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Review Article

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A Review of Adverse Effect of Heavy Metals on Fish

NDA A.A.[1], Abubakar M.I.[2], Musa M.A.[1], Masaya A.H.[1]

[1]Department of Fisheries and Aquaculture, Federal University, Gashua, Yobe State
[2]Department of Aquaculture and Fisheries, University of Ilorin, Ilorin, Kwara State

Abstract Water is an essential natural resources needed by fish for their survival. Heavy metals surface in water bodies either through natural or anthropogenic source. The feeding and living of fish in aquatic environments make them vulnerable and heavily exposed to heavy metals because of their inability to flee from the harmful effects of contaminants. While some heavy metals affect fishes by causing disorganization of hepatic cells with edematous hepatocytes, swollen of hepatic nuclei and devoiding many cells of cytoplasmic contents others cause typical patho-anatomical appearance includes a large amount of mucus on body surface, under the gill covers and in between gill filaments, visible external lesions such as discoloration and necrosis on livers at higher dose. Since fish is at the higher level of the food chain accumulating great deal of heavy metals which pose health issues to humans when consumed, there is need for organizations to intervene by creating awareness on the hazards of heavy metals on fish health and its consumers.

Keywords Adverse, effect, heavy metals, pollutant, fish

Introduction

Water is an essential natural resources needed by aquatic animals for their survival. Apart from water forming the vital basic components of aquatic organisms, it also serves as a medium for biochemical reactions sustaining the existence of organisms. Of all the water bodies on earth, freshwater resources are the most vulnerable and threatened aquatic ecosystem with the threats stemming from heavy metal, sewage and agricultural pollutants [1]. Schüürmann and Markert [2] showed that the tissues of fish can bioaccumulate heavy metals considered as a pollutants needing great attention in water bodies due to its environmental persistence. Unlike invertebrate, fish are sensitive and vulnerable to heavy metal pollutants and are therefore used to screen the health of ecosystem [3]. Heavy metals in aquatic environment originate from either anthropogenic or natural source [4]; also through mediating free radicals oxygen species [4]. Javed *et al.*, [5] stated that heavy metals can cause oxidative stress or carcinogenesis. Ebrahimi and Taherianfard [6] and Krishnani, [7] observed that small amount of heavy metal pollutants shows no conspicuous ill effects on the fish however, it may induce decline in the fecundity of fish population leading to reduction and eventual extinction of fish. Researches revealed that the amount of heavy metals found in the tissues of fish is heavily dependent not only on the concentration of metals but also period of exposure [8]. The level of heavy metal pollution in the water bodies can be checked using fish as biomonitors [9]. In the aquatic food chain, fish are found at the top accumulating heavy metals by passing it to humans through feeding causing chronic diseases [10], through intakes of contaminated diet and skin penetration, heavy metals bioaccumulate in fish [11]. The ingested heavy metals are then transported to the different organ via blood flow [12]; while heavy metals cause damage to fish at the cellular level, it can also interfere with the ecological stability. Consumption of fish polluted with heavy metals can cause serious health challenges such as reproductive and neurological problem [13]. This review therefore intends to survey the adverse effects of heavy metals on fish

Heavy Metal Pollutants

The term "heavy metal" has no unified definition. Several definitions have been given, some on the bases of relative density while others on the bases atomic weight, chemical properties and toxicity. However, each definition has its own limitation. For instance, Jarup, [14] defined heavy metal as those metals possessing specific density of more than 5 g/cm^3. In the fundamental review paper written by Duffus [15], 13 different works were cited that used lower limits on the density of a "heavy" metal ranging from 3.5 to 7 g cm−3. The author stated that the threshold varied depending on the author, and that "it is impossible to come up with a consensus". Moreover, he concluded that "any idea of defining "heavy metals" on the basis of density must be abandoned as yielding nothing but confusion". Apart from the specific weight, the atomic weight, the atomic number, specific chemical properties and the toxicity were all mentioned as a possible basis for classification and then rejected for good reasons. It was mentioned by Seema *et al*, [16] that more than 50 heavy metals have been recorded out of which 17 are extremely harmful. Examples of heavy metals include mercury, lead, cadmium, nickel, manganese, chromium, cobalt, copper, zinc, vanadium, iron etc. Heavy metals occur naturally in aquatic system in small amount. The increase in industries, agricultural activities and advancement in technology in both developed and undeveloped countries has resulted in the pollution of water bodies with heavy metals. Not only do heavy metals impede the growth rate, physiology of fish but also contribute to fish mortality [17]. Heavy metals are diluted and affected by various surface water components (carbonate, sulphate, organic compounds. humic, fulvic, amino acids) that formed insoluble salts or complexes. These salts and complexes are predicted to be not harmful to aquatic organisms. Part of them sink and are accumulated in bottom sediments. However, when water pH has declined (during acidic rains or other acidic episodes) heavy metals can be mobilized and released into the water column and become toxic to aquatic biota.

Sources of Heavy Metal Pollutants in Water Bodies

Basically, the source of heavy metals entering aquatic environmental can be subdivided into two: natural and anthropogenic source. Heavy metals occur naturally through geological weathering of rock and soil which are transported with sediments into the water bodies while sewage effluents, effluents resulting from industrial activities and agricultural effluent harboring pesticides and fertilizers are anthropogenic source of heavy metals supplying water body [18]. The quantity of heavy metals released by anthropogenic source into aquatic environment superimposed that of natural source [16]. The table below shows the anthropogenic source of some essential and non-essential heavy metals.

Table 1: showing heavy metals and their anthropogenic source [20]

Metal	Manufacturing Industries
Arsenic	Phosphate and Fertilizer, Metal Hardening , Paints and Textile
cadmium	Phosphate and Fertilizer, Electronics, Pigments and Paints
chromium	Metal Plating , Tanning, Rubber and Photography
copper	Plating, Rayon and Electrical
Lead	Paints and Battery
Nickel	Electroplating , Iron and Steel
Zinc	Galvanizing, Plating Iron and Steel
Mercury	Chlor-Alkali, Scientific Instruments and Chemicals

Classification of Heavy Metals

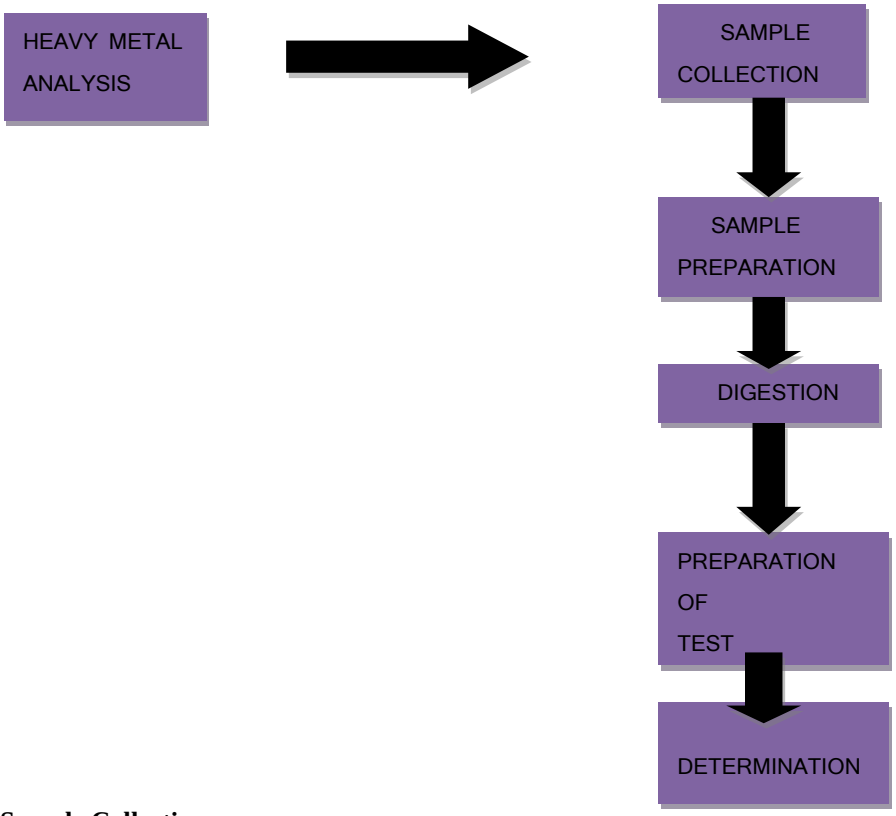
Generally, heavy metals are categorized into biological essential and non-biological essential elements which have a specific importance in ecotoxicology and can be harmful to both aquatic and terrestrial organisms depending on concentration and the category of heavy metals [20]. The essential heavy metals which include copper, manganese, cobalt, zinc, iron, and nickel are required in small amount by fish for biochemical and physiological functions to maintain good health. However, when the concentration of these essential metals is in excess it poses threat to fish and its consumers. On the other hand, non-essential metals such as Lead, Mercury, Arsenic, Chromium, Mercury and Cadmium lack biological importance or significance in fish, therefore their accumulation in the body leads to severe health problem or even death.

Forms of heavy metals in aquatic environment

Generally heavy metals in aquatic environment have two forms: particulate (insoluble) and soluble form. The forms include labile and non-labile fraction. The labile heavy metal compound are the most dangerous to fish. these include various ions [21].

Heavy metals analysis in fish

The heavy metals in fish tissues or organs can be analyzed following the procedures below:



Sample Collection

Fresh fish samples are collected either from market, fishermen or directly from water bodies using fishing gears. These samples are then transported either in clean containing polyethylene bags Eastward, [22] or ice-box Mustafa *et al.*, (2013) to laboratory for immediate sample preparation or preserve by freezing at -20 °C Benzer *et al*., [23] for later sample preparation. The ice in the container reduces tissue decay and also maintains moist condition. While awaiting sample preparation, fish sample can be washed with deionized water prior to freezing to remove contaminants Mustafa *et al*., [24] though authors like Ismaniza and Idaliza, [25], Eastwood, [22] and Benzer *et al*., [23] preserved the sample in freezer unwashed with deionized water

Sample Preparation

It involves homogenizing the test portion of fish such as gill, liver, muscles, kidney etc, weighing and then drying with oven in acid washed Petri dishes at a temperature of 80 °C for two days to obtain dry weight sample Abdulali *et al*., [26] and Mustafa *et al*., [24]. The samples are then crushed into fine powder by using porcelain mortar and pestle

Digestion of Sample

Sample digestion is a method of dissolving samples into solution by adding acids and heating until the complete decomposition of the matrix for release of analyte or for analysis of matals. There are two methods of digesting samples containing heavy metals: the microwave digestion method and conventional acid digestion method.

Microwave Digestion Method

The method is an alternative for conventional acid method because it retains volatile compound in solution, shorter time and less acid consumption Azaman et al., [27]. After crushing, 0.5g DW powder form of muscle gill and 0.1g DW powder form of liver because the liver is tiny compared to the gills and muscles in fish body is digested by using closed vessel microwave digestion with nitric acid (65%) and hydrogen peroxide (35%) mixture 3:1 in ratio at a temperatures (150^{o}) for 20 min. in the micro\wave [26]. Turkmen *et al.*, [28] using microwave digested sample tissues in 10ml of HNO_3 in Teflon vessels. Then, 1.5ml of 30% of H_2O_2 was added to break down organic matter that may be undigested during the acid digestion. Furthermore, Murthy *et al*. [29] had studied about fish meal and digested in Teflon containers using a microwave digester. Dried powder sample (3 g) was weighed into Teflon vials (100 ml) and digested overnight with 7mL of pure HNO_3 and 3 ml of H_2O_2. In another study, Ozparlak *et al*. [30] studied 1g of the homogenate was digested by the microwave digestion system. Digestion conditions for microwave system for the samples were applied as 2 min for 250 Watt, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, and 8 min for 550 W.

Conventional digestion method

Olawusi-Peters *et al*. [31] used FAO methods cited by Dybem [32] in order to dissect fish samples. 0.5 g of each fish sample was ashed in electric muffle furnace at 550 ºC for 24 h and diluted in 5 ml of mixture of concentrated HNO_3 and concentrated HCl with ratio 1:3. In other research, Bat *et al*. [33] has performed their study by using about 20 g of fish samples to digest the samples in hot concentrated HNO_3. Another study performed by Tao *et al*. [34], used 0.5 g of fishes and put into 50 ml Bunsen beakers and added to 10 ml nitric acid and 1 ml Perchloric acid. Then, 5 ml of HCl was added and transferred to 50 ml volumetric flasks for analysis. Besides, Ashraf *et al* [35]. Also studied about classical acid digestion and the methods used according to Moller *et al*. [36]. The samples were ashed for 4 h until a white ash residue was obtained. Then, the residue was dissolved in 5 ml of 25% HNO_3, transferred to a 25 ml volumetric flask and made up to volume. ICP-OES was used to analyze the level of heavy metals in fish tissue samples.

Table 2: Comparism of microwave and conventional digestion system [27]

Microwave digestion	Conventional digestion
highly expensive method	Less expensive method
Digestion period is less	Duration of digestion is high
Low probality of contamination	high probality of contamination
Low acid consumption	High acid consumption

Preparation of Test Solution

Following the digestion of test portion, test solution is prepared by diluting the digested solution with ultrapure water to a known volume. The test solution should be clear and colorless to slightly yellow. Occurrence of turbidity or a deep color often signifies incomplete digestion.

Toxicity of biological and non biological essential heavy metals on fish and their adverse effects on human by fish intake

The feeding and living of fish in aquatic environments make them vulnerable and heavily exposed to heavy metals because of their inability to flee from the harmful effects of contaminants. Unlike invertebrate, fish show more sensitivity to toxicants and are convenient test subject for indication of ecosystem health [37]. Below are some of the toxicity of biological essential and non essential heavy metals in fish.

Toxicity of biological essential heavy metal on fish Zinc toxicity on fish

Zinc serves as a cofactor in many enzyme systems, including arginase, enolase, several peptidase, as an active component for many important enzyme systems, zinc plays a vital role in lipid, protein and carbohydrate metabolism; being particularly active in the synthesis and metabolism of nucleic acids (RNA) and proteins. However at higher concentrations it creates unfavorable effects in fish by damaging structures which can hinder growth and survival of fish [38]. Also it aggregates in the respiratory structure of fish leading to hypoxia which

results in death [38]. Olaifa *et al.* [39] mentioned that zinc can cause changes in heart physiology. Sub-lethal levels of zinc have been known to unfavorably affect hatchability, existence and hematological structures of fish. Zinc could cause sub-acute effects that change fish behaviors, such observed behaviors include deficiency of balance since most fins are stationary in the affected fish, restless swimming, air guzzling, periods of dormancy and death [40]. Zinc poisoning of fish is mostly encountered in trout culture. Rainbow trout and brown trout, and especially their fry, are extremely sensitive to zinc and its compounds. The lethal concentrations are around 0.1 mg per litre for salmonids (some authors even suggest a level of 0.01 mg per litre) and 0.5 to 1.0 mg per litre for cyprinids. Resistance to zinc compounds increases with age. The toxicity of zinc to fish is influenced by the chemical characteristics of water; in particular, increasing calcium concentrations reduce the toxicity of zinc. The clinical symptoms and patho-anatomic picture of zinc poisoning in fish are similar to those found for copper [41]. In a study conducted by Bhatkar [42] the liver of *Labeo rohita* was exposed to zinc chloride, after ten days hepatic nuclei become swollen, disorganization of hepatic cells with edematous hepatocytes and many cells were devoid of cytoplasmic contents. Furthermore, after thirty days the entire liver tissue became a necrotic spongy mass with degeneration of sinusoids. Most of the hepatocytes lost their cell boundaries and some of them showed indistinct cell boundaries. Hepatocytes showed disintegration and at several places the nuclei could not be seen distinctly [42].

Effects of Iron on Fish

Iron assumes two forms in water surface, ferrous state II (soluble compounds) or ferric state III (mostly insoluble compounds). Oxygen concentration in the water, the pH and other chemical properties of the water dictates ratio of these two forms of iron in water. Ferrous iron (Fe^{2+}) is considered to be more toxic to fish than the ferric (Fe^{3+}) form [43]. Fish may be harmed by iron compounds in poorly oxygenated waters with a low pH where the iron is present mainly in the form of soluble compounds. Because the gill surface of the fish tends to be alkaline, soluble ferrous iron can be oxidized to insoluble ferric compounds which then cover the gill lamellae and inhibit respiration. At a low water temperature and in the presence of iron, iron-depositing bacteria will multiply rapidly on the gills and further contribute to the oxidation of ferrous iron compounds. Their filamentous colonies cover the gills. At first they are colourless but later the precipitated iron gives them a brown colour. The precipitated iron compounds and tufts of the iron bacteria reduce the gill area available for respiration, damage the respiratory epithelium and may thus suffocate the fish. In a similar toxic action, iron compounds can precipitate on the surface of fish eggs which then die due to a lack of oxygen. The lethal concentration of iron for fish is not easy to measure because it depends to a large extent on the physico-chemical properties of the water. In cyprinid culture, it is generally accepted that the concentration of the soluble ionized forms of iron should not exceed 0.2 mg per litre; for salmonids this limit is 0.1 mg per litre [41]. The highest bioconcentration of iron in fish tissues was found in the liver and gonads, decreasing in brain, muscle and heart [44]. In banded tilapia (*Tilapia sparrmanii*), iron caused hyperplasia and necrosis of the secondary lamellae [45]. A scanning electron micrograph study on the gills of *T. sparrmanii* after exposure to sublethal iron concentrations for 72 hrs in a continuous flow system, revealed collapse of the gills as well as increased amounts of mucus cells [44]. Gill collapse reduces the diffusion distance between the water and blood, and benefits the oxygen consumption of fish. Also, iron compounds can precipitate on the surface of fish eggs causing death due to a lack of oxygen [46].

Toxicity of Copper to Fish

Although copper is highly toxic to fish, its compounds are used in fish culture and fisheries as algicides and in the prevention and therapy of some fish diseases. The physical and chemical properties of the water exert a strong influence on the toxicity of copper to fish. In water containing high concentrations of organic substances copper can become bound into soluble and insoluble complexes. In very alkaline water it forms hydroxides of low solubility, and in waters with a high bicarbonate/carbonate concentration copper precipitates as poorly soluble or insoluble cupric carbonate. The typical patho-anatomical appearance includes a large amount of mucus on body surface, under the gill covers and in between gill filaments [46]. Higher doses of copper caused visible external lesions such as discoloration and necrosis on livers of *Cyprinus carpio*, *Carassius auratus* and

Corydoras paleatus [47]. Exposure of Nile tilapia (*Oreochromis niloticus*) to sublethal levels of Cu has been shown to cause histopathological alterations in gills (edema; vasodilation of the lamellar vascular axis) and livers (vacuolation and necrosis) [48]. In High concentrations of copper have been reported to inhibit catalase (CAT) enzyme in liver, gill and muscle after 24 hr of exposure in carp (*Cyprinus carpio* L.) [49]. Cyriac *et al.*, [50] showed that fish acutely exposed to copper showed an increase in both hematocrit as well as hemoglobin content in blood, possibly due to changes in blood parameters which result in erythrocyte swelling or by release of large red blood cells from the spleen. Reproductive effects are noted at low levels of Cu and include blockage of spawning, reduced egg production per female, abnormalities in newly-hatched fry, reduced survival of young, and other effects [51]. The maximum admissible copper concentration in water for the protection of fish is in the range of 0.001 to 0.01 mg per litre, depending on the physical and chemical properties of water and on the species of the fish [41]. The characteristic clinical symptoms of fish poisoned by copper ions and copper compounds include laboured breathing and, in cyprinids, gasping for air at the water surface [41].

Toxicity of Non Essential Heavy Metals

Cadmium Toxicity

Cadmium is the non-essential and most toxic heavy metal which is widely distributed in the aquatic environment and earth's crust. *Oreochromis mossambicus* exposed to Cd showed liver alterations in the form of hyalinisation, hepatocyte vacuolation, cellular swelling and congestion of blood vessels [52]. Epithelial swelling of the renal tubules and mitochondrial and endoplasmic reticulum (ER) swelling (cloudy swelling) were observed in kidney of *Dicentrarchus labrax* exposed to cadmium [53]. Moreover, cadmium inhibits calcium uptake in gills [54] and may alter the metabolism of essential trace element by affecting normal tissue distribution of trace elements as Zn and Cu [55]. Fish exposed to cadmium revealed disturbances in blood constituents and differential blood count. Cadmium causes the destruction of erythrocytes, decreases the hematocrit value and hemoglobin concentration and leads to anemia [56]. Also, cadmium altered the metabolism of carbohydrates, causing hyperglycemia in some marine and freshwater fish species [57]. Also, adverse influence of long exposure to cadmium upon the maturation, hatchability and development of larvae was recorded [53]. Effects of accumulation of Cd on indicators of oxidative stress in several tissues of *Sparus aurata* were investigated by Souid *et al.* [58]. After exposure to 0.5 mg Cd/L for 24 h, concentration in intestine was 0.4 while that in liver was 0.13 mg/kg wet mass (wm). Witeska *et al*. [59] studied the effects of Cd (100 µg/L) on the embryonic, larval or both stages of the ide, *Leuciscus idus*. Their results showed that metal toxication affected mortality, body size, various body morpohometrics and deformities (vertebral curvatures and yolk sac deformities).

Lead Toxicity

Lead (Pb) is a persistent heavy metal which has been characterized as a priority hazardous substance [60]. Lead toxicity to fish and other aquatic organisms is significantly influenced by the water quality and depends on the solubility of lead compounds and on the concentration of calcium and magnesium in water.Acute lead toxicity is characterized initially by damage to the gill epithelium; the affected fish are killed by suffocation. The characteristic symptoms of chronic lead toxicity include changes in the blood parameters with severe damage to the erythrocytes and leucocytes, and degenerative changes in the parenchymatous organs and damage to the nervous system. In trout, a characteristic symptom is a blackening of the caudal peduncle; a biochemical effect is the inhibition of amino levulinic acid dehydrase (ALA-D) in fish blood [41]. The maximum admissible lead concentration in water is 0.004 to 0.008 mg per litre for salmonids and 0.07 mg per litre for cyprinids. When *C. batrachus* exposed to 5 ppm of lead nitrate for 150 days, it exhibited marked inhibition of gonadal growth and showed decrease in cholesterol and lipid levels in brain, testis and ovary whereas the liver showed an elevation of both [61]. Hepatocyte vacuolization, hepatic cirrhosis, necrosis, shrinkage, parenchyma degeneration, nuclear pyknosis and increase of sinusoidal spaces were the distinct changes observed in the liver of lead-exposed fish (Olojo *et al.*,2005). The necrosis and desquamation of gill epithelium as well as lamellar curling and aneurisms were the direct deleterious effects reported in chronic lead exposed *Clarias gariepinus* [62]. Shah and Altindağ

[63] reported significant increase in immunological metrics following Pb exposure, which suggests that Pb may weaken the immune system, resulting in increased susceptibility to infections.

Chromium Toxicity

In surface waters, the most stable forms of chromium are the oxidation states III and VI. Of these two forms, chromium III is poorly soluble and is readily adsorbed onto surfaces, so that the much more soluble chromium VI is the most common form in freshwater. Chromium compounds in the trivalent state (III) are more toxic to fish and other aquatic organisms than are those in the hexavalent state VI. From the LC50 data obtained for different fish species, chromium III compounds are among those substances with a high toxicity to fish (LC50s of 2.0 to 7.5 mg per litre) whereas chromium VI compounds are among those substances of medium toxicity (LC50s of 35 to 75 mg per litre) [41]. With acute poisoning by chromium compounds, the body surface of the fish is covered with mucus, the respiratory epithelium of the gills is damaged and the fish die with symptoms of suffocation. Fish suffering from chronic chromium intoxication accumulate an orange yellow liquid in their body cavity. The overall toxic impact on organs like gill, kidney and liver may seriously affect the metabolic, physiologic activities and could impair the growth and behavior of fish [64]. Chromium compounds Virk and Sharma, [65] assessed the effects of acute toxicity of chromium on fingerlings of the *C. mrigala*. After 45 days of exposure significant decline in the protein and carbohydrate content of gills was observed. Reduced locomotor activity has been reported in chronic Chromium-exposed *Gambusia affinis* [66].

Mercury Toxicity

The acute lethal concentrations of commonly found organic mercury compounds are from 0.025 to 0.125 mg per litre for salmonids and from 0.20 to 0.70 mg per litre for cyprinids. For salmonids the maximum admissible concentration of inorganic forms is about 0.001 mg per litre and for cyprinids about 0.002 mg per litre. For fish in general, the maximum admissible concentration of mercury in organic compounds has been suggested to be as low as 0.0003 mg per litre. The chronic data about mercury toxicity indicated that the organic form of Hg, methylmercury is the most chronically toxic of the mercury compounds [67]. Some reports showed that mercury compounds could be retained in the tissues of animals for long periods, resulting in irreversible damages, such as neurological impairment and lesions, behavioral and cognitive changes, ataxia, as well as convulsions, in addition to its harmful effect on reproduction [68]. Necrosis and fibrosis of renal tubular lumen was reported earlier in chronic mercury exposed *Clarias batrachus* kidney, at very low concentrations mercury reduces the viability of spermatozoa, reduce egg production and affect the survival rate [69].

Histopathological Alterations Due to Heavy Metal Pollution in Fish Organs/Tissues

It is the microscopic examination of biological tissues to observe the appearance of diseased cells or tissues in very fine detail. A change in tissue may signal either the presence of toxic substance or disease. According to the research carried out in Bulgaria by Velcheva *et al.* [70] using Bleak Rudd fish and Perch captured from Dame Lake pathological alteration in both gills and liver leading to adverse effects were vacuolar hydrpoic, degeneration of cytoplasm in hepatocytes, which were finally necrotic and infiltrated with inflammatory cells. The gills showed lamellar hyperplasia, edema, separation and fusions as well as expansion of the cartilaginous base of the gill arches as seen figure 1-6.

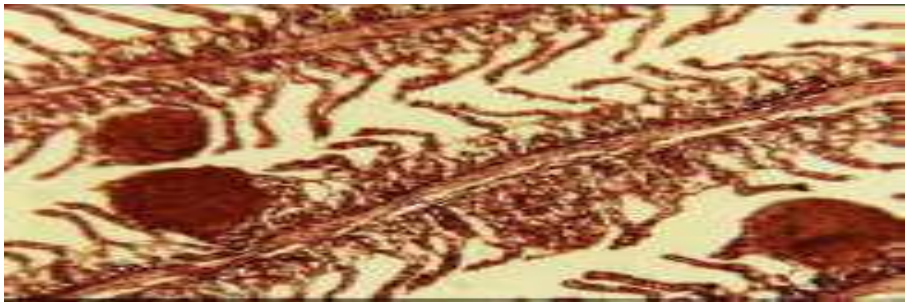
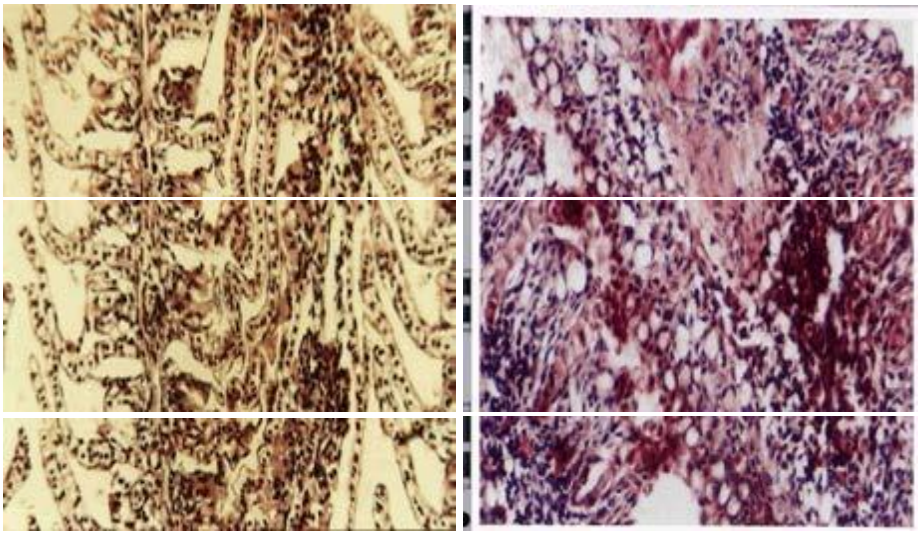


Figure 1: Gills of Tilapia fish reared in heavily polluted area with heavy metals showing lamellar telanejctasis (H&E, X400) [21]



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Figures 2 & 3: Gills of Tilapia fish reared in heavily polluted area with heavy metals showing lamellar necrosis (H&E, X400) [21]

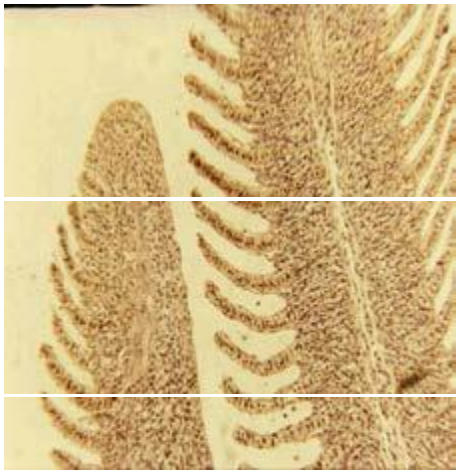

Figure 4: Gills of Tilapia fish reared in heavily polluted area with heavy metals showing lamellar hyperplasia and fusion (H&E, X400) [21]

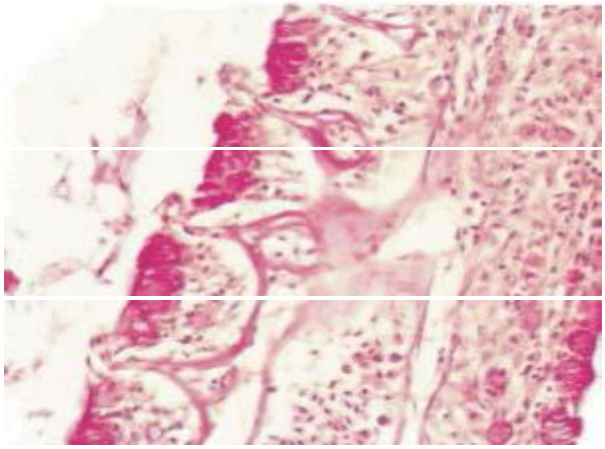

Figure 5: Hyperactivation of goblet cells (PAS stain, X400) [21]

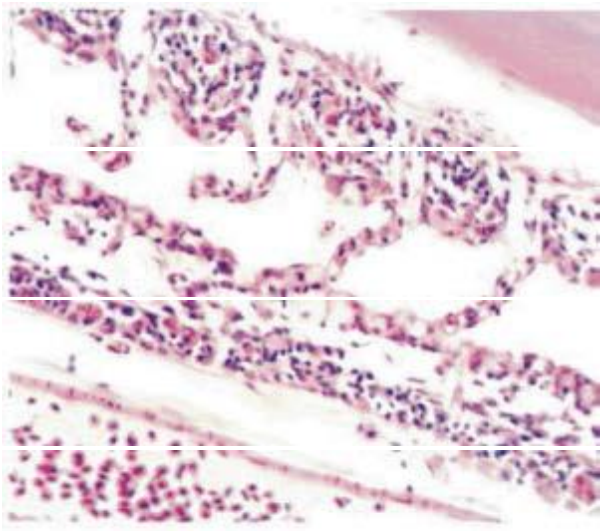

Figure 6: Gills of Tilapia fish reared in heavily polluted area with heavy metals showing lamellar edema and separation (H&E, X400) [21]

Conclusion

Human activities discharging waste such as heavy metals into aquatic environment have negative impact on fisheries resources and its consumers. Fish being an organism at the top of aquatic food chain , accumulate a great deal of heavy metals from the water column and sediment through feeding. Since fish is an important human nutrition containing fatty acid that can reduce the risk of heart diseases and stroke due to their contribution in lowering the cholesterol levels in blood and also provides minerals and vitamins, the maximum permissible limits of heavy metal for intake of fish should be a concern. There is need for organizations to intervene by creating awareness on the hazards of heavy metals on fish health and its consumers.

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