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Research Article

Toxicity of Nickel and Chromium on the Mineral Content of the Indian Major Carp, *Cirrhinus mrigala*

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Abstract

Industries discharge their effluents in to the environment and some of them reach aquatic systems. Trace metals present in such effluents as mixtures affect fish. Hence in the present work, an attempt has been made to estimate the effects of nickel and chromium separately and in combination on the mineral content of the fish, *C.mrigala*. Both nickel and chromium individually resulted in the decline of Ca, Na, K and Mg but increase in P content. Both the combinations increased Ca, Mg, and P content in the fish. (Ni)+Cr combination alone caused an elevation in Na and K content of the whole fish under chronic exposure.

Introduction

Industrial growth, economic development and consumption indicate a country's progress and life standard of individuals. Due to industrialization, urbanization and population growth, the basic amenities of life, air, water and land are being polluted continuously. There has been an excessive use of metals in industries in India. They cause greatest threat to the health of Indian aquatic ecosystems [1,2]. The main pollutant from these industrial complexes is the effluent containing heavy metals such as Cu, Ni, Zn, Pb, Cr, Hg, and Cd [3].

When these substances make their way into the aquatic ecosystems, they remain stable in the aquatic environment [4,5]. The activities of life forms in water bodies are hindered and furthermore, they may also reduce the quality of water. Disposal of industrial effluents into freshwater bodies deteriorates water quality, which is necessary to sustain aquatic life, primary productivity and food chain [6]. The biomagnification of these trace metals in ecosystem is a major threat to human life [7,8]. All trace metals are potentially harmful to most organisms at certain level of exposure and absorption [9,10]. These trace metals are toxic because they cause DNA damage and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability [11].

Nickel is the important raw material in many industries. Occupational exposures may arise in nickel miners, smelters and refiners. Nickel processing industries are the main sources of nickel pollution. It is an important constituent of alloys and is also used to provide protective or decorative coating for other metals. It normally occurs in surface waters at low concentrations. It is employed in the industries of pulp and paper mills, paper board mills, fertilizers, petroleum refining, steel work foundries, motor vehicles, air craft plating and finishing [12]. Electroplating industry is the major contributor of nickel [13]. Nickel as a potential carcinogen, may cause skin allergies, lung fibrosis and cancer of respiratory tract [14].

Chromium is also used in metal plating, tanneries and oil well drilling [15]. Sewage and fertilizers are also the sources of chromium [16]. Hexavalent chromium is found in effluents of electroplating and textile industries [17,18]. Tanneries are the major industries that use chromium for the treatment of leather and nearly 40% of the chromium used is released into the environment as sludge which contaminates surface water as well as ground water. Chromium exposure and its impact on the health of individuals depend on many factors. These include chemical form, the amount, and the length of time, the individual exposed and route of exposure (ingested, inhaled, or absorbed through skin). When chromium enters the body, numerous biochemical changes occur. These changes are dependent on age, sex, weight and health of the individual [19].

Freshwater fishes are high in protein and low in saturated fat and cholesterol. Eating fish is part of a healthy diet and can reduce the risk of certain cancers and heart diseases [20]. *Cirrhinus mrigala* was selected for the present study because it is one of the Indian major carps and is an esteemed food fish. It is cultured in ponds and reservoirs. There are chances of industrial effluents having these metals reaching these systems. The major objective of the present study is to investigate the sublethal effects of nickel, chromium and their mixtures on Na, K, Ca, P and Mg concentration in *C.mrigala*.

Materials and Methods

For the present study, the fingerlings of *C.mrigala* were purchased from local aquafarm in Madurai, Tamil Nadu, India. The fish were acclimatized for more than ten days in large aquaculture tanks (75L). The fishes were fed with commercially available feed daily. The excreta and excess food were siphoned out to avoid contamination and ammonia stress. Once in a day, water was changed. From the laboratory acclimatized fishes, fishes were selected and they were again acclimatized for one or two days in experimental tanks prior to commencement of the experiment. The capacity of experimental tank was twenty liters. The tank was closed by net to prevent the jumping of fish. 4.5g of nickel sulphate was dissolved in one liter double distilled water to get 1000 ppm of nickel stock solution where as 2.8g of potassium dichromate was dissolved in one litre of double distilled water to get 1000 ppm of chromium stock solution. The acclimatized fishes were introduced into five experimental tanks. Among these five tanks, four tanks served as experimental tanks and the remaining one as control. The ground water was used in the present study. Each tank was filled with five litres of ground water with five fishes.

Based on the acute toxicity test results, the sublethal concentrations were fixed and prepared ($1/10^{th}$ 96 hr LC_{50} value of individual metals and metal mixtures) from the respective stock solutions [3 ppm of Ni, 2.5 ppm of Cr, (3ppm of Ni)+ 4 ppm of Cr and (2.5 ppm of Cr) + 7.6 ppm of Ni]. The control tank had only ground water with fish.

The experimental fishes were exposed to the sublethal concentrations of Ni, Cr and metal mixtures [(Ni)+Cr and (Cr)+NI] for ten days. The water was renewed daily and the concentrations of metal/metal mixtures in the experimental tanks were maintained. The fish were fed with commercial fish feed. For the estimation of minerals present in the fish body, the following routine procedure was followed.

Triple acid digestion

The dried and powdered fish samples were weighed (0.5g) and taken in clean boiling test tubes. The sample was then treated with triple acid mixture, nitric acid (HNO_3), Sulphuric acid (H_2SO_4) and perchloric acid ($HClO_4$) in the ratio of 9:2:1. To complete the digestion process, the digestion was done three times and the samples were evaporated to dryness. The sample residues were dissolved in 1% nitric acid cooled and made up to 50ml in volumetric flask with the help of double distilled water. Following the dilution, the samples were centrifuged at 2000 rpm for about 30 minutes and the supernatant liquid was decanted into polypropylene tubes that were then capped and stored pending analysis. It was then neutralized using ammonium hydroxide with the help of phenolphthalein as an indicator. The above procedure was carried out for preparing a blank.

Sodium and Potassium

The concerned element concentration of the present study (sodium and potassium) was found out using flame photometer (ELICO make). The triple acid digested samples were taken for analysis. For different elements specific filters were used. A monochromator which allowed to pass the light of the wavelength specific to that of particular element was used.

Calcium

Calcium was estimated by complexometric titration method. Exactly 10ml of the sample was taken in a clean Erlenmeyer flask. About 0.4ml of 1N sodium hydroxide solution and pinch of murexide indicator were added. The solution was titrated against 0.01N EDTA solution. The change of pink color to purple marked the end point. Calcium content was calculated employing the following formula.

$$\text{Calcium } (\mu g/g) = \frac{\text{Volume of EDTA} \times 400.8 \times 1000}{\text{Volume of sample}}$$

Magnesium

To find out the magnesium content of the sample, the total hardness of the sample was first estimated by complexometric titration using EDTA. A 50ml sample was taken in Erlenmeyer flask. About 0.4ml of buffer solution (pH 10) and approximately 10mg of Eriochrome black T indicator were added. The sample was titrated against 0.01N EDTA solution taken in the burette. The end point was the change from wine red to blue colour.

$$\text{Total hardness } (\mu g/g) = \frac{\text{ml of EDTA} \times 1000 \times 1000}{\text{Volume of sample}}$$

Based on the EDTA consumption in the total hardness and calcium determinations, the magnesium content was calculated using the following relationship.

$$\text{Magnesium } (\mu g/g) = \frac{(Y-X) \times 400.8 \times 1000}{\text{Volume of sample} \times 1.645}$$

Where, Y = volume of EDTA used in the total hardness determination; X = volume of EDTA used in the calcium determination.

Phosphorus

10 ml of sample was taken and to this 0.4ml of ammonium molybdate and 3 drops of stannous chloride were added. The color of the solution changed to blue. Then the reading was measured at 690nm in a spectrophotometer. The concentration of phosphorus was calculated from the standard curve.

Results

The effect of nickel, chromium and their mixtures on the mineral content of *C.mrigala* was analysed.

Effect on calcium content

The calcium content of *C.mrigala* exposed to metal/metal mixtures and control fish are given in Figure 1. The control fish exhibited 61.22 µg/mg dry wt. of calcium in their body. The nickel exposed fish had 11.56µg/mg dry weight. The chromium exposed fish had 13.44 µg/mg dry weights. Here both nickel and chromium caused reduction in the calcium content in fish body. [(Ni) + Cr] mixture exposed fish had 143.67 µg/mg dry weight of calcium content in their body. Here nickel and chromium interacted against each other. So drastically increased level of calcium was found in [(Ni) + Cr] exposed fish body. In another combination [(Cr) + Ni] exposed fish, the calcium level was 103.6 µg/mg dry weight. Both chromium and nickel increased the calcium content in fish body.

Effect on sodium content

The sodium content of *C.mrigala* exposed to metals/metal mixtures and control fish is given in Figure 2. The control fish had

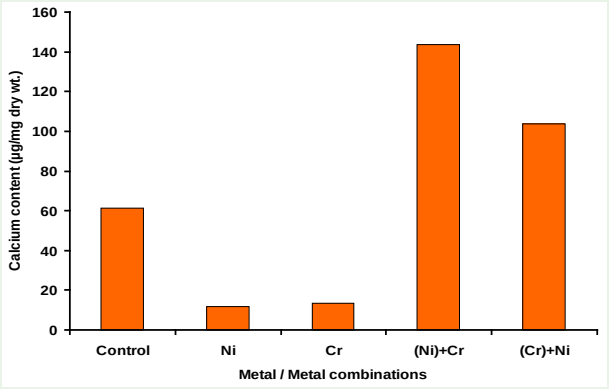

Figure 1: Effect of nickel, chromium and their combinations on the calcium content of *C.mrigala*.

5.86 µg/mg dry weight of sodium in their body. The nickel exposed fish had 0.7 µg/mg dry weight of sodium. Nickel decreased the sodium level in fish body and chromium exposed fish also showed decreased level of sodium (1.24 µg/mg dry weights) of fish. The metal combination of [(Ni) + Cr] exposed fish had 6.52 µg of sodium/mg of dry weight of fish. An increase in the sodium content was noticed in the combination of [(Ni) + Cr] whereas slight decrease in the sodium content (5.3 µg/mg dry weight) was observed in the combination of [(Cr) + Ni].

Effect on potassium content

The potassium content of *C.mrigala* exposed to metals/metal mixtures and control fish is given in Figure 3. The control fish had 9.18 µg/mg dry weight of potassium in their body. The nickel exposed fish had 0.66 µg/mg dry weight of potassium. The chromium exposed fish showed 0.60 µg/mg dry weight of potassium in their body. Here both nickel and chromium drastically reduced the potassium level in the fish body. In metal mixture [(Ni) + Cr] exposure, the fish had 9.68 µg/mg dry weight of potassium in their body. This shows a slightly increased level of potassium content than the control. But in [(Cr) + Ni] combination, the fish had 8.33 µg/mg dry weight of potassium. It

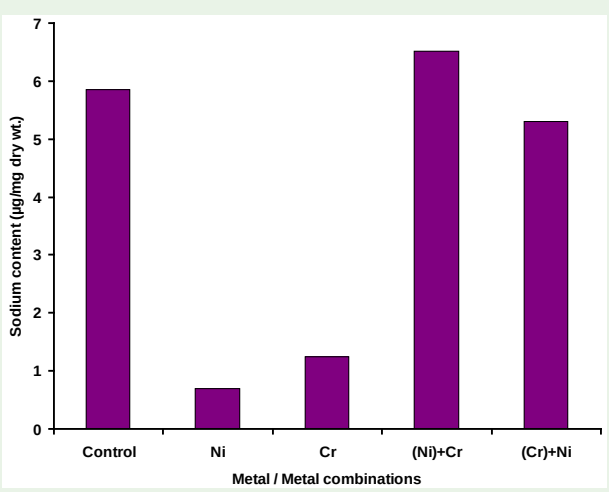

Figure 2: Effect of nickel, chromium and their combinations on the sodium content of *C.mrigala*.

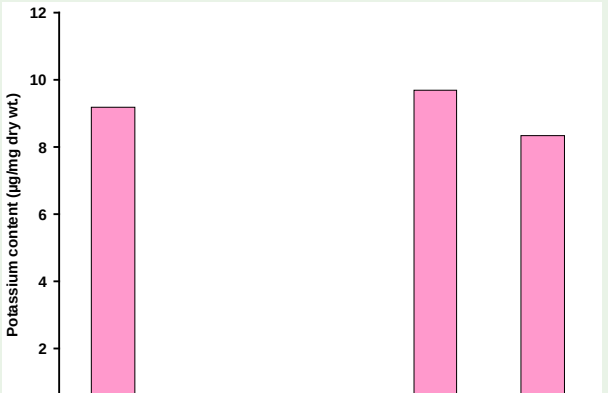

Figure 3: Effect of nickel, chromium and their combinations on the potassium content of *C.mrigala*.

is clearly observed that the combination of [(Cr) + Ni] reduced the potassium content of the fish body.

Effect on magnesium content

The magnesium content of *C.mrigala* exposed to metals/metal mixtures and control fish is given in Figure 4. The control fish exhibited 30.45 µg/mg dry weight of magnesium in their body. The nickel exposed fish showed 5.572 µg/mg dry weight of magnesium in their body while the chromium exposed fish exhibited 6.78 µg/mg dry weight of magnesium. Both nickel and chromium drastically reduced the magnesium content in the fish. In [(Ni) + Cr] exposure, the fish had 70.81 µg/mg dry weight of magnesium in their body. In the case of another metal mixture [(Cr)+ Ni], the exposed fish showed 51.9 µg/mg dry weight of magnesium in their body. Both nickel and chromium combinations increased the magnesium content of fish body to a large extent.

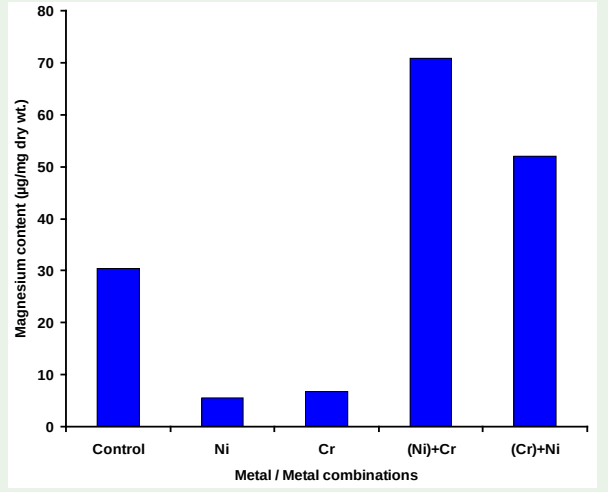

Figure 4: Effect of nickel, chromium and their combinations on the magnesium content of *C.mrigala*.

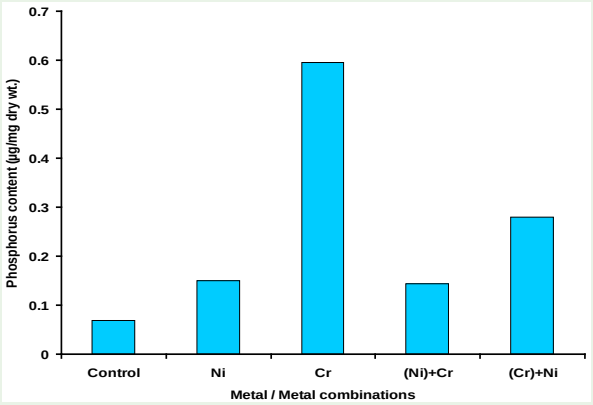

Figure 5: Effect of nickel, chromium and their combinations on the phosphorus content of *C.mrigala.*

Effect on phosphorus content

The phosphorus content of *C.mrigala* exposed to metals/metal mixtures and control fish is given in Figure 5. The control fish had 0.68 µg/mg dry weight of phosphorus in their body. The nickel exposed fish showed 0.150 µg/mg dry weight of phosphorus in their body and nickel caused an increase in the level of phosphorus content. The chromium exposed fish showed 0.596 µg/mg dry weights of phosphorus and it is clearly observed chromium drastically increasing the phosphorus content in *C.mrigala.* In [(Ni) + Cr] exposure, the fish exhibited 0.143 µg/mg dry weight of phosphorus in their body. In another combination [(Cr)+ Ni] exposure, the fish showed 0.280 µg/mg dry weight of phosphorus. Chromium individually and in combination with nickel drastically increased the phosphorus content of the fish.

Discussion

In animal body, the micronutrients interact with toxic metals at many areas; absorption and excretion of toxic metals, transport of metals in the body, binding to target proteins, metabolism and sequestration of toxic metals; and finally in secondary mechanisms of toxicity such as oxidative stress [21]. The amount of calcium present is more in the skeletal system of animals and very less amount of calcium is present in blood and muscle. For normal physiological function, narrow concentration of calcium in extracellular fluid is essential. It is an activator for several enzymes like acid phosphatase, cholinesterase, ATPase and dehydrogenases. Calcium stimulates the muscle contraction and regulates the transmission of nerve impulses from one cell to another through its control over acetyl choline secretion. In the present study, calcium level in the fish body drastically decreased, because of the exposure of nickel and chromium separately. But these two metals, in mixture interacted against each other. Hence increased level of calcium content in fish was observed. The exposure of nickel and chromium caused the depletion of minerals like calcium, magnesium and phosphorus in the vertebral column of scale carp [22]. Fox and coworkers [23] reported vertebral calcium depletion occurring due to the exposure of cadmium.

Sodium commonly found as sodium chloride in aquatic media is present in extra cellular fluid and is involved in maintaining osmotic pressure of the cell and acid-base equilibrium. Sodium is essential for the maintenance of membrane potential, blood volume and blood pressure. It also plays a role in water metabolism and muscle irritability. In the present investigation, the level of sodium increased with nickel in combination with chromium and these findings coincided with that of Shukla [24]. According to him, the plasma sodium increased in *Channa punctatus* exposed to Zn, Cu and Cd. But sodium level decreased with nickel and chromium exposure separately. It is contrary to the findings of Gupta and coworkers [25]. Kidney and muscle sodium level increased in *Channa punctatus* exposed to zinc. The metal mixture (nickel and chromium) decreased the percentage of alteration of sodium content in *C.mrigala.* Here the metals in mixture interacted with one another. Hence, the toxic effect is minimized than that of individual metals. The decrease of sodium was observed in *Channa punctatus* exposed to nickel and zinc mixture than that of individual metal [26]. The decrease of sodium level in gill was noticed in rainbow trout exposed to Ni, Cr and Cd by Hughes and cowokers [27].

Potassium regulates intracellular osmotic pressure. It is essential for the maintenance of membrane potential and it is a co-factor for enzymes. It is also required for glycogen and protein synthesis and the metabolic break down of glucose. The toxic effects are often due to physical changes in the tissue at the cellular or ultracellular levels and can only be speculated unless they are visualized [28]. In the present study, the level of potassium was drastically decreased when these two metals (Ni, Cr) were exposed individually. The level of potassium slightly increased in the (Ni) + Cr combination and slightly decreased in the (Cr) + Ni combination. The increased blood potassium level was noticed in *Channa punctatus* with the exposure of cadmium [24]. In contrast Schmid-Nielsen and co-workers [29] observed no effect from injected methyl mercury on Na-K-ATPase activity in the gills of *Pseudopleuronectes americanus.*

Magnesium is found in the skeleton, muscle and extracellular fluid. It is involved in energy production, synthesis of essential molecules like DNA, RNA and protein. It plays a structural role in the transport of ions across membrane and it is an activator of several key enzymes like kinases, mutases, muscle ATPase, cholinesterase, alkaline phosphatase, enolase, iso-citric dehydrogenase, arginase and deoxyribo nuclease. It plays an important role in the metabolism of carbohydrates, proteins and lipids. In the present investigation, the individual exposure of Ni and Cr to *C.mrigala* drastically reduced the magnesium content in fish body. In contrast, the mixture of (Ni) + Cr and (Cr) + Ni combination exposures increased the Mg content in fish body to a large extent. The result obtained in this work does not support Thatheyus [22], who reported the decline of magnesium content in the vertebrae of the scale carp, *Cyprinus carpio communis* exposed to nickel and chromium. The ethological responses of the fish *Labeo rohita* treated with industrial waste water were found to depend on its concentrations and duration of exposure time [30].

Phosphorus is an essential mineral which is required by every cell in the animal body for normal function. Phosphorus is present in the body as phosphates. It is an essential component of phospholipids, nucleic acids, phosphoproteins, high energy phosphate esters (ATP), hexose phosphates and creatinine phosphate. The inorganic phosphate serves as important buffer to regulate the normal acid-base balance of the animal body fluid. Phosphorus is also present in some amino acids. In the present study, the drastic increase of phosphorous in the exposure to chromium is observed in *C.mrigala*. Sensetlin and

co-workers [31] observed the concentrations of Cu, Fe and Zn being higher in the muscle tissue than in the waste water. Nickel, (Ni) + Cr and (Cr) + Ni combination could raise the body phosphorus content of *C.mrigala* to some extent. The exposure of nickel and chromium caused the depletion of minerals like calcium, magnesium and phosphorous in the vertebral column of the scale carp [22]. This observed differential toxicity as well as relative susceptibility has been reported by Oyewo and Don-Pedro [31]. It has been attributed to differences in the chemistry and mechanism of action of the different metals. Other reactions include nature of the cuticle (or) body covering with respect to penetrability, metabolic transformation capacities, optimal physico-chemical conditions, excretory capacity and the rate of elimination of the by-products of metabolism, availability of site of action; body size, age and life cycle stage as well as ecology with particular reference to location and activity co-efficient and possibly behavioral attributes. These attributes vary considerably between different metals and animals [32].

Since heavy metals are not naturally degraded, they are progressively accumulated in fishes and human body. According to Farkar and co-workers [33], the fish is the indicator organism for heavy metal pollution and the possible risk for human consumption. Heavy metals concentrations in the environment and their effects on human health must be regularly monitored. More researches are required to understand the mechanisms involved in heavy metal toxicity to fishes. Metal induced defense responses at molecular level have to be worked out for understanding the cascade of chemical mechanisms of heavy metal toxicity [34].

Conclusion

From the present study it is clearly observed that nickel, chromium and their combinations brought about changes in the mineral content in *C.mrigala*. So precautions have to be taken in order to avoid heavy metal pollution in aquatic bodies. Otherwise, these pollutants can be dangerous to fish and human health.

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