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Research Article

Behavioural effect of heavy metals on common carp (*Cyprinus carpio* L.) and Nile tilapia (*Oreochromis niloticus* L.)

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Abstract
The study aimed to investigate the effect of two heavy metals of cadmium and lead on the behaviour of two fish species, viz. *Cyprinus carpio* and *Oreochromis niloticus*. Two behavioural models, including the mirror-biting test and the dark/light test. The results showed that a significant relationship between the social behaviour of the fish species and the exposure to the selected heavy metals. Fishes exposed to heavy metals showed a significant decrease (P<0.01) in the number of mirror bites in the mirror biting test. Moreover, the results of the dark/light test showed that there was a significant reduction (P<0.01) in the total time spent in by fishes exposed to heavy metals in the light compartment.

Keywords: Common carp, Tilapia, Fish performance, Mirror test.

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Introduction

Social behaviour can be defined as any kind of interaction between conspecifics, including reproductive behaviour, cooperative interactions, shoaling and social hierarchy. Aggressive behaviour was mainly investigated due to its importance in wild life and neuro-hormonal background (Cruz 2002; Gonçalves-de-Freitas et al. 2019). Behaviour also works as the link between the ecological and physiological processes, as a suitable tool for studying pollutant effects. Pollutants negatively affect the physiology and survival of many organisms. Sub lethal concentrations of pollutants cannot cause mortality, but normal animals' behaviour is altered (Scott & Sloman 2004). According to Sloman et al. (2003), there is a significant relationship between the social behaviour of an individual fish and the accumulation of total heavy metals e.g. the uptake of copper was shown to be affected by the social status within the dominance hierarchy of rainbow trout (Sloman et al. 2003).

Human activities are the main sources of many pollutants, such as plastics, metals and pesticides. Metals are the major group of pollutants that disrupt the biochemical and physiological integrity of aquatic species, particularly fishes. Among these metals, copper, zinc, cadmium and lead have special concerns (Vieira et al. 2009). Some of the heavy metals such as Cu and Zn are essential elements with fundamental biological roles, but others such as Cd and Pb are more toxic in the aquatic systems without biological roles. World Health Organization (WHO) has classified Cd and Pb as two of ten pollutant substances that negatively affect the public health (Osredkar & Sustar 2011; Satarug et al. 2020).

Common carp, *Cyprinus carpio* and Nile tilapia, *Oreochromis niloticus*, are two main farmed fishes worldwide, listing in the top ten species in the global aquaculture (Abdul-Hassan 2010; ALKhafaji et al. 2012; Cai et al. 2019). Mammalian models especially

rodents have been successfully used to explore the neurotoxic effects of heavy metals. However rodent models have diverted the attention of the scientific community to other animal models in recent years. Fish can be used as a suitable alternative model for many reasons, including easy maintenance, prolific breeding, rapid development, and external development (Bui Thi et al. 2020). Therefore, the present study aimed to investigate the behavioural changes of *C. carpio* and *O. niloticus* after the exposure to two heavy metals of the Cd and Pb.

Materials and methods

Fish collection: Male and female fingerlings of *C. carpio* with a size range of 8.5-9.2g weight and 9.5-10cm total length and *O. niloticus* with 7.5-8.3 g weight and 8.5-9.0cm total length were obtained from fish farms in Al-Refaei City, Thi-Qar Province, southern Iraq. Fish were kept to acclimatize in 50 L. aerated tanks for 15 days before experiment under fasting conditions for the first 3 days. Fish samples were fed once daily with commercial pellets containing 38% proteins. In addition, the water in the aquariums was replaced every two days.

Exposure to heavy metals: Before the experiment, a preliminary study was conducted to determine the minimum lethal concentration of the heavy metals and the maximum tolerable concentration as well. Then A total of 140 healthy fingerlings from each species were used in the present study. The fingerlings were randomly divided in to eight groups for each species. The experiments were carried out at a density of 20 fish. Six groups for each species were exposed to heavy metals (Table 1). Two groups were served as control groups, one for each species was not treated with any heavy metals. Fishes were exposed to the selected concentrations of heavy metals for 30 days. On the 31th day, the behavioural study was conducted.

Behavioural study: At the end of the experiment, fingerlings from both species were individually subjected to two models of behavioural study:

Mirror biting test: Mirror biting test is a common test to study the aggressive behaviour. Fish from both species were individually introduced to a glass 25× 50×40cm) that a height of 25cm was filled with dechlorinated tap water. A mirror was inserted on the vertical side of the tank. Fishes were left to acclimate for 3 min. After the acclimation, number of times the fish bite the mirror were recorded for 5 min (Moretz et al. 2007).

Light/dark test: Fish of each species were individually transported to a tank divided into three equal sized compartments (25×25×30cm) using two opaque glass walls. The tank was filled by water to 20 cm height. Grey paint was used to cover the walls and the floor of the central compartment, which served as a neutral space for fish to choose either the light or dark ones. The floor and the walls of the dark compartment were covered with black paint. White paint was used to cover the walls and floor of the light compartment, and a 360-lumen light was mounted squarely above it. Each compartment had a rectangular door with dimensions of 5×7cm that permitted free movement between them. Individual fish were put in the centre compartment and allowed to move freely between compartments for 10min. The time that fish spent in light and dark compartments was recorded (De Pasquale & Leri 2018).

Statistical analysis: The data were analysed using SPSS using one-way ANOVA for variation. Least significant difference and independent t-test are used at $P<0.01$ and 0.05.

Results and Discussion

According to the results, there is a significant decrease in the number of times that the fish bite the mirror in both species ($P<0.01$) compared to the control group (Table 2). Furthermore, significant differences were found between the two fish species, except in K1, K3 and P3. The selected metals appeared anxiety effect on the studied fishes. The total time that the fish spent in light part was significantly reduced ($P<0.01$). There is a significant

Table 1. Fish groups of the current study and the used concentration of heavy metals.

Groups	Concentration of heavy metals (ppm)
K1	2.78
K2	4.17
K3	8.35
P1	2.35
P2	3.53
P3	7.07

Table 2. Effect of the heavy metals on the number of the times that the fish bite the mirror.

Groups	Mean ± SD		T test
	O. niloticus	*C. carpio*	*P*
Control	22.5±3.4^{c}	12.0±3.0^{b}	< 0.01
K1	12.2±3.4^{b}	7.50±2.0^{ab}	> 0.05
K2	10.5±2.0^{b}	6.50±2.0^{a}	< 0.05
K3	4.00±1.2^{a}	3.75±1.2^{a}	> 0.05
P. value	< 0.01	< 0.01	
LSD	4.9	4.6	
Control	22.5±3.4^{c}	12.0±3.0^{b}	< 0.01
P1	13.2±1.7^{b}	7.75±1.7^{ab}	< 0.01
P2	9.75±1.5^{b}	5.75±1.5^{a}	< 0.05
P3	5.50±1.3^{a}	5.00±1.6^{a}	> 0.05
P. value	< 0.01	< 0.05	
LSD	3.8	4.7	

Values represent mean ±SD. Values with nonidentical superscripted letters (a-c) are considered significantly different (P<0.01).

difference (P<0.05) between the two studied fish species in K3 (Table 3).

Metals and their compounds have negative impacts on the fish's nervous system. They interfere with synaptic transmission processes as well as synaptic plasticity. Metals have the ability to interact with specific targets in the synaptic pathways. Aluminum for example blocks voltage gated calcium channels and inhibits calcium ATPase causes a decrease in the release of excitatory neurotransmitters glutamate and aspartate i.e. it causes an increase in release of inhibitory neurotransmitter y-Aminobutyric acid (GABA) (Narahashi et al. 1994; Minami et al. 2001; Sadiq et al. 2012; Hussain et al. 2020). During embryotic development, exposure to Pb above 100ppb alters a number of genes associated with nervous system development. These changes are possibly associated with the decreased neuronal axon length (Lee & Freeman 2016; Zhu et al. 2016).

The results of the current study showed a significant reduce in the aggressive behaviour in *C. carpio* and *O. niloticus* treated with Cd and Pb. Similar to the present study, long-term exposure to $PbCl_2$ and $AlCl_3{\cdot}6H2O$ significantly suppress of zebrafish of aggressiveness (Capriello et al. 2021). Also, aggregation of *O. niloticus* was significantly higher than those of *C. carpio*. This may be associated to the high and complex interaction between individuals of *O. niloticus* (Gonçalves-de-Freitas et al. 2019). Aggression is an adaptive behavior that is essential for the establishment of social hierarchies, mating, and for territory and food competition. Aggression at a certain degree can be helpful to an individual's or species' survival and fulfill a social function. A deficit in aggressive behavior may impair the survival of animals in natural environments and reinforcing the deleterious effects of pollutants (Oliveira et al. 2011; Teles et al. 2013).

Dark/light test is a behavioural model for fish developed for all species that are important in

Table 3. Effect of heavy metals on the total time spent in the light compartment in dark/light test.

Groups	Mean ± SD		T test
	O. niloticus	*C. carpio*	
			P-Value
Control	224.3±4.0[d]	215.0±4.5[c]	> 0.05
K1	166.6±4.1[c]	167.6±7.5[b]	> 0.05
K2	158.6±4.7[b]	150.6±6.1[b]	> 0.05
K3	147.3±3.0[a]	124.0±15.5[a]	< 0.05
P. value	< 0.01	< 0.01	
LSD	7.6	17.6	
Control	224.3±4.0[c]	215.0±4.5[c]	> 0.05
P1	161.0±1.0[b]	161.6±4.5[b]	> 0.05
P2	159.3±3.5[b]	153.6±4.0[a]	> 0.05
P3	151.3±0.5[a]	150.6±1.5[a]	> 0.05
P. value	< 0.01	< 0.01	
LSD	5.1	7.2	

Values represent mean ±SD. Values with nonidentical superscripted letters (a-c) are considered significantly different (P<0.01) and (P<0.05).

neurology. It is based on scototaxis behaviour (fish natural preference for dark places in detriment of bright ones). This behaviour is a defence strategy to avoid predators (Maximino et al. 2010; Sandoval-Herrera et al. 2019; Tahmasebi et al. 2021; Shabgah et al. 2021). The present study showed a significant reduction on the total time that the fish spent in the light compartment of the dark/light apparatus in the fish groups exposed to the heavy metals. In line with the current study, Nabinger et al. (2018) and Tudor et al. (2019) revealed the exposure to nickel and arsenic induced anxiogenic-like behaviour making fishes spent more time in the dark portion.

It can be concluded that the exposure to heavy metals has a negative impact on the fish behavior. Further studies are essential to study the effect of heavy metals on genes associated with nervous system functions.

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