dependent on the life stage tested (hardness=23 mg/l). From the available information, EPA has determined the acute and chronic Cd criteria (3.7 μg/l=acute, 1.0 μg/l=chronic, with hardness of 100 mg/l CaCO3) and Cu (17 μg/l=acute, 11 μg/l=chronic, hardness of 100 mg/l CaCO3). Cadmium toxicity of gold fish, *Carassius auratus* was studied in hard and soft water. The proportion of these animals that died in a fixed time increased with the increase in the toxicant concentration and the median tolerance limit value decreased with the exposure period. The route of entry of the toxicant is generally agreed to be via gills and thus enters directly into the circulatory system. This caused damage to tissues as a result of which there is depression in active metabolism.

Copper (Cu) is an essential nutrient for life. As a micronutrient, it is necessary for haemoglobin synthesis and a component of Cytochrome oxidase. Higher concentrations of Cu have however been introduced into the environment due to anthropogenic activities such as mining, electroplating, paint and pigment industries textile factory effluents and pesticides. Due to its ability to exist in two oxidation states, Cu is an important redox cofactor for many copper-dependent enzymes, including cytochrome-c-oxidase, ceruloplasmin, copper/zinc superoxide dismutase and lysyl oxidase. Redox cycling between Cu (II) and Cu (I) can catalyse the production of highly toxic hydroxyl radicals, with subsequent damage to lipids, proteins and DNA. The acute toxicity of Cu to various freshwater fish is well documented, with 96-h LC50 values ranging from 2.58 mg Cu/l (Arctic grayling, *Thymallus arcticus*) to 7340 mg Cu/l (blue gill, *Lepomis macrochirus*). Although some fish and crawfish may survive at Cu concentrations of 0.03–0.8 mg l⁻¹, Cu exceeding of 0.1 mg/l in water is usually toxic to fish. A 96-h LC50 of 51–65μg l⁻¹ at 16 mg l⁻¹ total hardness and 1,084–1,880μg l⁻¹ at 287 mg l⁻¹ total hardness for channel catfish was also reported.

Behavioural toxicology study is a rewarding tool for hazard assessment of water pollution. Behavioural changes in animals are indicative of internal disturbances of the body functions such as inhibition of enzyme functions, impairment in neural transmission, nervous impairment due to blockage of nervous transmission between the nervous system and various effector sites, and disturbances in metabolic pathways. The development of response criteria in animals varies from detailed physiological measurements to whole animal response, especially preference/avoidance behaviour. Majority of behavioural changes investigated in fish deal with the locomotor or feeding activity, and with the avoidance or attraction responses. Studies on the effects of copper and zinc salts on carp species *Cyprinus carpio* and *Ctenopharyngodon idellus* revealed that the body and the gills of dead fish were covered...
by a veil like film which looked like coagulated mucus and which was formed by the heavy metal ions reacting with some constituents of the mucus secreted by the gill\textsuperscript{19}. Golden shiner, \textit{Notemigonus crysoleucus}, when exposed to 5 ppm copper piped at the surface, became restless, failed to school, became sluggish and finally lost equilibrium\textsuperscript{20}. The locomotor activity of Bluegill sunfish, \textit{Lepomis macrochirus}, treated with 0.04, 0.08 and 0.4 ppm Cu, decreased to 67, 61 and 44\% respectively\textsuperscript{21}. Lethargic response and frequent surfacing along with gulping of air in exposure to 0.25 ppm Cu were observed in \textit{Heteropneustes fossilis}\textsuperscript{22} and Cd exposed \textit{Tilapia mossambica}\textsuperscript{23}. \textit{Etroplus maculatus} on exposure to copper, mercury and selenium showed irregular erratic swimming, frequent surfacing, gulping of air, revolving, convulsions and accelerated ventilation with rapid arrhythmic opercular and mouth movements\textsuperscript{24}. The fish swam upside down and died with mouths opened as observed in adult \textit{Clarias gariepinus} exposed to copper\textsuperscript{25}.

**CONCLUSION**

From the present study it could be concluded that Copper, although an essential metal, is more toxic than Cadmium to Indian flying barb, showing acute affects at much lower doses compared to Cd\textsuperscript{26}. Behavioural changes in animals on both the metal exposure are indicative of internal disturbances of the body functions, mostly impairment in neural transmission. Behavioural manifestations of acute toxicity were mostly grouping, loss of equilibrium, erratic swimming, surfacing and darting movements, irregular collision to the inner glass walls of the aquarium along with circular movements. Effects were more severe, leading to death of fish, at higher doses or longer exposure duration.

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