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Effect of heavy metal pollution on *Carassius auratus*: A behavioral study

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Abstract: Heavy metal contamination in fresh water bodies is of great concern due to their toxicity, perseverance and bioaccumulation. The purpose of this study was to evaluate behavioral responses induced by Lead (Pb), Mercury (Hg) and its Synergistic effect (Pb+Hg) in the ornamental fish *Carassuis auratus*. Fishes were exposed to 1/10th of LC50 concentrations for 7,14,21 and 45 days along with a recovery set. The major behavioral responses observed in fish after exposure were restlessness, jumping, erratic swimming, gulping of air at the surface, loss of equilibrium, sluggishness, altered opercular movements and frequent surfacing were observed. After a longer period of exposure, the fish became sluggish and occasionally became motionless and sometimes swam vertically. Mercury was found to be more toxic compared to Lead and Synergistic regimens.

Index Terms: ***Carassuis auratus*, Heavy metals, Lead, Mercury, behavior**

I. Introduction

The goldfish, *Carassius auratus* (Linnaeus) belongs to the Cyprinidae family and is reared all over the world as an ornamental fish. It has a scaly and laterally compressed body. Their mouths are small, without barbels. It has a long dorsal fin and a slightly serrated third spine. It shares some common features with another species of crucian carp *Carassius carassius* (Linneaus) except that it is larger and has a slightly concave dorsal fin and larger scales (Lelek 1987). Taxonomically, there is a confusion regarding *C. auratus*. There are two subspecies in its native range: *Carassius auratus auratus* (goldfish, Chinese goldfish, or Asian goldfish) from Asia, and *Carassius auratus gibelio* Bloch (Prussian carp, gibele carp, or European goldfish) from Eastern Europe (Hanfling *et al*. 2005). Nico and Schofield (2006) found that the goldfish typically seen in US waters are hybrids of crucian carps and goldfish.

As a model system, goldfish can provide valuable insights into the molecular basis for skeletal and organ morphology and body colouration of vertebrates. Unlike zebrafish or medaka mutants, goldfish strains have biologically interesting phenotypes, such as the formation of hoods on the head, narial bouquets, body proportions, fin shapes, scale morphologies, and a variety of body colourations. The goldfish can grow 45 cm long (TL) and weigh up to 3 kg, but they are more likely to be 20 cm TL and weigh 100–300 g. Life span of goldfish is between 6 and 7 years, though they have been known to survive as long as 30 years (Menasse` 1974). They do not show any parental behavior; their eggs and larvae are left unattended. Water bodies with submerged aquatic vegetation, such as weedy ponds, shallow lakes, and slow-flowing rivers, are their natural (Lelek 1987, Maitland 2004). Goldfish have been bred to produce many different varieties through selective breeding for variation in size, colour and shape. These fish usually revert to their natural olive-bronze colour and fin shapes after being released from captivity.

Goldfish are considered to be extremely hardy fish, able to survive the abuse of many novice aquarium hobbyists. While they may survive in extremely harsh conditions, they thrive well in their ideal conditions. Fish behavior represents the fish physiological response towards the environmental factors. Behavioral changes in animals are indicative of internal disturbances of the body functions (Moreira-Santos *et al*., 2008). Moreover, the interaction of fish behavior related to the ecology can be easily observed (Scott and Sloman, 2004). For instance, the existence of metal ion in the environment increases mucus like secretion from gill, excessive excretion, anorexia and also the fin movement (Ezeonyejiaku *et al*., 2011).

Fish behavior, can be defined as an adaptation of fish to external and internal environments and to natural and artificial stimuli. Fish behavior entails the response of fish to the different physical and chemical stimuli associated with any kind of toxicants. The information on fish behavior helps in sustainable harvest and research. Behavioral changes in fish can help in determining and understanding the change in the environment (Kristiansen et al., 2004; Amiard-Triquet, 2009; Hellou, 2011). Such behavioral changes brought about by the changed environment may cause morphological changes too (Mazon et al., 1999). Environment, particularly where pollutants occur in chronic and sublethal concentrations induce stress which includes changes in the behavior, structure and function of aquatic organisms more frequent than the mass mortality (Poleksic and Mitrovic-Tutundzic, 1994). Stress can be initiated by inadequate water quality, stocking density, diet or feeding technique, infestation by parasites or a disease and toxicity by pollutants (Ashley, 2007). Physiological modifications in the body occurs with chronic metal exposure includes increased oxygen consumption, reduced mean swimming velocity or speed, elevation of ionic parameter, increase in neutrophils, changes in the immune system, adjustment of Cu-dependent enzyme activities, and absorption of epithelial cells in the gills or intestine (Handy, 2003). An affected fish shows altered behavior towards toxicants, especially pesticides and heavy metals (Patil and David ,2010).

II. RESEARCH METHODOLOGY

2.1 Experimental Animals:

Healthy and active adult carp *Carassius auratus* (Family: Cyprinidae), were purchased from the local vendor, Kalyan (19.2403º N, 73.1305º E), Maharashtra, India. *C.auratus* was acclimatized in the laboratory conditions as per the OECD, EPA guidelines. On the arrival, the fish were placed in large tanks with aerated tap water. Fish were acclimatized for 2 weeks prior to the experiment in 20L glass aquaria (39"X39") containing dechlorinated tap water. They were exposed to natural photoperiod of 12-12 h. It was ensured that the water used for aquaria later during experiment had a pH of 7±0.2 and a total hardness of 20 mg $CaCO_3$/L. Water was renewed every day. The temperature was maintained between the physiological tolerable limits of 25-30°C.

Adult Carp (7 in each aquarium) of 25 ± 5 gms weight irrespective of their gender were selected for the exposure. The fish were fed regularly with fish food pellets available commercially during acclimatization and test periods.

Toxicity studies:

The LC50 with 95% confidence limits for Lead and Mercury were estimated for 96 h by probit analysis (Finney, 1971). The fish were then exposed to analytical grade lead acetate and mercuric chloride solutions at the concentration ten times lower (Table.1). The fish were exposed for four different durations of 7, 14, 21 and 45 days. The control and recovery set were maintained separately.

Table 1. LC50 for three regimens

Metal-pollutant	LC50	Sub-lethal concentration (1/10th LC50)
Lead acetate $[Pb(C_2H_3O_2)_2]$	29.07mgL^{-1}	2.9mgL^{-1}
Mercuric chloride $[HgCl_2]$	4.65mgL^{-1}	0.46 mgL^{-1}
Synergistic dose $[HgCl_2+Pb(C_2H_3O_2)_2]$	6.02 mgL^{-1}	0.6mgL^{-1}

III. RESULTS AND DISCUSSION

Table 2. Effect of Lead on behavioral responses of fresh water fish *Carassius auratus.*

Sr. No.	Behavioral changes	Control	Duration of exposure (Lead)				
			7 days	14 days	21 days	45 days	Recovery
1	Loss of equilibrium	-	+	++	+++	++++	+++
2	Gulping air at surface	-	+	++	+++	++++	++
3	Irregular and Dart Swimming	-	+	++	+++	++++	++
4	Opercular movements	-	+	++	+++	++++	+++
5	Restlessness	-	+	++	+++	+++	++
6	Jumping	-	+	+	++	++++	+++
7	Sluggishness	-	+	+	++	++++	+++

(-) Normal, (+) Nil, (++) Less Change, (+++) Moderate Change and (++++) Noticeable Change.

Table 3. Effect of Mercury on behavioral responses of fresh water fish *Carassius auratus.*

Sr. No.	Behavioral changes	Control	Duration of exposure (Mercury)				
			7 days	14 days	21 days	45 days	Recovery
1	Loss of equilibrium	-	+++	+++	++++	++++	+++
2	Gulping air at surface	-	++	+++	+++	++++	++
3	Irregular and Dart Swimming	-	++	+++	+++	++++	++
4	Opercular movements	-	++	+++	+++	++++	+++
5	Restlessness	-	++	+++	+++	+++	++
6	Jumping	-	+++	+++	++++	++++	+++
7	Sluggishness	-	++	+++	+++	++++	++

(-) Normal, (+) Nil, (++) Less Change, (+++) Moderate Change and (++++) Noticeable Change.

Table 4. Effect of Synergistic regimen (Lead +Mercury) on behavioral responses of fresh water fish *Carassius auratus.*

Sr. No.	Behavioral changes	Control	Exposure (Lead acetate + Mercuric Chloride)				
			7 days	14 days	21 days	45 days	Recovery
1	Loss of equilibrium	-	++	++	+++	++++	+++
2	Gulping air at surface	-	++	+++	+++	++++	++
3	Irregular and Dart Swimming	-	++	+++	+++	++++	++
4	Opercular movements	-	++	+++	++++	++++	+++
5	Restlessness	-	++	+++	++++	++++	+++
6	Jumping	-	+	++	+++	++++	++
7	Sluggishness	-	+	++	+++	++++	++++

(-) Normal, (+) Nil, (++) Less Change, (+++) Moderate Change and (++++) Noticeable Change.

Discussion And Conclusion:

Toxicity studies are necessary for understanding the action and effects of toxicants. In aquatic ecosystems, swimming performance is considered as one of the behavioral parameters to assess the physiological status of fish under the influence of contaminant (Ballesteros *et al*., 2009; Cailleaud *et al*., 2011; Almeida *et al*., 2012). Swimming performance has been previously studied to assess the toxicity of the compound by the measurement of swimming velocity, critical swimming speed, maximal swimming speed and exhaustion time (Waser *et al*., 2009; Almeida *et al*., 2010).

Carassius auratus was exposed to lead acetate and mercuric chloride toxicity individually as well as synergistically. It was reported here that exposure to increasing durations to the fixed amount of all the three regimens resulted in various behavioral changes. The parameter for behavior alterations were measured in terms of swimming performance, avoidance behavior and feed intake.

The behavioral changes are considered to be directly related to complex physiological responses and have often been used as a sensitive indicator of stress (Elia *et al*., 2003). In the present investigation in both control and test groups, the behavior of fish was observed during the entire experiment. When fish were exposed to sublethal concentration of all three regimens, they showed notable changes while in control group such changes were not reported. After introducing the fishes to the test aquaria for the first seven days they did not show any significant changes. However, progressive treatment groups, showed the symptoms of swimming disability like uninhibited irregular, erratic and dart swimming movements. In addition to this restlessness, loss of equilibrium and drowning was observed for 21 days treatment group. On 45th day of the treatment, fish were found to be hitting against the wall and trying to jump out of the aquaria to avoid the chemical. Hesni *et al*. (2011) studied the acute toxicity of lead nitrate on behavioral changes of milk fish.

Other changes observed were hyper excitability, disturbed schooling, disrupted shoaling, reduced feeding and after few hours of exposure lethargy was seen. An apparent sluggishness at the end of the exposure period may be caused by irregular swimming and jumping, as well as restlessness and fatigue. Similar behavior patterns were seen in fish, trout and *Labeo rohita* exposed to an insecticide, Fenvelrate (Murthy, 1987). Changes in the behavioral activities increased by 45th day of exposure period. In lead acetate treatment group, the fishes showed lethargy and settled down at the bottom of the tank after 21 days of exposure while similar behavior was observed after 14 days of exposure in mercuric chloride treatment group. Fishes exposed to synergistic regimen showed almost similar changes as shown by the fishes exposed to Mercuric chloride treatment. With the increase in the number of days the fishes showed less activity and after 21 days they seem to get adapted to the toxic environment up to some extent. However, it was observed that whenever water was changed in the treatment aquaria, the fishes showed normal behavior for a few minutes to an hour and after some time they became lethargic. The effects are generally displayed as behavior responses and morphological changes which become cytologically visible in the tissues. Maruthanayagam *et al*. (2002) and Laovitthayanggoon (2006) reported that with the increase of concentration of cadmium compounds on freshwater snake headed fish C. *punctatus*, showed various behavioral responses and morphological changes. Raised swimming activity stimulated by the toxicant would increase the requirement for oxygen, to meet the energy demand of elevated muscular activity.

Fast opercular movements, gulping of air and surfacing were observed after 21 days in aquaria treated with mercury and synergistic regimen. After 45 days of treatment of Synergistic regimen, the important observations made were gradual decrease in opercular movement, loss of equilibrium and excess of mucus all over the body surface. Borah and Yadav (1995) observed increased opercular movements, loss of equilibrium, erratic swimming, jerky movement and mucous secretion all over the body in *Heteropneustes fossilis* after exposure to rogor and endosulphan pesticides.

Irregular, erratic and darting movements with imbalance in swimming activity and attempt to jump out of the toxic medium were observed by 45 days of the exposure to all the three regimens. Such a behavior is suggestive of intolerance to the pollutant. Jumping out of the water was indicative of avoidance behavior. Studies conducted by Ullah et al.2014, on mahseer, *Tor putitora* have shown similar results. Similar observations were recorded by (Santhakumar and Balaji, 2000) in *Anabas testudineus* after exposure to monocrotophos wherein the animal showed sluggishness, decrease in opercular movement and death.

Conclusion:

Lead and Mercury affect the behavior of *C.auratus* adversely in a dose dependent manner as seen from the present investigation. It has been seen that the toxic effect caused by Mercury affects the behavior more unfavourably than the Lead while the synergistic regimen showed less harmful effects then Mercury but more than Lead. Nevertheless, on 45th day of treatment in all the three regimen the behavioral changes were much more pronounced than the other 3 durations.

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