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# Impact of Water Pollution with Heavy Metals on Fish Health: Overview and Updates

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**Abstract:** Heavy metals (HM) are natural trace components of the aquatic environment, but their levels have been increased due to industrial wastes, geochemical structure, agricultural and mining activities. All these sources of pollution affect the physicochemical characteristics of the water, sediments and biological components, thus negatively affecting the quality and quantity of fish stocks. Environmental pollution is a worldwide problem; heavy metals constitute one of the most important pollutant challenges. The progress of industry has led to increased emission of pollutants into ecosystem. Environmental pollution can cause poisoning, diseases and even death to fish. The absorption and accumulation of different pollutants vary among different biological systems. Therefore, the aims of the present review article are three-way; first to high lighten the impact of the bioaccumulation of heavy metals in different organs of fish and the factors affecting their dissemination. Second, to monitor the biomarkers that is used in the determinations and diagnoses of heavy metal toxicity and pollution. Finally, the role that is played by the histopathological studies on the diagnosis of fish diseases caused the heavy metals.

**Key words:** Heavy Metals · Fish · Pollution · Biomarkers · Histopathology

## INTRODUCTION

Due to the insufficiency of protein sources for the vast increased population in the third world countries, fish farming has gained popularity as alternative source of a cheap protein. However, in several areas of these countries they use recycled water from agricultural drainage, industrial drainage or even sewage. These sources of recycled water comprise a health challenge, either to the fish raised in it, to the ecosystem or to the human consuming the fish.

Therefore, water is the most natural resource that exists on our planet and is essential for survival and the development of modern technology. Thus, rapid industrialization is one of the main causes for aquatic pollution. Discharged wastewater has been used in different regions of the world for fish raising [1-3]. Fish are one of the most widely distributed organisms in the aquatic environment and, being susceptible to metal contamination, may reflect the extent of the biological effects of metal pollution in waters [4].

The nutritive and economical values of fish are attributed to its good and cheap source of protein and minerals, richness in non-saturated fatty acids and Omega-3 that help in reduction of the blood cholesterol and prevent heart malfunction (arteriosclerosis) [5].

Environmental pollution is a worldwide problem as heavy metals belong to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems [6]. Environmental pollution can cause poisoning, diseases and even death for fish and the absorption and the accumulation of different

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biological tissues on pollutants are different. The absorption of heavy metal elements of various biological tissues on pollutants is an important biomedical problem [7]. Pollution describes the introduction of foreign substances into the biosphere. As xenobiotics, some of these pollutants sometimes find their way into the human system through the food chain. In the body, pollutants may undergo biotransformation, metabolism and excreted without the risk of toxicity depending on the chemical characteristics of these compounds and their dose. However, some of the pollutants resist chemical and biological transformation and accumulated in the tissues including, liver, kidney and nerve, to cause toxicity [8].

Water pollution is defined as introduction by man, directly or indirectly, of substances or energy to the aquatic environment resulting in deleterious effects such as hazards to human health, hindrance of fish activities, impairment of water quality and reduction of climate amenities. Contamination also, caused when an input from human activities causes an increase of a substance in fresh or seawater, sediments and organisms above the natural background level for that area and for those organisms [9]. Industrial development in the developing and undeveloped countries has resulted in heavy metal contamination of local water. Metal pollution may damage aquatic organisms (either fresh or marine water) at the cellular levels and possibly affect ecological balance. Exposure and ingestion of polluted aquatic marine products such as seafood can cause health problems in human and animals including neurological and reproductive problems [10]. Contamination is usually measured as parts per million (ppm=µg/L) or parts per billion (ppb=ng/L) and measured as "wet weight" (contamination in moist water containing-tissues) or as "dry weight" (contamination in dehydrated tissues) as water content can vary considerably, weight was found to be a better measure [9].

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (AS), chromium (Cr), thallium (Ti) and lead (Pb). Heavy metals are natural components of the earth' crust. They can't be degraded or destroyed. Heavy metals (HM) are natural trace components of the aquatic environment, but their levels have been increased due to industrial wastes, geochemical structure, agricultural and mining activities [11]. All of these sources of pollution affect the physicochemical characteristics of the water, sediments and biological components and thus the quality and quantity of fish stocks [12]. Although there is no clear definition of what a heavy metal is, density is in most cases taken to be the define factor. HM are commonly defined as those having a specific density of more than $5g/cm^3$. The main threats to human health from HM are associated with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid, but is usually classified as heavy metal). Heavy metals have been used in many different areas for thousands of years. Lead has been primarily used at least 5000 years ago in building materials, pigments for glazing ceramics and pipes for transporting water. In ancient Rome, lead acetate was used to sweeten old wine and some Romans might have consumed as much as gram of lead a day. Mercury was alleged to be used by the Roman as a salve to alleviate infants teething pain and was later ($13^{th}$-$18^{th}$ century) employed as remedy for syphilis. Adverse health effects of heavy metals have been known for a long time, due to continuous exposure to HM and working in the gold mines in many parts of Latin America. Arsenic is still a common constituent in wood preservatives and tetraethyl lead remains a common additive to petrol, although this use has decreased dramatically in the developed countries. Since the middle of the $19^{th}$ century, production of heavy metals increased steeply for more than 100 years, with concomitant emission to the environment [13].

To a small extent, these HM enter our bodies via food/drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. HM poisoning could result, for instance, from contaminated-drinking water (lead pipes), high ambient air concentrations near emission sources, or intake via the food chain. Heavy metals are dangerous because they tend to bioaccumulate in the body. Bioaccumulation means an increase in the concentration of a chemical in a biological organism (fish for example) over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. HM can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, rivers and ground water [14]. Heavy metal is a member of a loosely defined subset of elements that exhibits metallic properties. It mainly includes the transition metals, some metalloids, lanthanides and actinides. Different definitions are proposed, some based on density, some on an atomic number or atomic weight and some on chemical properties or toxicity [15].

Heavy metals are known toxicants, which reflect acute disorders in aquatic organisms. Uptake of heavy metals through food chain in aquatic organisms may cause various pathological disorders like hypertension, sporadic fever, renal damage, cramps in human [8]. The fish represent an important target for biomagnifications of metals as they are at top of the food pyramid and act as a possible transfer media to human beings [16]. Studies on various fish species showed that heavy metals accumulated mainly in the liver as a metabolic organ that stores metals to detoxification by producing metallothioneins [17]. Also, metals are concentrated in the gills, could be due to the element complexion with mucus, which is impossible to remove completely from and between the lamellae [18]. The pronounced heavy metal effluents were delayed embryonic development, malformation and reduce growth of adults of fish, mollusks and crustaceans [19].

Fish living in polluted waters tend to accumulate heavy metals in their tissues. Generally, accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, PH, hardness, salinity) and intrinsic factors such as fish age and feeding habits. Various metals show different affinity to fish tissues. Most of these metals accumulate mainly in liver, kidney and gills. Fish muscles, compared to the other tissues; usually contain the lowest levels of metals. Accumulation of heavy metals in various organs of fish may cause structural lesions and functional disturbances [20]. Living organisms require varying amounts of heavy metals, for example, human body requires iron, cobalt, copper, manganese, molybdenum and zinc. Excessive levels can be damaging to the organism. Other heavy metals such as mercury, plutonium and lead are toxic metals and their accumulation overtime in the bodies of animals can cause serious illness. Certain elements that are normally toxic might be beneficial for certain organisms or under certain conditions, of these are vanadium, tungsten and even cadmium [21]. Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Allergies are not common and repeated long-term contact with some metals may cause cancer. Heavy metals have an effect on the stability of colloids and prokaryotic and eukaryotic cells are of colloidal nature [22]. Colloids are sensitive to change of ion or heavy metal concentration and it leads to violation of colloid stability and subsequent disintegration [23]. In medical usage, heavy metals are loosely defined [15] and include all toxic metals irrespective of their atomic weight. Heavy metal poisoning can possibly include excessive amounts of iron, manganese, aluminum, mercury, cadmium, beryllium or arsenic. Minamata disease results from mercury poisoning and Itai-Itai disease from cadmium poisoning.

**Sources of Heavy Metals Pollution:** The sources of heavy metals were reported by The FAO [24] as follows; mining effluents, industrial effluents, domestic effluents, urban storm-water, leaching of metals from garbage and solid wastes dump, metal inputs from rural areas, batteries, pigments, paints, glass, fertilizers, textiles, dental and cosmetics, atmospheric sources and petroleum industrial activities. Moreover, heavy metal pollution can arise from many sources as smelting of copper, preparation of nuclear fuels, electroplating with chromium and cadmium. Cadmium, lead and zinc are released into tiny particles as dust from rubber tires on road surface. These small size particles allow these toxic metals to raise on the wind to be inhaled, or transported onto top soil or edible plants. Cadmium compounds are used as stabilizers in PVC products, color pigments, several alloys and now most commonly in rechargeable nickel-cadmium batteries. Metallic cadmium mostly used as an anticorrosion agent. Cadmium is present also as a pollutant in phosphate fertilizers. The anthropogenic sources of cadmium, including industrial emission and application of fertilizers, sewage sludge to farmland, may lead to contamination of soils to increase cadmium uptake by crops and vegetables grown for human consumption. Cigarette smoking is a major source of cadmium exposure. Food is the most important source of cadmium exposure in the general non-smoking population [25-27]. General population is exposed primarily to mercury via food, fish being a major source of methyl mercury exposure and, dental amalgam. A major use of mercury is in the chlor-alkali industry, in the electrochemical process of manufacturing chlorine, where mercury is used as an electrode. Organic mercury exists as methyl mercury, which is very stable and accumulates in food chain. Methyl mercury was commonly used for control of fungi on grain seeds [28, 29]. Occupational exposure to inorganic lead occurs in mines and smelters as well as welding or lead painters and in the battery plants. Low or moderate exposure may also take place in the glass industry. High levels of lead in air emission may pollute areas near lead mines and smelters. Airborne lead can be deposited on soil and water, thus reaching human via the food chain [30]. Lead emissions are related mainly to road transport and thus most uniformly distributed over space. Arsenic is a widely distributed metalloid, occurring in the rocks and in water used for drinking in several countries all over the world (e.g. Bangladesh, Chile and China), whereas organic compounds (such as arsenobetain) are primarily found in fish causing human exposure. Smelting of non-ferrous metals and the production of energy from fossil fuel are the two major industrial processes that lead to arsenic contamination of air, water and soil, smelting activities being the largest single anthropogenic source of atmospheric pollution. Other sources of contamination are the manufacture and use of arsenical pesticides and wood preservatives [31].

**Bioaccumulation of Heavy Metals:** Metals in natural waters occur in particulates or in soluble forms, including labile and non-labile fractions. The labile metal compounds are the most dangerous to fish. They include various ionic forms of different availability to fish. Data show that the amounts of metals in the labile fraction and the share of various metal ions strongly depend on environmental conditions [32, 33]. The results of many field studies of metal accumulation in fish living in polluted waters show that considerable amount of various metals may deposited in fish tissues without causing mortality. Various metals are accumulated in fish body in different amounts. These differences result from different affinity of metals to fish tissues, different uptake deposition and excretion [34]. Generally, the higher metal concentration in the environment, the more it may be taken up and accumulated by fish. Relationship between metal concentrations in fish and in water was observed in both, field and laboratory studies [35, 36]. Metals level in live fish usually follow the ranking; Fe >Zn>Pb>Cu>Cd>Hg. The levels of Zn may be very high, up to over 300 µg/g dw. The maximum concentrations of lead and copper are lower and usually don't exceed 10 µg/g dw. Metal accumulation in fish depends on pollution and may differ for various fish species living in the same water stream [37].

Poleksic and his colleagues stated that the accumulation of Cd, Pb, Cu, Fe, Zn, Mn, Hg and As in Danube Basin (Hungary and Serbia) in fish liver, gills and skin was in response to the presence of these pollutants in the environment and together with other pollutants [38]. Schludermann group used the fish macroparasites as bioindicators of heavy metal pollution at selected river stretches in Austria [39]. Firstly, changes in the diversity of endoparasites of the cyprinid barbell, *Barbrus barbus* (L.) were tested in relation to heavy metal contents in the aquatic system. Secondly, the bioaccumulation potential of Cd, Pb and Zn was assessed in the acanthocephalan. The group of Wan used a laser-induced breakdown spectroscopy (LIBS) method to analyze heavy metals quantitatively. Analayze heavy metal elements in various tissues of some contaminated fish samples [7]. Experimental results showed that there was heavy metal accumulation in fish liver, mouth and gills...etc, however the heavy metal content in fish meat is very low. This method can be used for assessment studies of the influence of pollution on the fish and can be promoted in biomedical fields. Contrariwise, Saleh and his group used graphite furnace atomic absorption spectrometry for the determination or measurement of the concentration of four heavy metals (Zn, Cr, Cd and Pb) in fish, water and sediments from samples collected from Caspian Sea in Iran [6]. The results showed that the concentrations of heavy metals were higher in water and fish than the recommended limits. In India, various studies have been conducted on heavy metal bioaccumulation in the muscle tissues of fish collected from different fresh water aquatic systems in relation to their concentrations in water [40, 41]. The environmental factors affect the uptake and accumulation of heavy metals in fish. Cadmium and lead levels in *Salvelinus alpinus* liver and kidneys indicate higher uptake rates of both metals in summer when water temperature was high [32]. The authors attributed that to the increased metabolic rate. Many data indicate that water acidity (pH) directly affects metal accumulation rates by the fish. Comparison of data concerning metal levels in fish from various lakes indicates the concentrations of cadmium and lead, but not zinc, are considerably higher in fish from acidified lakes [41].

Accumulation of copper is also higher at lower pH [33]. Water acidity affects bioaccumulation of metals by the fish in an indirect way, by changing solubility of metal compounds or directly, due to damaging of gill epithelia which become more permeable to metals. On the other hand, competitive uptake of $h^+$ ions may inhibit metal absorption. Water hardness (mainly due to calcium carbonates) considerably affects metal transport across the gill epithelium. Water hardness reduced copper accumulation in the gills of fish [40]. It has been reported that elevated dietary $Ca^{2+}$ protected against both, dietary and waterborne Cd uptake [42]. Also, salinity reduced uptake and accumulation of metals by the fish. Salinity decreases copper accumulation in fish tissues and inversely proportional to lead accumulation [43]. In fish, concentrations of most metals (except mercury) are usually inversely related to the age and size [44].

Recently, Mahino and Nazura [45] collected water samples from 22 Km segment II of Yamuna River from Okhla barrage, India. The water samples were used to determine the presence of Cr, Ni and Pb through the atomic absorption spectrophotometery. They found that the concentrations of the heavy metals were much above the maximum permissible limits set by WHO. This was bound to have its influence on the river flora and fauna. Also, the concentrations of these metals were higher in liver, kidney, gills and muscles than the permissible limits recorded by both WHO and FDA. These high levels of metals caused severe damage to the liver and kidneys. Bioaccumulation factor (BAF) used to evaluate the heavy metal concentrations in different fish tissues and calculated as the ratio of concentration of pollutant (heavy metals) accumulated in the tissue of organism with respect to the concentration of that polluted using the following formula:

BAF = Concentration of heavy metal in fish tissue $\mu g\ g^{-1}$/ Concentration of heavy metals in surrounding water ($\mu g\ L^{-1}$).

Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances [34].

Adverse effects of metals on fish are not only related with material accumulation but also to cumulative toxic effects. Lethal disturbances are resulted due to exceeding certain values of heavy metals concentration in fish [35]. In most cases, fish from metal–contaminated water are safe for human consumption due to low metal accumulation (except for mercury) in the muscle tissues. However, such contaminated fish may constitute a potential risk for predatory fishes, birds and mammals feeding on it [46,47].

## Heavy metal Determination/Detection

**Wet Digestion (Microwave Digestion):** The edible parts of fish (i.e. muscles) or other organs (liver, kidney, gills …etc.) separated and washed with tap water followed by doubled-distilled water, weighed and then oven-dried at 101 °C for 3 hours. The dried fish samples are crushed and powdered in agate mortar and then stored in polyethylene bottles at 30°C until analysis. The dried tissues samples of 2.5±0.5 g from each fish are digested using a microwave digestion over the classical method include shorter time, lowered acid consumption and the retention of the volatile compounds in the solution. After digestion, the residues are diluted to 25 ml with 2.5% of $HNO_3$. Approximately 1 ml of concentrated $HNO_3$ is added to the water samples to prevent the microbial utilization of heavy metals. Suspended particles matter in water samples are separated by filtering through 0.45 mm Whatman GF/C filters. River water samples are acidified to 0.5% (v/v) separated using concentrated nitric acid for sample precipitation. The digested water and fish samples are assessed with atomic absorption spectrometry (AAS) (GBC-AVANTA, Victoria, Australia) [48,49]. Gato *et al.* [50] described the multi-element analysis (MEA) to determine the lead in blood, liver, kidney and bone marrow. MEA measurements are carried out by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). Approximately 1g each of blood, liver, kidney and marrow is weighed into Teflon carousels containing 10 ml of 50% nitric acid (Ultra trace purity) and digested at high pressure in a microwave oven. After digestion, the samples are transferred to 50 ml conical tubes and diluted with 3% nitric acid to the 50 ml mark and further samples are diluted to a ratio of 1:10, 3 % nitric acid for final analysis. A 10 µl yttrium internal standard (10 µg/ml) is added to each sample just prior to ICP-MS analysis.

**Detection of Heavy Metals by Histopathology:** Also, the effect of heavy metals can be detected through the histopathological techniques. The histological analysis of fish organs (liver, kidney, gills, skin, muscles...etc) is done by the means of classical methods described by Romies [51], in which tissue samples are put in a series of ascending grades of ethyl alcohol (70-100%) for dehydration. Clarification is done in xylol or chloroform. The treated samples are embedded in paraffin wax (45°C). The paraffin blocks are prepared and with a microtome cuts at thickness of 0.6 µm are done. The tissue sections are stained with hematoxylin and eosin (H&E). For long-term examination, the stained slides are covered using Canada balsam and examined with a light microscope (OLIMPUS CX21), using a reference control tissue and photographed using a digital camera [52].

## Hazards of Heavy Metals

**Mechanisms of Pathogencity:** Lead (Pb) and cadmium (Cd) are industrial pollutants which have strong negative effect on human and animal health. These metals are accumulated in the organism, mainly in the liver and kidneys. The exposure to toxic elements could be minimized by regular control of food and feed and setting maximum levels for heavy metals in these products [53].

Lead exerts its effect, physiologically and biochemically as a mimetic agent substituting for essential elements participating in metabolism such as calcium, iron and zinc. For instance, it directly interferes with zinc and iron in the biosynthesis of heme, in the function sulfhydryl group rich-protein enzymes and in protein synthesis in general either directly or indirectly [54, 55]. Lead binds to different kinds of transport proteins including, metallothionein, transferrin, calmodulin and calcium-ATPase.

The maximum Cd and Pb levels permitted for sea fish are 0.1 and 0.4 mg/g, respectively. The levels of Cd and Pb in the underground water were reported as 0.003 and 0.01 mg/L, respectively [56]. WHO/FAO [16] reported that Cd in surface water is usually found together with zinc but at much lower concentrations. The Cd present in surface water may be either dissolved or insoluble. Of the dissolved forms, those which may be poisonous to fish, include the simple and various inorganic and organic complex ions. Its acute toxic action damage to central nervous system and parenchymatous organs, very small concentrations of Cd may produce specific effects after a long exposure period [57].The acute lethal concentration of Cd for different species of fish ranges from 2-20 mg/L [55]. The Cd is deposited in soft tissues of the body with 50-70% accumulation in both kidneys and liver. In whole blood, Cd is bound to the erythrocytes [58]. The Cd-induced renal damage in human beings is represented by proteinurea, renal tubular cell damage, decreased proximal tubular reabsorption and increased creatinin levels.

## Health Hazards

**Human:** Inhalation of cadmium fumes or its particles can be a life threatening and although acute pulmonary effects and death are uncommon, sporadic cases still occur. Also, Cadmium exposure may cause renal damage. Long-term high cadmium exposure may cause skeletal damage. First reported from Japan, where the Itai-Itai (Ouch-Ouch) disease (a combination of osteomalacia and osteoporosis) was discovered in the 1950s. Animal experiments have suggested that cadmium may be a risk factor for cardiovascular diseases. Cadmium is highly toxic metal that was the cause of death, serious illness, rheumatoid arthritis (RA), full skeletal deformities, depressed growth, hypertension and fetal deformity. The International Agency Research on Cancer (IARC) has classified cadmium as human carcinogen (group I). It was found to be associated with prostate cancer and renal cell carcinoma [58, 59].

Acute mercury exposure may give rise to lung damage. Chronic mercuric poisoning is characterized by neurological and physiological symptoms, such as tremors, changes in personality, restlessness, anxiety, sleep disturbance, depression, kidney damage, contact eczema and nervous system damage. Mercury toxicity include visual field construction, behavioral changes, memory loss, headaches, tremors, loss of fine motor control, spasticity, hair loss, mental retardation to fetus and fetal deformity, cerebral polsy, blindness, deafness and muscular rigidity [60]. The Minamata catastrophe in Japan in the 1950s was caused by methyl mercury poisoning from fish contaminated by mercury discharge to the surrounding sea resulting in an increased risk of coronary heart disease (myocardial infarction) [60].

The toxic effects of lead include anemia, proximal renal tubular damage, hypertension, cardiac disease, immune system suppression (antibody inhibition) and neurological damage. Also, the symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system, i.e. lead encephalopathy, neurotoxicity (sleepless and restlessness), disturbance of hemorrhagic sun thesis [61, 62].

Inorganic arsenic is acutely toxic and intakes of large quantities result in adverse symptoms to gastrointestinal tract, severe disturbances of the cardiovascular and central nervous systems and eventually death. In survivors, bone marrow depression, hemolysis, hepatomegaly, melanosis, polynephropathy and encephalopathy may be observed. Ingestion of inorganic arsenic may induce peripheral vascular disease, which in its extreme form leads to gangrenous changes (black foot disease in Taiwan). The latest WHO evaluation concludes that, exposure to arsenic via drinking water is causally related to cancer in the lungs, kidneys, urinary bladder and skin [31].

**Fish:** The toxic effect of mercury in marine environment was identified as health risk for humans (Minamata disease, 1956), neurological damage (brain damaged-fish), fetal deformity of the fingerlings, permanent disabilities, fatty liver and degeneration and immune system damage [60]. In fishes, it is observed that the external organs are affected due to the toxic chemicals (heavy metals for example), causing loss of equilibrium, increased opercular movement, irregular vertical movements, finally leading to death. Cadmium, lead, mercury and arsenic cause severe damage to the renal and nervous systems of fish as well as gill damage (severe destructive pathological changes, i.e. structural lesions) [13,37,45,64-67].

**Histopathological Alterations Due to Heavy Metal Pollution in Fish Organs/Tissues:** Histopathology deals with the study of pathological changes of the microscopic structure of the body tissue. Any peculiar type of alteration of cells may indicate the presence of the disease or the effect of toxic substances. Thus, study of histopathology of prime importance in the diagnosis, etiology and prevention of diseases in fish. Histopathological studies give us useful data concerning tissues prior to external manifestation [68]. The clinical histological changes in the liver of *Tilapia nilotica*, reared in area polluted with heavy metals, were cloudy swelling, vacuolar and hydropic changes of the hepatocytes as well as prominent coagulative necrosis, beside severe congestion and hemorrhage [68-70].

Velcheva *et al.* [64] reported the pathological changes in both gills and liver of Bleak Rudd fish and Perch captured from Dame Lake in Bulgaria polluted with heavy metals. These 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 (Figures 1-6) besides congestions of the cardiovascular system (CVS). Similar lesions were also recorded in *Tilapia nilotica* fish [68-70]. Recently, it has been stated the histopathological changes in liver, kidney and skeletal muscles of cyprinids fish reared in Kor River known as polluted with heavy metals as biliary canalculi dilatation, hemosiderosis, perivascular edema and melanophages hyperactivation [45,65]. Renal tubular cells suffered cloudy swelling, hydropic degeneration and necrosis infiltrated by interstitial inflammatory cells; skeletal muscles degeneration and necrosis were also noticed. Similar lesions in Tilapia fish were recorded [68-70].

**Gills:**

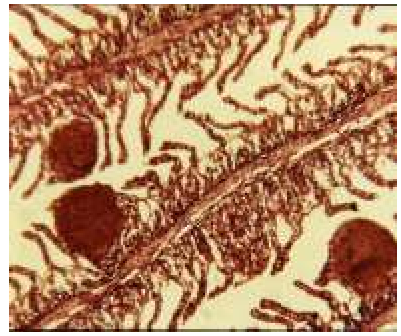
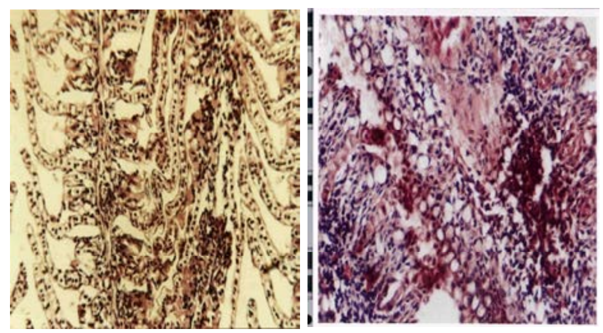


Fig. 1: Gills of Tilapia fish exposed/reared in areas heavily polluted with heavy metals showing lamellar telanejctasis (H&E, X400)



Figs. 2 & 3: Gills of Tilapia fish exposed/reared in areas heavily polluted with heavy metals showing lamellar necrosis (H&E, X400)

Fig. 4: Gills of Tilapia fish exposed/reared in areas heavily polluted with heavy metals showing lamellar hyperplasia and fusion (H&E, X400)

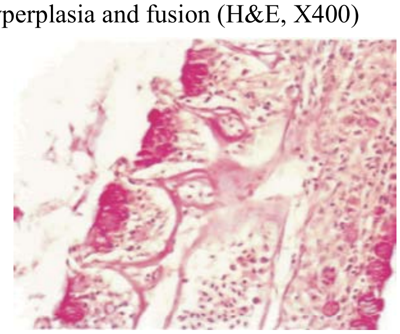


Fig. 5: Hyperactivation of goblet cells (PAS stain, X400)

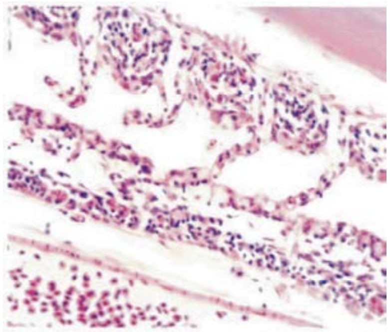


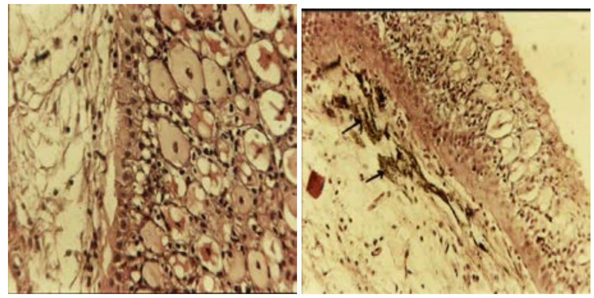
Fig. 6: Gills of Tilapia fish exposed/reared in areas heavily polluted with heavy metals showing lamellar edema and separation (H&E, X400)

**Skin:** Also, Tilapia fish skin was adversely affected by heavy metals pollution showing hyperactivation of goblet cells and dermal melanosis (Figs. 7 & 8) and dermal granuloma (Fig. 9).

**Kidneys:** In the polluted kidneys of carp fish there appears hyaline casts (Fig. 10), interstitial nephritis (interstitial mononuclear cells infiltration, Fig. 11) and renal necrosis and mononuclear cells infiltration (Fig. 12).

**Brain:** Sections of polluted carp brains exhibited symptoms of meningitis (Fig. 13) and gliosis (Fig. 14).

**Biomarkers:** Fish species were recently suggested as environmental biomarkers. Quantification of fish metallothionein transcript levels in absolute units has recently been presented [71]. Also, fishes are considered as early warning for the degradation of environmental quality, but also specific measures of the existence of toxic, carcinogenic and mutagenic compounds in the biological materials [72]. Liver and gills as main organs for metabolism and respiration are target organs for contaminants accumulation as reported by many authors concerning structural damage to organs and tissues related to the exposure of fish to petroleum derivatives [73]. The gills, liver and kidneys are commonly the primary target organs for pollution. Histopathological lesions and increase in size were reported in various fish exposed to heavy metals [73]. Measuring heavy metals in aquatic organisms may be used as bioindicators of their impact on organism and ecosystem health. Biomarkers are more efficient than bioindicators as measurements of heavy metals contamination because they deal with chemical and physiological changes on the organism level and assess contamination based on a direct measure of change in the organism [74]. Research overtime has focused on various



Figs. 7 & 8: Skin of fish reared in polluted area with heavy metals showing hyperactivation of goblet cells and dermal melanosis (H&E, X400)

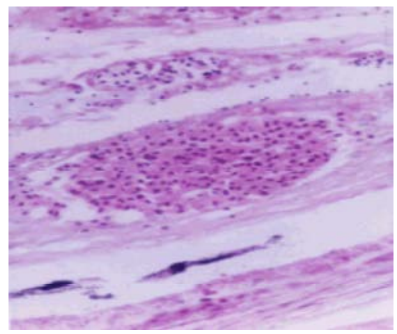


Fig. 9: Skin of fish reared in polluted area with heavy metals showing dermal granuloma (H&E,X400)

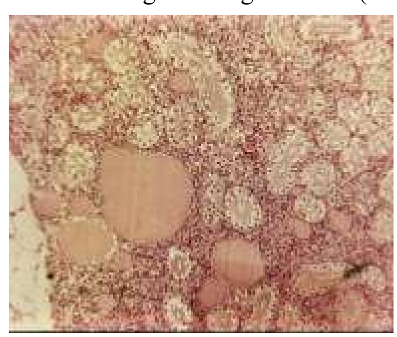


Fig. 10: Kidney of carp fish reared in polluted area with heavy metals showing hyaline casts inside the renal tubules (H&E, X400)

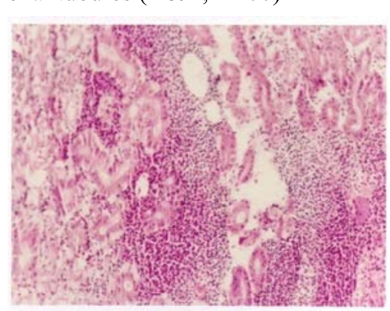


Fig. 11: Kidney of carp fish reared in polluted area with heavy metals showing an interstitial mononuclear cells infiltrations (H&E, X400)

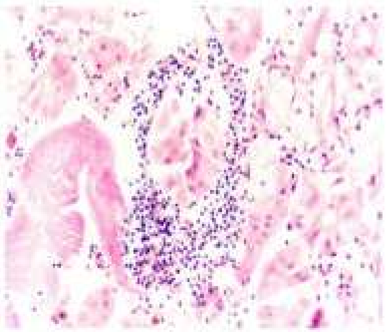


Fig. 12: Kidney of carp fish reared in polluted area with heavy metals showing renal necrosis with mononuclear cells infiltrations (H&E, X400)

Fig. 13: Brain of carp fish caught from area polluted with heavy metals showing meningitis (H&E, X400)

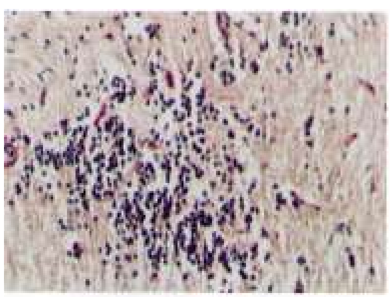


Fig. 14: Brain of carp fish caught from area polluted with heavy metals showing gliosis (H&E, X400)

species and various biomarkers to determine the amount of heavy metal toxicity in aquatic environment as sea anemones, sea urchins, grass shrimp and fish [75]. Biomarkers in marine fish such as glutathione (GSH) and metallothionein are often used to evaluate heavy metal contamination. Barry [76] used aquatic insects as effective biological monitors of heavy metals pollution (Zn, Cu, Pb and Ag). Diagnosis is a label given for a medical condition or disease identified by its signs, symptoms and from the results of various diagnostic procedures.

The term "diagnostic criteria" designates the combination of signs, symptoms and test results that allow the clinician to ascertain the diagnosis of the respective disease. Riji and George [77] reported that the diagnostic tools used in the diagnosis of fish diseases should be as follows:

- Case history (including water quality, feeding percentage, feed intake, fertilization, time of last algal bloom, liming details, treatment details and source of seed, stocking details); 2-Microscopic diagnosis (including light and electron microscopic slides preparations (H&E stain and special stains) for histological diagnoses; and 3-Immunological and molecular diagnoses; 4-Microbiological diagnoses; 5-Wet digestion (Microwave digestion) and 6-Biomarkers.

## CONCLUSION

This review highlights the causes and consequences of heavy metals contamination on fish organs.

There was a strong evidence of a correlation between heavy metals concentration in different fish tissue and those of the surface water of the polluted areas. The accumulation of metals in fish tissues depends on numerous factors, such as environmental concentrations, environmental conditions (i.e. pH, water temperature, hardness...etc), exposure duration and specie-specific living and feeding habits. Industrial wastes are potential source of heavy metal pollution in aquatic environments. In recent years, the anthropogenic pollution of aquatic ecosystems increased the need for studies to identify the impact of heavy metals on the species living there. Monitoring programs for bioaccumulation measurements serve as a biomarker for fish from contaminated places and provide information about the environmental conditions. Histological changes are more sensitive and occur earlier than any other evidence. They provide a better assessment technique of fish health and to the effects of pollution on each biochemical parameter. Metal pollution may damage aquatic organisms at the cellular level and possibly affect the ecological balance. Metallothionein is a low molecular weight protein that binds heavy metals in aquatic organisms, therefore, it is considered as a strong biomarker of heavy metal pollution in aquatic environments. Monitoring of environmental parameters (natural, man-made chemicals, biological and microbiological characteristics) is a key activity in managing the environment restoring polluted environments and anticipating the effects of man-made changes on the environment.

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