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Research Article

Mixture Toxicity of Nickel and Chromium to the Indian Major Carp, *Cirrhinus mrigala*

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Abstract

Background and Objective: Several industries release their waste water directly in to the aquatic ecosystems without appropriate treatment. The toxic heavy metals present in that waste water cause water pollution and also affect the aquatic organisms. Hence, the present work has been aimed to find out the acute toxicity of nickel, chromium and their combinations to the fingerlings of the Indian major carp, *Cirrhinus mrigala*. **Materials and Methods:** The fingerlings of *C. mrigala* were subjected to static bioassays to determine the acute toxicity of chromium, nickel and their combinations. Using probit analysis, 24, 48, 72 and 96 h LC_{50} values were determined along with 95% fiducial limits. The results were subjected to Chi-square test to find out the goodness of fit. **Results:** The 96 h LC_{50} values of chromium, nickel, Ni+Cr and Cr+Ni were 21.3, 25.8, 42.4 and 76.0 ppm, respectively. Chromium was more toxic to the fish than nickel. When all the tests are compared, Cr+Ni combination was the most toxic to the fish. **Conclusion:** Among the metals tested chromium were more toxic to the fish than that of nickel.

Key words: Heavy metals, nickel, chromium, mixture toxicity, *Cirrhinus mrigala*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The aquatic systems are continuously disturbed by human activities. The discharge of untreated and partially treated waste water from various industries like chemical, pesticides, fertilizer, pulp, paper and sugar have polluted the aquatic bodies such as rivers, ponds and ditches[1]. The pollutants of major concern in aquatic ecosystems are those which reach the environment in large amount, toxic to aquatic organisms, accumulate within the organisms and persist for long periods. They alter the physico-chemical properties of the aquatic environment and adversely affect the biota. After studying the limnology of major rivers of India, researchers revealed that no river or stream is completely free from industrial pollutants[2]. So, it is very much essential to have a periodic monitoring of water quality in aquatic systems. Heavy metals presented in the industrial effluents are the major factors which are responsible for fresh water pollution[3].

They constitute a variety of heterogeneous group of elements widely varied in their chemical properties and biological functions. The toxicity of heavy metals may be attributed to their binding quality with biologically active molecules[4]. The main pollutant from the industrial complexes is the effluent which contains heavy metals such as Cu, Ni, Zn, Pb, Cr, Hg, Cd and various organic compounds such as phenols and formaldehyde[5]. Heavy metals have been recognized as strong biological poisons because of their persistent nature, toxicity, tendency to accumulate in organisms and undergo food chain accumulation[6].

Nickel is the important raw material in many industries. It is listed by the EPA as one of the 129 priority pollutants and is considered to be one of the 14 most noxious heavy metals. It is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites[7]. It causes conjunctivitis, eosinophilic pneumonitis, asthma and local or system reaction to Ni containing prostheses such as joint replacements, pins, cardiac valve replacements, cardiac pacemaker wires and dental inlays[8]. Nickel is a potential carcinogen for lung and may cause skin allergies, lung fibrosis and cancer of respiratory tract in occupationally exposed populations[9].

Chromium is a toxic metal which is found in various forms in the environment. It is an essential element in trace amounts; however, it is toxic above permissible limits[10]. The sources of chromium in environment are both natural and anthropogenic, while natural sources include burning of oil and coal, petroleum from ferro-chromate refractory material, chromium steels, pigments, oxidants, catalysts and fertilizers. The most commonly reported effects of chronic chromium exposure in human are contact dermatitis, irritation and ulceration of the nasal mucosa[11,12].

Water pollution affects fisheries and aquaculture industries. The changes in the quality of water alter the behaviour of fishes besides causing mortality. The behavioral changes in fishes have been considered to be sensitive indicators of toxicity and among aquatic fauna, fishes are more sensitive to pollutants[13]. Very limited reports are available on the mixture toxicity of nickel and chromium to freshwater fishes. It is a freshwater fish belonging to the carp family Cyprinidae, found commonly in rivers and freshwater lakes in and around south Asia and south-east Asia. It is a bottom feeder feeding on decaying organic and vegetable debris; however young feed on zooplankton[14,15]. Nickel and chromium are present together in electroplating industrial effluents. Their individual effects and interaction effects have not been studied in detail using fish. Hence; the present study has been designed to determine the acute toxicity of nickel and chromium individually and in combination to the Indian major carp, *C. mrigala*.

MATERIALS AND METHODS

The present study was conducted for one year from June, 2017-May, 2018 in the laboratory of the Department of Zoology, The American College, Madurai, India. For the present study, the fingerlings of *C. mrigala* were purchased from local aqua farm in Madurai, Tamil Nadu, India. The fish were acclimatized for more than 10 days in large aquaculture tanks (75 L). 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 1 or 2 days in experimental tanks prior to commencement of the experiment. The capacity of experimental tank was 20 L. The tank was closed by net to prevent the jumping of fish.

About 4.5 g of nickel sulphate was dissolved in 1 L double distilled water to get 1000 ppm of nickel stock solution where as 2.8 g of potassium dichromate was dissolved in 1 L of double distilled water to get 1000 ppm of chromium stock solution. The acclimatized fishes were introduced into 5 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 5 liters of ground water with five fishes.

Determination of LC_{50}: After preparing the stock solutions for nickel and chromium, the wide range of these two metals were identified by using three fish in each concentration. The fish were not fed for 1 day before starting the experiment to avoid the change in toxicity of metals due to excretory products[16]. Then narrow range was identified from wide range. Different concentrations of the metals were prepared and in each of them 10 fish were exposed separately. The percentage mortality of fish in different concentrations was noted after 24, 48, 72 and 96 h of exposure. The LC_{50} values for different exposure periods were obtained after computing probit analysis[17].

Determination of LC_{50} value for metal mixtures: The combination of metals were prepared, in which one metal concentration was kept constant (i.e., 1/10th of 96 h LC_{50} value) and the other was varied. Different concentrations of metal mixtures were prepared and in each of them 10 fishes were exposed separately. The percentage mortality of fish in different metal mixture concentrations was noted after 24, 48, 72 and 96 h of exposure.

Statistical analysis: The LC_{50} values for metal mixtures were obtained employing probit analysis. In probit analysis, the concentrations were converted in to log concentrations and percentage mortality values were converted in to probit values. The LC_{50} values were derived after regression analysis. Chi-square test was applied to compare the observed Y values and expected Y values.

RESULTS

The percentage mortality values of *C. mrigala* exposed to different concentrations of metals and metal mixtures were observed. Using probit analysis, 24, 48, 72 and 96 h LC_{50} values along with 95% fiducial limits were derived after applying regression analysis.

LC_{50} determination for nickel and chromium: The LC_{50} values of nickel for 24, 48, 72 and 96 h were 75.3, 45.6, 30.5 and 21.3 ppm, respectively (Table 1). The LC_{50} values observed decreased with the increase in the duration of exposure to nickel. The LC_{50} values of chromium for 24, 48, 72 and 96 h were 37.9, 29.3, 27.5 and 25.8 ppm, respectively (Table 2). Here

Table 1: Acute toxicity test results of nickel to *Cirrhinus mrigala*

	Lethal concentration (ppm)							95% fiducial limits of LC_{50} (ppm)		Probit regression equation		Chi-square values		
Time (h)	LC_5	LC_{10}	LC_{16}	LC_{50}	LC_{84}	LC_{90}	LC_{99}	Lower	Upper	Y = a+bx	Slope function (S)	Observed	Table	Level of significance at 0.05 level
24	60.7	63.8	66.1	75.3	85.8	89.0	102.1	63.08	90.01	Y = -26.07041+16.5551x	1.138696	33.31218	5.99	S
48	18.9	22.9	26.7	45.6	77.9	91.0	159.6	38.39	54.35	Y = -2.207815+4.3427x	1.707300	17.41206	9.49	S
72	10.9	13.7	16.3	30.5	55.8	66.6	127.2	15.81	57.46	Y = -.9161072+3.987931x	1.850913	9.112652	5.99	S
96	14.8	17.4	19.8	21.3	49.1	56.0	90.11	21.94	44.55	Y = -2.872229+5.235402x	1.572273	12.84697	5.99	S

S: Significant

Table 2: Acute toxicity test results of chromium to *Cirrhinus mrigala*

	Lethal concentration (ppm)							95% fiducial limits of LC_{50} (ppm)		Probit regression equation		Chi-square values		
Time (h)	LC_5	LC_{10}	LC_{16}	LC_{50}	LC_{84}	LC_{90}	LC_{99}	Lower	Upper	Y = a+bx	Slope function (S)	Observed	Table	Level of significance at 0.05 level
24	23.1	25.8	28.1	37.9	51.1	55.7	76.1	34.99	41.12	Y= -6.894102+7.53276 x	1.347335	11.59468	9.49	S
48	17.1	19.3	21.2	29.3	40.6	44.6	62.8	28.27	30.52	Y= -5.442711+7.102896 x	1.384138	3.216171	9.49	NS
72	16.5	18.4	20.2	27.5	37.4	40.9	56.5	56.50	28.59	Y = -5.648381+7.409303 x	1.360824	4.805069	7.81	NS
96	15.4	17.3	18.9	25.8	35.2	38.5	53.2	53.20	26.93	Y= -5.459236+7.411866 x	1.365286	7.671631	7.81	NS

S: Significant, NS: Not significant

Table 3: Acute toxicity test results of Nickel+Chromium to *Cirrhinus mrigala*

	Lethal concentration (ppm)							95% fiducial limits of LC_{50} (ppm)		Probit regression equation		Chi-square values		
Time (h)	LC_5	LC_{10}	LC_{16}	LC_{50}	LC_{84}	LC_{90}	LC_{99}	Lower	Upper	Y = a+bx	Slope function (S)	Observed	Table	Level of significance at 0.05 level
24	35.1	40.2	44.8	65.2	94.9	105.7	156.6	62.05	68.66	Y= -5.985616 +6.051483 x	1.453981	2.579094	11.07	NS
48	32.4	35.7	38.6	50.5	66.2	71.5	94.9	46.67	54.84	Y= -10.32646 + 8.97338 x	1.308929	14.85321	9.49	S
72	31.3	34.3	36.8	47.1	60.3	64.7	83.8	44.37	50.10	Y= -10.61003 + 9.322058x	1.278979	9.875885	9.49	S
96	30.5	32.8	34.7	42.4	51.8	54.8	67.7	41.38	43.52	Y= -13.69793+ 11.48619x	1.220981	.2477417	7.81	NS

S: Significant, NS: Not significant

Table 4: Acute toxicity test results of Chromium + Nickel to *Cirrhinus mrigala*

	Lethal concentration (ppm)							95% fiducial limits of LC_{50} (ppm)		Probit regression equation		Chi-square values		
Time (h)	LC_5	LC_{10}	LC_{16}	LC_{50}	LC_{84}	LC_{90}	LC_{99}	Lower	Upper	Y = a+bx	Slope function (S)	Observed	Table	Level of significance at 0.05 level
24	70.7	78.0	84.2	109.9	143.4	154.8	204.8	103.43	116.8	Y=-12.92262+8.785142x	1.304891	2.519585	9.49	NS
48	57.4	62.1	66.0	81.9	101.5	108.0	135.4	75.69	88.64	Y=-16.92263+11.43384x	1.23975	37.45111	11.07	S
72	57.5	61.4	64.6	77.1	92.0	96.8	116.5	72.54	81.99	Y=-19.58254+13.01582x	1.193157	22.49	11.07	S
96	62.3	65.1	67.4	76.0	85.6	88.7	100.6	71.32	80.99	Y=-30.79543+19.01819x	1.127503	17.24042	7.81	S

S: Significant, NS: Not significant

also the LC_{50} values observed decreased with the increase in the duration of exposure to chromium.

LC_{50} determination for metal mixtures: In the mixture of nickel and chromium, the LC_{50} values for 24, 48, 72 and 96 h were 65.2, 50.5, 47.1 and 42.4 ppm, respectively (Table 3). In the mixture of chromium and nickel, the LC_{50} values for 24, 48, 72 and 96 h were 109.9, 81.9, 77.1 and 76.0 ppm, respectively (Table 4). Also in the above experiments, the LC_{50} values observed decreased with the increase in the duration of exposure.

DISCUSSION

The present study revealed that nickel and chromium being acutely toxic to the fish *Cirrhinus mrigala* and the mortality rate increased with increasing concentration of nickel and chromium. The LC_{50} value of nickel for 96 h was 31.3 ppm. The LC_{50} value of nickel for 96 h was about 6 times higher than that of fresh water fish *Hypophthalmichthys molitrix*[18]. The 96 h LC_{50} value to *Cyprinus carpio* for nickel was 47.5 ppm[19]. The 96h LC_{50} value of chromium to *Cirrhinus mrigala* was 25.8 ppm and the LC_{50} value of chromium to the freshwater mussel, *Lamellidens marginalis* was 11.74 ppm[20]. The LC_{50} value of Zn, Cu and Cd to adult *Channa punctatus* exposed for 96 h was 18.62, 0.56 and 11.8 ppm, respectively[21]. The 96 h LC_{50} value of zinc to *Labeo rohita* fingerlings was 156 ppm[22]. The 96h LC_{50} value to the fingerlings of *Cirrhinus mrigala* exposed to mercury was 240 ppm[23].

Assessment of mixture toxicity began as an art, but it has developed into a science used in many disciplines, pharmacology, toxicology, physiology, human and veterinary medicine, agriculture and especially pest control. However, some chemical mixtures pose a greater hazard to non-target organisms and to the environment[24]. Most of the industrial effluents that are discharged into the aquatic systems are mostly the blend of heavy metals and other chemicals. Regarding the acute lethality of metal combinations in the present study, decline in LC_{50} values were noted with the increase in the duration of exposure. The relative toxicity of Mn and Cu against *Tilapia guinensis* and *Tympanotonus fuscatus* showed that Cu was evidently more toxic than manganese[25]. Metal accumulation in fish depends on the metal species, exposure concentration and period and other factors, like salinity and temperature[26].

On chronic exposure to Ni, the liver exhibited several pathological changes including reduction in the size of fish liver[27] and the ultrastructural changes in the liver were characterized by severe enlargement of hepatocytes[28]. The kidney plays a principal role in the accumulation, detoxification and excretion of Ni and is considered to be a target organ for Ni toxicity[29,30]. The most marked histological alterations were observed in posterior kidneys of white fish fed high dose diets indicating that kidney may be a target organ for Ni toxicity[31]. Similarly chromium also exhibited toxic effects on the fish body[32]. Lowest contents of chromium were found in muscle while gill, liver, kidney and digestive tract contained most[33].

Penetration of epithelial membranes by uncomplexed metal ions appears to involve special transport associated with a carrier molecule. Such a mechanism is necessary for toxicants that lack sufficient lipid solubility to move rapidly through cell membranes. Ingested food is a significant source of metals assimilated by aquatic organisms and metallothioneins also regulate the form of metal that passes from mucosal cells into the circulatory fluid. The results of the present study can be used to understand the interaction effects of metals on organisms in the field studies and waste water treatment[34,35].

CONCLUSION

Among the individual metals tested, Chromium was more toxic than nickel to the fingerlings of *C. mrigala.* Cr+Ni was more toxic to the fingerlings than that of Ni+Cr*.*

SIGNIFICANCE STATEMENT

This study discovered the mixture toxicity of nickel and chromium to the Indian major carp, *C. mrigala* that can be beneficial for metal interaction studies. This study will help the researchers to uncover the critical areas of effects of mixtures of metals that many researchers were not able to explore. Thus a new theory on interaction effects of nickel and chromium on fish may be arrived at.

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