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Chromium toxicity in fish: A review article

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Abstract
The aim of this review paper was to assess the toxicological impacts of Chromium in fish. Chromium apart from being an important metal presents a substantial threat to aquatic life. It destabilizes the ecosystem due to their toxic impact on biota and bioaccumulation in certain organisms. Occurrence of chromium varies in fishes, depending upon their age, development as well as other physiological variables. It also produced cytotoxicity and detrimental impact on behavior of fish such as hypertrophy and paraplegia at gill epithelium, uneven swimming and suspended feeding. Various research studies indicated adverse effects of chromium in fish at hematological level like anemia, thrombocytopenia, decrease in hemoglobin and total erythrocytes count. At bio-chemical level, mostly decline in the contents of glycogen, lipids and proteins was observed. The review paper will be helpful for scientific community in restoring the chromium contaminated water bodies.

Keywords: Chromium, toxic effect, hematology, behavior, review paper

1. Introduction

As a consequence of human activities like mining, improper waste disposal and fuel combustion, our environment is getting to be more and more contaminated with toxic heavy metals. The aquatic environment receives wastes and might be the ultimate depository for these anthropogenically remobilized heavy metals [1]. Heavy metals are defined as metallic elements that have a relatively higher density in contrast to water [2]. They are also regarded as trace elements [3]. Many factors enhances their detrimental effect and may involve the age of particular species, sex of an individual, concentration of dose, route of exposure as well as various biological and physiological adaptations perform an essential part [4].

On account of their high level of toxicity chromium, lead, mercury, arsenic and cadmium, rank among the priority metals that are of great health significance. These metallic elements are viewed as systemic toxicants that are known to incite numerous organ damages, even at lower levels of exposure [1]. Heavy metals influence cellular organelles and various enzymes involved in metabolic process, detoxification, and damage repair [5]. Metal ions also damages DNA molecule and nuclear proteins that may possibly lead to carcinogenesis or apoptosis [6].

Heavy metals are environmentally present everywhere, readily get dissolved in water and are the major persistent element in the aquatic ecosystem. The major component of most aquatic habitats is fish and they are considered as bio-indicator of heavy metal levels in aquatic environment [7, 8]. The fresh water ecosystem occupies an extremely small area in comparison to marine ecosystem. Fresh water resources now a day’s degraded at a very large scale, due to water pollution [9]. Developing countries are facing the problem of water pollution due to rapid spread of industrialization and civilization. These industries produce large amount of polluted products especially heavy metals that are constantly drained untreated into nearby rivers. The impact of heavy metals on water ecosystem has turned out to be a global concern [10].

Fishes have actually been utilized as test organisms for acute toxicity bioassays due to several benefits. According to U.S. Environmental Protection Agency, they are conveniently maintained under research laboratory conditions, and are sensitive to a variety of pollutants as well as readily available throughout the year from both commercial as well as natural resources [11]. This section of review gives an analysis of the sources of chromium in different environments and its mechanism of toxicity to fish fauna.

Sources

1.1 Natural occurrence

Chromium is a glossy, steel-gray, crystalline metal having an atomic number 24 and density 7.14 g/ml [12, 13]. In nature it is present in Earth Crust in several oxidation states.

But most abundantly present in trivalent (+3) and hexavalent (+6) oxidation states in the environment [14]. Concentration of chromium in soil varies from 1 to 3000 mg/kg, 5 to 800 µg/L in sea water, and 26 µg/L to 5.2 mg/L in rivers and lakes [15]. Chromite ($FeOCr_2O_3$ or $FeCr_2O_4$) is the most crucial ore mineral [16]. It can also be found as outcome of bothmarine or terrestrial volcanic eruption [17].

1.2 Commercial importance
Various forms of chromium like sodium chromate, ferrochrome, dichromates are used on commercial scale [18, 19]. Its various compounds are used in stainless steel welding process, tanning leather, dyes and pigments, timber preservation [20, 21]. It also acts as an anticorrosive agent in boilers [22].

1.3 Bioaccumulation
The bioaccumulation of chromium depends upon size and organs. With subsequent increase in size and dimension, the concentration of chromium in soft tissues and shell is reduced substantially. Chromium accumulation occurs differently in various sorts of tissues. Its concentration have found to be highest in gills, kidneys and liver of fish while hardly any tendency for chromium accumulation in muscular tissues. Both physical and chemical properties of water and seasonal changes are the main factors accountable for the intensification of heavy metals in various types of fish tissues [23].

1.4 Toxicity and effects
Beside the role of Cr (III) in metabolism of glucose, fats and proteins in animals and humans it has distinct toxicological features [24]. In humans and animals high level of chromium (VI) in drinking water causes tumors in stomach [25]. Cr (VI) can be reduced to Cr (III) and in this form toxicity is not found as it cannot be transported inside the cells [26]. Cr (VI) enters many types of cells and under various physiological conditions produces reactive intermediates that may interrupt cellular integrity and numerous functions [27, 28].

1.5 Abiotic and biotic factors
Chromium toxicity in aquatic ecosystem depends on both biotic as well as abiotic factors. Biotic factors consist of age, developmental phase of an individual and also type of species. While the abiotic factors comprises concentration and oxidation state of Cr, temperature, pH, alkalinity and hardness of water. Furthermore, Chromium toxicity is directly related with the concentration and temperature, any increase in these parameters boosted its toxicity i.e. raised with the increase of concentration as well as temperature but declines with increasing salinity and sulfate concentration [5]. Cr toxicity is also influenced by the pH of the water. In contrast to seawater, interstitial waters and estuaries may experience variation in pH and represents different levels of chromium toxicity [16].

The concentration of chromium in lakes and rivers ranges from 1 to 10 ug/L and EPA proposed level for protection of aquatic life and human health are 50 to 100 ug Cr/L. Numerous fish species have fatal effect of chromium like lymphocytosis, anemia, eosinophilia, bronchial and renal lesions. Its high concentration can harm the gills of fish that swim near the point of disposal of metal products in surface waters [27]. This section of review evaluates the acute and chronic hazardous effects of Cr to numerous fish species.

1.6 Acute Effects
Various research studies indicated that chromium exerts its toxicity across various fish species at different functional levels. Different species of fish namely dace, perch, stickle back, roach and rainbow trout was investigated for Cr (VI) sensitivity and it was found that even at minute concentration rainbow trout is 1.16 to 2.52 times more sensitive as compared to other test species [29]. The root cause of fatality is multiple and depends on time-concentration combinations. Tables 1 summarize the results of various researchers which clearly demonstrate the acute effects of Chromium to fish in fresh water ecosystem.

Table 1: Acute effects of chromium in various fish species.

Fish species	Chromium	Acute effects	References
Salmo gairdneri	$0.005 mgL^{-1}$	Impact on fertilization	[30]
Tilapia sparrmanii	$0.098 mgL^{-1}$	Blood clotting time decreases	[31]
Tilapia sparrmanii	$0.0098 mgL^{-1}$ at pH 7.4-9.0	White blood cells, red blood cell counts & Hb concentration decreases. ALA-D activity also increases	[32]
Sccobranchus fossilis	$0.1\text{-}3.2 mgL^{-1}$	Increases in spleen to body ratio, Hb, WBC, PVC, RBC, MCV and splenocytes. Decreased antibody production and susceptibility to bacteria increases	[33]
Periophthalmus dipes	$5\text{-}15 mgL^{-1}$	Decrease in ion dependent ATPase activity	[34]
Labeo rohita	$39.40 mg L^{-1}$ 96 h-LC50	Decrease in glycogen content, total lipid content and protein content of muscle, gill and liver.	[35]
Carassium auratus	250 µM	Decrease in cell viability. Increase in ROS.	[36]
Colisa fasciatus	$60 mg L^{-1}$	Liver glycogen content decreases. Hyperglycemic response.	[37]

1.7 Effect of pH
The degree of toxicity is also related to the pH of water. In a recent research study conducted on *Nuria denricus* (teleost fish) chromium toxicity was found to be greatly affected by slight changes in pH [38] and these results are consistent with another study conducted on the rainbow trout representing variable vulnerability to Cr at various pH values respectively [39]. These studies signify that at pH 7.8 a significant amount of Cr mount up in the internal organs while at pH 6.5 the gills retained enormous amount of Cr [40]. Similarly another study on young rainbow trout revealed that Cr toxicity was 50-200 times much higher at pH 6.4 to 7.4. Also several relative studies evidently revealed that Cr accumulates more in gills as compared to other organs [41].

1.8 Cytotoxicity
Cr wastes exposure to fish in aquacultures demonstrates cumulative harmful effects. Steinhagen *et al*. examined the carp (*Cyprinuscarpio*) derived immune cells and subjected these cells to Cr (VI). The results revealed a decrease in the activation of mitogen induced lymphocytes, a remarkable change in neutrophils shape and phagocyte function. The altered lymphocyte and neutrophil functions reflected the diminish power of resistance to certain pathogens under chronic Chromium challenges in fishes [42].

Another study was conducted on rainbow trout and the effect of cyanide and several heavy metals on fertilization was investigated, the sperms of Cr-exposed fish even at the lowest concentration of 5 μg L−1 showed relatively high sensitivity, while the ova were only a little sensitive, representing differential toxicity of the chromium metal to gametes [30].

Tan *et al.* [43] assessed the sensitivity of six fish cell lines GCF (grass carp fins), CIK (*Ctenopharyngodon idellus* kidney), EPC (*Epitheliomapapulosum, cyprini*), CCO (channel catfish ovary), BB (brown bullhead caudal trunk), and FHM (fathead minnow muscle) to four heavy metals cadmium, chromium, zinc, and copper. After 24h exposure to selected metal concentrations, the cells were differentiated on the basis of their morphology, viability and proliferation. The results demonstrated that both Chromium and Cadmium exerts a more distinct cytotoxic effect as compared to other two metals. Furthermore the EPC cells reflect more sensitivity to Chromium and Zinc.

1.9 Biochemical toxicity

Various studies revealed that at both biochemical and enzymatic levels Cr induces deleterious effects. Bozcaarmutlu and Arinç [44] carried out study on *in vitro* effect of five metals namely chromium, mercury, nickel, cadmium and zinc on the properties of an enzyme NADPH-cytochrome P450 (CYP450) reductase in leaping mullet (*Liza saliens*). These findings indicated that Cr inhibits the activity of CYP450 reductase in fish.

Chromium exerts its effect on the epithelial cells of the intestine and can also modify the rate of glucose transport. One of the study conducted on the intestine of rainbow trout reflected a low rate of glucose absorption by epithelial cells [45]. In another research study on the impact of different concentrations of Cr on glucose intake in *Channa punctatus* highlighted enormous absorption rate at 0.001 mM [46]. On the contrary, *Colisa fasciatus* exposed to Cr (VI) displayed depleted glycogen levels in liver and hyperglycemic conditions in blood [47]. Some biochemical profiles were investigated at lethal concentration of 39.4 mg L−1 Cr in various organs of *L. rohita* like gill, liver, and muscle. The lipids, glycogen and protein levels were significantly diminished as a result of metallic stress, hypoxic or anoxic conditions in all three organs [35]. The outcomes are in agreement with an earlier study who reported that *L. rohita* exposed to Cr show evidence of hypoxia with lower oxygen utilization [48].

Osmoregulatory function of various species of fish is affected under the influence of trivalent chromium. Fish *Cyprinus carpiovar communis* was exposed to chromium sulphate to test the levels of plasma electrolytes and it was found that the metal has the potential to bring fluctuations in the osmoregulatory functions of fish [49]. Cr (VI) also retards the action of enzyme ATPase in gills, kidney, and intestine of coastal teleost [43]. Similar results were also reported on the failure of osmoregulatory and respiratory functions in rainbow trout [50].

1.10 Detrimental impact of chromium on fish hematology

Various modifications in the hematologic indices of freshwater fish subjected to Cr (VI) are well recognized. Studies on *Labeo rohita* revealed a significant decreases in the hemoglobin percentage, anemic state of the fish (attributable to iron deficiency) and the total erythrocyte count after exposure to Cr (VI) at concentration 39.4 mg L−1 [51]. Blood coagulation studies on *Tilapia sparrmanii* indicated that when it is exposed to potassium dichromate it results in an increase of clotting time at different pH levels [31]. It also causes thrombocytopenia, a deficiency of blood platelets.

Mamta *et al.* [52] investigated hematological parameters of freshwater fish Channa after exposure to chromium trioxide. The exposed fish represented an increase in Hb % while shown diminishing level of TLC. TEC and PCV boosted between 7 to 30 days but it turn down after 60 days. Comparatively CT and ESR revealed a declining tendency from 7 to 30 days but abruptly increase in these parameters were noted after 60 days.

In another study lethal effect of Cr was noted in fresh water fish, *Labeo rohita* for 7 and 30 days. The decrease in hematological parameters recommended that the exposed fishes after exposure to Chromium become anemic and their glycogen, protein and cholesterol level decreased significantly [53].

1.11 Chronic Effects

Long lasting impact of chromium in several species of fish at various functional levels is shown in Table 2 which clearly reflects concentration-duration relationships. The very early life phase of *Oncorhynchus tshawytscha* (Chinook salmon) from a polluted ground water subjected to Cr (VI) was assessed for 98 days [54]. No substantial change was observed in the behavior, survival rate, growth and development of fish. Survival of the fish and its growth depends upon the dose and period of exposure to chromium. Farag *et al.* [55] reported that Chinook salmon exposed to increasing Cr concentrations for 105 to 134 days influenced both growth and survival rate. Physiological modifications as well as DNA damages occurred at a concentration of 24 μg L−1.

The African catfish (*Clarias gariepinus*) when exposed to ≥ 36 mg L−1 Cr for five days affects the embryo survival and decreased the larval growth [56]. In another research study, increasing Cr concentrations at 0.02, 0.2, or 2 mg L−1 in rainbow trout confirmed more metal susceptibility at lower pH (6.5) rather than at higher pH (7.8). pH slightly affected the duration of embryo hatching, but no growth was affected in that experiment [57].

Hexavalent chromium is more detrimental and it may suppress the *in vivo* immune response more effectively as compared to trivalent chromium. *Oreochromis mossambicus* (African mouth breeder) was exposed to both Cr (VI) as well as Cr (III) but weight reduction in the spleen was more in Cr (VI) as compared to Cr (III). Both the groups have reflected decline in lymphocyte and leukocyte counts [56]. Continual exposure to Cr can alter the enzyme activities like succinate dehydrogenase, pyruvate dehydrogenase, and lactate dehydrogenase in brain, kidney and liver etc.

Table 2: Chronic effects of chromium in different fishes.

Exposure type	Fish species	Chromium concentration	Chronic toxicity	References
In vivo	*Oreochromis mossambicus*	7.5 μg/fish Cr(VI) 100 μg/fish Cr(III)	Decrease in antibody production. Reduction in splenic weight. Decrease in lymphocyte count	[56]
In vitro	*Oncorhynchus tshawytscha*	24-120 μg L^{-1}l 54-266 μg L^{-1} 24 μg L^{-1}	Decrease in survival rate. Decrease in growth rate. DNA damage	[55]
In vitro	*Cyprinuscarpio*	1010 μgL^{-1} 38 wk	Diminished humoral responses. Reduced serum proteins level by 25%.	[58]
In vitro	*Salmogairdneri*	0.2 mg L^{-1} pH 6.5; 2 mg L^{-1} pH 7.2 and pH 6.5	Induction of mortality rate. Effect on embryo hatching.	[57]
In vitro	*Clarias gariepinus*	>/ = 36 mg L^{-1} >/ = 11 mg L^{-1}	Decreased embryo survival rate. Decreased larval growth.	[57]
In vitro	*Nuria denricus*	0-100 mg L^{-1}	Erosion of fin and fin rays	[59]
In vitro	*Channa punctatus*	2.6 mg L^{-1} 60 days exposure 120 days exposure	Increased muscle and blood lactic acid. Decreased liver lactic acid and glycogen. LDH activity inhibited in liver and kidney. PDH and SDH activities inhibited in all the tissues except muscle Glycogen increased in liver but decreased in muscle. LDH inhibited in all six tissues. Decreased activity of PDH in liver, intestine, gill, muscles; SDH elevated in muscle but inhibited in other tissues	[52] [52]

1.12 Effect of chromium on fish behavior

When fish is initially encountered to chromium it undergoes various behavioral modifications like suspending feeding behavior, uneven swimming and accelerated operculum. It may triggered structural changes such as hypertrophy and paraplegia at gill epithelium and weakens the body immune system [60, 61].

Nisha *et al.* [62] determined the impact of chromium trivalent and hexavalent toxicity on the behavior of *Danio rerio* (zebra fish). Erratic motion, mucus discharge, opening mouth for gasping, color and shade alterations, irregular swimming was usually pointed out. Ali *et al.* [63] investigated the behavioral changes in gold fish (*Carassius auratus*) and noted that all the fingerlings come to the corner of the aquarium and there was also appetite decrease due to chemical effects.

In another study after exposure to hexavalent chromium modification in behavioral patterns of *Channa punctatus* was studied. The exposed fish showed irregular swimming and became sluggish. The changes in gills were illustrated by epithelial hyperplasia, oedema, epithelial lifting and necrosis [64].

2. Conclusion

Aquatic pollution is going to be a substantial threat to the ecosystems. This review indicates a direct correlation between the survival and concentration of Cr-III and Cr-VI to fish population. Cr (VI) is comparatively more toxic to fresh water fish species. Cr toxicity was greatly affected by slight change in pH. There is a need for monitoring the industrial effluents for Cr concentration level. The heavy metals have toxic effects on various organs of fishes but higher toxicity of Cr was noted in liver and gills. Various research studies indicated adverse effects of chromium in fish at hematological and bio-chemical level, subsequently declining the level of proteins and glycogen. The decrease in total protein content in turn affects the enzyme mediated bio defense mechanisms of the fish. Continual exposure to Cr changes various enzyme activities like succinate dehydrogenase, pyruvate dehydrogenase, and lactate dehydrogenase in kidney, brain, and liver. While ATPase activity diminished in gills, kidneys and intestine. Chronic exposure to Cr may also induce irregular behavioral responses in various species of fish. Therefore, the root causes of fish death are multiple but Cr-induced toxicological pathology is significantly affected by particular factors as species type, age, environmental conditions, exposure time and concentration. This review offers a base for comprehending the potential impact, as well as for advancing our data about the ecotoxicology and risk assessment of chromium.

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