International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 20-09-2019; Accepted: 23-10-2019
www.entomologyjournals.com
Volume 4; Issue 6; November 2019; Page No. 118-121

Nickel Chloride Induced alteration in acid and alkaline phosphatases in liver and kidney of the freshwater fish *Labeo rohita*

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Abstract
Quantitative assessment of enzymes is reliable to understand the stress imposed on the organism by environmental pollutants. In the present investigation, the effect of sublethal concentration of nickel chloride on acid and alkaline phosphatases in liver and kidney of the fish *Labeo rohita* was studied the result showed in general, a significant ($P < 0.05$) reduction in the activity of both the enzymes in liver and kidney, but more pronounced in kidney than liver. The alteration in enzymatic changes indicated that nickel chloride is very hazardous to the fish.

Keywords: nickel chloride, *Labeo rohita*, enzymes, pollutant

Introduction
Most of the pollutants produced by industrial, agricultural, commercial and domestic waste end up into oceans, rivers, lakes and wetlands, threatening the development and survival of aquatic organisms because of their toxic and non-biodegradable properties. (Bosch *et al.*, 2016) [8]. One of the most important characteristics of toxic pollutants such as heavy metal is that they can be accumulated in organs of the organisms (Palaniappan and Karthikeyan, 2009). Heavy metals are high density elements occurring naturally in the environment in a minute quantity (Lu *et al.*, 2017) [16]. Heavy metals have been shown to affect developmental, reproductive, hepatic, hematological and immunological functions of fish (Tarasco *et al*., 2019) [32]. Fish have been largely used in evolution of the quality of aquatic systems because aquatic organisms are often at the top of the aquatic food chain and may concentrate large amount of metals from the surrounding water (Rajkowska and Protasowicki, 2011) [25]. The accumulation of metals in aquatic systems suggests that fish may sever as a useful indicator of contaminating metals in aquatic systems, since they respond with great sensitivity to changes in the aquatic environment (Mansouri *et al.* 2011) [18]. Nickel emisions may stem from power plants, waste incinerators and metal industries and is very hazardous pollutant because nickel and its compounds have acute and chronic toxicity to aquatic life (Atef, 2007) [2]. Even at sublethal concentrations, nickel chloride has a cumulative polluting effect and could cause serious disturbances on fish by altering or inhibiting the activities of several enzymes involved in metabolism. (Vinodhini and Narayanan, 2008) [34]. Impact of nickel chloride on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver and kidney of fish that respond specifically to the degree and type of contamination (Barhoumi *et al.* 2012) [5]. The liver of fish is succesptible to damage from a variety of toxicants and kidney clear pollutants from blood hence they are considered as indicator of aquatic environmental pollution (Soufy *et al.* 2007) [30]. The present study aimed to investigate the effect of sublethal concentrations of nickel chloride on the fresh water fish *Labeo rohita*. Physiological parameters such as acid phosphatase and alkaline phosphatase activities of liver and kidney are selected to evaluate the response of treated fishes to nickel intoxication.

Materials and Methods
Collection of fish
The fresh water fish, *Labeo rohita* were collected from the fish farm located in Puthur, Nagai District, 10 Kms away from Chidambaram. These fishes were brought to the laboratory and transferred to the rectangular tanks (100 x 150 cm) of 400 litres capacity containing chlorine free aerated well water. Fishes were acclimatized to the laboratory conditions in rectangular tanks with unchlorinated well water for 3 to 4 weeks at a room temperature of 28 ± 3 C. Water was changed in alternate days. Tanks were covered with fish netting to prevent the escape of fishes.

Estimation of LC_{50} values
The LC_{50} values.were determined by following the method of Litchfield and Wilcoxon (1949). 96 hours LC_{50} value obtained 32.64 ppm of nickel chloride. According to Sparage (1971), $1/10^{th}$ of the LC_{50} was taken as sublethal concentration. 100 percent survival observed upto 30 days was considered as sublethal concentration of nickel chloride 3.264 ppm (LC_O). In the present investigation, $1/15^{th}$ (2.176 ppm), $1/10^{th}$ (3.264 ppm) and $1/5^{th}$ (6.528 ppm) of the LC_{50} of nickel chloride of low, medium and high sublethal concentrations were selected respectively for this study.

Experimental design
The freshwater fish *Labeo rohita* with a size range of 12-14 cm and weighing 18-20g irrespective of their sex have been chosen as the experimental organisms in the present study. The experimental fishes were divided into four groups. The

fishes belonged to the first group were maintained in a medium free from nickel chloride was served as control. The second group exposed to 1/15th (2.176 ppm) of the LC_{50} of low sublethal concentration of nickel chloride for the period of 10, 20 and 30 days. The third group exposed to 1/10th (3.264 ppm) of the LC_{50} of medium sublethal concerntration of nickel chloride for the period of 10, 20 and 30 days. The fourth group exposed to 1/5th (6.528 ppm) of the LC_{50} of high sublethal concentration of nickel chloride for the period of 10, 20 and 30 days. The medium was renewed daily with sublethal concentration of the nickel chloride. At the end of experiment, the control and experimental fishes were sacrificed. The liver and kidney were removed from both control and treated fishes for enzymatic assays. Estimation of tissue alkaline phosphatase and acid phosphatase were done using the method of and the values expressed in mole of PNP/mg protein/hr.

Statistical analysis

The values are expressed as mean ± SD. Data were statistically analyzed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) (Duncan, 1957) [11] which was applied to find out significant difference between various treatment means and control means for the observed parameters.

Results and Discussion

The results of the acid phosphatase and alkaline phosphatase in liver and kidney of *Labeo rohita* exposed to low, medium and high sublethal concentrations of nickel chloride after the period of 10, 20 and 30 days exposure were presented in Table I and Table II. The activity of ACP and ALP were observed to be significantly decreased ($P<0.05$) in liver and kidney of *Labeo rohita* after 10, 20 and 30 days exposure periods, with a corresponding increase in the concentrations of nickel chloride. The most significant decrease was found in the 30 days exposure.

In toxicological studies, alkaline phosphatase (ALP) and acid phosphatase (ACP) are important biochemical enzymes to be used to detect the alteration of physiological metabolism of animal induced by metal exposure (Orue and Uner, 1999) [22]. The toxic effect of heavy metals on the enzyme system deponds on the toxicants to react with ligands (Bharathi *et al.,* 2014) [6]. The heavy metal may cause injury to the organism and the damaged tissue shall dysfunction which results in altered enzyme activity (Parthiban and Muniyan, 2011) [24]. Thus, enzyme bioassay can provide diagnostic tool to asses a change or damage caused to aquatic organism due to administration of heavy metal (Arul Jothi, 2013) [1].

Table 1: Alkaline phosphatase activity (µg inorganic phosphate liberated mg/hr) in liver and kidney of *Labeo rohita* exposed to sublethal concentration of nickel chloride.

Tissue	Treatment Groups	Exposure Periods		
		10 days	20 days	30 days
Liver	Control	0.560 ± 0.04 ^{c}	0.570 ± 0.04 ^{d}	0.580 ± 0.04 ^{d}
	Low concentration	0.540 ± 0.04 ^{c}	0.510 ± 0.04 ^{c}	0.440 ± 0.03 ^{c}
	Medium concentration	0.501 ± 0.03 ^{ab}	0.450 ± 0.03 ^{b}	0.350 ± 0.02 ^{b}
	High concentration	0.460 ± 0.03 ^{a}	0.360 ± 0.02 ^{a}	0.210 ± 0.01 ^{a}
Kidney	Control	0.450 ± 0.03 ^{d}	0.460 ± 0.03 ^{d}	0.450 ± 0.03 ^{d}
	Low concentration	0.410 ± 0.03 ^{c}	0.390 ± 0.03 ^{c}	0.360 ± 0.02 ^{c}
	Medium concentration	0.350 ± 0.02 ^{b}	0.300 ± 0.02 ^{b}	0.240 ± 0.01 ^{b}
	High concentration	0.270 ± 0.01 ^{a}	0.220 ± 0.01 ^{a}	0.130 ± 0.008 ^{a}

*All the values mean ± SD of six observations; Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$); Duncan multiple range test (DMRT).

Table 2: Acid phosphatase activity (µg inorganic phosphate liberated / mg / hr) in liver and kidney of *Labeo rohita* exposed to sublethal concentration of nickel chloride.

Treatments	10 days	20 days	30 days
Liver			
Control	0.502 ± 0.04 ^{d}	0.490 ± 0.03 ^{d}	0.510 ± 0.04^{d}
Low concentration	0.440 ± 0.03 ^{c}	0.400 ± 0.03 ^{c}	0.360 ± 0.02 ^{c}
Medium concentration	0.350 ± 0.02 ^{b}	0.270 ± 0.01 ^{b}	0.240 ± 0.01 ^{b}
High concentration	0.220 ± 0.01 ^{a}	0.180 ± 0.01 ^{a}	0.100 ± 0.008 ^{a}
Kidney			
Control	0.310 ± 0.02 ^{c}	0.320 ± 0.02 ^{d}	0.310 ± 0.02 ^{d}
Low concentration	0.300 ± 0.02 ^{c}	0.280 ± 0.02 ^{c}	0.240 ± 0.01 ^{c}
Medium concentration	0.270 ± 0.01 ^{b}	0.230 ± 0.01 ^{b}	0.130 ± 0.008 ^{b}
High concentration	0.220 ± 0.01 ^{a}	0.160 ± 0.008 ^{a}	0.040 ± 0.000 ^{a}

All the values mean ± SD of six observations. Values which are not sharing common superscript differ significantly at 5% ($p<0.05$), Duncan multiple range test (DMRT)

Alkaline phosphatose is a p - stress marker enzyme most effect in an alkaline environment that catalyzes the hydrolysis of phosphorus compounds from many types of molecules such as nucleotides, proteins and alkaloids and the transfer of phosphoryl groups to an acceptor molecule (Dyhrman and Palnik, 1999) [12]. ALP catalytic activity is inversely proportional to the concentration of inorganic phosphate in the amphient environment and this enzyme could serve as good indicator of intoxication because of its sensitivity to metabolic salts (Boge *et al.* 1992) [7]. Jiang *et al.,* (2012) [15] reported that ALP activity in liver and kidney increased at lower copper concentration but went down with increased copper concentration in fresh water fish Carassius auratus gibelio var. The activity of ALP was increased in liver of fish Cirrhina mrigala exposed to lead nitrate (Fernandes *et al.* 2016) [13]. Babu and Narendiran (2016) [3]

observed that the enhancement of ALP activity in liver and kidney of fish Cirrhina mrigala exposed to sublethal concerntrations of arsenic trioxide. Marr *et al.,,* (1995) [20] pointed out that a metal binding protein (Metallothionein) could be induced by heavy metals and it can attenuate cytotoxicity induced heavy metals and reducing their intracellular concentration in liver of fish. The increased ALP concentration in liver suggested that the hydrolysis of phosphate esters to release energy in view of the synthesis of Metallothionein and the enhancement of the detoxification function of liver. The elevated ALP activity in kidney it may be possible to facilitate transport and excretion of phosphate ions (Baby Shakila *et al.* 1993) [4]. In the present Study, *Labeo rohita* exposed to sub-lethal concentrations of nickel chloride for the periods of 10, 20 and 30 days showed decrease in the activity of alkaline phosphatase in liver and kidney. The present study results were supported by the findings of Babu and Jothi Narendiran (2016) [3]. They observed that reduction in the activity of ALP in both liver and kidney of fish Cirrhinus mrigala exposed to arsenic trioxide. Mariappan and Karupasamy (2014) [19] reported that inhibition of ALP activity in liver and kidney tissues of *Cyprinus carpio* exposed to the sub-lethal concentrations of copper and cadmium. Wani *et al.,* (2017) [35] reported that significant reduction in the activity of ALP in liver and kidney of *Labeo rohita* exposed to iron toxicity. In the present study, the activity of ALP was gradually depleted with a corresponding increase in the sublethal concentrations of nickel chloride suggested that more metal ions could enter the liver beyond the regulation capacity of metal binding protein by nickel-binding, the surplus metal ions (nickel) could direct bind to- SH groups of enzyme molecule and cause decrease of ALP. The present study results were supported by Sreekala and Zutshi (2010) [31]. They reported that the accumulation of toxicants beyond a tolerable level in the liver might causes reduction in ALP activity. Sharkoori *et al.* (1992) [26] have suggested the decrease of ALP activity in fish tissues might be taken as index of necrosis in hepatocytes and the detoxification capacity. The decreased activity of ALP reflects alteration in protein synthesis and uncoupling of oxidative phosphorylation (Baby Shakila *et al.* 1993) [4].

Acid phosphate is a phosphatase which frees attached phosphate groups from other molecules during digestion (Verma *et al.* 1984) [33]. Acid phosphatase (ACP) is a lysosomal, hydrolytic enxyme with an acid pH optimum and it takes part in the dissolution of dead cells which serve as a good indicator of stress condition in the biological system (Gupta *et al.* 1983) [14]. Fernandes *et al.,* (2016) [13] reported that ACP activity was increased in liver of Cirrhina mrigala exposed to increasing concentrations of heavy metal lead nitrate. Chaudhari (2017) [10] also reported that ACP activity was elevated in liver and kidney of Cirrhinus mrigala exposed to thermal power plant effluent. The increased activity of ACP was noticed in kidney of fish Gambusia affinis exposed to chlorphrifos toxicity. The elevated activity of ACP due to increase in protease activity which causes damage to the lysosomal enzyme into cytoplasm and the adverse effect of xenobiotics on the cell and its organells might be due to alteration in the enzyme activity (Bujjamma and Padmavathi, 2018) [9]. In the present study, *Labeo rohita* exposed to sub lethal concentrations of nickel chloride for the periods of 10, 20 and 30 days showed decrease in the activities of ACP in liver and kidney. The present study results were affirmative with the findings of Mohan Kumar *et al.,* (2017) [21] reported that a significant decrease in the activity of ACP in liver and kidney of Oreochromis mossambicus exposed to dye effluent. Mariappan and Karupasamy (2014) [19] observed that the inhibition of ALP activity in liver and kidney of fresh water fish *Cyprinus carpio* exposed to copper and cadmium. The results of Wani *et al.,* (2017) [35] showed significant alteration in the activity of ACP in liver and kidney of iron intoxicated fish and reduction was more pronounced in the liver. The results of present investigation showed significant reduction in the ACP due to oxidative stress, is a characteristic of tissue damage. This finding in agreement with various studies carried out on heavy metal toxicity on liver and kidney of fresh water fish (Sastry and Subhadra, 1985, Sastry and Gupta, 1979 and Sharma, 1999) [27, 28, 29]. The present study results showed that significant depletion of ACP activity was more pronounced in kidney than liver tissue at higher sublethal concentration of nickel chloride and long exposure period of metal treated groups. This results indicated that nickel toxicity induced more nephrotoxicity than hepatotoxicity in fish *Labeo rohita*. This result was more affirmative with the finding of Mariappan and Karupasamy (2014) [19] and disagreement with the findings of Wani *et al.* 2017 [35] and Mohan Kumar *et al.,* (2017) [21]. They observed reduction of ACP activity more remarkable in liver than kidney of intoxicated fishes.

Acknowledgement
The authors wish to thank the authorities of Annamalai University for providing the facilities to carry out the study.

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