Bioaccumulation and Depuration Dynamics of Nickel Chloride in Nile Tilapia (*Oreochromis niloticus*) Exposed to Sub-lethal Concentrations

ABSTRACT

The current objective of this research is to examine the bioaccumulation and subsequentdepuration levels of nickel chloride within vital organs such as the gills, liver, and kidney of *Oreochromis niloticus* under controlled laboratory conditions. The fish were subjected to exposure under two sub-lethal concentrations, *i.e.,* 1/5th (9.39 ppm) and 1/10th (4.69 ppm), for 28 days of absorption and subsequently transferred to uncontaminated, good aerated water for 28days of elimination (depuration). Following 28 days of exposure to lower sub-lethal and higher sub-lethal nickel chloride concentrations, the sequence of bioaccumulation of nickel chloride in organs was observed as kidney > liver > gills. The depuration trend for higher and lower concentrations was gills> liver > kidney. The kidney exhibited the highest accumulation of Ni.Meanwhile, the Ni depuration through in the gills was significantly ($p<0.05$) more when compared to other routes following exposure to both concentrations.

*Keywords: {*Bioaccumulation, depuration, nickel, *Oreochromis niloticus }*

1. INTRODUCTION

In aquatic systems, contamination by various pollutants has raised global concerns in recent decades[1]. Heavy metals, in particular, pose significant threats to organisms attributable to their deleterious impacts[2]. Increased population density, industrialisation, and agricultural practices have led to substantial waste discharge into freshwater bodies, exacerbating the issue[3]. Metals, among other pollutants, are especially worrisome due to their ability to accumulate in aquatic environments and cause harm [4, 5].

Heavy metals infiltrate aquatic ecosystems, causing stress symptoms in fish [6]. As top predators in aquatic food chains, fish can accumulate substantial quantities of certain metals[6]. They serve as vital bioindicators of environmental pollution, offering insights into contamination risks from agricultural activities, either directly through surface run-off or indirectly through the food chain [7]. By absorbing metals from water, fish offer a dependable gauge of metal pollution levels in aquatic environments [8].

The build-up of heavy metals in the tissues of organisms can result in long-term health issues and potentially threaten populations [9]. Fish gills are the primary route for toxicants to enter fish, making them sensitive indicators of environmental pollutant effects on fish[10]. The liver, crucial for primary metabolism, plays a pivotal role in accumulating, transforming, and excreting environmental contaminants or xenobiotics [10].

2. MATERIAL AND METHODS

Prior to the commencement of the experiment, the live fish were transferred to the laboratory and acclimatized for one week. The lethal toxicity study followed the standard method (APHA, 2005)[11] using the static bioassay method. Merck grade Nickel chloride hexa hydrate ($NiCl_2$. $6H_2O$)

was used as the toxicant to assess the toxicity and sub-lethal effects on the accumulation and recovery changes in fingerlings of Nile tilapia, *Oreochromis niloticus* length 8.5-10 cm, weight 9.5-12 g). No food was provided to the fish throughout the experiment. Water quality parameters, including temperature and pH, were assessed utilising a mercury thermometer for temperature measurement and a pH meter for pH determination. The standard methods were followed for the estimation of dissolved oxygen (Winkler's method), alkalinity and hardness of water were estimated by following standard method (EDTA method) and ammonia and nitrate were carried out by following standard methods (APHA, 2005)[11]. The concentration of $NiCl_2$, which resulted in a 96-hour LC50, was determined to be 46.98 mg/L for *Oreochromis niloticus,* as calculated using probit analysis.

Sub-lethal studies were carried out by selecting the one-fifth (1/5th) and one-tenth (1/10th) of the acute toxicity values (LC50) obtained during the present[12]. In the laboratory setting, the bioaccumulation and Ni's depuration in different Oreochromis niloticus tissues were observed over 56 days. The experimental fish were individually subjected to the specified sub-lethal concentrations for 28 days, representing the bioaccumulation period. The surviving fish were relocated to fresh aerated water for 28 days following this period.

Experimental fishes were sampled from both treatment and control groups at every seven-day interval. The fish specimens obtained were sacrificed by dissection of the gills, liver, and kidneys. The fish tissue samples were desiccated in a laboratory oven at 65^{o}C for a constant dry weight of 0.5 g. A tissue sample (0.5 g) was taken, and two drops of Q - Q-milli water were added; then, it was subjected to heating on a sand bath at about 200oC until the sample fumed. Subsequently, 5.0 ml of HNO3 (70%) was introduced and left to react for 20 minutes. Afterwards, 10 ml of Hydrofluoric acid (40%) and 10 ml of Perchloric acid (70%) were introduced, and the mixture was heated until it formed a dried residue. Afterwards, 10 ml of 6N HCl was incorporated and warmed for 15 minutes, followed by milli-Q water; the volume was up to 50 ml. Subsequently, the solution was stored in a bottle resistant to acid. Filtered samples were then poured into auto-analyzer cups, and the heavy metal (nickel) concentration in each was determined employing an Atomic Absorption Spectrophotometer (AAS; Thermo Scientific iCE 3000 series).

The data acquired from the sub-lethal investigation of heavy metal, encompassing both accumulation and depuration phases, underwent One-way analysis of variance (ANOVA), followed by post hoc Duncan's Multiple Range Test utilising the SPSS software package (version 20) (USA). Significance was declared for differences between the two values when $p < 0.05$.

3. RESULTS AND DISCUSSION

The accumulation of metal is a function of uptake and excretion in fish. Uptake is considered passive and involves diffusion gradients created by adsorption or binding the metal to the tissue and cell surface[13].

The fingerlings of *O. niloticus* were subjected to two sub-lethal concentrations of 1/10th of LC50 (4.69 ppm) and 1/5th of LC50 (9.39 ppm) during the accumulation and subsequent depuration phases, respectively. Ni accumulation in the kidney was significantly increased with the exposure duration and concentration for 28 days *(P*<0.05*)*. After 28 days of exposure time, Ni accumulation values were0.0650±0.0017 to 0.0470±0.0012, 231.1180±0.5753 to 218.3440±0.4276 and

270.9600±0.5724 to 270.9600±0.5724 in control, 1/10th of LC50 and 1/5th of LC50 respectively (Fig 1). Ni accumulation was significantly increased for the liver with the exposure period and concentration for 28 days *(P*<0.05*)*. After 28 days of Ni exposure, Zn accumulation range varied from 0.0620±0.0012 to 0.0430±0.0014, 25.6210±0.7650 to 38.5210±0.3432 and 15.3700±0.3978 to 20.2160±0.5791 in control, 1/10th of LC50 (4.69 ppm) and 1/5th of LC50 (9.39 ppm) respectively in Fig 2. Throughout the 28 days, there were significant fluctuations in Ni accumulation in the gill (P <0.05).Top of Form

After 28 days of treatment, the Ni concentration range varied from 0.054±0.0006 to 0.059±0.0010, 23.541±0.5774 to 25.921±0.5927 and 27.762±0.3352 to 25.712±0.7635 in control, 1/10th of LC50 (4.69 ppm) and 1/5th of LC50 (9.39 ppm) respectively in Fig 3. The Ni accumulation sequence for lower and higher sub-lethal Ni concentrations is kidney > liver > gill.

Following 28 days of depuration, the gills exhibited the highest reduction in Ni levels. Gills showed a decreasing trend in the Nickel concentration rate, and the range varied from 0.0500±0.0015 to 0.0200±0.0015, 15.9520±0.5995 to 2.3100±0.4140, 18.2130±0.5052 to 4.2100±0.5254 in control, 1/10th of LC50 and 1/5th of LC50 respectively in Fig 3. After the gills, the liver exhibited the highest reduction in Ni levels, ranging from 0.0600±0.0012 to 0.0430±0.0015, 21.3260±0.3988 to 3.7460±0.4397 and 16.3200±0.4415 to 2.8160±0.6072 in control, 1/10th of LC50 and 1/5th of LC50respectively in Fig 2. Following the cessation of Ni exposure, the kidney showed the least reduction in Ni levels, which ranged from 0.0600±0.0012 to 0.0430±0.0015, 21.3260±0.3988 to 3.7460±0.4397 and 16.3200±0.4415 to 2.8160±0.6072 in control, 1/10th of LC50 and 1/5th of LC50 respectively in Fig 1. The findings indicate that for both lower and higher sub-lethal concentrations of Ni, Ni's depuration patterns follow the gill > liver > kidney sequence.

Various external and internal factors influence the accumulation of trace metals in fish tissues. External factors encompass environmental conditions like metal availability, water temperature, and alkalinity. Conversely, internal factors involve species-specific traits, age, size, physiological condition, and feeding habits of the fish[14].

Bioconcentration factor of nickel in gills ranged from 5.754 to 11.125, and 3.855 to 2.784 and 3.8553 to 2.7842 was noted in 1/10th of LC50 and 1/5th of LC50, respectively. The bioconcentration factor of the nickel in the liver ranged from 3.757 to 8.676, and 3.558 to 4.171 was notedin 1/10th of LC50 and 1/5th of LC50, respectively. The bioconcentration factor of Ni in the kidney ranged from 56.494 to 93.709, and 37.628 to 29.474 was notedin 1/10th of LC50 and 1/5th of LC50, respectively, in Table 1. A significant difference was notedin the current investigation between the control and treatment groups. The bioconcentration factor showed an increase in the lower concentration compared to the higher concentration. Christine et al. (1997)[15] made a similar kind of observation and reported increased metal concentrations in fish. Levels of exposure influence the cadmium uptake and elimination in the liver and kidney of carp *Cyprinus carpio*. The cadmium distribution decreased in the order kidney > liver >gills.

The kidney can play an essential role in maintaining osmotic homeostasis. Additionally, renal tissues experience substantial blood flow and play a crucial role in excreting metabolites of diverse xenobiotics from the body, as they are directly exposed to toxic substances. During the present study,

the accumulation of nickel chloride in kidney of *O. niloticus* increased significantly during the accumulation period when fishes were exposed to 1/10th and 1/5th concentrations of nickel chloride over 28 days. The kidney emerged as the primary organ for detoxification and depuration of heavy metals due to its higher concentration in most aquatic organisms.

Tulasi *et al*. (1987). [16] also reported a high metal concentration in the kidney in *Barytelphusa guerini* in other organs. Since the kidney is the principal organ involved in metal storage, the highest concentration of the accumulation is observed during present experimental treatments. It is documented that the kidney exhibits a high capacity for metal accumulation. The proportional increase in copper accumulation in the kidney correlates with the duration of exposure across various size groups. The kidney showed a selective re-absorption of essential electrolytes, glucose, and essential metals such as copper from urine at the proximal.

The liver is the prime organ for removing xenobiotics tubules[17] and biocides in fish[18]. The liver was examined due to its pivotal role in metabolising and eliminating xenobiotic compounds, often exhibiting morphological alterations during toxic conditions[19].

The liver plays a crucial role in accumulating and detoxifying heavy metals[20]. Fish exposed to heightened concentrations of heavy metals trigger the production of metallothionein protein (MT). Metallothionein protein has a higher affinity for heavy metals and is found to concentrate and regulate the metals in the liver[21]. Studies have confirmed that MT binds and detoxifies the metal ion[22]. During the current investigation, the accumulation of the nickel chloride in the liver of *O.niloticus*was significantly increased during the accumulation period when fish were exposed to 1/10th and 1/5th concentrations over 28 days. Further, it was noted that the liver is the primary site for metal binding reflexes and plays a multifaceted role; the liver serves in detoxification processes. This may be attributed to the increased synthesis of metallothionein and its storage as a constituent of liver cytoplasm, resulting in higher metal accumulation in the liver.

Gills are commonly acknowledged as effective water quality indicators and are frequently utilised in environmental impact assessments[23] as gills come into contact with the environment. During the present investigation, nickel accumulation in the *O. niloticus* gills increased significantly during the accumulation period when exposed to sub-lethal concentrations of 1/10th and 1/5th of nickel chloride for 28 days. It is also known that gills are the primary organ and important sites for entering heavy metals, and gill surfaces are the first target of water-born metals.

The gills are primary organs for encountering environmental pollutants, making them vulnerable sites for entering heavy metals, leading to lesions and damage [1]. Hence, the metal concentrations observed in the gills mirror those found in the surrounding waters[24]; according to Jackim et al., 1973[25], gills serve as the primary site for nickel accumulation in M. gulio fish. This finding underscores the significance of gills as the primary entry point, maintaining consistent and direct exposure to the aquatic milieu, resulting in the fish's heightened uptake of trace metals.

The tilapia fingerlings (*O. niloticus*) present study transferred to nickel chloride-free freshwater during the depuration period. The nickel chloride concentration in the gills showed a rapid decrease, and the highest depuration rate observed in gills might be favoured due to their extensive exposure to the aquatic environment. Thus, when subjected to well-aerated water, it could excrete heavy metal

(nickel). The rapid excretion of nickel in the gill, when compared with other organs, may be because gills persist in the aquatic environment. They can excrete nickel while subjected to cleaner water conditions. Karuppasamy (2007) [26] noted that the gill is the primary organ for rapid arsenic elimination, rather than the liver and muscle tissues of fish. In conclusion, the accumulation and subsequent depuration of nickel in *Danio rerio* depends on the organ, concentration, and exposure time.

The liver exhibited a rapid decrease in nickel chloride concentration following the gills; it demonstrated the highest purification level for lower and higher sub-lethal concentrations. The liver is a more efficient organ than the kidney in eliminating nickel content, following the gill. Therefore, the liver plays a crucial role as a metal storage and detoxification organ in fish.

During the depuration period, in the kidney, the nickel chloride concentration of fingerlings of tilapia, *O. niloticus,* transferred to nickel chloride-free fresh water was observed. The decrease was linked to the osmotic flow of water through the gills. Thus, more material might be excreted via the kidney in freshwater fish.

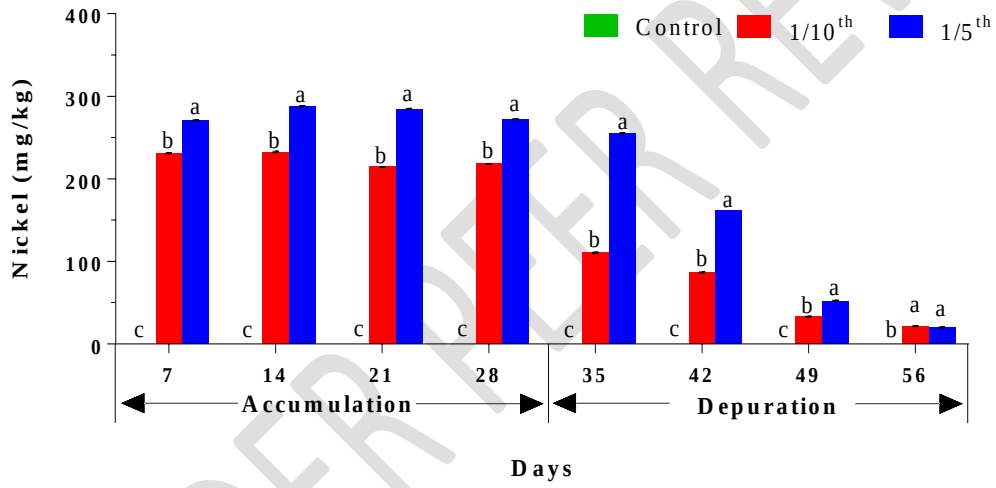

Fig 1. Nickel chloride concentration in kidney tissue of *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations

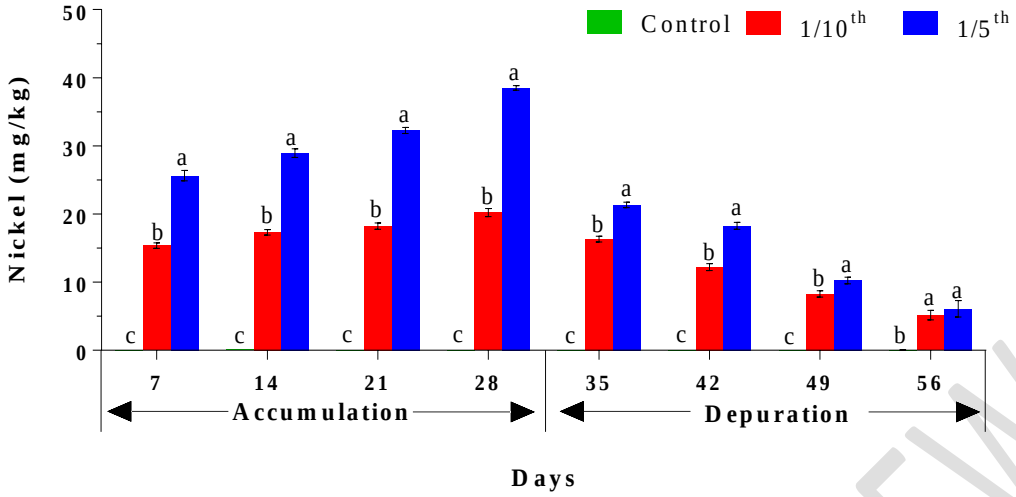

Fig 2. Nickel chloride concentration in liver tissue of *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations.

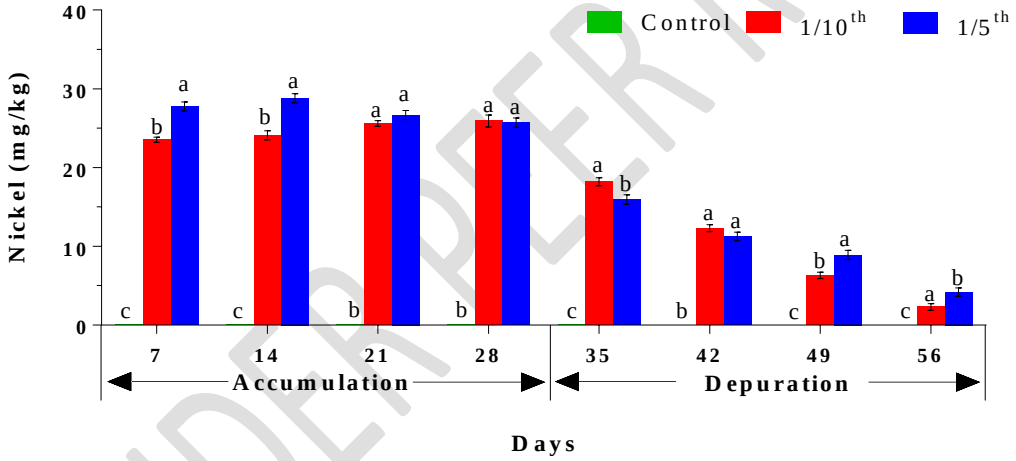

Fig 3. Nickel chloride concentration in gill tissue of *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations

Table 1. Bioconcentration factor of nickel chloride (mg/kg) in the gills of tilapia fingerlings exposed to different treatments under sub-lethal studies

Conc .(ppm)	**7**	**14**	**21**	**28**
Control	1.7475	1.7460	1.2277	1.5603

1/10th of LC_{50}	5.7543	7.0451	8.5880	11.1247
1/5th of LC_{50}	3.8553	3.5038	2.8900	2.7842

Table 2. Bioconcentration factor of nickel chloride (mg/kg) in the liver of tilapia fingerlings exposed to different treatments under sub-lethal studies

Conc .(ppm)	**7**	**14**	**21**	**28**
Control	2.007	2.717	1.734	1.639
1/10th of LC_{50}	3.757	5.063	6.116	8.676
1/5th of LC_{50}	3.558	3.518	3.497	4.171

Table 3. Bioconcentration factor of nickel chloride (mg/kg) in the kidney of tilapia fingerlings exposed to different treatments under sub-lethal studies

Conc.(ppm)	**7**	**14**	**21**	**28**
Control	2.098	2.402	2.492	1.243
1/10th of LC_{50}	56.494	68.002	71.935	93.709
1/5th of LC_{50}	37.628	35.051	30.873	29.474

4. CONCLUSION

The study highlights the significant impact of long-term exposure to sub-lethal concentrations of Nickel chloride on tilapia fingerlings, Oreochromis niloticus, evident through Ni bioaccumulation and depuration dynamics in their tissues over time and concentration gradients. Notably, the kidney exhibited a notable accumulation of Ni in Oreochromis niloticus. Additionally, distinct accumulation and depuration patterns of Ni were observed across various tissues. In conclusion, the kidney emerges as a critical site for nickel accumulation, while the gill is the primary organ for depuration in *Oreochromis niloticus*.

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