Physiological Changes in Nickel-exposed Nile Tilapia *Oreochromisniloticus* during Exposure and Recovery Periods

ABSTRACT

Heavy metals are a significant problem in aquatic ecosystems as they are toxic and tend to accumulate, immediately affecting fish physiology. The present investigation was carried out to evaluate the sub-lethality of Nickel chloride on fingerlings of Nile tilapia, *Oreochromis niloticus* on long-term exposure to it is below safe concentrations1/fifth (9.39 ppm) and 1/10th (4.69 ppm). The physiological studies revealed a significant reduction in Oxygen Consumption Rate (OCR), Ammonia-N Excretion Rate (AER), Oxygen Rate (O: R) and Food Consumption Rate (FCR) and behavioural changes during accumulation and depuration phases. Limited recovery was obtained in all the physiological parameters after depuration for 28 days from the end of the accumulation period.

Keywords: Nickel, *Oreochromis niloticus*, Physiology, Exposure, Recovery

1. INTRODUCTION

The integrity of the aquatic environment has been threatened severely by a broad spectrum of contaminants such as metals, pesticides, chemicals, and industrial discharge (Vinodhini & Narayanan, 2008) [1]. Given their capacity for bioaccumulation and potential toxicity, metallic contaminants are a focal point of concern within aquatic environments (Censi *et al*., 2006) [2]. Heavy metals are discharged into the environment through various sources, including domestic, industrial, and other anthropogenic activitiesthat may contaminate the natural aquatic system extensively (Velez & Montoro, 1998) [3]. Toxic effects of metals on organisms through accumulation in different organs, i.e. gill, liver, kidney, muscle, spleen, and brain (MacFarlane & Burchett, 2000) [4]. Nickel is the dominant chemical species pollutant that occurs in natural waters (US, EPA, 1980 [5]; 1986 [6]; Eisler, 1998) [7]. It does not break down in the environment and accumulates after exposure to low levels in aquatic organisms. Due to the persistence and bioaccumulation of Nickel, its compounds produce immediate and prolonged harmful effects on aquatic biota(Vieira *et al*., 2009) [8]. Fish physiology is an integral part of aquatic toxicology, and pollutants at sub-lethal concentrations can be considered an exciting environmental variable to which a fish will physiologically respond (Allen Heath, 1995) [9]. The heavy metals alter the fish's physiological parameters by posing a negative impact (Viella *et al*., 1999) [10]. To understand the toxicity mechanism of toxicant, it is important to know how a toxicant can enter into the organism, how it interacts with target molecules and exerts its effects and how the organism shows physiological responses, including behavioural changes, oxygen consumption rate, ammonia-N excretion rate and feeding activity with exposure to toxicant which gives a better understanding of fundamental physiological processes (Sheehan, 1984) [11]. Hence, efforts were undertaken tounderstand the knowledge of physiological changes in *O. niloticus* exposed to sub-lethal concentrations of Nickel chloride.

2. MATERIALS AND METHODS

A lethal toxicity study was carried out by following the standard guidelines (APHA, 2005)[12] to determine the lethal (LC_{50}) level of Nickel Chloride using the static bioassay method. Merck grade Nickel chloride hexa hydrate ($NiCl_{2.}$ $6H_2O$) is a toxicant. Uniform sizes of fish fingerlings of Nile tilapia, *Oreochromisniloticus*with length and weight range 8.5 to 10 cm and 9.5 to 12g were used to assess the lethal concentration of the toxicant. Water quality parameters were analysed following themethods mentioned in APHA (2005)[12] and found asfollows: water temperature: 24±2°C, pH: 7.1±0.2 at 24°C,dissolved oxygen: 9.3±0.8 mg/L, carbon dioxide: 6.3±0.4mg/L, total hardness: 23.4±3.4 mg as $CaCO_3$/L, phosphate: 0.39±0.002 µg/L and salinity: nil.All the tests were performed in triplicates and appropriate controls with 6 No. of fish in each replicate for 96 hours, and mortality was recorded atintervals every six hours.The LC_{50} valueswere estimated by the probit analysis method (Finney, 1971) [13].

Physiological study

Physiological variablessuch as oxygen consumption rate, ammonia-N excretion rate, Oxygen: Nitrogen (O: N) ratio, food consumption rate, and behavioural changes were studied. During the study period, the water quality parameters such as temperature, pH, DO, Alkalinity, hardness and nutrients (Ammonia and nitrite) were assessed following standardised procedures (APHA, 2005)[12].

Physiological parameters such as oxygen consumption by fish were determined by Zhen*et al.* (2010) method. The ammonia excretion rate was estimated every 24 hours by the phenol hypochlorite method (Solorzano, 1969)[14]. Absorbance was measured spectrophotometrically at 640 nm. The Oxygen: Nitrogen ratios were calculated from the oxygen consumption and ammonia excretion rates in *O. niloticus*. The Oxygen to Nitrogen ratio was determined by comparing the oxygen consumed to the nitrogen excreted within the specified interval (Bayne et al., 1958)[15]. The fingerlings were fed daily with prepared pelleted feed to determine the food consumption rate. The leftover feed was siphoned out after 60 minutes. The collected feed was dried overnight at 600C in a hot air oven and weighed to compare mean food consumption as per the methods of Broeck*et al*. (1997) [16].

Statistical analysis

The LC50 value was estimated using the probit analysis method (Finney, 1971)[13]. Physiological changes were tested using one-way ANOVA (analysis of variance). Post hoc tests were carried out using Duncan's multiple-range test comparison procedure. All the statistical analyses were performed via the SPSS 20.0 version.

3. RESULTS AND DISCUSSION

Oxygen consumption rate

The changes in the oxygen consumption rate are a good index of the metabolic capacity of an organism to experience environmentalstress (Jadhav et al., 2006)[17]. It is apparent from theresult that Nickel chloride influences oxygen consumption in fish. The normal respiratory metabolism can be altered because of its proximity to contaminated water, which decreases the oxygen-diffusing capacity of fish gills (Khoshnood, Z.

2017)[18].During the current study, Nickel chloride significantly reduced the oxygen consumption rate of fingerlings of O. niloticus under sub-lethal concentrations (1/10th and 1/5th of LC50). It showed a gradual increase trend during the depuration period (Fig.1).

A decrease in oxygen consumption in fingerlings of *Tilapia mossambica* on exposure to cadmium (Usharani & Ramamurthi, 1987)[19]**.** Their results indicate that the gills were the main target of pollutants and damaged the gills (AL-Yakoob *et al*., 1994)[20]. Also, Padmanabha *et al*. (2015)[21] observed a significant reduction in the rate of oxygen consumption in fish exposed to sub-lethal concentrations of cadmium (0.0022 ppm). Also, studies by Grinwis *et al*. (1998)[22] and Hartl*et al*. (2001)[23] have revealed that a decrease in the respiratory rate in sub-lethal concentrations was due to toxicant-induced stress on gills or membrane functions in the fish.

Ammonia-N excretion rate

The ammonia-n excretion rate indicates nitrogen balance and elucidates the influence of environmental and nutritional factors on protein metabolism in fish (Patricia & Land, 1998)[24]. In freshwater fish, most nitrogenous end products stem from protein catabolism, with ammonia-N being a primary end product. The assessment of ammonia excretion contributes to determining the total energy production of the fish, reflecting the extent of protein catabolism (Deboeck et al., 1997)[25]. In this investigation, ammonia- N excretion of *O. niloticus* decreased significantly during the accumulation period when subjected to one-tenth and one-fifth concentration of nickel chloride over 28 days, and the fingerlings of *O. niloticus* showed a gradual increase in the ammonia-N excretion rate when exposed to clean water (Fig.2). Parveen and Javed (2010)[26] observed an increase in ammonia excretion rate –N when *Catlacatla* was exposed to water-borne Copper for 90 days. In addition, Jisha *et al*. (2013)[27] recorded a significant increase in the ammonia quotient in Cadmium chloride-exposed tilapia, *O. mossambicus*, over seven days. Similarly, Padmanabha *et al*. (2015)[21] observed a significant decrease in ammonia excretion in the fingerlings of *O. mossambicus* subjected to a sub-lethal dose of Cadmium (0.0022 ppm). Further, they determined that the reduction of the ammonia-N excretion rate in both the sub-lethal concentrations was due to toxicants-induced stress, fish avoidance and the biotransformation process. Grosell *et al.* (2004)[28] recommended decreasing the ammonia excretion rate due to metal-induced stress on the excretory system in freshwater fish exposed to metal.

Oxygen: Nitrogen ratio

Oxygen consumption by aquatic animals is a sensitive physiological process. Therefore, alteration in respiratory activity is measured as an indicator of the stress of animals exposed to heavy metals (Jadhav *et al*., 2011)[29]. At the same time, ammonia excretion rates could indicate a higher reliance on proteins in stressed animals (Bayne *et al*., 1958) [15]. Pillai and Diwan (2002) [30] highlighted that the ratios of oxygen consumption to ammonia-N excretion in atomic equivalents offer insights into alterations in energy substrate utilisation across different environmental conditions.

It is apparent from the results that during In this investigation, the nickel chloride has affected the Oxygen: Nitrogen ratio in fingerlings of Nile tilapia (*O. niloticus)* under sub-lethal concentrations by showing gradually decreased values in the O: N ratio compared to the control group gradually increased values during accumulation and depuration phases respectively (Fig.3). Padmanabha *et al*. (2015)[21] observed a significant increase in Oxygen: Nitrogen ratio in fingerling of *O. mossambicus* subjectedto sub-lethal concentration of cadmium (0.0022 ppm) and Inhibition of oxygen consumption and increase ammonium excretion by Cd has been reported in *Litopenaeus vannamei* (Xuan et al., 2013) [31] because it diminze the efficiency of gaseous exchange.

Studies during the present investigation revealed that increased sub-lethal concentration of nickel chloride resulted in decreased respiration and decreased excretion in *Oreochromis niloticus*. Consequently, O: N ratios decreased with increased concentration of nickel chloride.

Food consumption rate

Changes in feeding behaviour are considered a sensitive indicator for detecting pollution due to metal nickel chloride. The results revealed that food consumption decreased significantly in the fishes exposed to nickel chloride. In the depuration period, it gradually increased (Fig.4).

The sub-lethal concentration of copper in water (0, 0.15, 0.3 and 0.5 ppm.) on the feed consumption of *O. niloticus*was studied (Ali *et al.*, 2003) [32]. Fish refused to accept the feed immediately after exposure and began taking it up after 4 hours compared with the control. The fish exhibited significantly reduced feed consumption when exposed to varying concentrations of copper in water compared to the control group.Further, they recommended that the lowered food intake of fish could bethe effect of copper on the central nervous system. Similarly, the fingerlings of Nile tilapia (*O. niloticus*) exposed to nickel chloride consumed less food than the control group. Thus, heavy metals might disturb the development of locomotor abilities that might severely affect the feeding ability of fish.

The sub-lethal effect of concentrations of cadmium (1/10th and 1/5th, i.e. 17ppm, 34 ppm) significantly decreased the food consumption rate(feed consumption body wt.) in *O. mossambicus* over 48 hours (Padmanabha*et al*., 2015)[21].Reduction in feeding behaviour under toxic environmental conditions might lower the energy costs of digestion (Halappa and David, 2009) [33]. The effects of three different sub-lethal concentrations (0.20 µM, 0.55 µM and 0.80 µM) of copper on food consumption in juveniles of common carp, C. carpio for 28 days was studied (Broeck et al., 1997)[16] and noticed a decrease in food consumption rate compared to control. The decreased food intake may be attributed to damage caused to taste receptors, as reported by Gerking, S. D. (2014) [34]. In this context, Heath (1987)[35]thinks that the fish subjected to long-term exposure to pollutants exhibited a reduction in appetite.

Behavioural changes

Physiological changes are vital attributes that indicate the health condition of organisms. All behavioural modifications are observed as aquatic organisms serve as sensitive indicators of stress induced by environmental chemicals. Behavioural changessuch as erratic swimming, restlessness and surfacing phenomena, and mucous secretion are attributed to fish and may protect against ecological contaminants (Sharma, M. 2019[36], Svecevièus. G.) [37].

Throughout this study, the control group of fish exhibited natural behaviour, characterised by active feeding and heightened awareness of minor disturbances, demonstrated throughcoordinated movements.Nile tilapia (O. niloticus) exhibited disrupted shoaling behaviour in toxic media, localisationto the experimental tank's lower section and swimming independence. The described symptoms were accompanied by a loss of coordination among individuals and an increase in the occupied area of the control group, with sub-lethal concentrations indicating the early effects of nickel exposure in both.

Khunyakari *et al*., 2001[38] studied the conduct of fishes exposed to nickel, copper and zinc in *Poecilia reticulate*. They suggested that heavy metal exposure caused mucus-like discharge on gills, heightened excretion, anorexia, and escalated fin activity. A similar type of behavioural observation was recorded by Yaji *et al.* (2011) [39] in *Orechromis niloticus* treated with Cypermethrin. Janardhana Reddy and Reddy (2013)[40] noticed similar behavioural anomalies in the Cadmium exposed to *Catlacatla*, showing respiratory rate impairment,skin irritation, and coughing provoked by the toxicants. The air gulping may help avoid contact with a toxic medium, and the surfacing phenomenon might demandelevated oxygen concentrations throughout the exposure period (Katja Schmidt *et al*., 2005)[41]. Loss of fish balance might be due to neurological impairment in the central nervous system, as evidenced by Cadmium's inhibition of the Acetylcholine enzyme (Patro,2006) [42].

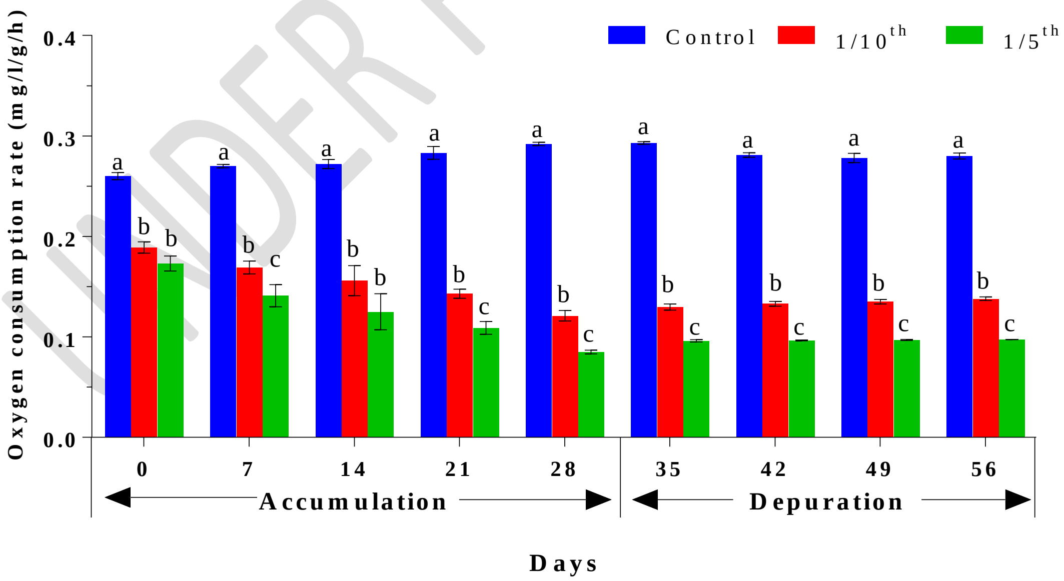

Fig. 1. Variation of the oxygen consumption rate (mg/l/g/h) in *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations of Nickel chloride.

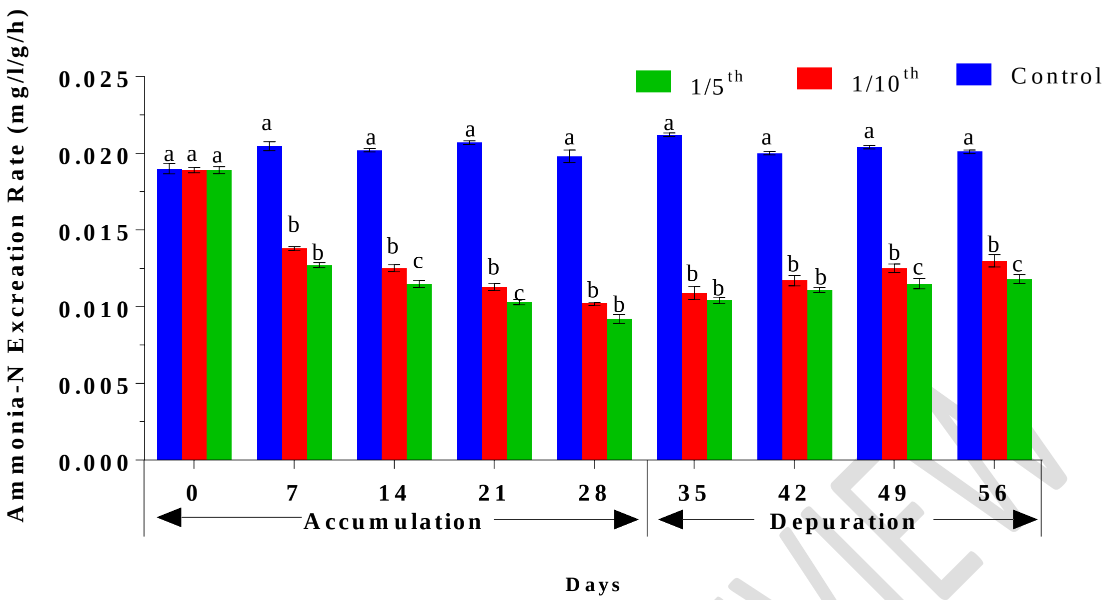

Fig.2. Variation of the rate of ammonia-N excretion (mg/l/g/h) in *Oreochromisniloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations of Nickelchloride.

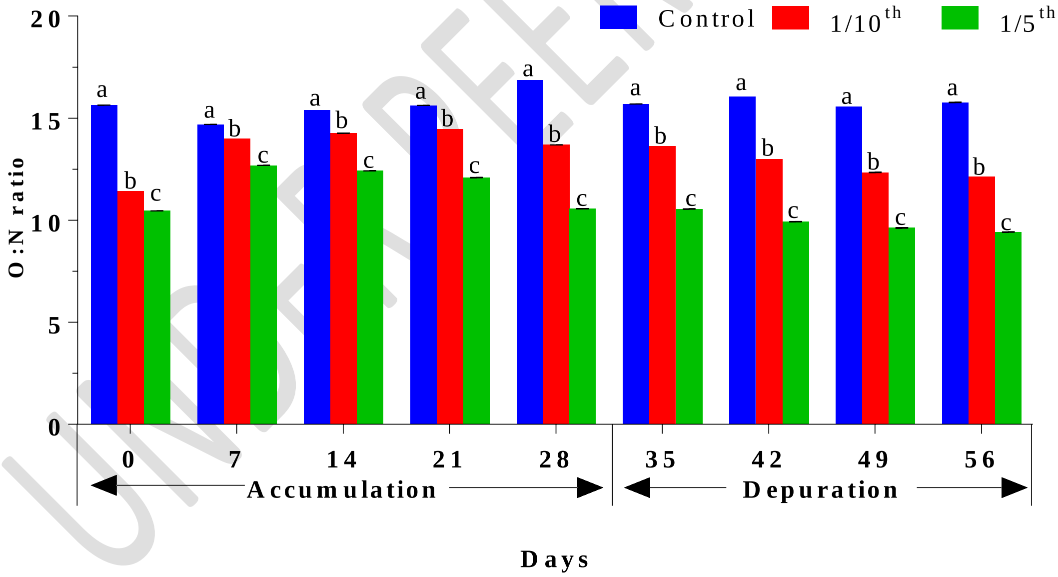

Fig. 3. Variation of the Oxygen: Nitrogen ratio rate in *Oreochromisniloticus* duringaccumulation and depuration phase on exposure to different sub-lethal concentrations of Nickelchloride.

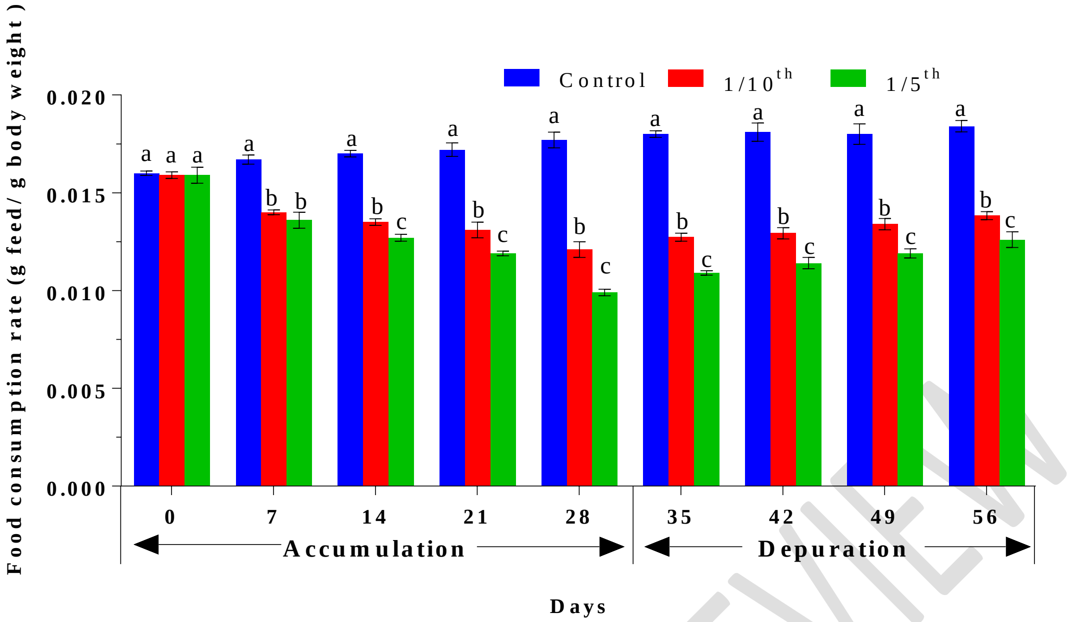

Fig. 4. Variation of the rate of Food consumption rate (g feed/ g body weight) in *Oreochromisniloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations ofNickel chloride

4. CONCLUSION

Exposure to nickel chloride significantly affects various physiological parameters, including oxygen consumption, ammonia excretion, oxygen-nitrogen ratio, food consumption rate, and behavioural patterns, in Nile tilapia fingerlings. These findings highlight the detrimental effects of heavy metal pollution on fish health and behaviour, underscoring the importance of environmental monitoring and conservation efforts to mitigate such impacts on aquatic ecosystems.

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