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Review on Some Heavy Metals Toxicity on Freshwater Fishes

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ABSTRACT

Heavy metals contamination is a serious problem in the aquatic environment. Some of them are very important, having biological roles for aquatic organisms, and called essential heavy metals. In contrast, other heavy metals are considered harmful even at low concentrations. The toxic levels of heavy metals may be of agricultural, industrial and mining activities. This will cause water pollution and changes in the physicochemical characteristics of the aquatic environment. This pollution has deleterious toxic effects on fish and raises concerns over its potential impact on human health. The most common heavy metals are arsenic, chromium, lead and mercury, which affect human health and are considered systemic toxicants. These metals induce organ damage even at low levels of exposure and according to the US Environmental protection agency and international agency for research on cancer, they classified as carcinogens. For all the above reasons, this review was written to contribute to heavy metals' role in the environment, toxic mechanism and toxic effects on fish.

Keywords: Freshwater fish, heavy metals, Pathological effects, toxic mechanism.

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INTRODUCTION

In the last few years, the aquaculture industry has been developed rapidly faster than the rest of other animal husbandry industries in the world. Aquaculture production represents about 17% of animal protein and nearly 6% of all protein consumed by the human population **(Domingo *et al.*, 2007)**. It provides a good source of animal protein containing vitamin B with low saturated fat and omega-3 fatty acids (**Hajeb *et al.*, 2009; Gu *et al.*,2017 and Özden et al., 2018).**

However, fish consumption may threaten human health through different types of pollutants; the most important and common ones are heavy metals. Heavy metals are metallic chemical elements with highly poisonous ability at low concentration, They are a natural component of the earth' crust and considered as a natural trace component of the aquatic environment with limiting permissible concentration table(1), which has been increased in levels due to industrial and agricultural activities (**Zhang *et al.*, 2011; Martin *et al.*,2015 and Li *et al.*,2017**).

There are different sources for water pollution as the paint on ships, stabilizers for packed cell volume (PVC) and agricultural activities like pesticides and fungicides **(Lee *et al.*, 2016**) these will increase environmental hazards to invertebrates and vertebrates including fish and humans (**Yi *et al.*, 2017; Bashir *et al.*, 2013**).

Table 1: Heavy metals permissible concentration ppm in water. **(Svobodová,1993 and Engwa *et al.*,2019)**

Heavy metals	Limiting concentration water (ppm)
Aluminum	0.05 - 0.2
Arsenic	0.01
Mercury	0.002
Lead	0.015
Chromium	0.1

ppm= part per million

Heavy metals accumulate in the aquatic environment, then affects fish and accumulates in their bodies. Freshwater fishes are more vulnerable and more exposed to heavy metals than marine fish. This is because freshwater fish tend to gain water and lose salts in contrast to marine water fish which tend to lose water and gain salts **(Nikinmaa, 2014).** Heavy metals enter the fish body through ingestion, gills and skin (Ion exchange) or adsorption by fish tissues **(Ahmed et al., 2014)**. Metal accumulation in varying parts of the body depends on many factors as water solubility, feeding behaviour, ecology and fish physiology including species, age, size, reproductive state, fish health, bioavailability and different habitats **(Perugini *et al.*, 2014 and Anandkumar *et al.*, 2017).** This study aimed to provide data about some heavy metals toxicity in freshwater fish.

Classification of heavy metals

1.Essential heavy metals

Copper, chromium, zinc-nickel, cobalt and iron are essential metals required for all vital processes inside the body with optimum level. Otherwise inadequate amount causes deficiency diseases and high-level causes toxicity (**Sivaperumal *et al.*, 2007 and Abadi *et al.*, 2014**).

2.Non essential heavy metals

Those haven't biological roles and also called xenobiotics. when they are increased in concentrations, it will cause toxopathic effects in tissue; those involve Aluminum, Mercury, Lead, Cadmium and others. (**Sfakianakis *et al.*, 2015**).

The bioaccumulation of heavy metals in different tissues of aquatic organisms leads to several harmful effects. It may have genotoxic, mutagenic, Immunosuppressive and cytotoxic effects. It may also result in histopathological changes, abnormalities in fish reproduction; and public health hazard effects for human consumption such as polluted fish **(Salamat *et al.*, 2015; Matos *et al.*, 2017**).

Heavy metals and their toxicity mechanisms

Heavy metals as metallic elements (mercury, arsenic, aluminum, lead and chromium) are systemic toxicants that can induce toxicity in human and other vertebrates even at low concentrations; they are interfering with cellular organelles (cell membrane, endoplasmic reticulum, mitochondrial lysosomes) causing disturbances in cell and organ detoxification, metabolisms and tissue repair **(Wang and Shi,2001)**. They can also attack and combine with DNA molecules and cause cell cycle modulation, carcinogenesis, and apoptosis **(Beyersmann and Hartwig,2008)**. The oxidative stress is the main toxic mechanism that results from imbalance regulation of the antioxidant system and production of free radical (ROS) due to disturbances of cell membrane Fig.. 1 and 2 **(Jaishankar *et al.* 2014 and Engw *et al.*,2019**).

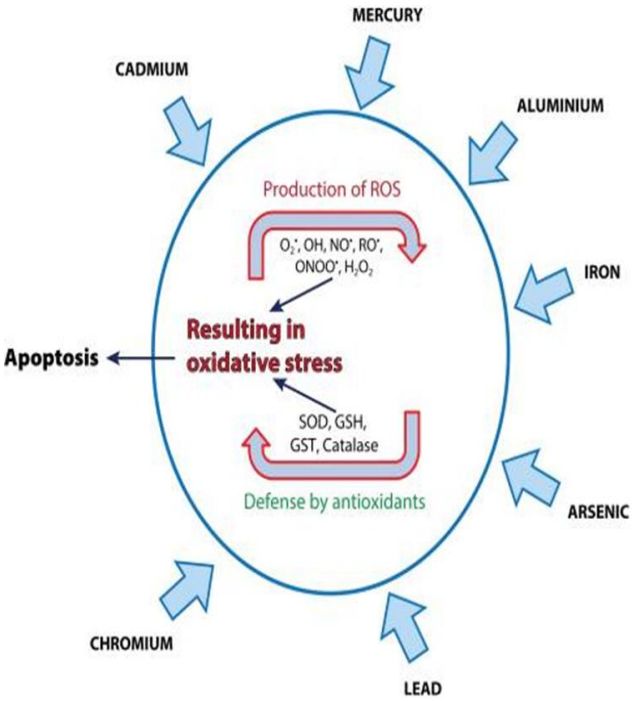

Fig.1: The effect of heavy metals on a cell and the balance between ROS production and the subsequent defense presented by antioxidants **(Jaishankar et al., 2014)**

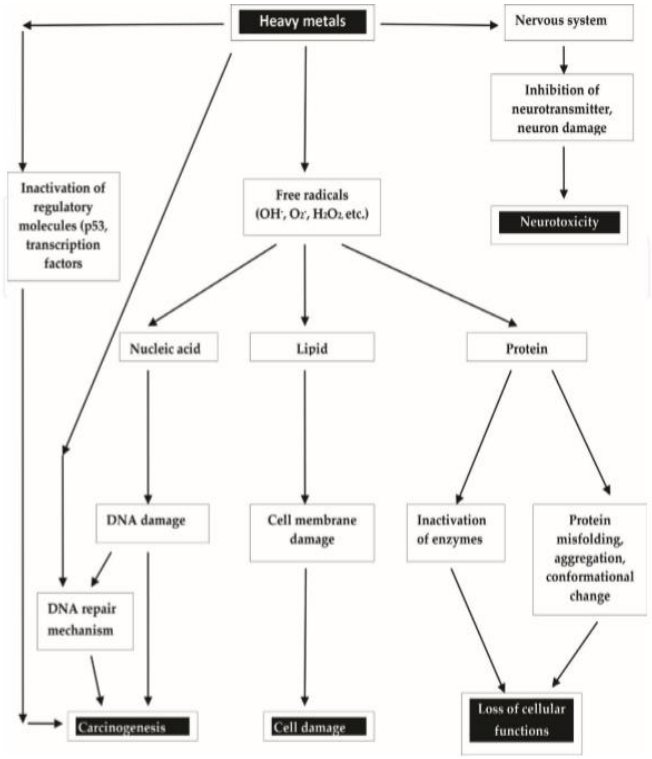

Fig.2: Pathway of heavy metals in living organisms' body **(Engw et al.,2019)**

Aluminum (Al)

Aluminum is the third most prevalent metal in the earth after oxygen and silicon with no biological role and considered a toxic non-essential metal for microorganisms (**Authman *et al.*, 2008; Olaniran *et al.*, 2013**). Aluminum may enter natural waters via water treatment facilities, coal strip mining activities, industrial wastes and acid rainfall. Metal toxicity is magnified in the aquatic environment, accompanied by high organic matter content with low water pH (**Svobodova, 1993).** The sublethal concentration of Al variable according to the aged of fish and species, as in *Catla Catla* the LC_{50} was (81.68 mg/l), while in fish *Labeo rohita* and *Cirrhina mrigala* and both fish aged (60 and 240) day the LC_{50} reached to (78.32 and 140.58mg/l) and (91.77 and 149.14mg/l) respectively**(Azmat *et al.*, 2012)**.

Aluminum interferes with most physical and cellular processes. The plasma membrane leads to ionic balance and osmoregulation disturbances and causes respiratory stress and coagulation of mucous on the gill and fusion of lamellae. Fish exposure to Al causes a pathological alteration in cardiovascular, respiratory ion-regulatory, changes in hemoglobin concentration, and reduction in fish growth besides endocrine disruption (**Abdel-Latif, 2008; Correia *et al.* et al. 2010; Barcacarolli and Martinez, 2004**). Also, it causes disturbances in calcium, phosphorus, iron and fluorine metabolism in living organisms. It has a high affinity to DNA and RNA and causes inhibition to Enzymes such as hexokinase, phosphodiesterase, alkalic phosphatase and phosphoxidase (**Barabasz *et al.*, 2002**).

Histopathological investigations have been considered a biomarker of stress in fish **(Yancheva *et al.*, 2016)**. It is valuable for evaluation of the health of fish exposed to contaminants as well as it gives a category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for respiration, excretion and the detoxification of xenobiotics in the fish **(Gernhöfer *et al.*, 2001**). The degree of the histopathological changes varies according to the concentration of the materials, fish species, length of exposure and other factors **(Paris-Palacios *et al.*, 2000).**

Histopathological changes in gills, liver and kidneys in the freshwater fish, *Tilapia zillii* exposed to three concentrations of aluminum (25, 50, and 100 µg/L) for 96 hours was demonstrated by (**Hadi and Alwan , 2012**), who refers to the cellular hypertrophy or hyperplasia with cellular degeneration which results in necrosis of gill epithelial tissues and alteration of the circulatory system involved oedema , congestion, inflammation in the liver with atrophy of pancreatic tissue and activation Melan macrophage (**Authman, 2011**) Fig.. (3), the author demonstrated toxic effects of Al in the kidney which lead to severe degeneration in the tubules cells, irregular diameters of renal tubules with glomerular expansion, renal corpuscle damage and haemorrhage.

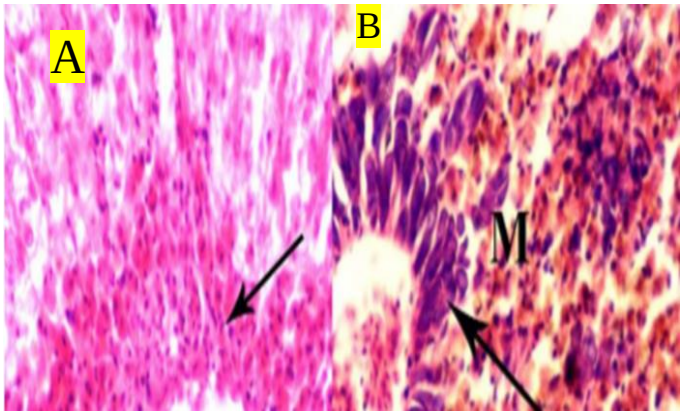

Fig.3: Microscopic examination of the liver of *Oreochromis niloticus* exposed to Al toxicity exhibit infiltration of the inflammatory cell (black row) in section **A**, with pancreatic tissue atrophy(black row) and activation of melan macrophage (**M**) in section **B,** (H&E,X160) **(Authman, 2011)**.

Arsenic (AS)

Arsenic reaches the aquatic environment through the industrial effluents as smelting operations and electric generators effusions and agricultural run-off, whereas the arsenic intake is through gills or contaminated feeds **(Ahmed *et al.*, 2008).** Arsenic has a high rate of metabolism and accumulates in different tissues and organs of several fish species as Rainbow trout, Medaka, Tilapia mossamica **(Suhendrayatna *et al.*, 2002_a).** The lethal concentration of AS in variable fish species ranged from 3-30 mg/l **(Svobodova, 1993), Suhendrayatna *et al.*, (2002_b)** reported the LC_{50} for *Oryzias Latipes* were 14.6 and 30.3 mg /l**,** acute exposure to arsenic causes sudden fish death because of increase mucous secretion, defect in gill epithelium and suffocation. In contrast, in chronic exposure, it may accumulate in different tissue and cause several pathological changes (**Hughes *et al.*, 2002**).

It is mentioned by **Sorensen (1991**) that Arsenic toxicity causes histopathological changes in different organs like degenerations in kidneys and liver with focal hepatic necrosis and bile duct proliferation with plugs with intranuclear arsenic inclusions in parenchymal hepatocytes. Apoptosis in the heart muscle of *Channa punctate* exposed to sodium arsenite with enlargement of intestine mucosa and submucosa (**Hossain, 2012).**

Arsenic also accumulates in the retina and interferes with the immune system, leading to depletion of both B and T lymphocytes from lymphatic organs with variant toxicity according to the time of exposure to **Arsenic (Liao *et al.*, 2004, Suhendrayatna *et al.*, 2002$_b$).**

Gills are in direct contact with the aquatic environment, so it represents a target organ for pollution. Exposed *Odontesthes bonariensis* to a toxic level of arsenic lead to pathological changes in the gills represented by oedema, desquamation of the epithelial cells with hyperplasia of mucus-secreting cells and telangiectasis combined with curling in the secondary gill's lamellae (**Puntoriero *et al.*, 2018)**, Fig. (4). Both (**Barnes *et al.*, 2002 and Gornati *et al.* .2002)** referred that arsenic is considered a genotoxic agent and causes changes in protein expression like heat shock protein.

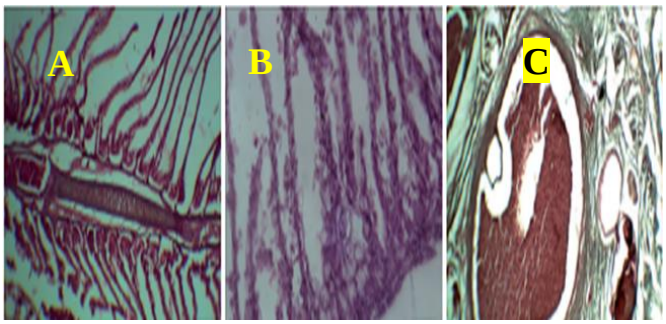

Fig.. 4: Microscopic examination of gills in *Odontesthes bonariensis* exposed to arsenic (A) Curling of secondary lamellae (10×). (B) Hyperplasia of mucous cells (40×). (C) Congestion and telangiectasia in blood vessels in gill filaments (40×), H&E. (**Puntoriero et *al.*, 2018).**

Mercury (Hg)

Mercury is widely used as a fungicide in farms whereas organic compounds of mercury are considered as their main source to reach the aquatic environment with a concentration mostly not exceeds 0.1 Mg/L (**Devlin, 2006**). Methyl mercury is one of the most toxic compounds to fish usually generated through methylation of inorganic mercury by anaerobic microorganism as sulfate-reducing bacteria SRB, methanogens MPA and iron reducers FeRP (**Pack *et al.*, 2014**). Methyl mercury is a highly lipophilic environmental contaminant, firstly reported in 1970 to cause pollution in Minamata Bay in Japan and massive human poisoning in Iraq **(Bakir *et al.*, 1973 and Tsubaki and Irudayaraj, 1977).**

The lethal concentration of Hg different according to fish species, so it ranged from 0.3-1mg/l in Salamon fish, while in cyprinid, it reaches 0.2-4mg/l and 0.20-0.70 mg/l with organic mercuric compound **(Svobodova, 1993)**.

The uptake of mercury is through ingestion where it passes to the circulation reaching its target organ (the liver). The liver is the site for binding, storage, and redistribution of mercury into the inter peripheral circulation for bioaccumulation in different tissues, mostly in the kidney, liver and gill tissues. Mercury toxicity is accompanied with behavioral and cognitive changes as convulsions and ataxia, cellular and structural changes as detachment in the gills with the fusion of secondary gills filaments of the fish *Clarias batrachus* Fig. (5)in addition to acute inflammation in the liver with degeneration in the blood vessels Fig.(6) **(Selvanathan *et al.*, 2013)**. Also, a prominent degeneration of the renal tubules was reported in the guppy (*Poecilia reticulata*) exposed to waterborne methylmercury **(Western and Canton, 1992).** Harmful effects also noticed at olfactory epithelium and blood components **(Gochfeld,2003; Oliveira et al., 2006 and Raihan *et al.*,2020)**

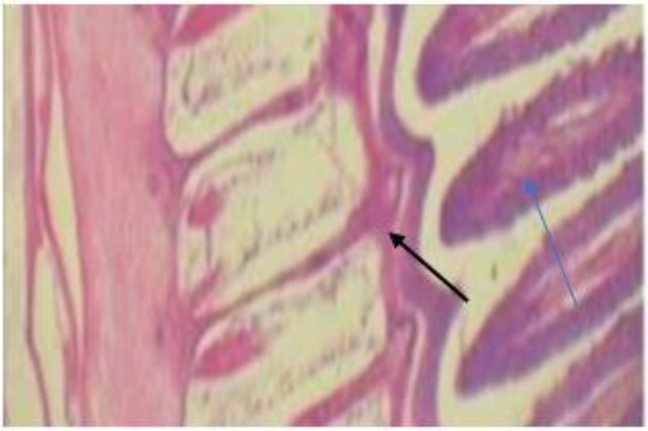

Fig. 5: Microscopic examination of gills in *Clarius batrachus* exposed to Mercury show detachment in the gills (black row) with the fusion of secondary gills filaments (blue row**) (Selvanathan *et al.*, 2013)**

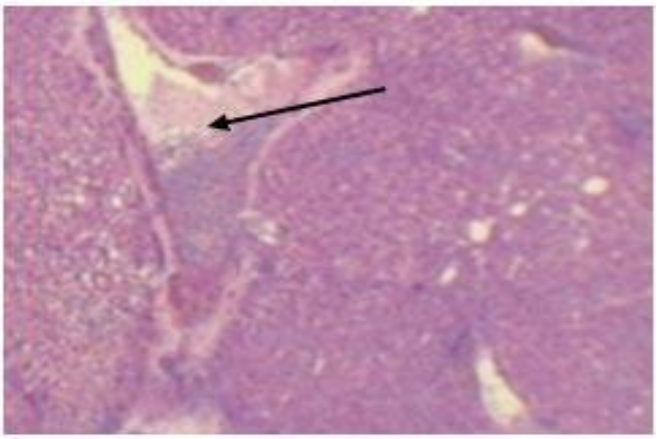

Fig. 6: Microscopic examination of liver in *Clarius batrachus* exposed to Mercury show degeneration in the blood vessels (black row) H&E. **(Selvanathan *et al.*, 2013**).

Raldua *et al*.,(2007) observed that the accumulation of MeHg in the and reproductive system even at low concentration might affect the survival rate of eggs and fry, degenerative in ovary tissue in zebrafish (**Zhang *et al*.,2016)** lead to reduce egg production, also affected spermatogenesis and availability of spermatozoa as a result of histopathological alteration in the testes structure, (**Vergilio *et al*., 2013)** demonstrated the disorganization of seminiferous tubules with a reduction in the germ cells with the proliferation of interstitial tissue in tropical fish *Gymnotus carapo* and disturbances in blood circulatory.

Lead (Pb)

It is one of the most hazardous heavy metals. Lead is naturally present in the environment, but its concentration is highly elevated by anthropogenic sources **(Monteiro *et al.* 2011 and Sfakianakis *et al*.,2015).** The acute lethal concentration in fish species ranged from 10-100 mg/l. The bioavailability of lead depends on the physicochemical properties of water as PH, alkalinity and hardness, and natural organic matter content in water **(Svobodova, 1993 and Sepe, 2003**).

Although it enters fish bodies through diet, the gills are the central part of the body for lead precipitation reflecting two different responses, one of them considered as physiological compensatory mechanism while other responses including epithelial damage, desquamation and aneurism are the main pathological effects of chronic lead toxicity in *Clarisas gariepinus* (**Olojo *et al*.,2005**). In addition, Vacuole formation, shrinkage of lamella and fusion of tips of gill lamella was noticed in lead toxicity **(Choudhary *et al*., 2019)**

A noticeable result by **Castro-González and Méndez-Armenta, (2008**) was a decrease in lipid cholesterol levels in both brain and gonadal organ with growth retardation in *C.batrachus* fish after exposure to lead nitrate for 150 days; also there was increase number of rodlet cells in the epidermis of common carp and rainbow trout exposed to lead polluted water with harmful effects on liver included hepatic cells vacuolation, nuclear pyknosis, sinusoidal congestion, Parenchymal cells degeneration and necrosis with liver cirrhosis it also causes oxidative stress as a result of its effect on depletion of antioxidant in the cell (**Ghosh *et al*.,2006**).

Hou *et al*. (2011) reported the effects of lead in Chinese sturgeon Acipenser *smensas* as deformities and spinal curvatures with reduced ability to locomotion. They compromised the immune system **(Shah and Altindağ, 2005).** Lead present in the aquatic system directly affects the fish's health, but it can also affect the reproductive system. Concerning this point, **Biswas and Ghosh, (2016)** reported the toxic effect of lead in *Mastacembelus pancalus*, which can destroy the oocyst, atresia and degeneration of yolk globules with necrotic oocyst Fig. (7) with necrosis in the tissue of the ovary Fig. (8)**.**

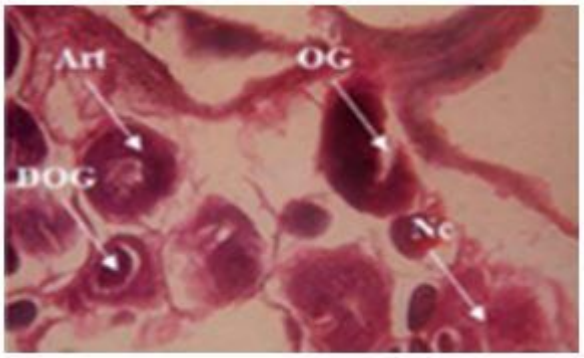

Fig.7: Microscopic examination of the ovarian structure of *Mastacembelus pancalus* exposed to lead toxicity exhibit atresia (Art) and degeneration of yolk globules (DOG), necrotic oocyst (Nc), H&E, X100.

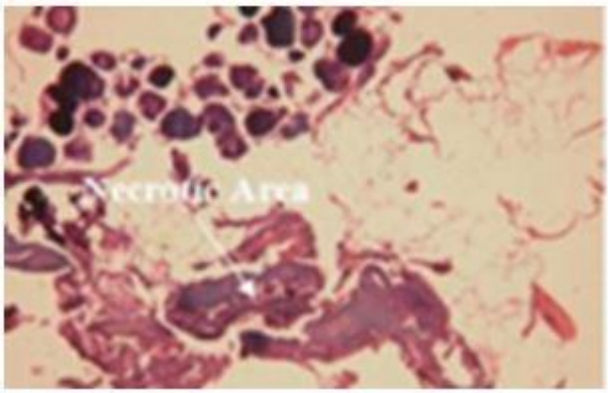

Fig.8: Microscopic examination of the ovarian structure of *Mastacembelus pancalus* exposed to lead toxicity show necrotic tissue of ovary, H&E, X10.

Chromium (Cr)

Chromium is an essential heavy metal that plays an essential role in the metabolism of carbohydrates (**Farag *et al*., 2006**). It is present in rivers and lakes with a concentration range between 1-10 microgram /L. The EPA reported that 50-100 microgram/ L of chromium is a permissible concentration for keeping human and aquatic animal health. (**Rashed 2001 and Has-Schön *et al*., 2006**). Those concentrations were elevated in the aquatic environment through effluent discharge from industrial products (**Abbas and Ali, 2007**).

According to the physicochemical properties of the surface water, two forms of chromium may be present in the water environment, the trivalent

Cr^{+3}(LC_{50} 2-7.5mg/l), which is a more stable state in oxidation, and hexavalent Cr^{+6}(LC_{50}35-75mg/l) which is more toxic because it can pass cell membrane and then reduced to trivalent. These will be combined with macromolecules as genetic materials and cause mutation **(Svobodova, 1993; Bakshia and Panigrahi, 2018**). It is considered a vital role for carcinogenesis, and it will stimulate oxidative stress (. **Eisler, 2000; Lushchak *et al*., 2009**).

Like other heavy metals, chromium enters the body through gills or digestive tracts, but it is known that it has a lower ability to accumulate than others (**Rashed,2001**). Sublethal effects of Cr^{+6} in fish are mainly related to inhibition of vital metabolic and physiologic processes and histopathological in gills, kidneys, liver and gonads of *Oreochrmis massambicus* exposed to sublethal concentration of Cr^{+6} was explained by (**Ackermann, 2008**). Hyperplasia of epithelial cells and epithelial lifting of secondary lamellae in Rainbow Trout have been demonstrated by (**Roberts and Oris, 2004**) High Lamellar degradation. Necrosis in epithelial cells. Thickening of blood vessels, Atrophied central axis in *Labeo rohita* (**Muthukumaravel and Rajaraman,2013**). Exposure of fish to acute toxicity may cause an increase of mucous secretion and suffocation and death.

The liver has multiple biological functions in fish as detoxification, metabolism and excretion of toxic substances from the body, so the lesions result from the chromium toxicity have been detected and described as hepatocellular vacuolization, atrophy and pyknotic nuclei in acute and chronic chromium toxicity in *Channa punctatus* **(Mishra and Mohanty, 2008).**

In chronic toxicity, chromium may severely affect kidneys causing reduction of renal function and renal tubular hypertrophy, glomerular necrosis and fibrosis with stenosis of the tubular lumen in *Chinook salmon* Fig.(9), (**Mishra and Mohanty, 2009**).In addition to hypertrophy and vacuolization of the head kidney's internal cells, which play essential roles in innate immunity with necrosis in hematopoietic tissue**(Mishra and Mohanty, 2008)**. Also, (**Begum *et al*.,2009**) observed a reduction of locomotor activity in *Gambusia affinis.* The conclusion of this study the non-essential heavy metals may present naturally in the aquatic environment and cause toxicity and pathological changes in fish tissue when their permissible limiting concentration was elevating as a result of agriculture and industries activities.

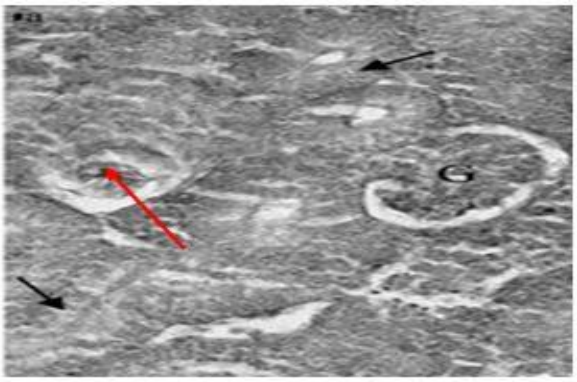

Fig. 9: Microscopic examination of kidney in *Chinook salmon* exposed to chromium toxicity, showing epithelial hypertrophy and reduction of lumens (black row), necrosis in the glomerular tissue (red row), X400. (Mishra and Mohanty, 2009).

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