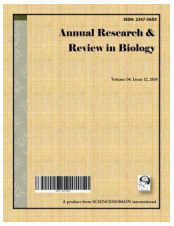

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Impact of Certain Heavy Metals on Histology and Physiology of Fishes: Interpretative Study

Basim M. Jasim[1*]

[1]Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Heavy metals, as essential or non-essential ones, are present in aquatic systems at different levels. The over limited concentration of metals has a negative effects as physiological, histological and morphological patterns. The levels of contaminants in fish are of particular interest because of potential risk to human consuming them. Heavy metals bring about their effects as single or mixture on organs or tissues of fish. The influence starts at cellular or molecular level, then being at population level, particularly through early stages of development, and may result in species extinction, due to tendency towards accumulation and reduced biodegradation. The liver, gills and kidney seem to be the most interested organs included in current studies due to their sensitized feature towards metal impact. Fishes could be employed as biomarker indicator for environmental contamination, in particular with long term monitoring, and with trace levels of pollutants. Overlap between different factors, along with integrated responses must be considered in determination of heavy metals impact, in complementary of water quality parameters; and so, standardized methods being necessary for comparison among variable results obtained. This study is an interpretative effort of some aspects included in toxicity of five heavy metals for fish species.

Keywords: Heavy metals; histopathology; accumulation; contamination; biomarker; fish.

**Corresponding author: E-mail: basimj180@gmail.com;*

1. INTRODUCTION

Aquatic system is a facile solvent, and so enables most pollutants to dissolve in it and contaminate it, along with inhabitant organisms.

Many people depend on aquatic products as a main source of food, therefore, the heavy metals in these products can threaten human health as these metals have the ability to accumulate in animals and human. The fishes are a good source of omega-3 fatty acids, which associated with human health due to cardiac protection [1].

Some heavy metals such as Cu, Zn, and Fe act as essential elements of enzymes and metabolism functions in fish whereas Cd, Pb, and Hg are toxic and may adversely affect DNA and enzymatic processes [2]. The aquatic organisms take up these metals and incorporate them in their body. Still, the higher concentrations of the metal may be converted as toxicant element. Heavy metals bioaccumulation in fishes limits their consumption by human due to threats they bring about health; therefore, assessment of heavy metals in fishes inhabiting different aquatic bodies is a prerequisite.

[3] assumed both biochemical parameters and histopathological observations in liver of freshwater fish (Cyprinidae) as sensitive indicators of liver metabolism for polluted areas. [4] suggested a rational use of biomarkers of oxidative stress in biomonitoring of aquatic pollution, since that alternation in the antioxidant enzymes, glutathione system (GSH) and induction of lipid peroxidation reflects the presence of the heavy metal which may cause oxidative stress in the fish.

Histology provides a rapid method to detect the effects of irritants in various tissues at different level, and so, the harmful effect is indicated among histopathological change in fish organs [5]. The histological alternations concern either entire organ or specific units of organ construction. [6] suggested standardized assessment to lead a better understanding of the significance of histological findings in fish after contamination exposure to heavy metals, and to compare different studies. Gills, kidney, liver and skin seem to be suitable sites in fish for histological investigation in order to detect the pollutant effect [6,7]. Grey mullet (*Mugil* sp.) showed protection for oxidative stress and low malondialdehyde at contaminated Huelva Estuary compared with that from reference area, and accordingly, it was proposed to use antioxidant enzymes and glutathione – S – transferase (GST) as tools for biomonitoring of aquatic pollution [8]. In this manner, the scientific research can identify possible environmental pollution early, prior to attaining serious levels.

Copper and zinc affect the species distribution in estuaries, although they are less obvious than that observed in experimental data [9]. Also, they may cause toxic effects in fish through disturbing the physiological activities [10], or disturbing reproduction [11]. [12] observed a destruction of spleen and kidney components in grass carp (*Ctenopharyngodon idella*) following exposure to sub-lethal doses of Cd.

Many species of fishes came under extinction as a result to metal pollution, in addition to other factors [13].

In this review we selected two essential heavy metals (Cu & Zn) and three non-essential ones (Cd, Pb & Hg) to demonstrate their impact on histological and physiological aspects of fishes, along with consequences follow that influences. Then the data were argued to manifest the interference among different factors, and prospects could be concluded.

1.1 Copper (Cu)

Copper is one of essential metals in the diet; still, it being harmful when large intake occurs. The cupric forms (Cu^{+2}) act as toxic matter for tissues; whereas the decrease of ion below the natural level in fish can affect physiological functions like osmoregulation [14]. Measured gill metal concentrations in fathead minnows (*Pimephales promelas*) correlated with free metal ion concentrations, not with total metal [15]. It was indicated that Cu^{+2} bound to ketones and aldehydes (C-II groups) are bioavailable, therefore, the higher proportion of C-II groups, or lower Cu^{+2}, had high mortalities for larval fathead minnows [16]. Copper is component element of some oxidative enzymes [17], and catalyzes the oxidation of unsaturated fats and oils [18]. Exposure of freshwater fish (*Labeo rohita*) to 0.5 ppm CuSO4 induced mild oxidative stress in the experimental samples with concomitant elevation of GSH and argininosuccinic aciduria (AsA) content of the muscle; where the 2.0 ppm CuSO4 in the ambient water led to severe oxidative stress, which forecast to suppress immune response in the fish [19]. There was significant decline in the protein level of the liver,

muscle and gills of *Channa punctatus* after 96h of experimental exposure to 5 mg/L CuSO4; that means an effect on vital role in the physiology of the fish [20].

The bioconcentration factor (BCF) of Cu attained 3.93 ppm in liver and 3.87 ppm in muscles of freshwater tilapia (*Oreochromis niloticus*) from Nile tilapia farms, where BCF is denoted as the ratio of chemical concentration in the organ to that in the surrounding water [21]. The acute lethal concentration dose LC_{50}/72h of CuSO4 attained 40.6 mg/L in farm fish *O. niloticus*, and caused apparent gill histological alteration represented by lamellar epithelial hyperplasia and desquamation of gill epithelium from underlying basement membrane. The liver showed degenerated and necrotic hepatic cells with pyknotic and karyolitic nuclei; that is during 6 weeks of treatment. The kidneys revealed severe degenerative changes of the renal tubular cell, but a decrease number of melanomacrophage centers [22].

The lowest level of Cu was found in the muscle and skin parts of *Capoeta capoeta umbla* in comparison with gills, gonads, kidney and liver tissues [23]. The condition was interpreted as a result to lower activity in these two tissues. However, there was no clear relationship between metal concentration and fish weight. Copper concentration amounted 0.38-1.07 μ g/gm in whole Antarctic fish (*Pagothenia borchgrevinki*), whereas it amounted 0.92-5.88 μg/gm in liver [24]. It is suggested that high accumulation of an element in an organ is related to the tendency of the organ in accumulation and detoxification [25]. Nile tilapia showed many epithelial lifting and dilated primary lamellae with congested blood vessels in its gills after 10 days of treatment with Cu, in addition to hyperplasia of interlamellar epithelium [26]. The hepatocytes in the same treated specimens showed vacuolar degeneration with a faint eosinophilic cytoplasm and aggregation of nuclei toward blood sinusoids. After 21 days of recovery some damage also observed in forms of a moderate disorganization of hepatic cords, damage of cell membrane and dilated, congested blood vessels. According to [6] these alterations in liver tissue could be classified as marked pathological importance. The Cu-treated Common Carp (*Cyprinus carpio*) eggs revealed distinct anomalies during developing cleavage. There are irregular distributed and uneven blastomeres [27]. Accordingly, most formed embryos (and some normally ones) died during embryonic development.

It was conducted that salinity acclimation have a drastic effect on Cu toxicity, when the euryhaline sheepshead minnow (*Cyprinodon variegatus*) exposed to different Cu concentration after acclimation to various concentrations and durations of salinity. Copper exposure had more effect on samples acclimated for shorten periods and lowest salinities [28]. Similarly, the exposure of sturgeon (*Acipenser naccarii*) to 35‰ salinity, for 20 days, caused changes in antioxidant enzyme activities and osmoregulatory processes [29]. Also, the fluctuations in salinity can influence the interactions between rainbow trout (*Oncorhynchus mykiss*) juveniles and pollutants in water, and therefore, affect toxicity of xenobiotics [30]. On the other hand, the toxicity of Cu to native fish (*Capoeta fusca*) decreased with increasing water hardness, determined as acute toxicity in hard and very hard waters, in comparison with that of soft water [31].

Copper, together with zinc, was found to be the principal toxicant polluted the trout streams in the catchment of the R. Mawddach in Wales, UK, where the water hardness was about 20 mg/L as $CaCO_3$. Analogous results were observed in Canadian lakes in the Flin Flon area [32]. The metal concentrations in common fish species from Dammam city ranged 8.76 ± 0.35 – 10.3 ± 0.11 μg/gm dry weight (DW); whereas it ranged 2.30 ± 0.08 – 9.74 ± 0.33 μg/gm DW in samples from Jazan (in the same country) during 2011 [33]. It points a drastic variance in concentration. The concentration of Cu in 10 marine fishes from Arabian Sea were in range of 0.006 – 0.052, 0.030 – 0.108 and 0.042 – 0.189 μg/kg in tissues of heart, skin and fillet respectively, where metal accumulation was highest in *Acanthopagrus berda* (in fillets) and lowest in *Pomadasys olivaceum* [34].

1.2 Zinc (Zn)

Zinc is component of enzymes, principally oxidases, so its deficiency may cause inborn error of metabolism [17,35]. Increased Zn and Cu concentrations observed in heart tissues of *Epinephelus microdon*, and justified as a result of specific metabolism process, a cysteine-rich copper binding protein and enzyme catalyzed reactions involving Zn and Cu occurrent in the heart tissues [36]. Zinc cannot be destroyed biologically but are only transformed from

oxidation state or organic complex to another through blood stream to different organs [37].

The range amount of Zn attained 138.13 – 170.40 mg/kg DW in liver of *Carassius auratus*, *Cyprinus carpio*, *C. damascina* and *C. aculeata* from Zayandeh-rood river, where it had the highest level in *C. carpio*, as the amount of metal depends on type of species and sampling area [35]. [24] indicated the existence of low concentration of Zn at skin by the pale colour of *P. borchgrevinki* from Antarctica. However, the biochemistry of the pigments, the source of colour, may be built on genetic control of metabolism, with consideration of diet [38]. Freshwater fish *Channa punctatus* exposed to sub-lethal concentrations of $ZnSO_4$. $7H_2O$ (6.62 and 13.24 mg/L) for 45 days. Concentrations calculated in fish organs at low and high doses were found to follow the order of liver (49.22 and 67.31 μg Zn/gm respectively) > kidney > intestine > gill > muscle (4.95 and 5.29 μ g Zn/gm respectively) [37]. This metal (that is among five heavy metals determined) was the highest in trend of accumulation in the heart, gills, kidney and liver of African catfish (*Clarias gariepinus*) from Nigeria Ogun River [4]. Zinc occasions accumulative action of toxicant on blood and ultimately to other cellular structures [37]. It's concentration increased gradually in the gills of *C. punctatus* and then decreased again by the end of exposure period (45^{th} day) compared to level at 30^{th} day, which indicate the induction of regulatory process [37]. Gills of *O. niloticus* showed moderate hyperplasia, lifting of lamellar epithelium and elongate secondary lamellae with curling in many parts after 10 days of treatment with Zn [26]. After 7 days of recovery period, the gills showed large spaces between secondary lamellae due to oedema leading to hypertrophy of chloride cells. The hepatocytes in the same individuals suffered some alternations as that caused by Cu treatment. The exposure of Indian common carp (*L. rohita*) to 2 mg/L $ZnCl_2$ for 10 days in laboratory prompted swelling of hepatic nuclei and disorganization of hepatic cells. After 30 days, the entire liver tissue became necrotic spongy mass with degeneration of sinusoids, and most of hepatocytes lost their cell boundaries [39]. High levels of Zn can affect circulatory, nervous and respiratory systems in fish body [35].

Zinc attained 88.88% bioavailable as supplementary material at Panethi reservoir which receives heavy metal-loaded waste water [40].

The accumulation rate of Zn was evaluated in *C. carpio, O. niloticus and P. hypophthalmus* from Cirata Dam. The contamination of Zn in fish meat ranged 7.3985 – 10.4972 ppm, which is within limits do not exceed the standard quality (40 ppm) of the food and drug administration of Republic Indonesia (FDA RI) [41]. The metal concentration attained 49.43 $\pm$1.58 μg/gm DW in sardines meat, whereas 16.79 $\pm$ 0.51 μg/gm DW in grouper collected from Dammam city, during 2011, which reflect high fluctuation among fish species [33]. Zinc showed the highest order of accumulation in tissues of six edible fishes from upper stretch of the Ganga River at West Bengal, compared to Cu, Cr, Cd and Pb [42].

The quantity of Zn in gills, liver and muscles of common carp sampled from River Kabul was 0.074 $\pm$ 0.01, 0.07 $\pm$ 0.009 and 0.018 $\pm$ 0.004 μg/gm respectively; whereas in *L. rohita* it was 0.058 $\pm$ 0.009, 0.088 $\pm$ 0.008 and 0.02 $\pm$ 0.008 μg/gm respectively [43]. Hence, the data point a variance in accumulation among organs of the two species studied, but the levels considered within the US recommended daily dietary allowances (RDA) limits. Likewise, [23] found that Zn levels was significant in kidney and liver of *C. c. umbla*, in relation to fish length, but insignificant in gill and skin.

1.3 Cadmium (Cd)

Cadmium in one of non-essential trace metals with high toxicity [18]. The complex species of Cd are inactive to ligand substitution, and so, it forecast to accumulate scarcity to metalloprotein complexes [36]. International guidelines restrict Cd concentration to 1mg/L water; whereas by Romanian guidelines the level is 0.01 ppm for salted fish [44].

There was a separation between the epithelium cells and the underlying pillar system in gill filaments, and dilated primary lamellae with severe congestion of blood vessels in freshwater tilapia after 10 days of treatment with Cd [26]. It seems that disturbances in gill structure, subsequent to the metal exposure, are invariably accompanied by drop in the electrolyte concentrations of blood plasma, particularly Na^+ and Cl^- with increased number of chloride cells. This drop was observed in cichlid fish (*O. mossambicus*) following exposure to 1000 μg/L Cd [45]; that is in addition to significant increased rates of necrosis and apoptosis in gills after 4 weeks of treatment.

Cadmium have obvious damage on the hematopoietic organs (spleen & kidney) of grass carp at doses 20-60 μg/L [12]. There was linear proportion between the degree of destruction and concentration of toxicant, as well as, the period of exposure. Cadmium resulted in severe disorganization of the hepatic cords with damage in cell membrane and hyperplasia of nuclei in liver of freshwater tilapia, as there was focal necrosis and dilated sinusoids after 21 days of recovery from the metal stress [26].

When coho salmon (*Oncorhynchus kisutch*) exposed to water-born Cd (150 ppb) the concentration of metal in scales increased more slower than that of water-born lead due to relatively rapid discharge of Cd through the mucus. The metal exposure induced a substantial increase in mucus production at the fish [46]. In the discus fish (*Symphysodon* spp.), Cd transfer from parental epidermal mucus to frys where they feed on epidermal secretion for several weeks after hatch. Then, fry body Cd concentration matched parental mucosal Cd concentration during 2-3 weeks [47].

Exposure of juvenile rainbow trout to acute dosage of $CdCl_2$ (2.4 mg/L for 2-4h) caused an increment in both plasma cortisol and T_4 levels, but had no effect on plasma T_3; whereas exposure to 0.8 mg/L dosage for 1 week gave rise to increase of plasma cortisol level and decrease of plasma T_4, but plasma T_3 remained stable [48]. When juvenile rockfish (*Sebastes schlegedi*) was treated with sub-chronic dietary Cd for 60 days, the weight and length of juveniles revealed significant inverse relationship in front dietary concentration Cd at 25 and 125 mg/Kg [49]. Also, there was decreased haemoglobin which appeared dose dependently, along with lower total protein in serum. Concentration of 9 ppm Cd in aquarium caused hypertrophied hepatocytes and sometimes loss of boundaries with shrunken pyknotic nuclei in liver of freshwater catfish (*Clarias batrachus*), that is after 28 days of exposure [50]. The Cd binding sites on gills of fathead minnows, as that for Cu, depend on free metal ion concentration rather than total metal, which successively, determine the toxicity to fish [15].

Cadmium affected early spermatogenic stages in the dogfish (*Squalus acanthias*), where it specifically activates a cell death program in spermatogonial clones, as passively affects blood-testicular barrier function [11]. Moreover, Cd accumulation and binding *in vitro* was testicular stage-dependent in dogfish, as its binding was specific where Hg could replace bound Cd. The biphasic apoptotic response was in PrM in spermatocytes, with compromise of blood-testis barrier function in PoM cysts [51]. It is worth mentioning that the differences and similarities between stocks and individuals, and the population of origin of single fish are determined through molecular markers, which resulting in numerous researches and applications in practical fisheries [52]. Cadmium accumulated in tissues of discus fish after 7 days of experimental exposure (400 mg/Kg through diet or 3 μg/L through the water) and caused significant alterations in ATPase activities of intestine and kidney, where epidermal mucus showed a high accumulation, particularly from the diet [53].

It was observed that the higher Cd concentration in some fishes from Kelantan River, Malaysia was in the station whish had more agricultural activity along the river bank, as the concentration was found to be elevated in the wet season [54]. However, the average Cd concentration was the lowest among 8 metals determined in 7 fish species from some lakes in Tokat/Turkey, and varied in the range of 0.1 -1.2 μg/gm [55]. Also, it was the lowest among 7 metals analyzed in kidney and heart tissues of marine fish *E. microdon* collected from the Arabian Gulf [36]. Like this, Cd was the lowest metal in order of concentration in heart, kidney, gills and liver of African catfish from Nigeria Ogun River [4]. Analyzed Cd content in different fish tissues from Arabian Sea was 0.00 – 0.41 , 0.00 – 0037 and 0.00 – 0.020 mg/kg in heart, skin and fillet respectively, where it was following the lowest order in comparison to Pb, Cu & Fe metals in *E. chlorostigma , L. rohita* and *P. argenteus*. However, it was not detected in the skin, heart and fillet of *Lutjanus argentimaculatus* [34]. Cadmium was estimated in the range of 1.17 $\pm$ 0.04 (in blackspot emperors) – 2.55 $\pm$ 0.15 μg/gm DW (in sardines) collected from Dammam city during 2011 [33].

Despite its presence in very low concentration in natural waters, Cd may accumulate in phytoplankton, the normal food source of many fish species, and enter fish body to accumulate there [44]. Omnivorous fish, in turn, were detected with elevated concentrations of Cd [54]. It is known that Cd containing products are rarely re-cycled [56]. The concentration of Cd increased in liver and kidney tissues of rainbow trout along with age, on the contrary of lead and arsenic

which showed a dilution with growth [57]. Still, it didn't alter significantly in muscle tissue. On the other hand, [24] concluded that Cd accumulation in *P. borchgrevinki* depends on exposure period or age rather than the metabolic turnover.

It was found that Cd is less toxic for rainbow trout while exposed to 2.6 mg Cd/L for 96 hours in hard water, in comparison with exposure 1.3 mg Cd/L in soft water; although without differences in Cd uptake among the two treatments [58]. In this meaning, the elevated aqueous Ca protected against acute respiratory and osmoregulatory action in rainbow trout due to exposure to combination of the (0.18 μM Cd and 0.80 μM Cu) in soft water, but for limited period, not for longer term [59]. Now, there may be variable results in hard water.

1.4 Lead (Pb)

Lead was put on the top of 6 pollutants or 4 metals poison the freshwater resources and may constitute a public health problem when the contaminated fishes are consumed as food [60]. According to animal data and insufficient human data in 1978, international agency for research on cancer (IARC) classified Pb as a possible human carcinogen [56,61]. Trace metals such as Pb can interfere with essential nutrients of similar characteristics like Ca and Zn [54].

After 10 days of treatment, Pb resulted in large subepithelial spaces with thin but much elongated secondary lamellae of gills in freshwater tilapia. Besides, severe damage was observed after recovery period. The liver in the same treated specimens showed a moderate to severe dystrophy in the form of hepatic necrosis [26]. The treatment for the African cat fish *C. gariepinus* juveniles with 0.006 – 0.008 mg/L of Pb $(NO_3)_2$ resulted in drastic reduction in the activity and feeding rate of the fish, with slower swimming. After 9 days of treatment with 0.006 mg/L the gills showed a gradual process of cyto-architectural distortion of the lamellae with primary and secondary lamellae overlapping, as there was a decrease in the size of gill because of shrinkage in cartilaginous supporting mass [5]. The liver in the treated specimens revealed a degree of hepatic cirrhosis with density of fibrous connective tissue, as there was a damage of biliary columnar epithelial cells. It is suggested that high accumulation of Pb in the liver of *C. gariepinus* is related to the function of the liver in accumulation and detoxification [4]. Trail for study of sublethal concentrations of lead acetate (0.4 – 0.7 mg/L) indicated different impacts on selected organs in adult Nile tilapia. The gills showed curling in secondary lamellae, lamellar swelling, shorter lamellae and lifting of the outer layer of lamellar epithelium. The ovary revealed increased number of atretic follicles, where the relatively older oocytes are more affected. The liver showed disarrangement of hepatic cords, nuclear pyknosis and cytoplasmic vacuolation; inside the loss of contact between hepatocytes and pancreocytes [62]. Gills of farm fish *O. niloticus* revealed congestion, edema and extravasaed erythrocytes with lymphocytic infiltration in gill arch after exposure to lethal dose of lead acetate for 2 weeks. There were severe degenerative changes after 6 weeks [22]. Yet, liver, under same experience, exhibited severe diffuse vacuolar degeneration of hepatocytes with necrosis of some hepatocytes after 6 weeks of exposure. The kidney, in turn, revealed vacuolar degeneration and extensive necrosis of epithelial renal tubules in addition to hyaline cast. The spleen had focal parenchymal edema and depletion of lymphoid follicles.

Lead concentration in scales of coho salmon exposed to water-borne Pb (150 ppb) increased more rapidly than concentration of Cd in the water-borne Cd exposed fish due to preferential uptake of Pb; as the metal induced a substantial increase in mucus production at the fish [46]. Sublethal concentrations of lead acetate (28.2 and 14.1 ppm) caused an increase of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in fish *Cirrihinus mrigala* which indicate liver damage. The gills showed lamellar degeneration, epithelial lifting, dilation with congestion in blood vessels of primary filaments and necrosis of lamellar epithelial cells [63].

It was observed that the range amount of lead in liver tissue was 1.30 – 3.65 mg/Kg DW in cyprinidae fish species (*C. carpio , C. aculeata , C. damasciana*) collected from Zayandeh – rood river, which receive waste waters [35]. The accumulation rate of Pb was evaluated in common carp, Nile tilapia and striped catfish (*Pangasianodon hypophthalmus*), from Cirata Dam, in the range of 2.0318 – 3.1553 ppm in fish meat, that exceeds the allowance standard quality (2 ppm) of the FDA R1 [41]. The concentration of Pb analyzed in 10 marine fishes from Arabian Sea were in range of 0.00 – 0.569 , 0.012 -0.529 and 0.008 – 0.218 mg/Kg in heart, skin and fillet respectively; where it followed variable orders among fishes in comparison to

Cd , Cu and Fe metals [34]. It comes out that fillet have lower concentration of metal than skin or heart. In fish meat of certain common species brought from Dammam city, Pb concentrations ranged 7.40 $\pm$ 0.70 – 9.17 $\pm$ 0.53 μg/gm DW, which exceeded the permissible levels (0.4 – 0.5 mg/kg DW) [33].

All tissues of fish (*Barbodes* sp.) that lives in the upstream and downstream of the Brantas River / Indonesia contain Pb, and revealed damage with necrotized cells. The highest level in succession, are found in the tissues: liver, gonads and gills [64].

1.5 Mercury (Hg)

Mercury is one of non-nutrient elements, as presents in waters at very low concentrations [65]. It scored the lowest concentration determined in common fish from Dammam city during 2011. It ranged 0.014 $\pm$ 0.003 – 0.055 $\pm$ 0.011 μ g/gm DW in comparison with higher values recorded for Cu, Zn, Pb and Cd [33]. However, these concentrations obtained are below the permitted mercury limit of 1.0 µg/gm DW. It is generally considered that 0.5 ppm of Hg in fish is the maximum permitted level [18].

It was observed that mean values of liver enzymes, GOT and GPT, were higher in carp *C. mrigala* exposed to sublethal concentrations of $HgCl_2$, that in coincidence comply with metal concentration. In addition, there was loss of cellular architecture in hepatocytes, along with haemolysis due to destruction of erythrocytes and prominent focal necrosis [63]. The gills in the same treated samples showed degenerative changes in the epithelial cells of secondary lamellae and moderate necrosis in inter lamellar cells. Experimental study for immature male yellowfin seabream (*Acanthopagrus latus*) to test the stress of $HgCl_2$ on gills indicated that the lower doses (10 and 20 μg/L) gave rise to fusion of some filaments, increase of mucus due to increased mucous cells, epithelial lifting of lamellae with edema and hyperplasia of the epithelial cells. The higher doses (35 and 50 μg/L) caused severe disorganization of filaments, severe increase of mucus and epithelial rupture [66].

It was found that the concentration of Hg, as that of Cd, increased in regards of age – related variation, in liver and kidney tissues of farmed rainbow trout, but the concentration did not alter significantly in muscle tissue with growth. Application of Hg mass balance model showed that the fish have a ratio concentrations as in feed with about 1:1 [57]. On the other hand, there was significant positive correlations existed between Hg concentration in muscle tissue and length, body mass and age in perch (*Perca fluviatilis)* and northern pike (*Esox lucius*) during three seasons of sampling, in addition to fluctuations of metal concentration among seasons [65]. Similarly, [67] noticed that the highest Hg level was ascertained in the oldest age group of rainbow trout at liver, muscles and kidney. These consequences may clear intake / excretion relationship through developmental stages in fish. In comparison study, it was revealed that Hg has assimilation efficiencies (AEs) higher than that of polychlorinated biphenyls in goldfish (*C. auratus*), while both chemicals were similar in elimination coefficients; simultaneously, Hg has a bioaccumulation potential in the fish 118% higher than the highest observed for polychlorinated biphenyls [68].

In *P. borchgrevinki* inhabited Antarctic ocean the metal was higher in the liver and low one in the muscle, in comparison to other organs [24]. The BCF of Hg attained 0.79 and 50 ppm in liver and muscles respectively, in freshwater tilapia cultured in Nile farms [21]. Hence, it is noticed that the metal still higher in liver than in muscles. It was observed that the Hg concentration in caudal fin clips samples from largemouth bass (*Micropterus salmoides*) was more than a factor of 20 greater than in scale samples (0.261 opposite to 0.012 μg/gm DW) [69].

To assess the bioaccumulation kinetics of inorganic mercury (Hg [II]) and methylmercury (MeHg) by assessment of assimilation efficiency (AE) of ingested prey in marine fish, the sweetlips (*Plectorhinchus gibbosus*), it was found that the AE ranged between 10-27% for Hg (II) and between 56-95% for MeHg. In general, the two types of Hg were assimilated at higher efficiency when associated with copepods (*Acartia spinicauda*), and at lower efficiency with brine shrimp (*Artemia* sp.). Besides, a much lower uptake of Hg (II), from aqueous phase, was demonstrated with increasing content ration of metal, whereas increasing uptake of MeHg with increased concentration of compound [70]. Therefore, it is stated that MeHg type is more poisonous than Hg (II) type. Also, the food habits must be considered to assess the toxicity of heavy metals, along with chemical composition. Inorganic mercury is converted to MeHg, which is very stable and accumulates among the food

chain [56]. Mercury is marked by conversion into highly toxic methyl mercury compounds in sediments or river and lake bottoms, principally at alkaline pH [18].

There was a highly significant relationship between biopsy and whole-fish Hg concentration determined in 13 piscivorous and nonpiscivorous fish species from 12 western states [71]. Mercury is found in high levels in tuna and swordfish, this may be due to the predatory lifestyle and long lives of these fishes [18].

The levels of Hg accumulation in fingerling tilapias *O. niloticus* were in order: liver > gills > muscles, where the maximum level recorded was 0.799 mg Hg/kg. After 96 h of exposure to 0.3 mg/L concentration there was a severe disorganization of epithelial cells and modification of the structure of secondary lamellae of gill, whereas liver was slightly affected [25]. Mercury concentrations increased in some tissues of silver crucian carp (*C. a. gibelio*) after 21 days of 0.25 ppm $HgCl_2$ exposure, as it was highest in gills (0.022 mg/kg WW) and lowest in cord (0.002 mg/Kg WW) [72]. Exposure of freshwater catfish (*Clarias batrachus*) to 12 ppm Hg for 28 days in aquarium prompted hypertrophied hepatocytes with pyknotic and displaced nuclei. Also, there were distinct intercellular spaces around most of the hepatocytes, with loss of normal shape of liver construction [50]. The exposure of *C. punctatus* to chronic nonlethal levels of $HgCl_2$ (16.7 ppb) caused degeneration and dispersion of interrenal and chromaffin tissue with necrosis in haemopoyetic elements in the head kidney, whereas the trunk kidney revealed highly decreased dimensions of Bowman's capsule and glomerulus; that indicate the effect on endocrine and excretory parts of the kidney [73].

It was determined that Hg concentration varied from 0.04 mg/kg (WW) in whitefish (*Coregonus lavaretus*) to 2.5 mg/Kg (WW) in northern pike from Lake Heddalsvatn, southern Norway at different seasons. As well, it increased by the trophic magnification factor (TMF) of 4.29 per trophic level in the lake food web [65]. The data reflect apparent variation of metal accumulation among fish species, along with season significance.

1.6 Mixture of Metals

In general, the mixed effects of toxicants on responses of fishes for lethal or sub-lethal ones cannot be interpreted by variations in exposure to every individual toxicant. The acutely lethal concentrations to freshwater fishes of mixtures of toxicants, present in sewage and industrial wastes, indicate that they being 0.4 – 2.6 times those predicted from the sum of the proportions of the respective toxic units of the predicted values [32]. The heavy metals (Cr, Ni, Pb and Cd) were elevated in catfish (*Heteropneustes fossilis*) and *Channa striatus* compared to recommended values of the Federal Environmental Protection Agency (FEPA), 1999 for edible fish, although the heavy-metal load is beyond the World Health Organization (WHO) maximum permissible limits. There were reduced activities of superoxide dismutase (SOD), catalase (CAT) and GSH in both species, as there were significant distortions in histology of liver, kidney and brain of affected fishes [74]. This phenomenon may indicates the bioaccumulation pattern.

The metals Hg, Cd, Cu and Pb resulted in hydropic swelling of proximal and distal tubules in the kidney of freshwater tilapia with many necrotic areas. Simultaneously, there were several histopathological alterations in muscles included degeneration in muscle bundles with aggregation of inflammatory cells between them and focal areas of necrosis. Still, Hg was most bioaccumulated and biomagnified in the muscles than other metals (Cd, Cu, Pb) [21]. Metal solution of combined (Pb + Cd + Cr + Ni) containing 1.25 mg/L of each metal ion occasioned increased activity of connective tissues, particularly near the kidney tract, in common carp after 32 days of exposure. There was a selective dystrophic change in kidney tubules, together with hyper secretion of mucus cells in the affected regions and pronounced activity of macrophages. In addition, the flesh samples showed disappearance of striations, with necrosis and homogenous liquid appearance [75].

Adult African freshwater fish (*Oreochromis mossambicus*) was studied to determine the toxic effect of mixture 5% and 10% concentrations of Cd and Zn on the histology of the liver. The results indicated hyalinization, vacuolation, cellular swelling and congestion of blood vessels at both concentrations; with an adaptive, regenerative response after prolonged exposure [76].

Concentrations of Cr, Ni and Pb in Yamuna River, which receives sewage drains, were much

above the maximum permissible limits (MPL) set by WHO, and the accumulations of these metals in liver, kidney, gills and muscles of *C. striatus* and *H. fossilis*, which inhabit the river, were above MPL. As a result, there were histopathological damages observed in liver and kidney of both species [77]. The concentrations of Zn, Cd, Pb and Fe in the liver of fish *Auchenoglanis occidentalis* sampled from Tiga dam, Nigeria, were higher than that in the water of dam; and degree of tissue change (DTC) attained 14.06 $\pm$ 9.46 , 9.75 $\pm$ 3.00 and 26.36 $\pm$ 11.16 in the liver, gills and kidney respectively. The histological investigation revealed epithelial hyperplasia, complete lamellae fusion, mucous cells hyperplasia and epithelial detachment in the gills. The liver has cellular infiltration and vacuolation, along with necrosis. Yet, the kidney showed glumeral and tubular necrosis; besides, a tubular vacuolations that indicate fatty degenerative changes due to metabolic disorders [78]. The activity of SOD and GST enzymes in African catfish from polluted river (by heavy metals) was found to be higher in liver, kidney and heart, but lower in gills, compared to that in individuals from Agodi fish farm (as control specimens); while CATA activities were reduced in above organs in comparison with control [4]. The heavy metal loaded waste water of Panethi reservoir catalyzed multiple biomarker responses in *C. punctatus* represented by low glucose content, lower albumin, higher serum globulin value, but lower albumin: globulin ratio (about 0.8), high total lipids levels, higher levels of SOD, CAT and GST, with higher levels of lipid peroxidation (LPO) in gills, liver and kidney. The histological sections showed increased incidence of oedema and hyperplasia in lamellae of gills, as well as, lipid granules and vacuolation with hemorrhage in the liver and prominent damaged pancreas [40]. It was observed that the metals concentrations, generally, in northern Ontario lakes affected the function of chemical alarm systems in fishes. However, the contamination wrong on such function has no apparent effect for heterospecific non-darter prey-guild or predator-guild species [79].

In Indus River, Pakistan, the order of bioaccumulation of Cd, Ni, Zn and Co in tissues of *Wallago attu* was downstream > middle stream > upstream. The bioaccumulation among tissues of the fish was highest in gills followed by liver, skin and muscles [80].

Heavy metals pollution negatively affected the histopathological structure of gonads in Indian Major Carp *L. rohita* from Harike wetland. There were atretic oocytes with broken membrane and vacant space in the ovary. The maturing follicles showed atresia with loss of inter follicular connective tissue, and larger inter follicular spaces [81]. Metals exposure of fish may lead to contamination of gonads and negatively affect spawners fertility and embryonic development. Early developmental stages of fish are being particularly sensitive to toxicants, although when embryo is protected by egg shell; this may be due to stimulation of energy-consuming detoxification operations, therefore, there will be less growth rate due to less energy used in intoxicated fish [22]. A study of the effect of mixtures of Cu, Zn and Cd on ovary of fathead minnow showed that the interaction decreased egg production by the fish [32].

Metal concentrations in organs, tissues and whole body of fish vary somewhat with reproductive activities, then they vary with life stage and sex [24]. The contamination with heavy metals has been associated with alternation of immune function, in addition to, reproductive abnormalities in fishes. The prolonged exposure to these effects my influence fish quality due to alterations occur that induce tissues and biochemistry of body, and at longer time, may impair reproductive success and reduce lifespan, then posit threat to sustainability of fish population, and later on may cause species extinction [13,82]. Some studies revealed DNA damage in gills, liver and kidney in fishes exposed to heavy metals pollution [40].

In Sardaryab River, Kabul, the recorded quantities of Zn in gills, liver and muscles of common carp were 0.074 $\pm$ 0.01, 0.07 $\pm$ 0.009 and 0.018 $\pm$ 0.004 μg/gm respectively; whereas the quantities of Cu were 0.024 $\pm$ 0.004, 0.089 $\pm$ 0.007 and 0.016 $\pm$ 0.008 μg/gm respectively. In *L. rohita* the quantities of Zn, Cu, and Pb attained higher of lower values in different organs. Still, overall concentration of the above heavy metals was higher in common carp as compared to *L. rohita* [43]. The metals Zn, Cu, As, Fe, Pb and Ni in the gills, liver and kidney of river Hayle brown trout, UK, had 30-60 times higher levels than that of metals in fishes living in nearby river Teign (as control). It was confirmed that Hayle brown trout fishes are truly tolerant of such high metal contamination, that may be due to adaptation over several generations [83]. The water in urban stream sites differentiate from that of reference sites, for example, the Neotropical fish (*Prochilodus lineatus*) caged in disturbed urban

stream underwent histological alterations in gills, kidney and liver that not found in samples from reference sites [84]. It is known that metallothioneins are cysteine-rich proteins, that has the capacity to bind various heavy metals, through the thiol group of their cysteine residue [44]. It is possible that such bind may interfere with organs functions and cause that alterations. In polluted Yuriria lake (due to wastewater and agricultural activities) no significant differences relative to controls were in LPO or the activity of SOD and CAT , at gills or liver of the native fish (*Goodea Atripinnis*), which may refer to adaptation of the fish. Still, there was significant difference in the activity of glutathione peroxidase (GP_x) in liver and gills tissues, as the two organs varied in activity of mentioned enzymes, but the integrated biomarker responses (IBR) in liver were lower than in gills. Histopathologically, the gills in individuals of 6 cm showed hypertrophy in the lamellar epithelium, manifested as epithelium thickening and fusion of primary lamellae. The liver of > 0.1 – 3.9 cm individuals showed presence of bleeding, mainly in areas closed to blood vessels; whereas in larger individuals there were some vacuoles in liver tissue [85].

The accumulation pattern of heavy metals in kidney of marine fish *E. microdon* from Arabian Gulf followed the order: Zn (47 $\pm$ 13.26 ppm wet weight) > Cu > Pb > Ni > Co > Mn > Cd (0.41 $\pm$ 0.16 ppm wet weight); whereas, it followed the order Zn (34.53 $\pm$ 9.96 ppm wet weight) > Cu > Pb > Co > Ni > Mn > Cd (0.36 $\pm$ 0.23 ppm wet weight) in heart tissue [36]. There were few significant correlations according to Kendal tau (below 0.4) among As, Cd, Pb, Mn, Hg, and Se in yellowfin tuna, bluefish and flounder collected from commercial sources in New Jersey, America. No fish types had the highest levels of more than two metals [86]. In the same manner, [24] noticed plain variations of heavy metals concentrations in the Antarctic fish *P. borchgrevinki* in comparison with those of fishes from other oceans. Otherwise, in different areas close to the Antarctic Peninsula, the Hg and Cd levels in fishes were below the detection limit [87].

The heavy metals in Fayoum Governorate, Egypt, showed differential bioaccumulation in fish organs. The accumulation pattern, as total heavy metal residues, was at highest level during summer and at lowest level during spring [88]. Also, it was indicated that heavy metal concentration in the tissues of six edible fishes, collected from upper stretch of the Ganga River, tended to vary significantly among season, and monsoon period showed particularly high metal concentration compared to pre-monsoon and post-monsoon [42].

It seems that the lower concentrations of heavy metals in polluted waters can achieve similar stress for higher concentrations, but including lower portion of affected individuals [27].

2. DISCUSSION

Gills represent the target for the toxicity of dissolved metals, for they are the main site of entry for the surrounded elements, as they have large surface. Moreover, the gills have the thinnest epithelium of all organs (a single or double layer of cells), and so metals can penetrate through it [89]. Gills showed higher levels of LPO in *G. atrippinis* due to their continuous and direct contact with water and contained pollutants. The destruction of the morphology of gills epithelium be capable of alterations in blood ionic levels, gill Na^+/K^+ - activated ATPase and ionic fluxes [90]. The activity of Na^+/K^+ - ATPase, along with the numerical density of chloride cells seem to express osmoregulatory capacity of the fish [45]. On the other hand, the destruction of gills can disturb the respiratory function and osmoregulation, then it can reduce the activity of swimming of the fish due to oxygen deficiency, and finally, the growth rate. The accumulation of toxicant could be used as indicator of physiological dysfunction interruption in detoxification or osmoregulation. The chronic disturbance of gills will have negative effects on fish growth and reproduction due to irregular excretory and diffusional ions losses with elimination of metabolic waste products [45]. However, the branchial responses serve to impair the entry of more toxicants, although they inhibit the excretion rate occur through gills [85]. It seems that histopathological alterations in the gills may be an adaptive response to prevent more entry of heavy metals. Hyperplasia in gill lamellae may point evidence or initiation of cancer, in addition to impaired capillary circulation and inhibition of ion exchange activity.

Kidney renal tissue receives large supply of blood flow and serves as a major rout of excretion for metabolites of various xenobiotics [91]. It was observed that the kidney in the plaice (*Pleuronectes platessa*) is a major site of phagocytosis; and the phagocytic cells are

specialized macrophages of the reticulo-endothelial (RE) system, and so better referred to as RE cells [92]. In contrast, [22] observed a decrease in the number of melano-macrophage centers in the kidney of *O. niloticus* exposed to two environmental pollutants. This condition may be due to decreased blood supply, since the macrophages derived from the blood stream [93].

There was an adduct in liver cells of common carp, exposed to heavy metals effect, as a result of their metal chelating proteins that target the hepatocytes to release lipofuscin produced by lipid peroxidation and pigment hemosiderin because of internal bleeding [75].

In this manner, the bioaccumulation of heavy metals in liver may reaches a proportion which impedes the organ function due to degenerative hepatocytes and decrease in blood supply through hepatic artery, in companions with reduction of liver pump blood. It could be concluded that the decrease in antioxidant enzyme levels because of long-term exposure of fish to heavy metals may lead to lack of elimination of toxic compounds and then the more accumulation of these metals. It seems a condition of overlapping of related factors, that influence the lipid regulation by the liver.

Heavy metals can inhibit the activity of the acetyl-cholinestrase (AChE) enzyme which found in the brain and controls physiological and behavioral responses in the fish, and so, the decreased levels of the enzyme in polluted sites was used as specific biomarker in assay of exposure to pollutants [94], not to mention, some enzymes are not readily inducible, and the biotransformation enzymes are seen in homogenous distribution [7]. Several pollutants being or mediate reactive oxygen species (O_2, OH^-, H_2O_2) at water ecosystem and interact with critical macromolecules such as DNA, proteins and lipids, resulting in physiological disruption [85]. The heavy metals are usually bond to proteins while they transport through circulatory blood. It was noted that the metalo-binding proteins were accumulated in the nuclei of hepatocytes of the examined *O. niloticus* which showed histopathological alterations [21]. It was stated that the fish liver has high affected enzyme activity for SOD & CAT at the polluted sites [3], which metabolize the reactive oxygen species (ROS) [85]. In common dentex (*Dentex dentex*) the larval stage acquires enzymatic equipments such as glycogen stores, first zymogen granules, non-specific esterase and alkaline phosphatase activities, which insure digestive processes [95]. It may confirm the start of toxicants bioaccumulation since early stages of development at polluted systems.

Effects of heavy metals pollution start as tissue of cellular damage and so they could be an evidence before explicit changes can be identify in external appearance or fish behavior. Otherwise, metals bring about cellular change in affected organ, as initiation for arising to specific enzyme that provoke changes in metabolism, which cause intoxication and death at cellular level [91]. It was concluded that glucose, hepatic alanine transaminase (ALT) and aspartate transaminase (AST) levels along with erythrone profile are more convenient biomarkers of water pollution and can be used for early detection for pollution effects on fishes [96]. The enzymatic changes are quick adaptive responses, although their compensation mechanisms may be not enough to avoid damage at the histological level [85].

The histological evaluation is usually required to test the existence or extent of non-neoplastic liver toxicity in fish, because biochemical assays are not routinely used in this scope [7]. To identify the impact level of each metal, for monitoring process, it is required to select a specific organ or tissue, along with respect to age, sex, size and technique used in the study. For example, mercury concentrations were high in the muscles > liver > gills in *O. niloticus* (100 – 150 gm weight) [21], while they were high in liver > gills > muscles in fingerling of the same species [25]. In another aspect, some proteins like vitellogenin is detected only in liver of female fish, and induced even in males by an increasing number of industrial compounds [6]. Moreover, some chemicals may present in concentration below the detection limit of the analysis.

Macrophages aggregate and act as phagocytic cells which arise with pathologic infections [97]. It could be concluded that the higher degree of macrophage aggregation in stressed organ, in comparison to control, may possibly indicate a higher content of cellular debris resulting from necrosis in tissue or a higher susceptibility of fish to pathologic infection due to decreased immunity. Besides, it is worthy to know that increased connective tissue may give arise to more diffusely scattered macrophages, which may also be found in increased number of liver, as they proliferate [98]. Just the same, the fibrosis results after substantial tissue destruction

or when inflammation occurs in tissues that do not regenerate; hence, the repair occurs by replacement of non-regenerated parenchymal cells with connective tissue. After the recovery period the organ may shows an extent of structure damage as observed in gills of *O. niloticus* treated with Zn, like fusion of adjacent secondary lamellae and detachment of the epithelium from pillar system [26].

Heavy metals may introduce their damage through indirect process, for example, they can disrupt alarm system responses [79], and as a result, the population being more vulnerable for predation which decrease occasion to persist in nature.

Heavy metals may release the toxic effect by converting natural compounds in body to harmful ones. For example, the production of the free radicals by heavy metals may make the fishes from polluted estuary unable to cope with available antioxidant defences due to decreasing in activities of antioxidant enzymes such as superoxide dismutase, that observed in grey mullet (*Mugil cephalus*) [99].

It was determined that 20% of accumulated MeHg was lost by the fish, whereas 73-81% of the accumulated Hg (II) was lost over the 28 days of elimination period [70]. It means a variation in the equation or in balance calculated for uptake/elimination in species of fish and species of element, or the equilibrium between the uptake rate of metal and the sum of excretion rate by the fish. Also, the age of fish must be considered in relation to this equilibrium [57]. In this case, the fishes being a major source of MeHg exposure, particularly certain fishes, as shark, swordfish and tuna [56].

Most articles deal with heavy metals impact on fishes are in agreement that the duration of exposure and the concentration level of the specific metal are intensifying the harmful effect [32,100].

It is mentionable that different organs in fish have positive or negative allometry with metabolic activity through fish development [101]. Hence, it is necessary to compare alterations, under similar stress, in samples from convergent life stage of fish, considering organ mass. The integrated biomarker response (IBR) method helps resolve complication resulting by assessing several biomarkers in an organism yield a set of responses [85]. In this manner, [34] recommended that the fish skin, in some species from Arabian Sea, should be discouraged as food for human or animals.

Aquatic life is potentially able to magnify metal residues through food chain to higher levels in fishes tissue, particularly predator species [9]. The fishes are located at the top of the aquatic food chain, as they accumulate heavy metals at polluted habitats, and pass them to human existence through consumption resulting in subclinical of acute diseases. In respect of this, the Pb concentration in fishes collected from Kelantan River was significantly higher in carnivorous species compared with herbivorous or omnivorous species [54]. It appears as a result of higher biomagnification through food chain.

The physiological functions of each fish species may be susceptible to specific type of element. Different fish species seem variable in their response towards variable mixtures of toxicants; accordingly, such agents bring about some departure for values of joint action of toxicants from that of additive effect [32].

Season is considered as an important factor in comparison of samples from different geographical sites when migrate and assemble in sampling locations which targeted for study [23]. To compare samples from different sites, it is recommended diet sampling within the same season, preferably for fish on their primary resident feeding ground [91]. This seems a significant factor for sampling fishes from shallow waters of the intertidal zone that experience fluctuations in ambient conditions. There were seasonal differences in the types and severity at organ histopathological alterations at brown trout and loach (*Barbatula barbatula*) exposed to polluted streams during the year [102]. The same phenomenon found in grey mullet from Ennor estuary [98].

Some bioindicator responses at lower level of organization (as biochemical or individual levels) seem to be useful for environmental hazard assessment procedure due to their sensitivity and apparent relations to higher levels (as population level) [103]. As a result, we get better predictive prospects for assessment of aquatic system hazard. It was concluded that the evolution of resistance to toxicants appears to take place in most populations of fishes, but not all, in polluted habitats, to consider that possibility in evaluating the monitoring programs [104]. It may be a sign to different responses in populations due to variation in physiological and

biological aspects. In this way, when replaced conditions emerge rapidly, fish have less time to adapt [105]. It is known that fewer studies dealt with physiological mechanisms that underlie toxicant effects on behavior of fish, particularly so early in life stage [106].

It seems difficult to identify particular substances responsible for sub-lethal morphological effect, and even clearly distinguish between morphological or physiological abnormalities, since one may be caused by the other, as they are often related [45]. In addition, the overlapped environmental conditions must be considered [4]. Here, the dangerous metal concentration in specific media may be less or more in damage at another media, particularly for sub-lethal and chronic effects. In addition, the chemical concentrations in water and sediments may not constant overtime, therefore, they cannot provide direct indication of the effects of contaminants on aquatic organisms. Moreover, the half-life of chemical compound must be regarded, along with qualities of surrounding water [70]. In the aquatic phase, the substance may interact with other constituents in the water, for example, the humic acids will form complexes with some heavy metals, particularly Cu [32].

In relation to season, it was found that the accumulation of heavy metals in tissues of *C. c. umbla* is higher in spring and summer, companion with physiological activity during these seasons [23]. This state may be related to modulation of temperature upon enzyme systems which set energy levels for membrane events, or they could directly on the excitable membranes [107]. There was higher concentration of metals in fish during dry season due to high temperatures, which elevated the metabolic rate [80,108]. On the other side, it was found that the metal concentration of Cd, Ni and Pb in fishes from Kelantan River, Malaysia were higher during wet season due to increased surface runoff and farm drainage [54]. It appears that different conditions play havoc in different modes in relation to heavy metals impact. Water temperature has an effect on plasma osmality, Na, K and Cl concentrations in common carp. The changes in plasma ion level was resulted by altered Na pump activity and hormonal control of branchial permeability to water and ions. There was lower activity in Na^+/K^+ -ATPase at 15°C and higher activity at 29°C, as well as, lower control over permeability for prolactin mRNA expression [109]. Hence, the disturbance in blood ion regulation, due to different ambient temperature, causes the dysfunction in enzymes and hormones activity, and then, may be different impact for soluble minerals. It is well-known that the poikilothermic animals (as fish) are experiencing temperature fluctuations in their bodies.

It is apparent that lethality of heavy metal to fish decreases with increasing water hardness due to low toxicity of created complexes, in addition to competition for active sites in fish tissues between the toxic cations and those of Ca and Mg [110]. In another study, it was noted that bluegill sunfish (*Lepomis macrochirus*) exposed to water soluble zinc (13.5-32 mg Zn^{+2}/L) showed 90-100% mortalities, but no fish died in water containing insoluble Zinc. The mortalities were more at low pH, and at slower rate under low temperatures [111].

Diverse factors, such as personality differences, contribute to variation in the individual resting metabolic rate, and as a result, responses to chemical effectiveness. Accordingly, standardized assessment method is urgency needed to compare different histological changes in different studies. For instance, many fish species have the tendency to assemble triglyceride in their livers; thus it seems difficulty to determine the extent for being excessive and potentially deleterious [7]. In this field, the histological stains responses or histochemical aspects being a device which reflect developmental enzymes activities through successively life stages of fish.

Authors are rather divergent to explain metals variance in accumulation among fishes tissues or organs [23,44,57]. Therefore, the author may shown a degree of confidence intervals for his results [42]. Different studies may consider the factor in relation, for example, the Kilantan River was categorized as polluted river in relation to suspended solid materials [54]. Some authors interested in the role of enzymes as significant biomarker of contaminant – induced stress in fish [112,113], or employment of plasma glucose and blood parameters [114]. It was observed that glucose levels in *C. Carassius* individuals from polluted areas of Sitnica River, Kosovo are higher than that from the reference sites. Therefore, the studies employing several biomarkers may provide a complementary results to evaluate the pollutants action in the same species. The integrated biomarker response (IBR) method helps to resolve the complication resulting by assessing several biomarkers in an organism yields a set of responses [85].

Table 1. Some fish species, heavy metal and concentration, target organs, expression symptoms and the way for avoidance of the heavy metal contamination

Fish species	Heavy metal and concentration	Target organs	Expression symptoms	The way for avoidance of heavy metal contamination
L. rohita	0.5 ppm $CuSO_4$ (Experimentally)	Muscle	Mild oxidative stress	Reduction of exposure period
	2.0 ppm $CuSO_4$ (in ambient water)	Muscle	Severe oxidative stress	Dilution of metal concentration
	2 mg/L $ZnCl_2$	Liver	Swelling of muclei and necrosis of hepatocytes	Reduction of exposure period
C. punctatus	5 mg/L $CuSO_4$	Liver, muscles & gills	Decline in protein level	Dilution of metal concentration
	6.62 & 13.24 mg/L $ZnSO_4$	Liver, kidney, intestine & gill	Precipitation in the organ tissue	Reduction of exposure period
	16.7 ppb $HgCl_2$ (as chronic treatment)	Kidney	Destruction of endocrine and excretory parts	Reduction of exposure period
O. niloticus		Gills	Lamellar epithelial hyperplasia	
		Liver	degeneration& necrosis of hepatocytes	
	40.6 mg/L $CuSO_4$	Kidney	Severe degeneration of the renal tubular cells	Dilution of metal concentration
	1 mg/L Cu	Gills	Epithelial lifting & dilated primary lamellae	Dilution of metal concentration and reduction of exposure period
		Liver	Vacuolar degeneration of hepatocytes	Dilution of metal concentration and reduction of exposure period
	1 mg/L Zn	Gills	Hyperplasia & lifting of lamellar epithelium	Dilution of metal concentration and reduction of exposure period
		Liver	Alterations in hepatocytes	Dilution of metal concentration and reduction of exposure period
	1 mg/L Cd	Gills	Separation between the epithelial cells	Dilution of metal concentration and reduction of exposure period
		Liver	Disorganization of the hepatic cords with focal necrosis	Dilution of metal concentration and reduction of exposure period
	1 mg/L Pb	Gills	Large sub-epithelial spaces with thin secondary lamellae	Dilution of metal concentration and reduction of exposure period
		Liver	Hepatic necrosis	Dilution of metal concentration and reduction of exposure period
	0.4 – 0.7 mg/L $Pb(CH_3COO)_2$	Gills	Curling in secondary lamellae	Reduction of exposure period
		Ovary	Increment in atretic follicles	Dilution of metal concentration and reduction of exposure period
		Liver	Disarrangement of hepatic cords	Dilution of metal concentration and reduction of exposure period

	0.5 LC50 of $CuSO_4$ or $Pb(CH_3COO)_2$	Gill, liver, kidney & spleen	Increment in bands (as electrophoretic patterns)	Dilution of metal concentration
O. niloticus (fingerling)	0.3 mg/L Hg	Gills Liver	Severe disorganization of epithelial cells Slight effect	Dilution of metal concentration Dilution of metal concentration
C. carpio	0.5 -0.1 mg/dm^3 Cu	Eggs	Anomalies during developing cleavage	Keeping the eggs away during developmental stages
	(Pb+Cd+Cr+Ni) as mixture containing 1.25 mg/L of each metal	Kidney Flesh	Dystrophic change in tubules Disappearance of striations, with necrosis	Keeping away from mixed effect
C. variegatus	14.6 – 16.6 µM Cu^{+2}	Whole body	Enzymes activity & glucose levels	Increasing of ambient salinity
C. fusca	1.1 mg/L Cu	Whole body	Response to toxication	Increasing of water hardness
O. mossambicus	1 mg/L Cd 5% and 10% for $CdCl_2$ and $ZnCl_2$	Gills Chloride cells Liver	Drop in the electrolyte concentration Increase in density Hyalinization, vaculation and cellular swelling	Dilution of metal concentration Reduction of exposure period
C. idella	20 – 60 µg/L Cd	Spleen & kidney	Obvious damage in both organs	Reduction of exposure period and dilution of metal concentration
O. kisutch	150 ppb Cd (as water – born)	Scales	Increase in mucus production	Discharge through the mucus
O. mykiss	2.4 mg/L $CdCl_2$ 1.3 – 2.6 mg/L Cd Combination of (0.18 µM Cd & 0.80 μM Cu)	Thyroid gland Whole body Whole body	Increment of T_4 levels Response to toxication Response to toxication	Dilution of metal concentration Exposure at hard water Exposure at hard water & for limited period
S. schlegedi (as juvenile)	25 – 125 mg/kg	Whole body (juveniles)	Reduction in growth	Dilution of metal concentration &choosing larger size for treatment
C. batrachus	9 ppm Cd 12 ppm Hg	Liver Liver	Hypertrophied hepatocytes Hypertrophied hepatocytes with pyknotic nuclei	Reduction of exposure period Reduction of exposure period
Symphysodon sp.	400 mg/kg Cd through diet or 3 µg/L Cd through the water	Intestine & kidney	Alterations in ATPase activities	Dilution of metal concentration
C. gariepinus (juvenile)	0.006 – 0.008 mg/L $Pb(NO_3)_2$	Whole body Gills	Reduction in activity Distortion of lamellae	Reduction of exposure period
C. mrigala	14.1 & 28.2 ppm $Pb(CH_3COO)_2$ 0.0206 – 0.0402 ppm $HgCl_2$	Liver Gills Liver Gills	Increase of GOT & GPT activities Lamellar degeneration & epithelial lifting Increase of GOT & GPT activities Degeneration & necrosis	Dilution of metal concentration Dilution of metal concentration Dilution of metal concentration Dilution of metal concentration
A. latus (Immature)	10 & 20 µg/L $HgCl_2$	Gills	Fusion of some filaments & increase of mucus	Dealing with larger specimens & with less metal concentration
	35 & 50 µg/L $HgCl_2$	Gills	Severe disorganization of filaments with severe increase of mucus	Dilution of metal concentration
C. a. gibelio	0.25 ppm $HgCl_2$	Gills & cord	Accumulation in tissues	Reduction of exposure period

3. CONCLUSIONS

Heavy metals can affect physiology, histology, reproduction and immunity functions of fishes; mostly, at direct proportion to concentration and period of exposure. As a result, the heavy metals produce impact upon population stability, and may lead to species extinction.

The metals may affect the fish indirectly, as to reduce the food sources, or it converts to more poisonous form.

The fishes could be used as a biomarker for habitat pollution, particularly with trace elements, and so, it being a fine predictive mean for assessment of aquatic system hazard.

Season must be considered in determination of metal impact, along with environmental conditions such as temperature, hardness and pH of water, and on the whole, the overlapped agents.

Standardized methods seem to be required to compare variable results come out from different researches. Besides, it is difficult to identify a single metal responsible for alteration could be occur; in particularly with existence of undetectable metals. The authors have to imply a degree of confidence for their achieved outcomes.

It is suggested to select a certain organ or tissue in fish for monitoring a specific metal impact. Table 1 summarizes the effect of heavy metals, in different concentrations, on specific organs within certain studied fishes, with recommended ways to avoid that effects.

The effluent from waste water must be treated before disposal to aquatic system, and different projects are necessary to remove heavy metals residuals. Also, the long-term monitoring seems to be required for future anticipation, employing developed technique.

Finally, Enforcement Of Laws And Legislations Are Demanded To Protect The Aquatic Habitats And Living Organisms Inside Them.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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