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Influence of pH and water hardness upon nickel accumulation in edible fish *Cirrhinus mrigala*

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Abstract: *The freshwater fingerlings of Cirrhinus mrigala were exposed to Nickel in acidic medium pH = 6.0 (5.8 – 6.2), alkaline medium pH = 9.0 (8.8 – 9.2) and water hardness (40 mg/l) of $CaCO_3$. The study indicates that nickel accumulation was significantly influenced by pH and hardness of water. The concentration was found to be significantly higher at pH = 9.0 than at pH = 6.0. Also the presence of hardness in water results in reduced toxicity of nickel.*

Key words*: Cirrhinus mrigala, Bioaccumulation, Nickel, pH, Hardness*

Introduction

Pollution of marine ecosystem by toxic metals like nickel, chromium, cadmium, mercury, lead is a widespread problem. Metals after entering the water may precipitate or adsorb on solid surface, remain soluble or suspended in water or may be taken up by fauna and flora. These heavy metals may accumulate in marine organisms that are consumed by human. Nickel is ubiquitous and is abundant in the earth crust. Natural concentrations of nickel in soil and water are not considered as biological hazards, however higher concentration can be toxic to man and biota (USEPA,1975). Aqueous speciation and chemical partitioning are the key process regulating available trace metal levels in environment. Knowledge about the fate of chemicals in aquatic environments is essential for the understanding and production of possible ecotoxicological effects. Bioconcentration of chemicals in aquatic biota is an important factor in the assessment of the potential hazard of chemicals to the environment. This parameter can be used to quantify bioconcentration in aquatic biota and is defined as the ratio of the concentration of the chemicals in the biota (C_B) to that of water (C_W) at equilibrium (Taizo Tsuda *et al.*,1989). The bioconcentration factor (BCF) is usually measured in the laboratory using the test animals and is defined as :

$$BCF = \frac{k_1}{k_2} = \frac{C_B}{C_B} = \frac{\text{Chemical concentration in each part of the fish } (\mu g/g \text{ wet weight})}{\text{Chemical concentration in water } (\mu g/l)}$$

The availability of pollutants to organisms is a key determinant for bioconcentration and toxicity. Several environmental factors including pH (Meador,1991) and hardness (David *et al.*,1986) can alter the bioavailability in water primarily controlling speciation and complexation. Water pH affects metal toxicity in several ways (Champbell and Stokes,1985). Firstly, the speciation and the bioavilability of the metal may change in form between pH values of 4-8 (Pynnonen *et al.*,1987). Secondly the uptake and toxicity of the metal can be affected by the changes in the sensitivity of the cell surface. It has been established that acid water (Doherty *et al.*,1987) as well as heavy metals affects the rhythmic valve movements in *Anodonta cygna* and may possibly change the amount of metal coming into contact with the soft part of the clam (Florence,1976; Oshea and Mancy,1978). Elements of specific effects on pH on the bioaccumulation by freshwater invertebrates have been encountered in several field and laboratory studies (Robert *et al.*,1984; Lewis and Mc Intosh,1986; Stephenson and Mackie,1988). Physical characteristics of water such as temperature, pH and hardness also influence the rate of bioconcentration and depuration. For some metals the toxicity is related to alkalinity as well as hardness, the effect is most prevalent in hard water. However the role of such influencing factors remains limited for heavy metals especially nickel. Hence, the present work is aimed to determine the influence of pH and hardness on the accumulation of nickel in various organs of freshwater fish *Cirrhinus mrigala* fingerlings.

Materials and Methods

Cirrhinus mrigala fingerlings with an average length of 6cm (n=200, SD=0.9) and average weight of 8g (n=200, SD=2.0) obtained from local fish farm, Puthur, Tamil Nadu were used in the experiments. During the period of study they were fed with groundnut cake, rice bran and earthworm pieces on alternate days. The desired pH was obtained by adding appropriate amount of 0.5M HCl and 1M NaOH. Maintenance of the more consistent pH was difficult without addition of appropriate

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Table - 1: Accumulation of nickel (mg/g) in the organs of *Cirrhinus mrigala* under varying pH and hardness

Organ	Control	Nickel treatment (1.05 ppm)		Nickel treatment (4.6 ppm)		Nickel treatment (3.91 ppm)	
	pH=7.0 (6.8 - 7.2)	pH =7.0 (6.8 - 7.2)	pH=6.0 (5.8 - 6.2)	pH=7.0 (6.8 - 7.2)	pH=9.0 (8.8 - 9.2)	Soft water	Hard water
Gill	11.625±0.930	31.413±3.162	24.826±2.410	143.726±8.625	168.237±10.252	108.725±5.152	97.458±4.268
Liver	7.8636±0.425	38.536±3.210	31.453±3.145	297.635±10.240	358.168±11.530	213.431±5.416	197.636±6.225
Kidney	10.714±0.628	62.728±4.162	42.862±2.892	381.331±11.893	436.137±12.863	245.656±8.318	232.552±9.680
Muscle	3.652±0.226	17.462±3.456	9.814±1.682	92.766±5.635	143.435±8.463	72.225±1.426	68.426±2.146

The differences between controls and exposures are statistically significant ($p<0.005$) (n=4)

buffer, but to exclude the buffering action, the pH was maintained without addition of buffering agent. The pH was measured at regular intervals using pH tester (accuracy ±0.2) and adjusted to the required level. Initially the hardness of the water found to 200± 12.5 mg/l was taken as control. The required hardness of the test water was obtained by the addition of appropriate amount (40 mg/l) of $CaCO_3$. For the toxicity of varying pH, the fishes were not acclimated (Lloyd and Jordan, 1964, Robinson *et al.*, 1976; Falk and Dunson, 1977; Mudge *et al.*, 1977) and for hardness toxicity study alone test animals were acclimated for a period of 7 days in hard water prior to the experiment. To obtain LC_{50} value, the animals were exposed to different concentration of nickel chloride for 96 hr and the value was determined by the method of Finney (1971) for pH = 6.0, pH = 9.0 and hard water. LC_{50} value obtained was 3.16 mg/l, 13.80 mg/l and 11.74 mg/l for acidic, basic and hard water respectively. For acute exposure 1/3rd of LC_{50} values were taken as 1.05 ppm, 4.60 ppm and 3.91 ppm respectively for pH = 6.0, pH = 9.0 and in hard water (40 mg/l $CaCO_3$). Test animals of 20 each were exposed to the respective concentrations of nickel for a period of seven days as follows :

Group 1: Animals maintained in normal water (pH = 7.1 0.2, total hardness as $CaCO_3$ = 200 ± 12.5 mg/l) without addition of any toxic metal, treated as control.

Group 2: Animals exposed to 1.05 ppm of nickel in normal water (pH = 7.1 ± 0.2 and total hardness as $CaCO_3$ = 200 ± 12.5 mg/l).

Group 3: Animals exposed to 1.05 ppm of nickel at pH = 6.0 (5.8 - 6.2) and total hardness as $CaCO_3$ = 200 ± 12.5 mg/l.

Group 4: Animals exposed to 4.6 ppm of nickel in normal water (pH = 7.1 ± 0.2 and total hardness as $CaCO_3$ = 200 ± 12.5 mg/l).

Group 5: Animals exposed to 4.6 ppm of nickel at pH = 9.0 (8.8 - 9.2) and total hardness as $CaCO_3$ = 200 ± 12.5 mg/l.

Group 6: Animals exposed to 3.91 ppm of nickel in normal water (pH = 7.1 ± 0.2 total hardness as $CaCO_3$ = 200 ± 12.5 mg/l).

Group 7: Animals exposed to 3.91 ppm of nickel in hard water (pH = 7.1 ± 0.2 total hardness as $CaCO_3$ = 224 ± 10.8 mg/l).

The experiments were carried out at (28 ± 1°C) in aerated plastic trough. At the end of the exposure periods (7days) the test animals were sacrificed, organs like gill, liver, kidney and muscle were dissected and kept in hot air oven at 85°C for 24 hours. The dried samples were digested with concentrated nitric acid and perchloric acid in the ratio of 3:1 (Topping, 1973). The concentration of the nickel in each organ was determined using inductively coupled plasma atomic emission spectrometer (ISA JOBIN YUON 24 Model) available in CAS in Marine Biology, Annamalai University. All the experiments were carried out in four replicates and the average of the values along with the standard deviation are presented in Table I. The data were analyzed using standard student- t test (Siegal, 1956).

Results and Discussion

The accumulation of nickel in selected organs of the fish, *Cirrhinus mrigala* under different pH and hardness were presented in Table 1. The Table 2 shows the bioconcentration factor (BCF) determined using the standard procedure (Taizo Tsuda *et al.*,1989) on different organs. It is observed from the Tables 1 and 2 that for all the treatments (pH and hardness) the accumulation of nickel was found to be in the order: Kidney > Liver > Gill > Muscle.

Among the selected organs highest level of nickel was observed in kidney (BCF = 40.82 ±3.45, 94.81. 6.27 and 59.48± 4.76) and this may be due to strong irrigation and in relation to the function of excretion as suggested by Dallinger *et al.* (1997). Next to kidney, higher concentration of nickel was observed in liver. Since liver tissues is a major producer of metal binding proteins, the induction of low molecular weight metal binding proteins, such as metallothionein can be closely related to heavy metal exposures and this metal taken up from the environment

Table - 2: Bio-concentration factor (BCF) of various organs of the freshwater fish Cirrhinus mrigala at different pH and hardness

Organ	Bioconcentration factor		
	Acidic medium	Alkaline medium	Hard water
Gill	23.64±1.86	36.57±2.87	24.93±2.10
Liver	29.96±4.64	77.86±5.66	50.55±5.24
Kidney	40.82±3.45	94.81±6.27	59.48±4.76
Muscle	9.35±1.57	31.23±3.84	17.50±2.72

can be detoxificated by binding on these proteins (Roesijadi and Robinson, 1994) which results in higher concentration of metal.

A considerable amount of nickel has also been observed in gill tissues in all treatment and this may be due to passive exchange of metals that occurs between animals and its environment through bronchial epithelium of gills (Ay *et al.*, 1999). In addition, the gill tissues play an important role on ion regulation, gas exchange, acid balance, nitrogenous wastes and excretion which signifies the key role it play at the interface with the environment. The muscle tissue showed least concentration of nickel (BCF=9.35 ±1.57, 31.23 ±3.84 and 17.50± 2.72) in all the treatment. This can be explained by the very fast rate of decontamination in the tissue (Cinier *et al.*, 1971). Also it is generally accepted that freshwater fish muscle is not considered as a metal accumulating tissue (Milklovics, *et al*., 1985 and Romeo, 2006 ; Ana Martin Gonzalez *et al.*, 2006) whereas liver and kidney are considered as good biomonitors (Mount, 1966). These results are in agreement with earlier findings (Hamza Chaffai *et al.*, 1993 ; Reichert *et al.*, 1979) that the lowest accumulation observed in muscle and highest level in kidney and liver.

The results of the present study also show that accumulation of nickel was significantly increased at the higher pH = 9.0 than at pH = 6.0. This variation may be interpreted in terms of pH dependent changes in the speciation of nickel. The dissociated cation Ni^{2+} may predominates at pH = 6.0 whereas the undissociated neutral $Ni(OH)_2$ predominates at pH = 9.0, which can penetrate biomembranes much more easily than Ni^{2+}. Similar results have been reported for *Daphnia magna* exposed to tributyltin chloride (Roesijadi and Robinson, 1994). The influence of pH on metal speciation has also been stressed by a number of authors (Eckhart Wildi *et al.*,1994). It is generally considered that under acidic conditions more energy is required for maintenance of basic functions than under non-acidic conditions (Rosseland and Stournes, 1994). Additional exposure of nickel may display another severe chemical stress to organisms. These results make it probable that a change in pH has a major impact on the diffusion of molecules within aqueous stagnant layer. This is consistent with the fact that gills and muscles increases the mucus secretion at lower pH. Pentreath (1973) and Cunningham (1979), have suggested that mucus layer of the gills may be an important matrix influencing metal uptake. Since the pH of the ambient environment can influence mucus secretion or formation (Mount, 1966) reduction in the pH may altered the mucus, leading to reduced metal uptake. These results support our finding, that is increase in pH results in a proportional increase in metal concentration.

It is also observed from the Table 1. That addition of $CaCO_3$ results in decrease in accumulation of nickel by 5-10%. The slightly lower toxicity of nickel in hard water could be explained by reduced intake of nickel into the gills, the prime site of lethal action for metals and other pollutants. This reduced toxicity of nickel observed may be explained chemically by a reduction in concentration of soluble metal due to changes in speciation and the formation of insoluble complexes in hard water. Mc Carty *et al.* (1978) have observed a similar reduction in toxicity of cadmium to the goldfish, *Carassius aurates* in hard water. Skidmore and Towel (1972) and Lloyd (1965), has also observed that copper toxicity was reduced by the calcium hardness.

From the present study, we can conclude that the nickel accumulation was significantly influenced by pH and hardness of water. The bioconcentration was found to be significantly higher at pH = 9.0 than at pH = 6.0. Also, the toxic effect of nickel was reduced due to hardness.

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