Effects of Heavy Metal Nickel Chloride on Enzyme Succinate Dehydrogenase of an Air Breathing Fish *Channa Gachua*

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Abstract

Nickel (Ni) is the 24th most abundant element in the earth's crust comprising about 3% of the composition of the earth. It is the 5th most abundant element by weight after iron oxygen magnesium and silicon. It is a member of the transition series and belongs to group VIII B of the periodic table along with iron, cobalt, palladium, platinum and five other elements. Nickel is a naturally occurring element that can exist in various mineral forms. As a member of the transition metal series it is resistant to corrosion by air water and alkali but dissolves readily in dilute oxidizing acids. Natural nickel is a mixture of five stable isotopes and nineteen other unstable isotopes are known. Succinate dehydrogenase is the oxidative enzyme which was drastically affected by the action of heavy metals. Succinate dehydrogenase is chosen as a representative of metabolic enzyme. It is a marker enzyme for detecting the presence of TCA cycle in tissues. The aim of the present study was to assess the enzyme succinate dehydrogenase activities in gill liver kidney brain and muscle of the air breathing fish *Channa gachua* exposed to sub lethal concentration of nickel chloride $1/5^{th}$ (high) 1/10th (medium) and 1/15th (low) of the 96 hour of LC 50 values for the period of 15, 30 and 45 days. The fish exposed to nickel chloride showed the decrease of enzyme succinate dehydrogenase activities for 15, 30 and 45 days in gills liver kidney, brain and muscles. However no information is on record concerning the three different sub lethal concentration of heavy metal nickel chloride on the enzyme succinate dehydrogenase of the fish *Channa gachua*. The objective of the work was to observe the effect of nickel on succinate dehydrogenase activities in gills liver and kidney of an air breathing fish *Channa gachua*.

Keywords: Nickel chloride, succinate dehydrogenase, sub lethal conc. *Channa gachua*.

Introduction

Nickel is one of many trace metals widely distributed in the environment being released from both natural sources and anthropogenic activity with input from both stationary and

mobile sources. It is present in the air, water, soil and biological material. Environmental pollution due to toxic heavy metals in air soil and water is a major global problem. Heavy metals cannot be degraded or destroyed and hence they are persistent in all parts of the environment. The reduction amount of these metals from effluents to the permissible limit before discharging them into streams and rivers is very important for human health and environment (Srividya and Mohanty, 2009 Qaisur and Sadhu, 2011). Water pollution is thus cosmopolitan problem that needs urgent attention and prevention (Ali and Soltan, 1996; Handy, 1994). It resulted from many sources from different ways of chemical wastes discharge of industrial or sewerage effluents agricultural drainage domestic waste water and gasoline from fishery boots (Handy, 1994; Ali and Soltan, 1996). Water pollution is one of the principal environmental and public health problems (Osman and Kloas, 2010). The aquatic habitats are being contaminated with heavy metals due to industrialization and other anthropogenic activities (Muthupriya and Altaff, 2010). Aquatic animals inhabiting polluted water bodies tend to accumulate many chemicals in high concentrations even when the ambient environmental contamination levels are low potentially hazardous situation for the entire food chain. Among several elements of the periodic table there are 35 metals are associated with community and occupational exposure. Out of these 23 are described as heavy metals. These elements are generally released in small amounts into the environment by processes like weathering of rocks volcanic eruptions and their intake on exposure is necessary in trace amounts for good health. But presently there is steady increase in their concentration in all habitats owing to mining electroplating paints and dye battery making industries. The release is rapidly growing technology and heavy metal application in these industries (Sopha, *et al.,* 2007). The contamination of fresh waters with the wide range of pollutants has become matter of great concern over the last few decades (Al Weher, 2008). Heavy metals are natural trace components of the aquatic environment but their levels have increased due to domestic industrial mining and agricultural activities (Leland *et al.,* 1978; Mance, 1987; Kalay and Canli, 2000). Aquatic organisms such as fish and shell fish accumulate metals concentrations many times higher than present in water or sediments (Olaifa *et al.,* 2004, Gumgum *et al.,* 1994). Discharge of heavy metals into river or any aquatic environment can change both aquatic species diversity and ecosystems due to their toxicity and accumulative behavior (Heath, 1987). Nickel is very abundant element. In the environment it is found primarily combined with oxygen as oxides or sulfur as sulfides. Nickel has properties that make it very desirable for combining with other metals such as iron copper chromium and zinc to form alloys. These alloys have important uses such as in the making of metal coins and jewelry and in industry for making items such as valves and heat exchangers. Most nickel is used to make stainless steel (Javed and Abudullah, 2006). Nickel compounds are used for nickel plating to colour ceramics to make some batteries and as substances known as catalysts to increase the rate of chemical

reactions. Nickel is released into the atmosphere during nickel mining and by industries that convert scrap or new nickel into alloys and nickel compounds by industries that use nickel. These industries may also discharge nickel in waste water. However the major sources of nickel exposure are tobacco smoke, auto exhaust, fertilizers super phosphate, food processing hydrogenated fats, oils industrial waste, stainless steel, cook ware testing of nuclear devices, baking powder combustion of fuel oil, dental work and bridges. High exposure can cause cough shortness of breath and fluid in the lungs which is sometimes delayed for 1 to 2 days after exposure (Farombi, *et al.,* 2007). Single high and repeated lower exposures may damage the lungs with scarring of lung tissues and may cause damage to heart muscle liver and kidney (Al-Attar, 2007). Fish has been the main supply of cheap and healthy protein to the large percentage of the world's population. In most countries especially those in South East Asia fish is a main protein of the diet. It is particularly valuable for providing proteins of high quality comparable with those of good source of omega-3 fatty acids calcium and phosphorus iron as trace elements like copper and fair proportion of the B-vitamins. Beside good health benefits of fish there were many reports on contamination of fish by chemical in the environment. The fish as bio indicator species plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution the effects of exposure to sub lethal levels of pollutants can be measured in terms of biochemical physiological or histological responses of the fish organism (Mondon *et al.,* 2001). Changes in age and species distribution in a stock fish population are general indicators of water pollution but there are responses specific to single pollutant or group of contaminants. Bio chemical are induced in the presence of a specific group of contaminants that have the same mechanism of toxic activity (Iroka and Drastichova, 2004). Succinate dehydrogenase is the primary enzyme in the oxidative catabolism of sugars (Lehninger *et al.,* 1993) and as such is used effectively as a marker of mitochondrial abundance and activity to identify any possible physiological disturbance in fishes. Hence in the present investigation has been made to find out the effect of sub lethal concentration of nickel chloride on succinate dehydrogenase activities in gills liver kidney brain and muscle of air breathing fish *Channa gachua.*

Material and methods

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25º 59'N and Longitude 85º 22'E) and maintained in large glass aquaria size (90x60x60cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 20 days in the laboratory. Routine oxygen consumption from air and still water was measured in a closed glass respirometer containing 3 litres of water (initial O_2 content = 6.5 mg O_2/ litre; pH = 7.2) and 0.51 mL of

air. The fish were acclimated to the respirometers for at least 12 hours before the readings were taken. The experiments were conducted at 29.0 ± 1.5°C. The pH of the ambient water was measured by an electronic pH meter systronics. The respiratory chambers were thermostated by immersion in a temperature controlled water bath. Fishes from 90-100 grams were used during the experiment. They were checked thoroughly for injury and disease conditions and only healthy fishes were used for this study. After washing with 0.01% $KMnO_4$ solution for 15 min, they were placed in nine plastic pools (200 L) containing non chlorinated water. Prior to the start of the experiment the fishes were acclimatized to the food and laboratory conditions with 12 hours dark and 12 hours light cycles. The nickel chloride was used in this study and stock solutions were prepared. Nickel chloride LC 50 was found out for 96 hours 33.65 ppm (Sprague, 1971) and 1/5th high 1/10th medium and 1/15th low of the LC 50 values were 6.538, 3.364 and 2.276 ppm respectively taken as sub lethal concentrations for this study. Sixty fishes were selected and divided into 4 groups of 15 each. The first group was maintained in free from nickel chloride and served as the control. The other 3 groups were exposed to sub lethal concentration of nickel chloride in 20 liters capacity. The 2nd 3rd and 4th groups were exposed to nickel chloride for 15 30 and 45 days respectively. At the end of each exposure period the fishes were sacrificed and the required tissues were collected for succinate dehydrogenase activity estimation. Fishes were exposed to three sub lethal concentrations of Nickel chloride separately in plastic bags and control fishes were also maintained separately. The medium was renewed daily with sub lethal concentration of the Nickel chloride. The succinate dehydrogenase activities of the tissues were estimated by the method of Nachales *et al.,* (1960). The data were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

Results

Depletion of succinate dehydrogenase activities of the gills liver kidney brain and muscle of *Channa gachua* exposed to the nickel chloride for 15 30 and 45 days in 1/5th 1/10th and $1/15^{th}$ of the LC 50 values of sub lethal concentrations were estimated. Among these the maximum depletion of succinate dehydrogenase was observed in liver during 45 days. General depletion in succinate dehydrogenase activities is directly proportional to the exposure period of the toxicant. The obtained biochemical estimation values of the gills liver kidney brain and muscle were subjected to statistical analysis and showed significant values at $P<0.05$ (**Table 1**).

Table 1: Succinate dehydrogenase activity in gills liver kidney brain and muscle of *Channa gachua* exposed to sub lethal concentration of nickel chloride.

Sl. No.		Treatment	15 Days	30 Days	45 Days
1.	Gill	Control	0.056±0.004	0.057±0.004	0.055±0.004
		Low conc.	0.054±0.004	0.050±0.003	0.046±0.003
		Medium conc.	0.052±0.004	0.045±0.003	0.039±0.003
		Medium conc.	0.049±0.003	0.041±0.003	0.030±0.002
2.	Liver	Control	0.047±0.003	0.048±0.003	0.047±0.003
		Low conc.	0.044± 0.003	0.039±0.003	0.032±0.002
		Medium conc.	0.040±0.003	0.032±0.002	0.026±0.001
		High conc.	0.033±0.002	0.024±0.001	0.018±0.001
3.	Kidney	Control	0.040±0.003	0.039±0.003	0.038±0.002
		Low conc.	0.037±0.002	0.035±0.002	0.031±0.002
		Medium conc.	0.036±0.002	0.036±0.002	0.028±0.002
		High conc.	0.031±0.002	0.023±0.001	0.016±0.008
4.	Brain	Control	0.052±0.004	0.051±0.004	0.050±0.003
		Low conc.	0.048±0.003	0.043±0.003	0.038±0.002
		Medium conc.	0.045±0.003	0.039±0.003	0.032±0.002
		High Conc.	0.040±0.003	0.031±0.002	0.019±0.001
5.	Muscle	Control	0.059±0.004	0.058±0.004	0.059±0.004
		Low conc.	0.057±0.004	0.050±0.003	0.043±0.003
		Medium conc.	0.053±0.004	0.046±0.003	0.037±0.002
		High conc.	0.047±0.003	0.038±0.002	0.024±0.001

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

Discussion

Pollution by heavy metals is an important problem due to the metals' persistence in the environment. Since the aquatic environment is the ultimate recipient of the pollutants produced by natural and anthropogenic sources accumulation and persistence of heavy metals in the aquatic environment constitute the formidable threat to biological life (George, 1989; Gagne *et al.*, 1996; Fleeger *et al.*, 2003; Aramphongphan *et al.*, 2009). Heavy metals are some of the most active polluting substances they can cause serious impairment to circulatory metabolic physiological

and even structural systems when high concentrations are present in aquatic ecosystems (Shugart *et al.,* 1992). Although heavy metals are often referred to as common group of pollutants individual metals pose different problems in fresh water environments and therefore they have to be considered separately (Lloyd, 1992). Much more extensive biochemical toxicological research has been conducted in mammals than in fish. However it is not surprising that many bio chemical similarities exist among vertebrate species (Hochachka and Mommsen, 1995). Occurrence of some heavy metals in all the environmental compartments including food chain of aquatic medium despite their declining trend as the distance from the point of source increased and remained within the permissible limit was responsible for the heavy metal toxicity that perhaps affected the succinate dehydrogenase enzyme activity of fish (Mukherjee and Jana, 2007). The use of biochemical approaches have been advocated to provide an early warning of potentially damaging changes in stressed fish. In toxicological studies of acute exposure changes in concentrations and enzymes activities often directly reflect cell damage in specific organs (Casillas *et al.,* 1983). The succinate dehydrogenase is an important enzyme of kreb's cycle whose qualitative changes are significant during certain pathological conditions (Harper *et al.,* 1978). Succinate dehydrogenase is the oxidative enzyme which was drastically affected by the action of heavy metals. Succinic acid dehydrogenase is chosen as representative of metabolic enzyme. It is a marker enzyme for detecting the presence of TCA cycle in tissues. The impact of contaminants on aquatic ecosystem can be assessed by measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination (Petrivalsky *et al.,* 1997). Gills are the vital organs in fish which have direct contact with the medium through which pollutants enter into the body (Edwards, 1973). The succinate dehydrogenase enzyme is concentrated in chloride cells within the fish gills and has been used as an indicator of osmoregulatory activity (Langdon and Thorpe, 1984). Liver is one of the most multi-faceted and active organs in higher animals. In vertebrate body the liver is most important target organ as it is the chief metabolic and detoxification center (Bhattacharya and Mukherjee, 1976). Fish muscles are edible and economically important. The reduction in the succinate dehydrogenase enzyme activities in the present investigation in *Channa gachua* suggests that the fish is not in healthy condition. Many investigators have also recorded such reduction in succinate dehydrogenase enzyme activities in fishes exposed to different toxicants (Sastry and Sharma, 1980; Natarajan, 1984). In the

present study the activity of succinate dehydrogenase decreased in gills liver kidney brain and muscle of *Channa gachua* exposed to sub lethal concentration of nickel chloride. This suggests that an inhibited mitochondrial oxidation of succinate which may lead to drop in energy production and the suppression of succinate dehydrogenase activity indicates the impairment of oxidative metabolic cycle and hence relies on anaerobic glycolysis may be increased to meet its energy demands as revealed by Qaisur and Shamim (2014) respectively . There are evidences that the succinate dehydrogenase enzyme activities in the liver and muscle tissues of *Tilapia* decreased when they were exposed to pesticide thiodon. The inhibition of succinate dehydrogenase enzyme activities indicated the impairment of aerobic metabolism (Rajeswari *et al.,* 1989). Also there was decrease in the activity of succinate and lactate dehydrogenase in the gill liver and muscle tissues of fish *Oreochromis mossambicus* when exposed to pesticide methyl parathion which was caused by binding of endosulfan and methyl parathion with enzyme molecule and by blocking enzyme synthesis (Shukla, 1997; Qaisur, *et al.,* 2015). Similarly Sastry and Subhadra (1982) have reported the decrease in the succinate dehydrogenase activity in the liver tissue of *Channa punctatus* exposed to cadmium and copper. Mary chandravathy and Reddy (1994) have reported that a decreased in succinate dehydrogenase activity in the gill and liver tissues of *Anabas scandens* exposed to lead nitrate. James *et al.,* (1992) have observed that the level of succinate dehydrogenase activity decreased in the liver tissue of animals exposed to metal. They reported a metabolic shift from aerobiosis to an anerobiosis due to metal actions. More *et al.,* (2005) have observed that the level of succinate dehydrogenase activity decreased in *Lamellidens marginalis* exposed to heavy metal. They also reported that the anaerobic activity of the cells due to pollution stress has reversed physiological and biochemical adaptation. This decrease in succinate dehydrogenase activity might be suggestive of the weakening of bio chemical differences which in turn could be the results of tissue damage. Radhakrishnaiah *et al.,* (1992) have reported that the suppression in succinate dehydrogenase activities in liver tissues of *Labeo rohita* exposed to copper. The results of the present study clearly show significant alterations in succinate dehydrogenase enzyme due to intoxication of nickel chloride stress in *Channa gachua.* The decrease in succinate dehydrogenase enzyme activity may reflect decreased dependence on anaerobic carbohydrate metabolism by the gills liver kidney brain and muscle of fish *Channa gachua* that were exposed to nickel chloride.

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