Toxicology Reports 5 (2018) 440–447

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

# A comprehensive review on chromium induced alterations in fresh water fishes

A. Bakshi[a], A.K. Panigrahi[b,*]

[a] Ecotoxicology, Fisheries and Aquaculture Extension Laboratory, Department of Zoology, University of Kalyani, Kalyani, 741235, India
[b] Department of Zoology, University of Kalyani, Kalyani, 741235, India

ARTICLE INFO

*Keywords:*
Chromium
Fish
Toxicity
Lethal concentration
Fresh water
Acute
Chronic
Risk assessment
Hexavalent
Trivalent

ABSTRACT

Chromium is considered as one of the most common ubiquitous pollutants in the aquatic environment, but the pure metallic form is absent naturally. There are three oxidation states in case of Chromium *viz.*, Cr (II), Cr (III), Cr (VI). Among which Cr (II) is most unstable. Cr (III) and Cr (VI) are the stable oxidation state of Chromium in the environment. Being one of the commonly used metals Chromium and its particulates enter the aquatic medium through effluents discharged from different industries like textiles, tanneries, electroplating workshops, ore mining, dyeing, printing-photographic and medical industries. Among these, hexavalent chromium is considered as the most toxic form because it readily passes cellular membranes and then reduced to trivalent form. This trivalent chromium combines with several macromolecules including genetic material inside the cytosol, and is ultimately exposes the toxic and mutagenic alterations due of chromium toxicity. Chromium is taken up either through gastrointestinal tract or respiratory tract. The amount varies depending upon the medium and the form of chromium. In this review, an attempt has been made to accumulate the mammoth available data regarding impact of chromium on fresh water fishes into a systematic representation. The main objective of the review is to provide a future guideline for the scientific community and public officials involved in health risk assessment and management ensuring a better environmental condition for human health.

## 1. Introduction

Nowadays, pollution, especially in aquatic medium, due to heavy metal contamination has become a great issue of concern to the environmentalists. Extensive industrialization, and rapid urbanization have measurably imposed adverse impact on the water quality of lakes, ponds and rivers all over the world [1]. The problem has become more hazardous because the industries often release their wastes containing metallic contaminants into the environment which exceeds the permissible limit [2]. In spite of the progress in environmental waste management system, the complications due to heavy metal discharge are still posing immense adverse impact on aquatic biolife [3]. Especially lithophilic or class- B metals are marked to be more dangerous to the ecosystem [4] and core group of aquatic pollutants [5] because of their long persistence (or long half-life), properties of bioaccumulation, biomagnification and non-biodegradibility [6] as they can destroy the framework of species diversity [7,8]. Heavy metals can show high toxicity even in low concentration producing cumulative deleterious effects in an aquatic ecosystem [2].

Chromium, one of the most common ubiquitous metal pollutants in the environment [2], enters the aquatic system through effluents from industries like textiles, tanneries, mining, electroplating, dyeing, printing, photographic printing, pharmaceuticals, stainless steel manufacturing and rubber manufacturing industries [9–12,4].

As an element Chromium is very stable, but is not usually found pure in nature. The principle ore of Chromium is Chromite, from which ferro-chrom alloys and chromium metal are obtained. The chemical formula of the ore is $FeO{\cdot}Cr_2O_3$ [13,14]. The metal may be present in divalent ($Cr^{+2}$), trivalent ($Cr^{+3}$) and hexavalent form ($Cr^{+6}$) forms, $Cr^{+3}$ and $Cr^{+6}$ being the most predominant and stable forms [2]. In biological system, Chromium is usually found in the trivalent form [15], and this form ($Cr^{+3}$) is reported as an essential element in mammals as it takes effective role in glucose, lipid, and protein metabolism [16]. Due to poor membrane permeability, non-corrosiveness and very less tendency to biomagnify in the food chain, the toxicity of trivalent chromium is very low.

Hexavalent chromium is considered to be more toxic than trivalent form because of its easy permeability through the cell membrane [4]. Hexavalent Chromium has two main oxy-anion forms $CrO_4^{-2}$ and $CrO_7^{-2}$ which are involved in reversible transformation [14].

* Corresponding author.
*E-mail addresses:* avijit86@klyuniv.ac.in (A. Bakshi), apanigrahi@klyuniv.ac.in (A.K. Panigrahi).

https://doi.org/10.1016/j.toxrep.2018.03.007
Received 11 May 2017; Received in revised form 20 February 2018; Accepted 5 March 2018
Available online 06 March 2018

$$2\ CrO_4^{-2} + 2\ H_3O^- \leftrightarrow Cr_2O_7^{-2} + 3\ H_2O$$

After entering the cell, the hexavalent chromium readily reduces to its trivalent form and complexes with intracellular macromolecules even with genetic materials [17,18]. The easy permeability and biotransformation property of hexavalent chromium is ultimately responsible for its toxicity and mutagenic activity [19,20]. Beside these, hexavalent chromium is reported to be potential carcinogen and teratogen [13]. In nature, Chromium concentration in surface water limits between 1 and 10 μg/l, whereas provisional guideline value of Chromium in surface water is 50 μg/l [21] though chromium is not found in pure metallic form [2]. According to WHO and ISI, the permissible limit of chromium in drinking water is 0.1 mg/l and 0.05 mg/l respectively [22]. According to the reports of WHO and FEPA or Federal Environmental Protection Agency, the maximum allowable limit of chromium in fish food is 0.05–0.15 mg/kg body weight [23,24].

## 2. Materials and methods

Chromium pollution in aquatic environment is a major issue of concern as it is posing adverse impacts on human health and society directly or indirectly. An attempt was made to prepare an utmost consolidated manuscript on the research topic. This manuscript was prepared through extensive review to compile and consolidate the maximum number of scientific data to make the review process a comprehensive one. Data were collected, from science journals of repute, published reports from international agencies and doctoral theses. Importance was given to the reproducible articles which are indexed in science journal database like scopus, copernicus, PubMed *etc*. Articles featuring ambiguous working methodologies were excluded fastidiously. Keywords were meticulously chosen and searched based on methodological scientific strategies. Key words, for searching; were as follows: chromium; freshwater fish; histopathology; genotoxicity; beravioural; enzymological; alteration; *etc*. Data related to freshwater fishes were on chosen for the manuscript preparation. Scientific names of the experimental organisms were carefully screen before documentation and data related to test specimen other than freshwater fishes were strictly excluded. The work of the author was also described and incorporated throughout the manuscript.

Experimental findings were included at different parts of the present review to enhance the essence of the article.

## 3. Chromium in the environment

Chromium (atomic weight of 51.996), in the crystalline form, is a steel-gray, lustrous, hard metal characterized by an atomic number of 24, a density of 7.14, a melting point of 1900 °C, and a boiling point of 2642 °C [25]; Langard and Norseth 1979). Naturally, four Chromium isotopes are found *viz*., Cr-50 (4.3%), Cr-52 (83.8%), Cr-53 (9.6%), and Cr-54 (2.4%) and other seven are of anthropogenic origin [13].

Chromium is a widely spread industrial element coming from several industries like electroplating, alloy cast irons, paints, stainless steel manufacturing industries; chrome plating, rubber manufacturing industries; leather industry, wood treatment, dyeing factories *etc* [9–11]. It has also been reported that chromium is also released from textile, tanneries, mining, fertilizer, printing, photographic and pharmaceuticals industries [12,5]).

Chromium concentration in the air of small towns of India is reported to be about 0.02 μg/$m^3$ [14]. In commonly available natural water bodies, the concentration of Chromium ranges between 1 and 2 mg/l in dissolved form [26]. Trivalent and hexavalent, most available forms of chromium, can coexist in aquatic medium with little organic matter; whereas, hexavalent chromium is usually predominant form in marine water [25]. The hexavalent form of chromium exists in the form of chromate ($CrO_4^{-2}$), hydrochromate ($HCrO_4^{-1}$), or dichromate ($Cr_2O_7^{-2}$) in dissolved condition as a component of a complex anion that varies with pH [13].

Water quality assessment of river Rupsha and river Bhairab of Bangladesh have been 0.021 mg/l and 0.013 mg/l respectively [27]. Amount of chromium concentration at different sampling stations of River Ganges have been estimated by various authors. A number of samples from different sampling site like Bhagalpur, Buxar, Ballia have been found to contain chromium below detectable limit or BDL [28]. However, Chromium concentrations at sampling sites like Dakshineswar, Palta, Uluberia, Baharampur have been found to be 16–22 μg/l, 13–21 μg/l, 13–24 μg/l and 10–18 μg/l respectively [29,30,28]. According to WHO, average chromium concentration in river water sample is 0.050 mg/l [31]. Species Mean Acute Value (SMAV) for hexavalent chromium in case of freshwater fishes has been reported to be ranged from 30.0 mg/l to 139.90 mg/l [32]. According to FDA guideline limit of hexavalent chromium in fish tissue should remain within 12–13 mg/kg body weight [33].

## 4. Absorption, distribution, metabolism and excretion of chromium

As stated earlier, hexavalent chromium is the toxic form of chromium whereas, trivalent form is relatively non-toxic. The reason might be its higher solubility or higher mobility rate in aquatic medium [34]. Cr(VI) has been reported to be different toxicologically from other heavy metals by Doudoroff and Katz [35] as it can readily penetrate the gill membrane by the process of passive diffusion which is mediated by pH of the system. Cr(VI) is allowed to enter easily into the cytoplasm of aquatic organisms [36]. Thus, it can be said chromium enters the body of the fishes mainly through the gills [20]. Obasohan [37] has reported that chromium concentrations in fish tissue arise through bio-magnification at each trophic level and especially carnivorous bottom feeders concentrate higher amount of metal.

Dhara [38] has cited that chromium (VI) gets associated with the plasma protein and involves in transportation after passing the cell membrane through sulphate ion channel. Then the metal biologically gets accumulated in various organs. The general pattern of distribution of Cr(VI) in fishes is as follows: Gills > Liver > Skin > Muscles [39]. Bio concentration of chromium in the fish muscle, gills and liver has been reported to increase depending on the concentration in the medium and the exposure time [40]. It has been reported that range of pH has tremendous influence in determining the bioavailability of metal to the fish. This fact is substantiated by Abbasi et al. [41] on a teleost, *Nuria denricus*. Van Der Putte et al. [42] has experimentally shown that Chromium accumulation in the tissues of Rainbow trout (*Salmo gairdneri*) is highly effected by pH of the surrounding water. They have also reported that gill contains more amount of chromium at pH-6.5 than other internal organs whereas, reverse is evident at pH-7.8. Comparative studies have revealed that Chromium concentration remains higher in gill than other organs at same pH [43].

After getting entry, the hexavalent chromium undergoes metabolic reduction within the cell. The ultimate result of this phenomenon directs the predominance of trivalent chromium in the cytoplasm [44]. During these metabolic reactions, different reactive intermediates are released which are reported to be detrimental to ensuring the stability of DNA helix [45], causing fatal effects in the affected individual. The same authors have also reported that migration of various intermediate chromium metabolites to the nuclei and interaction with DNA are evident during this process causing the final negative effect.

The primary storage and detoxification site for chromium is said to be liver [46] in experimental condition. Higher concentration of metals is evident in bile of the experimental organism (*Clarias batrachus*) being exposed to metal contaminated food and environment [38]. Gauglhofer and Bianchi [47] reported that this storage is stabilized mainly by protein linkage or small peptide linkage such as glutathione linkage. In case of fishes the main elimination route of chromium or its compounds is through feaces [38].

**Table 1**
96 h $LC_{50}$ Values (mg/l) of Chromium toxicity for different experimental fishes.

| Sl. No. | Test Organisms | Time of Exposure | Test Conditions and Organism Size (if available) | 96 h $LC_{50}$ Values of Cr Concentration (mg/l) | References |
|---|---|---|---|---|---|
| 1. | *Labeo rohita* | 96 h | Renewal, (27.5 ± 1)°C | 39.40 | [53,2] |
| | | 96 h | Static; (22.7 ± 1)°C; fingerlings;Temp- (°C) 27.4- 33.7; pH 7.4–7.8; D.O. (4.6–6.2) mg/l; T.Alk (140–190) mg/l;T Hardness (120–230) mg/l | 30.36 | [14] |
| | | 72 h | | 38.19 | |
| | | 48 h | | 53.42 | |
| | | 24 h | | 58.76 | |
| 2. | *Pimephales promelas* | 96 h | Flow through; 25 °C | 61.00 | [54] |
| | | | Flow through; 15 °C | 48.00 | [55,2] |
| 3. | *Channa punctatus* | 96 h | Static; (29.8 ± 1)°C | 50.00 | [90,2] |
| 4. | *Salvelinus fontinalis* | 96 h | Flow through; 12 °C | 59.00 | [2] |
| 5. | *Catla catla* | 96 h | Static; (28 ± 1)°C | 100.00 | [2] |
| | | | NM | 31.41 | [56] |
| | | | Fingerlings;Age group 60 days | 57.49 | [50] |
| | | | Fingerlings;Age group 120 days | 71.97 | |
| | | | Fingerlings;Age group 240 days | 101.58 | |
| 6. | *Carassius auratus* | 96 h | Renewal; 26–28 °C; | 85.70 | [49,2] |
| 7. | *Salmo gardnerii* | 24 h | NM[a] | 180 | [57] |
| | | 96 h | Renewal; early stage fry | 44.00 | [58] |
| | | | Flow through; fry | 69.00 | [2] |
| 8. | *Cirrhinus mrigala* | 96 h | NM[a] | 34.00 | [59,60] |
| | | | Static; early stage fry | 79.56 | [50] |
| | | | Static; Age 60 days | 85.99 | |
| | | | Static; Age 120 days | 113.35 | |
| | | | Static; Age 240 days | 144.49 | |
| 9. | *Cyprinus carpio* | 96 h | Static; Early stage fry | 155.00 | [10] |
| | | 84 h | | 156.35 | |
| | | 96 h | Static; Age-60days | 87.96 | [61] |
| | | | Static; Age-90days | 102.87 | |
| | | | Static; Age-120days | 128.89 | |
| 10. | *Labeo bata* | 96 h | Static; Early stage fry | 7.33 | [56] |
| 11. | *Puntius sarana* | 96 h | Static; Early stage fry | 10.37 | [56] |
| 12. | *Heteropneustes fossilis* | 96 h | Static; Fingerlings; Temp-(°C) 27.4–33.7; pH (7.4–7.8) D.O. (4.2–6.1) mg/l; Total Alkalinity (120–180) mg/l; T. Hardness (100–220) mg/l. | 33.39 | [14] |
| | | 72 h | | 43.03 | |
| | | 48 h | | 53.42 | |
| | | 24 h | | 58.75 | |
| 13. | *Colisa fasciatus* | 96 h | NM[a] | 60.00 | [62] |
| 14. | *Oreochromis nilitica* | 96 h | Age gr. 60 days | 119.52 | [61] |
| | | | Age gr. 90 days | 139.29 | |
| | | | Age gr. 120 days | 164.36 | |
| 15. | *Puntius conchonius* | 96 h | NM[a] | 331.40 | [63] |
| 16. | *Clarius batrachus* | 96 h | NM[a] | 36.65 | [64] |

[a] NM–Not mentioned.

## 5. Toxicity of chromium

The aquatic toxicology of Chromium depends on several biotic factors *viz.,* experimental species, age and developmental stage as well as different abiotic factors *viz.,* temperature, concentration of Cr, oxidation state of Cr, pH, alkalinity, salinity, and hardness of water *etc*. However, lethal and sub-lethal concentrations of the metal and its speciation also regulate the sensitivity of the experimental organisms [2]. The later part of the review concentrates on the acute and chronic effects of exposure to various chromium concentrations on fishes.

### 5.1. Acute toxic effects of chromium

Short term exposure or acute exposure to different concentrations of chromium may bring alterations in fresh water fishes in various aspects.

#### 5.1.1. Effect on behavior of the fish

In an acute study with different concentrations of potassium dichromate solution, it has been revealed that Rohu fingerlings (*Labeo rohita*) lose its body balance after 24 h when exposed to 28.99 mg/l concentration. Activeness and swimming rate of the fingerlings have been found to vary after the 24 h when exposed to 56.59 mg/l concentration. Fingerlings are found to be restless with highly decreased body balance and higher rate of mucus secretion at the aforesaid condition [14]. In case of *H. fossilis* fingerlings, it is observed that their activeness increases slightly with elevated swimming rate after 24 h at 56.59 mg/l chromium concentration. Others parameters have been found to remain normal even in higher concentration (*i.e.*, higher than 56.59 mg/l concentration). Body balance of the fingerlings has been found to start decreasing after 48 h of exposure to 42.45 mg/l and 56.59 mg/l chromium concentration. After 96 h of exposure, fingerlings have been found to face the problem of imbalance in all the test concentrations. Mucus secretion rate has been found to increase in all concentrations after 96 h of exposure [14]. In another experiment with *Channa punctatus,* it has been reported that erratic swimming, hyperactivity, loss of balance, increased rate of swimming, tendency of convulsion and increased opercular beat rate (the parameters of behavioural study) are found to be altered than control system (0 mg/l) when exposed in 20 mg/l and 40 mg/l concentration of potassium dichromate [48]. Hyperactivity and erratic swimming of freshwater fishes have been found to be most phenomenon while exposed to chromium contaminated environment [49,2,50,4,38,14].

#### 5.1.2. Effect on mortality of fishes

To predict and prevent the immediate toxic effect of any xenobiotic in any aquatic system, short-term acute toxicity test on experimental organisms is regarded as one of the best tools. Acute toxicity test can provide environmentally relevant data very rapidly. Short term toxicity test on different aquatic organisms has shown that responses to any xenobiotic are especially organism specific. Bakshi [14] has reported that 96 h $LC_{50}$ Value is 30.36 mg/l and 33.39 mg/l in case of *Labeo rohita* (fingerlings) and *Heteropneustes fossilis* (fingerlings) respectively. Mishra and Mohanty [48] have reported that 50% lethal concentration value of chromium for the potassium dichromate salt is 41.75 mg/l in *Channa punctatus* (Bloch). The value is reported to be 39.40 mg/l in case of *Labeo rohita* [51]. Svecevicius [52] has designed an experiment to substantiate the aforesaid result. In the experiment five fish species *viz.,* rainbow trout, three-spined stickleback, roach, perch, and dace were exposed to acute concentrations of Cr(VI) which revealed that rainbow trout is 1.16–2.52 times more sensitive than the other experimental species to chromium. A review on acute toxicity of Chromium on different fish specimens has revealed that value of 96 h $LC_{50}$ Value is not fixed as changes occur according to metal type, different experimental conditions, age of experimental fish and different fish species. The exact causes of mortality due to heavy metal poisoning have been found to be multiple and depend on time concentration combinations [2]. Experimental condition also exerts some effect on the acute toxicity. For instance, fluctuations in pH, temperature and other water quality parameters have some influences on the value of $LC_{50}$ [42]. A List of 96 h $LC_{50}$ Value (mg/l) for different experimental fishes is given in Table 1.

#### 5.1.3. Effect on cellular system

Cytotoxicity has been reported by various authors in different studies along with bioconcentrating property of chromium. *In vitro* cytological investigations have revealed that metal toxicity exerts influences on several cytological parameters including measurement of death of cell, viability, cellular morphology, cell metabolism, cell attachment or detachment, cell membrane permeability, proliferation, and growth kinetics [65–67]. During a toxicological study on hepatocytes of gold fish, it has been established that exposure to 250 μM of hexavalent chromium secures reduction in cell viability and stimulates ROS production significantly, whereas, this experimental condition does not allow cellular calcium ion ($Ca^{2+}$) to alter homeostatic condition. In this study, lysosomal $Fe^{2+}$ pool and the mitochondria have been identified as the sources of ROS [68].

Tan et al. [69] has experimented the response of six fish cell lines to four heavy metal exposures. The cell lines have been indicated as GCF (grass carp fins), CIK (*Ctenopharyngodon idellus* kidney), EPC (epithelioma papulosum, cyprini), CCO (channel catfish ovary), BB (brown bullhead caudal trunk), and FHM (fathead minnow muscle). These cell lines are evaluated comparatively for their cytotoxic sensitivity to different metals *viz.,* cadmium (Cd), chromium (Cr), zinc (Zn), and copper (Cu). The cytomorphology, cell viability and proliferation after an exposure (for 24 h) to metal salts at selected concentrations have been estimated in that experiment. The test results have specified that all six fish cell lines are sensitive to all four metals. The inhibitory concentration (IC50) values for the metals have indicated that both Cr and Cd exert a more pronounced cytotoxic effect than the other two metal salts. Among the six experimental fish cell lines, the EPC cells have been found to be more sensitive to Cr and Zn exposure. The complete comprehensive study has established the fact that CIK, EPC, and CCO cell lines can serve as valuable bio-indicators for monitoring and assessing the acute toxicity of these metals at the cellular level in an aquatic environment.

#### 5.1.4. Effect on immune system

Prabakaran et al. [70] have designed an experiment to understand the immune response and non-specific immunity in the tilapia fish (*Oreochromis mossambicus*) exposed to sublethal concentrations of tannery effluent containing chromium (88.2 mg/l), calcium carbonate, and sodium sulphate. Both ELISA and bacterial agglutination assays have been used to determine the specific immune response of fish to heat killed *Aeromonas hydrophila.* Nonspecific immune mechanisms have been evaluated in terms of serum lysozyme activity, the production of intracellular reactive oxygen species (ROS), and reactive nitrogen intermediates (RNI) by peripheral blood leucocytes (PBL) during laboratory studies. The chronic exposure of fish to 0.53% concentration of tannery effluent significantly suppressed the antibody response, nonspecific serum lysozyme activity, and ROS and RNI production. Similar responses have also been detected in fish exposed to a low concentration of 0.053% (1% $LC_{50}$) concentration of tannery effluent, although to a lesser extent. The same authors have also stated that these kinds of studies can serve important factors discussing the role of immunological studies in monitoring fish health and risk assessment.

#### 5.1.5. Effect on biochemical condition of cell

Biochemical studies have revealed that alterations in concentration and activity of some enzymes are evident in chromium induced environment. Bozcaarmutlu and Arinc [71] have reported that chromium is a strong inhibitor of Cyt-$P_{450}$-reductase activity in fish independent of its oxidation states. Another study on Indian major carp has established

**Table 2**
Major histopathological alterations in different tissues of some fresh water fishes.

| Tissues | Major Alterations |
|---|---|
| Liver | Hyperplasia, Necrosis of hepatic cells, Cellular disorganization in *Labeo rohita* and *Heteropneustes fossilis* after 60 days exposure to 96 h 1/10th $LC_{50}$ [2,81,14]. Reduction of nucleus to cytoplasm ratio in liver cells in Rainbow Trout [80]. |
| Kidney | Highly fenestrated Bowman's capsule, Constricted lumen of Renal tube, Glomerular disorganization in *Labeo rohita* and *Heteropneustes fossilis.* after 60 days exposure to 96 h 1/10th $LC_{50}$ [14] |
| Intestine | Inner epithelial layers highly degraded in *Labeo rohita* and *Heteropneustes fossilis* after 60 days exposure to 96 h 1/10th $LC_{50}$ [2,14] |
| Muscle | Loosening of muscle fibre with increased space between fibres in *Heteropneustes fossilis* after 60 days exposure to 96 h 1/10th $LC_{50}$ [14] |
| Gill | High Lamellar degradation. Necrosis in epithelial cells. Thickening of blood vessels, Atrophied central axis after 60 days exposure to 96 h 1/10th $LC_{50}$ in *Labeo rohita* and *Heteropneustes fossilis* [82,2,81,14]. Hyperplasia of epithelial cells and epithelial lifting of secondary lamellae in Rainbow Trout [80] |

that Chromium is not significantly important for the activities of alanine amino transferase (ALT) or aspartate amino transferase (AAT) in *Labeo rohita* [5]. On the other hand, elevated levels of ALT and AAT have been reported at $LC_{50}$ (61 mg/l) of hexavalent chromium for 24 h and 96 h by Vutukuru et al. [5]. Glucose absorption is said to be dependent on chromium. The impact of Chromium on glucose uptake in *Channa punctatus* at different concentrations *viz.,* 10 mM, 1 mM, 0.1 mM, 0.01 mM, 0.001 mM, have been observed by Sastry and Sunita [72]. They have reported that the rate of glucose absorption becomes higher at 0.001 mM Chromium concentration. In an earlier study, Vutukuru [51] has estimated that the level of glycogen, lipids, and protein diminish in various organs like gill, liver, and muscle of *L. rohita,* exposed to lethal concentrations of chromium (39.4 mg/l). The possible reason may be metallic stress or the prevalence of hypoxic or anoxic conditions. Another study has revealed that trivalent chromium can alter the osmoregulatory function of various fish regulating the $Na^+/K^+$ concentration [91].

#### 5.1.6. Effect of chromium on endocrine disruption

Endocrine disruption tests are useful tools for toxicity study. Biomarkers for endocrine function like plasma cortisol, thyroid stimulating hormone (TSH), free triiodothyronine ($T_3$), and free thyroxin ($T_4$) have been observed to fluctuate in case of chromium exposure [2]. It has been experimentally established that plasma $T_4$ levels in eels decreases only when exposed to the metal [73].

Thus, it is evident that chromium contaminated aquatic environment induces several biochemical, cytological, physiological alterations in inhabitant's body even after a short-time exposure.

### 5.2. Chronic toxic effects of chromium

Long term exposure to hexavalent chromium exhibit several alterations in behavior, physiology, cytology, histology and morphology. Decrease in antibody production and lymphocyte count, reduction in spleen weight [74], DNA damage, decrease in Growth and survival rate [17], reduction in protein level, diminished humoral responses [75], increase in blood and muscle lactic acid [76,77], decrease in larval growth and embryo survival rate [78] and erosion in fin and fin-ray morphology [41] have been reported to be the major identified chronic effects of Chromium in different experimental conditions for different experimental fishes. Most of the aforesaid symptoms are found as concentration and duration dependent.

#### 5.2.1. Effect on physiology and growth

Farag et al. [17] have experimented with Chinook salmon exposed to a range of Cr concentrations (0–266 μg/l) and reported that the concentration of the metal seems to have an insignificant effect on growth. Increased concentration of chromium (from 24 to 120 μg and 54–266 μg/l) for 105–134 days exposure has been shown to affect both survival and growth rate significantly. Physiological alterations are also identified after exposure to ≥120 μg/l of chromium. Phenomenon of DNA breakage has been testified after exposure to a concentration of 24 μg/l. At the end of 105 days exposure during same experiment, lipid deposits have been observed with alteration in lipid peroxidation have been observed. In a separate study, Nguyen and Janssen [78] have observed that a five-day exposure to different experimental concentrations of chromosome results in significantly reduction in larval growth rate (≥ 11 mg/l) and also drops in embryo survival rate (≥36 mg/l) in case of African catfish (*Clarias gariepinus*). Van der Putte et al. [42] has reported that lower concentration of chromium at different pH values (0.2 mg/l at pH 6.5 and 2.0 mg/l at pH 7.8) induces mortality of embryo and mild problem in hatching. Glycogen content in gill, liver and muscles of fish *Labeo rohita* has been reported to decline after hexavalent chromium exposure [53]. In *Channa striatus,* Chromium intoxication lowers the glycogen level in gill, liver and kidney altering some biochemical mechanisms of the fish [79]. Sastry and Sunita [76,77] has reported about the increased muscle and blood lactic acid, decrease liver lactic acid and glycogen, inhibited LDH activity in liver and kidney, and inhibited PDH (pyruvate dehydrogenase) and SDH (succinate dehydrogenase) activities in all the tissues except muscle in *Channa punctatus* after 60 days exposure to 2.6 mg/l concentration.

#### 5.2.2. Effect on histopathology

Several authors have reported about the histopathological changes in the tissues like liver, gill, kidney, muscle *etc*. of experimental fishes after chronic exposure to sublethal concentrations [2,80,81,14]. Major alterations in different tissues of some fresh water fishes are listed in Table 2.

#### 5.2.3. Effect on hematology and immune system

Immuno-haematological study on African mouth breeder (*Oreochromis mossambicus*) by Arunkumar et al. [74] has revealed that several phenomena like decrease in lymphocyte and leucocyte count, reduction in spleen weight, suppression in *in vivo* immune responses *etc*. are evident in hexavalent chromium exposure. Two freshwater fishes (*Cyprinus carpio* and *Salmo trutta* L.) have been exposed for 38 weeks to 1–10 μg/l of potassium dichromate to find out the influence of chromium on humoral immunity by O'Neill [75]. The primary and secondary humoral responses have been found to be diminished for $MS_2$ bacteriophage in that experiment. In *Salmo trutta*, the primary antibody response has also been found to be diminished by 10%, whereas, in secondary antibody response the value rises by 50%. In carp, the serum proteins level has reported to be reduced by 25%. In the same study, common carp has appeared to be more sensitive to Chromium than trout. On the other hand, prolonged exposure to Chromium (VI) is shown to induce adaptability in fish. Haematological studies on chronically chromium (0.098 mg/l) exposed *Tilapia sparrmanii* have confirmed that no significant changes take place in leukocytes or erythrocytes counts but haemoglobin concentrations decrease significantly [92].

#### 5.2.4. Effect on enzyme activity

Long-term exposure of chromium exerts some dose-duration depended effects on different enzyme activities. In an experiment, Sastry and Sunita [76,77] have exposed *Channa punctatus* to 2.6 mg/l of the

metal for 60–120 days to determine the activity of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), pyruvate dehydrogenase (PDH) on its different organs like kidney, brain, liver, gill, intestine and muscles. They have reported that the activity of LDH decreases significantly in liver and kidney in case of 60 days exposure. They have also cited the inhibition in enzymatic activity of PDH in all selected organs during same study period. As a result, hyperglycemia and hyperlactamia have become evident in experimental fish after 120 days exposure. The activity of PDH has also been reported to be diminished significantly in liver, kidney and muscle during 120 days exposure. Chromium promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides which enhances the peroxides and reactive hydroxyl radicals. These lipid peroxides and hydroxyl radicals may cause cell membrane damage and thus destroy the cell [83].

Though, chromium is believed to be essential for some metabolic performances of living organisms but the ultimate necessity of chromium still remains as a debatable subject as huge number of laboratory studies have shown that, apart from various toxic effect of Cr(III), the trivalent chromium may also cause allergy, Some of the Cr(III) compounds have been reported to possess toxic even genotoxic effects for humans. It has also been reported that chromium possesses some feotoxic and embryotoxic effects. The metal may have some effects on reduction in implantation rate in case of exposed organisms [93]. It also exerts some effect on ovarian physiology and ovulation [94]. The oxidation of trivalent chromium to hexavalent form in cells and migration of $CrO_4^{2-}$ or $Cr_2O_7^{2-}$ ions through the bio membranes in all living organisms are still under scrutiny. Being environmentally toxic, discharge of hexavalent chromium should be restricted to limit in wastewater or sludge, especially when utilized for agricultural purposes. Similar attention should be paid to chromium discharge like the release of other potentially toxic trace elements like Mercury, Cadmium and Lead.

## 6. Biomarkers of chromium toxicity in fishes

Biomarkers are measurable indicators of some biological state or condition. There are several biomarkers of chromium intoxication in fresh water fishes at various investigatory levels. Stress proteins like metallothionine take longer time to express in case of chromium exposure at sublethal concentrations [84]. Heat shock protein (HSP-70) has been recorded to be over-stimulated in different fishes after chromium exposure at sublethal concentrations [84]. Significant reduction in carbohydrate and increase in lipid content in muscles has been reported in *Cirrhinus mrigala* by Virk and Sharma [59,60]. In *L. rohita*, a 96h-LC50 exposure to a concentration of hexavalent chromium (39.4 mg/l) significantly declines the tissue glycogen, total protein, and total lipid content in liver, muscle, and gill tissues of the fish [51]. Similar results have also been reported by Bakshi [14] in *Heteropneustes fossilis*. The enhanced utilization of glycogen and its subsequent depletion in tissues due to impaired glycogenesis and might be due in part to its utilization in the formation of glycoproteins and glycolipids is attributed to hypoxia [51]. Similar results have also been evident in *Catla catla* exposed to hexavalent Chromium at 20, 25, 30, and 35 mg/l over a period of 30 days [85].

A number of biomarkers of chromium pollution is also found through some genotoxicity studies (*viz.*, MN-study, BN-study *etc.*) as introduced by various authors [2]. Non-refractive, small, ovoid or circular chromatin bodies in the fish erythrocytes showing the identical staining pattern to the main nucleus has been considered as MN and cells with two nuclei have been considered as binucleated cell (BN) in an experiment with 60 days exposure to Cr (VI) by Bakshi [14]. MN-assay and BN- assay are referred to as a major tool of genotoxicity study. The report has revealed that significant ($p < 0.05$) increase of the MN frequencies at different exposure concentrations compared to control group. The MN frequency increased significantly ($p < 0.05$) with increasing concentration of potassium dichromate within treatment groups in case of both experimental organisms *L. rohita* and *H. fossilis*. In case of *L. rohita*, the micronucleuss percentage has been found to range from 0.16 to 0.32 in control group; whereas, a maximum of 2.48% has been found in case of a sub-lethal (1/10th 96 h LC50) concentration after 60 days exposure. On the other hand, *H. fossilis* has been found to adapt more successfully in chromium induced environment. The average value of micronuclei percentage has been found to be 2.208 ± 0.061 in case of a sub-lethal (1/10th LC50) concentration exposure.

Another genotoxic experiment inducing trivalent chromium has been studied using fish, *Pimephales promelas*, the fathead minnow, of 45–60 day-old, to assess the spontaneity of genetic damage through MN analysis in peripheral blood erythrocytes. The genotoxic effect of Chromium (VI) in experiments performed for 7, 14, and 21-d exposure periods has also been estimated and significant increase in micro-nucleated erythrocytes (MNE) induction has been detected in fish exposed for 7 d to 2.5 mg/l Cr(VI) whereas, the trend is found to be decreased after 21 days of exposure [86]. Significant increase in the frequency of micro nucleated erythrocytes and gills cells has also been reported in *Oreochromis nilotica* when exposed to hexavalent chromium [87]. Other symptoms like intra-strand cross-links and strand breaks in salmon sperm DNA with extensive DNA strand breakage has been evident in Salmon fish when exposed to 1 mM Chromium concentration [88].

Hexavalent chromium, a well known carcinogen, employs genotoxic effects in addition to endocrine disruption in freshwater fishes. For example, a short-term exposure to chromium has revealed genotoxic effects through physiologic and genetic responses in European eel (*Anguilla anguilla* L.) [73]. Authors have reported that alteration in plasma cortisol, thyroid stimulating hormone (TSH), free triiodothyronine ($T_3$), and free thyroxin ($T_4$) level can be used as biomarkers for endocrine function. The impact of the chromium exposure has been evident with decreased plasma $T_4$ levels in eels only when exposed to the metal. The genotoxicity has been recorded by the frequency of erythrocytic nuclear abnormalities (ENA) of the endocrine cells [73]. Histological alterations like cellular disorganization, hyperplasia, necrosis of hepatic cells, highly fenestrated Bowman's capsule, constricted lumen of renal tube, glomerular disorganization in Kidney, highly degraded inner epithelial layer of intestine, high lamellar degradation, necrosis in gill epithelial cells, thickening of blood vessels and atrophied central axis of gill can be used as remarkable biomarker of chromium exposure to fresh water fishes. Occurrence of enlarged nuclei, condensation of cytoplasm and disarray of hepatic cords blood congestion in sinusoids, vacuolation of hepatocytes and necrosis has also been cited as a potential biomarker in chromium contamination [89].

## 7. Conclusion

Although chromium is ubiquitous metal in the environment and trivalent chromium is also essential for biolife, hexavalent chromium is said to be a toxic metal with mutagenic, carcinogenic, and different deleterious impact on biota. Researchers have revealed that chromium affects the physiological, behavioural, histological, biochemical, genetic and immunological condition of the experimental organism. Trivalent chromium is essential component of different enzymes whereas, hexavalent chromium with the bio-membrane permeable capacity is found to have toxic impact on fresh water fishes. In case of acute exposure at 50% lethal concentration, fishes have been found to lose their body balance with restlessness, lowered breathing rate and higher rate of mucus secretion. Haematological alteration such as decreased haemoglobin percentage, decreased RBC count can be considered as biomarker. Breakage of DNA, presence of micronucleated (MN) and binucleated (BN) RBC have been reported as genotoxic impact of chronic chromium exposure in fishes. Significant histopathological deterioration has been found in gill, liver, kidney and intestine of experimental organisms when exposed to a sub-lethal concentration. Significant changes in total glycogen, total protein and total lipid

concentration in gill, muscle and liver tissues of experimental organisms have been found during chronic exposure study especially when exposed to sub-lethal concentrations. Altered endocrine function like alteration in plasma cortisol, thyroid stimulating hormone (TSH), free triiodothyronine ($T_3$), and free thyroxin ($T_4$) level is common marker of chromium exposures. Thus, it can be concluded that industrial effluent discharges with chromium contamination is imposing huge alterations in aquatic life though all the hazardous notations are dose-time dependent. This review can put forward the basic potential alterations of chromium pollution in aquatic ecosystem and will be helpful for the future researchers to gather advanced knowledge of the ecotoxicology and risk assessment of chromium.

## References


[1] M. Praveena, V. Sandeep, N. Kavitha, K. Jayantha Rao, Impact of tannery effluent, chromium on hematological parameters in a fresh water fish, *Labeo Rohita* (Hamilton), Res. J. Anim. Vet. Fish. Sci. 1 (July (6)) (2013) 1–5 ISSN-23206535.

[2] V. Velma, S.S. Vutukuru, P.B. Tchounwou, Ecotoxicology of hexavalent chromium in fresh water fish: a critical review, Rev. Environ. Health 24 (2) (2009) 129–145.

[3] S. Adhikary, L. Ghosh, S. Ayyappan, Combined effects of water pH and alkalinity on the accumulation of lead, cadmium and chromium to *Labeo rohita* (Hamilton), Int. J. Environ. Sci. Technol. 3 (3) (2006) 289–296 ISSSN-1735-1472.

[4] M.K. Ahmed, G.K. Kundu, M.H. Al-Mamun, S.K. Sarkar, M.S. Akter, M.S. Khan, Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging Catfish, *Heteropneutes fossilis*, Ecotoxicol. Environ. Saf. (2013) 1–7.

[5] S.S. Vutukuru, N.A. Prabhat, M. Raghavender, A. Yerramilli, Effect of arsenic and chromium on the serum amino-transferases activity in Indian major carp, *Labeo rohita*, Int. J. Environ. Res. Public Health 4 (3) (2007) 224–227 (PubMed: 17911661).

[6] H.S. Lodhi, M.A. Khan, R.S. Verma, U.D. Sharma, Acute toxicity of copper sulphate to fresh water prawns, J. Environ. Biol. 27 (2006) 585–588.

[7] N. Saha, M.R. Zaman, Concentration of selected toxic metals in groundwater and some cereals grown in Shibganj area of Chapai Nawabganj, Rajsahi, Bangladesh, Curr. Sci. 101 (2011) 427–431.

[8] R.K. Sharma, M. Agrawal, Biological effects of heavy metals, an overview, J. Environ. Biol. 26 (2005) 301–313.

[9] D. Bagchi, M. Bagchi, S.J. Stohs, Chromium (VI) induced oxidative stress, apoptosis cell death and modulation of p53 tumor suppressor gene, Mol. Cell. Biochem. 222 (1–2) (2001) 149–158.

[10] V. Sridhar, R.J. Katti, M.T. Laksmipathi, T.J. Ramesha, Behavioural alteration and biochemical composition of *Cyprinus carpio* exposed to hexavalent chromium, J. Inland Fish. Soc. India 32 (2) (2000) 18–24.

[11] T.K. Ghosh, Effects of chromic nitrate on fish and fish food organisms with special reference to river Churni, J. Nat. Conserv. 14 (2) (2002) 171–176.

[12] A. Kaviraj, Chronic effect of chromium on behavior: growth and reproduction of fish and on aquatic system, Environ. Ecol. 1 (1983) 17–22.

[13] R. Eisler, Chromium hazards to fish, wildlife, and invertebrates: a synoptic review, U.S. Fish and Wildlife Service Biological Report 85 (1.6), (1986) 60 pp..

[14] A. Bakshi, Analysis of Anthropogenic Disturbances and Impact of Pollution on Fish Fauna of River Churni with Special Reference to Chromium Pollution (Doctoral Dissertation), Kalyani University, Kalyani, India, 2016, p. 188.

[15] S. Langard, T. Norseth, L. Friberg, G.F. Nordberg, V.B. Vouk (Eds.), Handbook on the Toxicology of Metals, Elsevier/North Holland Biomedical Press, 1979, pp. 383–397.

[16] J.D. Steven, L.J. Davies, E.K. Stanley, R.A. Abbott, M. Ihnat, L. Bidstrup, J.F. Jaworski, Effects of chromium in the Canadian environment, Nat. Res. Coun. Canada (1976) 168 NRCC No. 15017. Avail. from Publications, NRCC/CNRC, Ottawa, Canada, K1A OR6.

[17] A.M. Farag, T. May, G.D. Marty, M. Easton, D.D. Harper, E.E. Little, L. Cleveland, The effect of chronic chromium exposure on the health of Chinook Salmon (*Oncorhynchus tshawytscha*), Aquat. Toxicol. 76 (2006) 246–257 (PubMed: 16330107).

[18] Z.H. Li, V. Zlabek, R. Grabic, P. Li, J. Machova, J. Velisek, T. Randak, Effects of exposure to sublethal propiconazole on the antioxidant defense system and Na + -K + Atpase activity in brain of Rainbow trout, (*Onkorynchus mykiss*), Aquat. Toxicol. 98 (2010) 297–303.

[19] M.S. Rahman, A.H. Molla, N. Saha, A. Rahaman, Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh, Food Chem. 134 (2012) 1847–1854.

[20] V. Velma, P.B. Tchounwou, Hexavalent chromium-induced multiple biomarker responses in liver and kidney of Gold fish, *Carassius auratus*, Environ. Toxicol. 26 (2009) 649–656.

[21] WHO, Guidelines for Drinking Water Quality, World Health Organisation, Geneva, Switzerland, 2003.

[22] M. Kumar, A. Puri, A review on permissible limit of drinkingwater, Indian J. Occup. Environ. Med. 16 (2012) 40–44.

[23] WHO (World Health Organization), Guidelines for Drinking Water Quality vol. 1, Recommendation WHO, Geneva, 1985 p. 130.

[24] Federal Environmental Protection Agency, Guidelines and Standards for Environmental Pollution Control in Nigeria, (2003) p. 238.

[25] L.E. Towill, C.R. Shriner, J.S. Drury, A.S. Hammons, J.W. Holleman, Reviews of the environmental effects of pollutants: III chromium, U.S. Environ. Protection Agency Rep. 600/1-78-023, (1978), p. 287.

[26] J.W. Moore, Ramamoorthy, Heavy Metals in Natural Waters: Applied Monitoring and Impact Assessment, Springer-Verlag, New York, USA, 1984, p. 268.

[27] T. Sarkar, M. Masibul Alam, N. Parvin, Z. Fardous, A.Z. Chowdhury, S. Hossain, M.E. Haque, N. Biswas, Assessment of heavy metals contamination and human health risk in shrimp collected from different farms and rivers at Khulna-Satkhira region, Bangladesh, Toxicol. Rep. 3 (2016) 346–350.

[28] D. Paul, Research on heavy metal pollution of river Ganga: a review, Ann. Agrarian Sci. (2017) 278–286 http://www.journals.elsevier.com/annals-of-agrarianscience.

[29] R.K. Sinha, S.K. Sinha, D.K. Kedia, A. Kumari, N. Rani, G. Sharma, k. Prasad, A holistic study on mercury pollution in the Ganga River System at Varanasi, India, Curr. Sci. 92 (2007) (2007) 1223–1227.

[30] D. Kar, P. Sur, S.N. Mandal, T. Saha, R.K. Kole, Assessment of heavy metal pollution in surface water, Int. J. Environ. Sci. Technol. 5 (2008) 119–124.

[31] WHO chronicles 38, Guidelines for Drinking Water Quality, F edition, (2011), pp. 104–108.

[32] US EPA, Ambient Water Quality Criteria for Chromium-1984. EpA-440/5-84-029, Office of Water Regulation and Standards, Washington, 1985.

[33] US FDA, Food and Drug Administrations, Guidance Document for Chromium in Shellfish, DHHS/PHS/FDA/CFSAN/Office of sea food, Washington, 1993.

[34] R. Vinodini, M. Narayanan, Heavy metal induced histopathological alterations in selected organs of *Cyprinus carpio L* (Common carp)? Int. J. Environ. Res. 3 (1) (2009) 95–100.

[35] P. Doudoroff, M. Katz, Critical review of literature on toxicity of industrial wastes and their components to fish. II. The metals, as salts, Sew. Ind. Wastes 25 (1953) 802–839.

[36] R. Vinodini, M. Narayanan, Impact of toxic heavy metals on the haematological parameters in Common carp (*Cyprinus carpio L*), Iran. J. Environ. Health. Sci. Eng. 6 (1) (2009) 23–28.

[37] E.E. Obasohan, Heavy metals concentrations in the offal, gill, muscle and liver of a freshwater mudfish (Parachanna obscura) from Ogba River, Benin city, Nigeria, Afr. J. Biotechnol. 6 (22) (2007) 2620–2627 ISSN 1684–531.

[38] K. Dhara, Hazardous Impact of Fly Ash and Some of Its Ingredients on Fish, Fish Food Organisms and Aquatic Ecosystem. Ph.D. Thesis, Kalyani University, Kalyani, India, 2014, p. 170.

[39] A. Avenant-Oldage, H.M. Marx, Bioaccumulation of chromium, copper and iron in the tissues of *Clarias gariepinus* in the Olifants River, Kruger National Park, Water SA 26 (4) (2000) 569–582.

[40] B. Mallesh, P.K. Pandey, K. Kumar, A. Vennila, S. Kumar, Bioconcentration of hexavalent chromium in *Cirrhinus mrigala* (Ham 1822): effect on haematological parameters, J. Bio. E. Sci. 5 (1) (2015) 59–67 ISSN-2084-3577.

[41] S.A. Abbasi, T. Kunhahmed, P.C. Nipaney, R. Soni, Influence of acidity of water of chromium toxicity–a case study with the teleost *Nuria denricus* as model, Pollut. Res. 14 (3) (1995) 317–323.

[42] I. Van Der Putte, J. Lubbers, Z. Kolar, Effect of pH on uptake, tissue distribution and retention of hexavalent chromium in Rainbow trout (*Salmo gairdneri*), Aquat. Toxicol. 1 (1981) 3–18.

[43] A.S. Hogendoorn-roozemond, J.J.H.M. Tenholder, J.J.T.W.A. Stirk, The influence of pH on the toxicity of hexavalent chromium to rainbow trout (Salmo gairdneri). Aquatic pollutants-transformation and biological effects, Proceedings of the Second International Symposium on Aquatic Pollutants, Oxford: Pergamon Press, 1977, pp. 477–478.

[44] J. Blasia, J. Kowalik, A comparison of the in vitro genotoxicity of tri- and hexavalent chromium, Mutat. Res. 469 (2000) 135–145.

[45] J.F. Wang, M. Bashir, B.N. Engelsberg, C. Witmer, C. Rozmiarek, P.C. Billings, High mobility group proteins 1 and 2 recognize chromium damaged DNA, Carcinogenesis 18 (1997) 371–375.

[46] A.G. Heath, Water Pollution and Fish Physiology, CRC Press Inc., Boca Raton, Florida, 1987, p. 245.

[47] J. Gauglhofer, V. Bianchi, Chromium, in: E. Merian (Ed.), Metals and Their Compounds in the Environment, Occurrence, Analysis and Biological Relevance, VCH Publishers Inc., New York, USA, 1991, pp. 853–878.

[48] A.K. Mishra, B. Mohanty, Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the fresh water fish, Channa punctatus (Bloch), Environ. Toxicol. Pharmacol. 26 (2008) 136–141.

[49] Brungs, Effects of pollution on freshwater fishes, J. Water Pollut. Control Fed. 49 (1977) 1425–1493 Washington D.C.

[50] H. Azmat, M. Javed, Acute toxicity of chromium to *Catla catla, Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions, Int. J. Agric. Biol. 13 (2011) 961–965.

[51] S.S. Vutukuru, Chromium induced alterations in some biochemical profiles of the Indian major carp, *Labeo rohita* (Hamilton), Bull. Environ. Contam. Toxicol. 70 (1) (2003) 118–123.

[52] Svecevicius, Acute toxicity of hexavalent chromium to European freshwater fish, Bull. Environ. Contam. Toxicol. 77 (5) (2006) 741–747.

[53] S.S. Vutukuru, Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major Carp, *Labeo rohita*, Int. J. Environ. Res. Public Health 2 (2005) 456–462.

[54] R.G. Reusink, L.L. Smith, Relationship of 96 h LC50 to lethal threshold concentration of hexavalent chromium, phenol and sodium pentachlorophenate foe fathead minnow, *Pimephales promelas* (Raf), Trans. Am. Fish. Soc. 104 (1975) 567.

[55] I.R. Adelman, Smith, Acute toxicity of sodium chloride, pentachlorophenol, guhion and hexavalent chromium to *Pimephales promelas* and *Carassius auretus*, J. Fish. Res. Board Can. 33 (1976) 203.

[56] T. Sanyal, A. Kaviraj, S. Saha, Toxicity and bioaccumulation of chromium in some freshwater fish, Hum. Ecol. Risk Assess. 23 (7) (2017) 1655–1667, http://dx.doi.org/10.1080/10807039.2017.1336425.
[57] G. Boge, P. N'Diaye, H. Roche, G. Peres, Effects of hexavalent chromium at non-lethal concentrations on the enzymology of the intestine of *Salmo gairdneri* and (Pisces), J. Physiol. 83 (2) (1988) 57–63.
[58] P.G. Stevens, G.A. Chapman, Toxicity of trivalent chromium to early stages of steelhead trout. Environ, Toxicol. Chem. 3 (1) (1984) 125–133.
[59] S. Virk, A. Sharma, Alterations in the biochemical constituents of muscles of *Cirrihunus mrigala* following exposure to and withdrawal from metals, Bull. Environ. Contam. Toxicol. 70 (2003) 106–111.
[60] S. Virk, A. Sharma, Changes in the biochemical constituents of gills of *Cirrhinus mrigala* (Ham.) following exposure to metals, Indian J. Fish. 50 (1) (2003) 113–117.
[61] T. Shaukat, M. Javed, Acute toxicity of chromium for *Ctenopharyngodon idella, Cyprinus carpio* and *Tilapia nilotica*, J. Agric. Biol. (2013) 1560–8530 ISSN Online: 1814–9596 12–589/2013/15–3–590–594 http://www.fspublishers.org.
[62] K. Nath, N. Kumar, Hexavalent chromium: toxicity and its impact on certain aspects of carbohydrate metabolism of the freshwater teleost, *Colisa fasciatus*, Sci. Total Environ. 72 (1988) 175–181.
[63] J.C. Pant, T.S. Gill, On the toxicity of hexavalent chromium to the fish and tadpoles from Nainital Lake, Himal. Res. Dev. 1 (1982) 149–152.
[64] C. Johnson, M.V. Radhakrishnan, Estimation of acute toxicity of chromium to the freshwater catfish *Clarias batrachus* (Linn.), Int. J. Res. Environ. Sci. (IJRES) 1 (July–September (2)) (2015) 30–37 www.arcjournals.org.
[65] Z. Li, In vitro cytotoxicity of the organophosphorus pesticide parathion to FG-9307 cells, Toxicol. In Vitro 15 (6) (2001) 643–647 (PubMed: 11698164).
[66] H. Segner, Fish cell lines as a tool in aquatic toxicology, EXS 86 (1998) 1–38 (PubMed: 9949875).
[67] K. Schirmer, D.G. Dixon, B.M. Greenberg, N.C. Bols, Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill, Toxicology 127 (1–3) (1998) 129–141.
[68] G. Krumschnabel, M. Nawaz, Acute toxicity of hexavalent chromium in isolated teleost hepatocytes, Aquat. Toxicol. 7 (2004) 159–167.
[69] F. Tan, M. Wang, W. Wang, Y. Lu, Comparative evaluation of the cytotoxicity sensitivity of six fish cell lines to four heavy metals in vitro, Toxicol. In Vitro 22 (1) (2008) 164–170 (PubMed: 17931828).
[70] M. Prabakaran, C. Binuramesh, D. Steinhagen, R. Dinakaran Michael, Immune response in the tilapia, Oreochromis mossambicus on exposure to tannery effluent, Ecotoxicol. Environ. Saf. 68 (3) (2007) 372–378 (PubMed: 17261332).
[71] A. Bozcaarmutlu, E. Arinc, Effect of mercury, cadmium, nickel, chromium and zinc of kinetic properties of NADPH-cytochrome P450 reductase purified from leaping mullet (Lizasaliens), Toxicol. In Vitro 21 (3) (2007) 408–416 (PubMed: 17113746).
[72] K.V. Sastry, K.M. Sunita, Effect of cadmium and chromium on the intestinal absorption of glucose in the Snakehead Fish, *Channa punctatus*, Toxicol. Lett. 10 (2–3) (1982) 293–296 (PubMed: 7080099).
[73] M. Teles, M. Pacheco, M.A. Santos, Physiological and genetic responses of European Eel (*Anguilla anguilla L.*) to short-term chromium or copper exposure-influence of pre-exposure to a PAH-Like compound, Environ. Toxicol. 20 (1) (2005) 92–99 (PubMed: 15712285).
[74] R.I. Arunkumar, P. Rajashekaran, R.D. Michael, Differential effect of chromium compounds on the immune response of the African mouth breeder *Oreochromis mossambicus* (Peter), Fish Shellfish Immunol. 10 (2006) 667–676 (PubMed: 11185752).
[75] G. O'Neill, The humoral response of *Salmo trutta L.* and *Cyprinus carpio L.* exposed to heavy metals, J. Fish Biol. (1981) 297–306.
[76] K.V. Sastry, K.M. Sunita, Enzymological and biochemical changes produced by chronic chromium exposure in a Teleost fish, *Channa punctatus*, Toxicol. Lett. 16 (1983) 9–15.
[77] K.V. Sastry, K.M. Sunita, Enzymological and biochemical changes produced by chronic chromium exposure in a Teleost fish, *Channa punctatus*, Toxicol. Lett. 16 (1983) 9–15.
[78] L.T.H. Nguyen, C.R. Janssen, Embryo-Larval toxicity tests with the African catfish (*Clarias gariepinus*): Comparative sensitivity of endpoints, Arch. Environ. Contam. Toxicol. 42 (2002) 256–262 (PubMed: 11815818).
[79] M. Muthulingam, M. Sivaraman, J. Sekar, R. Palaniappan, Impact of heavy metal, chromium on glycogen contents in gill, liver and kidney of fresh water fish, *Channa striatus* (Blotch), Int. J. Curr. Res Dev. (2017) 1–9 ISSN- 2321-0516(Online).
[80] A.P. Roberts, J.T. Oris, Multiple biomarker response in rainbow trout during exposure to hexavalent chromium, Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 138 (2004) 221–228.
[81] K. Muthukumaravel, P. Rajaraman, A Study on the toxicity of chromium on the histology of gill and liver of fresh water fish *Labeo rohita*, Int. J. Pure Appl. Zool. (2013) 122–126 ISSN Print/Online: 2320-9577/2320-9585.
[82] V. Sesha Srinivas, B.M. Rao, Chromium induced alterations in the gill of the freshwater teleost fish, *Labeo rohita*, Indian J. Comp. Anim. Physiol. 17 (1) (1998) 31–33.
[83] P. Madhavan, K. Elumalai, Effects of chromium (VI) on the lipid peroxidation and antioxidant parameters in the gill and kidney tissues of catfish, *Clarias batrachus* (Linnaeus, 1758) (Actinopterygii: Siluriformes), Int. J. Adv. Res. Biol. Sci. 3 (4) (2016) 249–255.
[84] E. Fulladosa, E. Deane, A.H.Y. Ng, N.Y.S. Woo, J.C. Murat, I. Villaescusa, Stress proteins induced by exposure to sub lethal levels of heavy metals in sea bream (*Sparus sarba*) blood cells, Toxicol. In Vitro 2006 (20) (2006) 96–100 (PubMed: 16054798).
[85] S. Vincent, T. Ambrose, L. Cyrill, M. Selvanaygam, Biochemical responses of the Indian Major carp, *Catla catla* (Ham.) to chromium toxicity, Indian J. Environ. Health 37 (1995) 192–196.
[86] C.T. De Lemos, P.M. Rodel, N.R. Terra, B. Erdtman, Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes, Environ. Toxicol. Chem. 20 (6) (2001) 1320–1324 (PubMed: 11392142).
[87] T. Cavas, S. Ergene-Gozukara, Induction of micronuclei and nuclear abnormalities in *Oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents, Aquat. Toxicol. 2005 (74) (2005) 264–271.
[88] D.R. Lloyd, D.H. Phillips, P.L. Carmichael, Generation of putative intrastrand cross-links and strand breaks in DNA by transition metal ion-mediated oxygen radical attack, Chem. Res. Toxicol. 10 (1997) 393–400 (PubMed: 9114975).
[89] N.V. Bhatkar, Chromium, nickel and zinc induced histopathological alterations in the liver of Indian common carp *Labeo rohita* (Ham.), J. Appl. Sci. Environ. Manag. (2011) 331–336 JASEM ISSN 1119–8362.
[90] I.R. Sonaraj, A.J.A. Ranjitsing, A. Pushparaj, G. Ramathilingam, Pesticidal stress influenced respiratory alterations in the freshwater fish, *Mystus vittatus*, Indian J. Environ. Ecoplan. 10 (3) (2005) 803–806.
[91] V. Subhashini, A.V.V.S. Swamy, D. Harika, K. Venkateswararao, Phytoremediation of heavy metals contaminated soils, Int. J. Curr. Microbiol. App. Sci. (5) (2017) 19–30 Special Issue.
[92] V. Wepner, J.H.J. Van Vuren, H.H. Du Preez, The effect of hexavalent chromium at different pH values on the hematology of *Tilapia sparrmanii* (Cichlidae), Comp. Biochem. Physiol. 101 (2) (1992) 375–381.
[93] R.K. Kanojia, M. Junaid, R.C. Murthy, Chromium induced teratogenicity in female rat, Toxicol. Lett. 89 (3) (1996) 207–213.
[94] M. Junaid, R. Murthy, D.K. Saxena, Embryotoxicity of orally administered chromium in mice: exposure during the period of oraganogenesis, Toxicol. Lett. 84 (1996) 143–148.