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REVIEW

Impact of Heavy Metal Toxicity on Hematology and Glycogen Status of Fish: A Review

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Abstract In the era of industries the problem of pollution of aquatic resources has become aggravated. Generally because the industries are constructed near the water bodies in order to get rid of waste generated. Mining, agricultural run-offs, domestic/sewage water etc. further add to the pollution. These waste/sewage waters contain heavy metals which in turn accumulate and affect the health of fishes. Heavy metals are readily available for uptake in cationic state, part of a hydroxyl complex or organometallic compound. Previous studies have reported that heavy metals accumulate in different organs of the fish without causing mortality and their effect first appeared in blood. These alterations make fish weak, anemic and vulnerable to diseases. Hence industrialization on the other hand is targeting the major protein source in the form of fish. The exposure to heavy metals causes increase or decrease in hematological indices, as well as decline in the glycogen reserves. Moreover haematologic parameters and glycogen may serve as suitable biomarkers of fish health and can be used as the bioindicators to monitor the quality of aquatic environment.

Keywords Aquatic ecosystem · Heavy metal toxicity · Fish · Hematology · Glycogen

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Introduction

It is now evident that growth of industries, urbanization and modernization leads to environmental pollution. The waste generated from these sectors is discharged or dumped into the water bodies. Industrialization and use of new technologies are essential for the quest of development and ease but on the other hand they are destroying the precious natural aquatic resources. Water is the main component of biosphere but fresh water is limited. Heavy metals form the category of pollutant, of wide occurrence because almost every industry whether it is fertilizer, petroleum, distillery, pharmaceutical, hardware/software, steel, chemical, power plants etc. uses a variety of heavy metals. Besides this they are also used in daily life products like detergents, shampoos, tube lights, batteries and cosmetics etc. Therefore the domestic wastewater and sewage water further adds to the contamination. These degrade the aquatic ecosystems and also have adverse impacts on the inhabiting flora and fauna. Heavy metals are of particular concern due to their low biodegradability, persistent properties and toxicity to fish and humans. Both acute and chronic exposure can cause the accumulation of heavy metals in tissues of flora and fauna and cause deleterious effects.

Heavy metal in general applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3. However a heavy metal has little to do with density but concerns mainly with the chemical properties. All heavy metals exist in surface waters in colloidal, particulate, and dissolved phases, although dissolved concentrations are generally low [1]. Their bioavailability largely depends upon the environmental conditions and it is the ionic state of metal which causes harm. Often free metal ions react with dissolved organic matter and with clay minerals to form complexes; this reaction is known as complexation.

This considerably reduces the concentration of metal ion and in turn their bioavailability and consequently bioaccumulation and toxicity by a factor of 100 or more. Thus in the presence of dissolved organic matter, higher concentration of total metal can be tolerated in solution before any adverse effect on organisms is observed. Only when metals are in a free cationic state, or part of a hydroxyl complex or organometallic compound, they are potentially available for uptake by organism. Many metals possess biological activity and, as opposite to organic compounds, do not undergo transformation in the tissues of aquatic animals. Consequently, heavy metals leave biological cycles very slowly [2]. Elements such as Hg, Cd, As, Pb, Cu, Ni, Fe, Co, Mn, Cr and Zn are considered most dangerous for aquatic ecosystem in the toxicological studies. Heavy metals (Hg, Cd, Pb) and metalloid (As) are particularly toxic for both aquatic animals and humans. At the same time, Cu, Ni, Fe, Co, Mn, Cr and Zn are essential micronutrients/metals included in the active centers of enzymes and serve as regulators of many biochemical functions. Therefore, the monitoring of concentrations of essential metals in the aquatic environment is also necessary. Aquatic animals are able to accumulate and biomagnify the heavy metals up to the concentrations that are tenths and even thousands of times higher than their concentrations in the environment through the aquatic food web [3].

The contamination of aquatic ecosystem with metals may cause toxic effects on both humans and aquatic organisms. When metal enters the cell, it gets distributed to a number of sites and by an unknown mechanism it induces the de novo synthesis of mRNA for the synthesis of metallothionein protein. This metallothionein protein leads to the formation of apometallothionein which competes with the metal to form metal-metallothionein complex. Metallothionein biodegrades the metals in order to detoxify them. There are three possible ways by which metals enter the body of fish: the gills, alimentary tract and the body surface. Gills are not only the main organs of gaseous exchange, but, as a highly specialized and exposed part of the body surface, also represent an important site of uptake of essential and non-essential metal ions from the water [4]. From the gills, the absorbed metals are distributed throughout the whole body and accumulate in specific organs. Heavy metals have also been reported to alter gill morphology. It thus seems that passage through the gills is an important pathway for the soluble fractions of heavy metals into fish. Secondly, uptake of particulate metal fractions by fish occurs from contaminated suspended matter, sediments, and small organisms serving as food sources, and for this the only route is the alimentary tract. In short the main carrier behind the transport of heavy metals within the organism after their uptake is the blood. Whatever fish uptakes is absorbed by the blood to transport it to different organs therefore it is said that metal concentrations in blood is much higher than other organs. Generally, heavy metal accumulates in all the vital organs of the fish. Most often highest concentrations of heavy metals are found in fish liver, kidney, gills [5, 6] and in some cases in the gut. Even at very low concentration heavy metals (Cu, Ni, Fe, Co, Mn, Cr, Zn, Hg, Cd, Pb) induce changes in morphology, physiological and biochemical parameters in fish. Such effects besides including decrease in immunity [7], changes in behavior, growth, digestive enzyme activities [2], efficiency of food assimilation [8], also affect state of carbohydrate metabolism [9–11]. However, little is known about the uptake of heavy metals through the skin. It can be assumed, however, that the body surface of fish is more or less impervious to harmful substances of the surrounding water [12]. There are some indications that mucus secretion may prevent heavy metals from entering the body of fish.

The development of analytical techniques/instrumentation over the past 30–40 years has allowed us to detect trace metals in very low levels that is at the parts per quadrillion (ppq). As early as 1960s, trace metal determinations were carried out by some traditional wet chemical methods such as volumetric, gravimetric, or colorimetric assays. It wasn't until the development of atomic spectroscopy (AS), in the early to mid-1960s that the clinical researchers realized that they had a highly sensitive and diverse trace metal technique that could be automated. Every time there was a major development in AS, trace metal detection capability, sample throughput, and automation improved dramatically, then comes flame atomic absorption spectrometry (FAA), Graphite furnace atomic absorption spectrophotometry (GFAAS) etc. The most common method for detection of heavy metals in food including fish is atomic absorption spectrophotometry (AAS). It is easy to operate and is a highly sensitive method which needs very simple previous preparation procedure. Good results are obtained at relatively low cost with this method.

Influence of Heavy Metals on Hematology and Glycogen Reserves of Fish

Hematologic parameters are considered as an important tool in evaluating fish health without killing the animal [13], as there are many ethical issues about the use of animals in experiments. Blood is a sensitive tissue which is affected with the environmental changes; therefore, hematologic evaluation can be used in monitoring the health status of fish. The abnormalities of erythrocytes, leukocytes, thrombocytes, and clotting factor may serve as an index in the toxicological studies. Similarly as for non-aquatic organisms, hematologic data have been used in

evaluating fish health and to test the effect of heavy metals. The changes in the fish blood prior to the onset of more striking morphological and physiological changes can be indicative of unfavourable aquatic medium [14]. It has been known in the vertebrates that the plasma proteins play a dominant role in metal transport. The blood plasma contains diverse proteins that transport a wide range of metals. Binding of metals to plasma proteins can be either specific (e.g., Fe by transferrin and Cu by ceruloplasmin) or nonspecific (e.g., Ca, Ni and Zn by serum albumin). Blood parameters are therefore considered as patho-physiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants [15]. In fish, exposure to heavy metals can induce either increase or decrease in the level of hematological parameters. Hematological indices like hemoglobin (Hb) content, total red blood cell count (tRBC), total white blood cell (tWBC) count/leucocyte count (TLC), hematocrit (Hct)/packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) may be changed in fish after exposure to heavy metals. Agrawal et al. [16] reported that metals are known to induce anemia in fish. Anemia might be one of the earliest indications of metal toxicity. According to Christensen et al. [17] hemoglobin value of many species of fish was reported to be a useful index of health. Hemoglobin is a very important component of erythrocytes which plays the important role in transport of the oxygen in blood [8]. Generally, the impaired erythropoiesis may cause anemia. There are several factors associated with impaired erythropoiesis. They include direct effect of metal on hematopoietic centers (kidney/spleen), accelerated erythroclasia due to altered membrane permeability and/or increased mechanical fragility, and lastly the defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions.

Studies on the effect of toxins on carbohydrate metabolism are inevitable, because glucose is stored as glycogen which plays a major role in the carbohydrate metabolism of all animals in general. The immediate energy demand of the body during starvation or stress is met by ready utilization of glucose. Changes in the environment due to pollution lead to changes in the glycogen reserve thereby affecting the entire carbohydrate metabolism. Glycogen content in the liver and muscle is one of the sensitive biochemical indicators which reflect changes in the normal activity of various functional systems. Metals may enter cell metabolism after flowing into a cell and attaching to the membrane. Usually heavy metal ions enter a cell either by means of simple diffusion or by interaction with transport proteins and ion channels in plasmatic membrane. Then the heavy metal gets distributed over all subcellular fractions. In cytoplasm free metal ion either interacts with high molecular (metal-enzymes) or low molecular (metal-lothioneins, glutathione) ligands or gets deposited in lysosomes or membrane granules, leaving the cell further by way of exocytosis [18]. Many toxicological studies have investigated the effects of heavy metals on the carbohydrate metabolism in fish. The increased concentration of heavy metals in aquatic ecosystem is usually reflected in higher uptake of pollutants. As the consequence the level of glucose in fish blood and glycogen content in muscle gets decreased.

Review

The important heavy metals showing their impact on fish health are discussed as under.

Cadmium (Cd)

Acute exposure to Cd (5 mg/L of Cd during 1 h) had no effect on Hct and TEC values in the blood of carp. However, exposure to Cd (10 mg/L of Cd during 1 and 3 h) increased the levels of these parameters [19]. An increase of RBC and Hb and a decrease in the Hct % was also observed in the blood of dogfish (*Scyliorhinus canicula*) after 24-h exposure to 25 mg/L of Cd [20]. However, after exposure of *Scyliorhinus canicula* to 50 mg/L of Cd, their hematological indices (RBC count, concentration of Hb, and MCV), which had changed in the fish after 24- and 48-h exposure, returned to the control level after 96-h exposure. However an increase in WBC in the dogfish was observed only after 4-day exposure to 50 mg/Lof cadmium [21]. A decrease in the WBC count was associated only with decreased level of small lymphocytes. An increase in the amount of neutrophils was found in *Oreochromis mossambicus* exposed to sublethal Cd concentrations (0.1–10.0 mg/L) [22].

Ghazaly [23] observed hyperglycemia in *Tilapia zilli* exposed to Cd which may be a physical response to meet the critical needs of the brain tissue for increased energy in the form of glucose during exposure to toxicants. The common carp (*Cyprinus carpio*) was exposed to sublethal concentrations of cadmium (0.05, 0.1, 0.5 and 1.0 mg/L) for 10 days. The levels of glycogen reserves in the liver and muscle tissues were significantly ($P < 0.05$) decreased as compared with the levels measured in the control groups. The decrease in glycogen levels in the liver and muscle tissues under the highest metal concentration (1.0 mg/L) were 24 and 29 %, respectively. The blood serum glucose level of fish exposed to Cd were significantly ($P < 0.05$) increased as compared to the levels measured in the control groups [24]. Sobha et al. [25] measured glucose and glycogen levels in muscle, gill, liver,

heart and kidney of fish, *Catla catla* exposed to cadmium chloride during 96-h to LC_{50} (Lethal) and sub-lethal concentrations (1/10th of the lethal dose) for 7 days and reported significant fall in glycogen in these tissues. However, glucose level was considerably increased which suggested that the fish cultured in the aquatic systems closer to the industrial locations would not have the expected nutritive value and are apparently indicative of the organism's response to the toxicant stress. Also, changes in serum glucose in response to Cd exposure were studied in the freshwater fish, *Oreochromis niloticus*. When fish were exposed to 1.0 mg/L Cd for 7 and 14 days then the elevation in blood glucose was observed which was due to the mobilization of glycogen from muscle and liver [26]. The acute toxicity of cadmium sulphate to *Colisa fasciatus* in terms of LC_0, LC_{50}, LC_{100} have been observed for 30 days and exposure to sub-lethal dose of cadmium sulphate caused significant reduction in glycogen in liver and muscle tissue [27].

Mercury (Hg)

Significant decrease in RBC and Hb content of fresh water fish, *Oreochromis niloticus* was observed when exposed to Hg [28]. Similarly, exposure to mercuric chloride caused decline in tRBC, Hb content and PVC value, while total WBC increased [29]. Saroch et al. [30] reported that mercuric chloride (0.1 mg/L) caused reduction in total count of RBCs and Hb in *Clarias gariepinus*. Furthermore, Karuppasamy [31] reported that lowering of tRBC coupled with low Hb content in *Channa punctatus* may be due to destructive action of phenyl mercuric acetate on erythrocytes and as the result of which the viability of the cells gets affected. In contrast, subacute toxicity of $HgCl_2$ resulted in considerable increase in Hb, Hct in *Acanthopagrus latus*. At the same time, WBC values were significantly lowered as compared to control and differential leukocyte count was changed [32]. In contrast leukocytosis was observed in *O. mossambicus* after exposure to Hg [33]. Also Saroch et al. [30] reported a significant increase in the total count of WBC in a time dependent manner after exposure to Hg. Total WBC count increased in *Tinca tinca* exposed to lethal and sublethal dose to Hg in a time dependent manner [34].

Glycogen level in the liver, muscle and gills of all Hg-exposed carps (*Cyprinus carpio)* significantly decreased. Compared with Ni and Cr, exposure to Hg caused the highest depletions of glycoge up to 96 % in the tissues [35].

Lead (Pb)

Zaki et al. [36] reported hazardous effects of Pb pollution on hematology of *Oreochromis niloticus* and found elevation in RBC, Hb, Hct and MCHC. In contrast, Cogun and Sahin [37] did not observe the changes in Hct, Hb, RBC and WBC levels in fish *Oreochromis niloticus* exposed to different concentrations of Pb for 10 days. However, at the end of 20 days exposure period, the levels of these hematological parameters showed a decrease.

The effects of Pb on Hct levels and sera glucose concentrations of *Anguilla anguilla* exposed to 0.06 and 0.12 ppm Pb were studied over 15 and 30 days. Lead concentration of 0.12 ppm did not cause any variation in Hct level, while 0.06 ppm increased it up to control level. Sera glucose level showed a linear decrease. These changes can be attributed to restoration of homeostasis against the stress conditions caused by the metal [38]. *Oreochromis mossambicus* exposed to sublethal (17.5 and 35 ppm) levels of Pb showed a time and dose dependent decrease in glycogen content of liver and muscle whereas blood sugar level got increased [39]. *Cyprinus carpio* when exposed to sublethal concentrations of Pb along with Ni, Cr and Cd caused decline in glycogen of fish liver [40].

Manganese (Mn)

Mn also has negative impact on hematological indices. Exposure to Mn caused reduction in the number of RBC in *Channa punctatus* [41]. After exposure of *Tilapia sparmanii* to Mn at pH 5 significant decrease was found in RBC, Hb, MCV, Hct and WBC [42]. The sublethal effects of Mn were determined by exposing the freshwater fish, *O. mossambicus* to this metal in an experimental flow-through system. The exposure to Mn (0.345 g/L) was characterized as acute (96 h) and (0.259 g/L) chronic (26 days), both at 23 ± 1 °C. Acute exposure to Mn caused significant increase in RBC count, Hb, Hct, changes in differential WBC between the control and exposed fish ($p < 0.05$). During chronic exposure to Mn, an oxygen deficiency developed which resulted in hypoxia and increased hematologic data related to RBC counts, Hb and Hct [43].

No dose response effects of Mn on carbohydrate/glycogen metabolism of fish was found. At high concentrations Mn is reported to be toxic to the fish, *Colisa fasciatus*, resulting in decreased liver glycogen level and increased blood glucose level [44]. Strydom et al. [45] reported that it has inhibitory effect on enzymes related to glycolysis and krebs cycle.

Copper (Cu)

Exposure to 3 mg/L of Cu for 96 h caused increase in hematologic data (tRBC, Hb and Hct) in fish *Colisa fasciatus*. Also this exposure showed significant decrease in the number of lymphocytes, blood clotting time (CT) and erythrocyte sedimentation rate (ESR) [46]. Acute exposure

to Cu (concentrations close to LC_{50}) had serious impact on erythropoiesis of carp and rainbow trout showing increased level of erythrocyte count, increased Hb, increased Hct and decreased WBC counts [47, 48]. Singh and Reddy [49] also reported increase of Hb in the blood of *Heteropneustes fossilis* when exposed to Cu. Hemolysis and anemia were also determined in the catfish *Clarias lazera* after 96-h exposure to 3.2 mg/L of Cu [50]. Although chronic exposure (3 months) to Cu (0.1 mg/L) slightly affected the RBC count in the blood of trout but no changes in Hct levels were observed. However, while exposing fish to a double concentration of copper (0.2 mg/L), the tendency of decrease in both these indices was observed [44]. Dharam Singh et al. [51] evaluated the hematologic data after acute exposure to Cu (sublethal concentrations, 0.36 mg/L) in *Channa punctatus* and reported a significant decrease in the Hb (from 10.73 to 6.60 %), in RBC (from 2.86 to $1.84 \times 10^6/mm^3$) and in PCV (from 31.00 to 23.33 %) at the end of 45th day as compared to control. Other parameters of erythropoiesis (MCHC, MCH and MCV) showed significant increase during 15 and 30 days exposures. At the same time, the WBC count increased from 60.00 to 92.48 $x10^3/mm^3$, clotting time (CT) was prolonged from 27.66 to 43.00 s and ESR was increased from 5.0 to 13.66 mm/h.

Although, reports of Cu toxicity have shown significant deleterious effects on almost all physiological systems of fish however different tolerance levels were recorded for different species. Acute exposure to Cu (0.032 mg/L) during 96 h caused an increase in blood glucose in the *Clarias gariepinus* [52]. During long-term tests, 0.1 mg/L of Cu did not have any impact on blood glucose of rainbow trout, while 0.2 mg/L of Cu caused a lowering in the glucose content [48]. However the long-term exposure (30 days) of low concentrations of Cu (3.4–104 μg/L) to brown bullhead (*Ictalurus nebulosus*) caused an increase in blood glucose level [50]. Furthermore, exposure to aqueous Cu is known to aggravate oxidative stress response in the fish, *Anguilla anguilla* which in turn leads to glycogen depletion [53]. In order to evaluate the impact of Cu on carbohydrate metabolism of a fish, the levels of glucose and glycogen have been monitored in *Cyprinus carpio* exposed to a sublethal concentration of Cu (0.08 mg/L at pH 7.5, 6.0 and 9.0) for 1, 7, 15 and 30 days. A progressive increase in glucose level and with the corresponding decrease in glycogen level indicates the occurrence of glycogenolysis [54].

Zinc (Zn)

Exposure to increased Zn concentrations had toxic effects on hematological and biochemical indices in fish. However, only high Zn concentrations (close to LC_{50}) are associated with decreased Hb and Hct % in the blood of *Cyprinus carpio*. Chronic exposure of fish to 30 mg/L of Zn had no effects on hematologic data [47]. However, according to other authors, after 1-day exposure of *Cyprinus carpio* to sublethal concentrations of Zn, the RBC count and Hb increase but no changes in the total WBC count were observed [55]. Significant decrease in total WBC count in *Cyprinus carpio* on exposure to 140 mg/L of Zn for 96 h was reported by Svobodova et al. [47]. It was due to a significant decrease in the lymphocyte count and increase in the amount of neutrophils. Hilmy et al. [56] studied the effect of Zn on hematological indices of two freshwater fishes, *Clarias lazera* and *Tilapia zilli*. Significant increase was observed in RBC count, Hct or PCV and Hb in both the fishes. Celik et al. [57] demonstrated the negative effect of Zn (1, 2.5 and 5 mg/L) on hematology of *O. mossambicus*. In all groups of exposed *O. mossambicus*, a decrease in the RBC count and lymphocyte percentage and an increase in Hb, MCV, MCH values and neutrophil percentage occurred ($p < 0.05$). A decrease in WBC count and an increase in MCHC values occurred with medium and high concentrations ($p < 0.05$). At high exposure dose Hct also decreases while at low and medium dose it increases.

An increase in glucose concentration was observed in rainbow trout after 7 day exposure to 214 mg kg/L of Zn [58]. Acute exposure of salmonid fish to LC_{50} of Zn increased the concentration of blood glucose by 80 % [59]. Long-term exposure to Zn solution results in depletion of liver and muscle glycogen in *Labeo rohita* [9]. *Salmo gairdneri* when intoxicated with lethal concentration of Zn for a short time, starts utilizing glycogen from dorsal white muscles which increased with time of exposure. However, exposure of trout to similar Zn concentrations (3, 11, 19 ppm) did not change the utilization of glycogen [60].

Chromium (Cr)

The alterations in the hematological indices of freshwater fish exposed to Cr(VI) are well documented. The metal is reported to induce reduction in most blood parameters. Effect of hexavalent Cr at different pH values on the hematology of *Tilapia sparmanii* showed more MCHC concentration [42]. In another study on freshwater fish *Saccobranchus fossilis*, exposed for 28 days to 0.1, 1.0 and 3.2 mg/L concentrations of Cr(IV), hematologic parameters (erythrocyte counts, Hb and PCV) got decreased indicating anemia [61]. Studies on *Labeo rohita* exposed to Cr(VI) (39.4 mg/L) revealed significant decrease in Hb and the TEC at the end of both 24 and 96 h [62]. However low concentrations of Cr for a long term exposure reported an increase in tRBC count, Hb and Hct levels in the fish, *Salmo gairdneri* [63] and *Barbus conchonius* [64]. Shaheen

and Akhtar [65] reported significant decline in Hb and RBC count and increase in WBC of *Cyprinus carpio* when exposed to Cr(VI). Similar findings were also reported in *Labeo rohita* to Cr [66].

Biochemical studies conducted on various species have revealed that Cr induces cumulative deleterious effects on biochemical parameters. Alterations in the biochemical constituents of the fish, *Cirrhinus mrigala* following exposure to Cr and withdrawal were reported by Virk and Sharma [67]. They observed that the muscle carbohydrate content was significantly reduced. In another such study on *Labeo rohita* a 96 h-LC_{50} exposure to a concentration of Cr(VI) (39.4 mg/L) significantly decreased the glycogen content in liver, muscle, and gill tissues of the fish [66]. The enhanced utilization of glycogen and its subsequent depletion in tissues is attributed to hypoxia. The author reasoned that the consistent decrease in tissue glycogen reserves observed in the study was due to impaired glycogenesis which might be due in part to its utilization in the formation of glycoproteins and glycolipids, which are essential constituents of various cells and other membranes. Abedi et al. [68] found significant increase of serum glucose level in *Cyprinus carpio* exposed to sublethal dose of trivalent Cr exposed for 28 days.

Nickel (Ni)

Colisa fasciatus, a freshwater teleost, was exposed for 90 h to 45 ppm nickel sulphate under static test conditions. The treatment resulted in leucopenia due to reduction in the number of small lymphocytes and polycythemia with concomitant increase in the Hct and Hb values, and in retardation of the ESR of the fish. No differences in total thrombocyte count and clotting time were found between the control and the treated fish [16]. The effect of Ni on *Tilapia nilotica* showed elevated RBC count, Hb and PCV along with leucopenia and lymphopenia [22]. The blood parameters (RBC, WBC, Hct, MCV, MCH and Hb) in *Clarias gariepinus*, decreased after exposure to median lethal concentration of Ni (8.87 mg Ni/L) for 96 h [69]. Short term exposure to high concentrations of Ni in *Cyprinus carpio* induces significant decrease in RBC, WBC, Hct, Hb, MCV, MCH, MCHC as compared to control [70].

Ghazaly [23] evaluated the effect of sublethal concentrations (19.2, 32 and 51.2 mg/L) of Ni in the fish *Tilapia nilotica* and reported prominent hyperglycemia associated with decrease in liver glycogen level. Similarly, glycogen level was decreased in the muscle of fish exposed to Ni at higher concentrations.

Chaudhary [71] investigated the effects of sublethal concentration (0.8 mg/L of LC_{50} 96 h) of nickel sulphate ($NiSO_4 \cdot 7H_2O$) on muscle glycogen level of the freshwater teleost, *Colisa fasciatus*, at time intervals of 3 to 96 h. Muscle glycogen levels showed a general decrease at all-time intervals as compared to control, with a maximum decrease of 35.2 % at 96 h ($p < 0.001$).

Vinodhini and Narayanan [72] examined the impact of mixture of heavy metals Cd, Pb, Cr, Ni, on hematological parameters in *Cyprinus carpio*. They found that the values of the Hb and PCV decreased while amount of RBCs showed elevation when compared to control. Fish *Oreochromis niloticus* exposed to metals (Cu and Pb) showed increase in serum glucose level, however, a return to control levels was observed at the end of the exposure period of 4 days [73]. The concentration of glucose in *Tilapia mossambica* showed increase after exposure to $HgCl_2$ and $ZnSO_4$ [74]. The Indian major carp, *Labeo rohita*, was exposed to 1/10th sub lethal concentrations of three heavy metals—Cadmium chloride ($CdCl_2$), Lead chloride ($PbCl_2$) and Mercuric chloride ($HgCl_2$) for a period of 3, 7, 15, 30 and 45 days. The glycogen content in gill, liver and muscle tissues of *Labeo rohita* under stress of these metals got decreased. The maximum percentage decrease (−48.31) was observed on day 45th, in liver tissue under mercuric chloride intoxication only [75]. Chandanshive et al. [76] examined the effect of heavy metal mixture of Cd, Zn, Pb and Hg in WBC, RBC and Hct of the laboratory acclimatized fish, *Labeo rohita* in which RBC count decreased significantly ($p < 0.005$) in the blood of fish exposed to almost all heavy metal mixture concentrations studied while alterations of Hct level depended on heavy metal mixture concentration. The concentration of heavy metal mixture which was 21.79 %, induced a drop in Hct level as compared to control. Vinodhini and Narayanan [72] exposed *Cyprinus carpio* to a mixture of heavy metals (Cd + Pb + Cr + Ni) and observed significant elevation in blood glucose.

Labeo rohita obtained from the Fe and Zn polluted Chowkalli Lake, Bangalore showed decline in RBC count, Hb content and leukocytosis [11]. Javed and Usmani [77, 78] in their study on *Mastacembelus armatus* thriving in water reservoir reported that fish exhibited the influence of effluents from coal fired Thermal Power Plant releasing Cu, Ni, Fe, Co, Mn, Cr and Zn. The exposed fish showed significant decrease in tRBC count (2.16×10^6 mm^{-3}), significant increase in tWBC count (3.84×10^3 mm^{-3}) and insignificant decrease in Hb (9.48 gdL^{-1}) when compared to control. Similar changes were observed in haematology of *Channa punctatus* dwelling in the same rivulet and sewage fed pond [79, 80]. Hanan et al. [81] reported much higher values of WBC count in fish, *Clarias gariepinus* inhabiting El-Rahawy delta of river Nile which receives industrial, domestic and agricultural waste. These wastes contained Fe, Mn, Zn,Cu, Pb and Cd.

Many researchers found disturbed carbohydrate metabolism in aquatic organisms due to pollution of aqua system. Levesque et al. [82] investigated the effects of heavy metals on intermediary metabolism in yellow perch (*Perca flavescens*), sampled from most polluted lake Dufault and lake Osisko situated along a contamination gradient of Cd, Zn and Cu in the mining region of Rouyn–Noranda, Quebec. Lower liver glycogen content in *Perca flavescens* from the most contaminated lakes was associated with the inability to increase plasma glucose following confinement. Fish with the highest tissue burdens of metals (Cd, Zn,Cu) might not show the capacity to increase their plasma glucose concentrations following an acute stress, because of low liver glycogen reserves. Mobilization of glycogen from liver resulted in glycogen depletion in yellow perch, *Perca flavescens* (Mitchill) sampled from more than 20 lakes located near metal smelter around Rouyn-Noranda, Quebec polluted by smelter effluents containing Cd, Cu, Pb, Ni and Zn [10]. Azmat et al. [83] sampled two marine water species, *Liza subviridus* and *Johnius belengerii* from Island Manora, Karachi and two fresh water fishes *Cyprius carpio* and *Pomodasy argyrew* from lake Halegyy. The water of Manora Island contains high concentrations of metals like As, Hg, Pb, Cd, Zn than lake Halegy. The glycogen content in muscles of marine fishes was lower than that of fresh water fishes. Zutshi et al. [11] sampled *Labeo rohita* from lake Hebbal receiving a storm water drain and lake Chowkalli receiving domestic sewage and industrial effluents having heavy metals like Fe and Zn. Serum concentration of glucose in *Labeo rohita* showed initial higher levels. Low concentration of glucose was reported in fish from lake Chowkalli as compared to those from lake Hebbal. A decrease in glycogen reserves of muscle and liver and associated increase in blood level of glucose was also reported in *Mastacembelus armatus* [78].

To summarize the results of various researchers, in general, red blood cells and hemoglobin got decreased in fish exposed to heavy metals either in experimental settings or caught from the polluted aquatic system. In contrast, white blood cells showed elevation as an immunogenic response. Rise or fall in other haematological indices like MCV, MCH, MCHC, ESR, PCV, oxygen carrying capacity depends upon the amount of RBC and Hb since they are derived from them. Very less work has been conducted on differential leucocyte count. Likewise haematological indices, on exposure to heavy metals, glucose level showed abrupt increase to meet the instant energy demand which leads to fall in glycogen reserves of muscle and liver. As the fish tries to adapt the environment, the glucose level comes down and glycogenesis and gluconeogenesis may have started to cope up with further degraded environment. Therefore measurements of various haematological indices and carbohydrate metabolites were serving as useful biomarkers to assess the fish health. The fish acts as the bioindicator to monitor the effects of heavy metals in the environment. This has also been observed that surplus literature is available on the laboratory induced studies however field studies on the similar works are very few. Therefore, authors encouraged field studies because every water body has the finger prints of anthropogenic activities. Inducing healthy fishes in laboratory either directly to salts of metals or to the effluents collected from the source makes no sense. However it leads to the rise of ethical issues. Tables 1 and 2 summarize the overall data of induced and field study respectively.

Table 1 Influence of heavy metals on hematology and Glycogen reserves of fishes, induced in the laboratory

Heavy metals	Fish species	Alteration in hematology and glycogen	Reference
Cd	*Cyprinus carpio*	5 mg/L (1 h) had no effect on % Hct and tRBC, while 10 mg/L (1–3 h) increased % Hct and TEC	[19]
	Scyliorhinus canicula	25 mg/L (24 h), increase RBC, Hb but decrease % Hct. 50 mg/L (96 h) bring these to normal levels	[20]
		WBC increase only at 50 mg/L (96 h)	[21]
	Oreochromis mossambicus	0.1 to 10 mg/L, increase WBC and Neutrophil	[84]
	Tilapia zilli	Hyperglycemia	[23]
	Cyprinus carpio	Hyperglycemia, decrease in liver and muscle glycogen reserves	[24]
	Catla catla	Hyperglycemia, significant fall in glycogen in liver, muscle, gill, heart and kidney after exposure to lethal and sublethal concentration	[25]
	Oreochromis niloticus	Hyperglycemia, decrease in liver and muscle glycogen	[73]
	Colisa fasciatus	Sublethal dose cause reduction in liver and muscle glycogen	[27]
Mn	*Channa punctatus*	Erythrocytopenia	[41]
	Tilapia sparmanii	Decrease in RBC, Hb, Hct and WBC	[42]
	Oreochromis mossambicus	Increase in RBC, Hb, Hct, DLC	[43]
	Colisa fasciatus	Hyperglycemia, decrease in liver glycogen	[44]

Table 1 continued

Heavy metals	Fish species	Alteration in hematology and glycogen	Reference
Cu	*Colisa fasciatus*	Increase in tRBC, Hb, Hct, thrombocytosis and decrease in large and small lymphocytes, ESR, blood clotting time (CT)	[46]
	Cyprinus carpio and *Oncorhynchus mykiss*	Increase in TEC, Hb, Hct and Decli decline in WBC in both fishes	[47, 48]
	Heteropneustes fossilis	Increase in Hb	[49]
	Clarias gariepinus	RBC and Hb initially decrease and after 48 h slightly increase	[52]
	Clarias lazera	Hemolysis and anemia	[14, 85]
	Oncorhynchus mykiss	Low dose (0.1 mg/L) for 3 months decrease Hb and no changes in Hct while double dose decrease both. Hyperglycemia	
	Channa punctatus	Decrease in Hb, RBC and PCV whereas WBC, CT, ESR, MCV, MCH, MCHC, DLC increased	[51]
	Clarias gariepinus	Acute exposure cause hyperglycemia	[52]
	Ictalurus nebulosus	Hyperglycemia	[17]
	Cyprinus carpio	Progressive hyperglycemia and corresponding decrease in liver glycogen	[54]
Zn	*Cyprinus carpio*	Low dose for long term had no effect on Hb and Hct. While high dose decrease Hb, Hct and WBC	[47]
	Cyprinus carpio	Acute exposure to sublethal dose increase RBC, Hb and no change in WBC	[55]
	Clarias lazera and *Tilapia zilli*	Increase in RBC, Hb, Hct and PCV	[56]
	Oreochromis mossambicus	Reduction in RBC, Hb, MCV, MCH, Lymphocyte, Neutrophil, WBC. Increase in MCHC and Hct at low and medium concentrations	[57]
	Oncorhynchus mykiss	Hyperglycemia	[58, 59]
	Labeo rohita	Depletion of liver and muscle glycogen	[9]
	Salmo gairdneri	Glycogenolysis in white muscle	[60]
Cr	*Tilapia sparmanii*	Elevation in MCHC	[42]
	Saccobranchus fossilis	Reduction in RBC, Hb, PCV, anemia	[61]
	Labeo rohita	Decrease in RBC, Hb and WBC increased	[62]
	Salmo gairdneri	Low dose for long term increase RBC, Hb and Hct levels	[63]
	Barbus conchonius	Low dose for long term increase RBC, Hb and Hct levels	[64]
	Cyprinus carpio	Decline in RBC, Hb content but WBC increased	[65]
	Cirrhinus mrigala	Significant reduction in muscle glycogen	[67]
	Cyprinus carpio	Significant reduction RBC, Hb, WBC, Hct, MCH, MCHC whereas, lymphocytes, neutrophils and MCV were insignificant	[68]
	Labeo rohita	96 h LC_{50} (111.45 mg/L) cause reduction in glycogen in liver, muscle and gill	[62]
	Cyprinus carpio	Hyperglycemia	[68]
Ni	*Colisa fasciatus*	Polycythemia with concomitant increase in Hb, Hct, leucopenia, retardation in ESR, no change in total thrombocyte count and CT	[16]
	Tilapia nilotica	Elevation in RBC count, Hb, PCV along with leucopenia and lymphopenia. Whereas, hyperglycemia and fall in liver and muscle glycogen	[23]
	Clarias gariepinus	At 96 h medial lethal concentration (8.87 mg/L) reduction in RBC, Hb, Hct, WBC, MCV, MCH and MCHC	[69]
	Cyprinus carpio	Acute exposure to high concentrations decrease RBC, Hct, Hb, WBC, MCV, MCH, MCHC	[70]
	Colisa fasciatus	Reduction in muscle glycogen	[71]
Pb	*Oreochromis mossambicus*	Hyperglycemia and depletion of liver and muscle glycogen levels	[39]
	Oreochromis niloticus	Elevation in RBC, Hb, Hct and MCHC.	[36, 37]
	Anguilla anguilla	Low concentrations (0.06 ppm) increase Hct while 0.12 ppm had no effect. In contrary sera glucose increased linearly with time	[38]
	Cyprinus carpio	Deplete glycogen in liver	[33, 40]
Hg	*Oreochromis mossambicus*	Leukocytosis	[33]
	Channa punctatus	Lowering in tRBC and Hb	[31]
	Oreochromis niloticus	Decrease in RBC and Hb content	[28]
	Clarias batrachus	Decline in tRBC, Hb and PCV while WBC increased	[29]
	Anabas testudineus	Variation in DLC, lymphocytosis, neutrophilia, monocytosis, eosinophilia and thrombocytopenia	[86]
	Tinca tinca	Sublethal and lethal concentration cause increase in tWBC	[34]
	Acanthopagrus latus	Considerable rise in Hb, Hct and monocyte count while WBC, lymphocyte and eosinophil decreased	[32]
	Clarias gariepinus	Drastic reduction RBC, Hb and Hct while WBC increased	[30]
	Cyprinus carpio	Deplete glycogen in liver, muscle and gill	[35]
Cd + Pb + Cr + Ni	*Cyprinus carpio*	Hb and PCV decreased while RBC count and blood glucose elevated	[72]
Cu + Pb	*Oreochromis niloticus*	Hyperglycemia at 4 days exposure and then return to control levels at the end of experiment	[26]

Table 1 continued

Heavy metals	Fish species	Alteration in hematology and glycogen	Reference
HgCl2 + ZnSO4	*Tilapia mossambica*	Hyperglycemia	[74]
Cd + Pb +Hg	*Labeo rohita*	Glycogen content of gill, liver and muscle decreased and maximum decrease observed under Hg intoxication	[75]
Cd + Zn + Pb + Hg	*Labeo rohita*	RBC decreased at all concentrations while alteration in Hct is dose dependent	[76]

tRBC total red blood cell, *Hct* hematocrit, *Hb* hemoglobin, *tWBC* total white blood cell, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *ESR* erythrocyte sedimentation rate, *CT* clotting time, *DLC* differential leukocyte count, *PCV* packed cell volume

Table 2 Influence of heavy metals on hematology and glycogen status of fishes from field studies

Heavy metals	Fish species	Site	Effluent type	Alterations in hematology and glycogen reserves	References
Cu, Zn	*Labeo rohita*	Chowkalli Lake	Domestic sewage, industrial effluents, run off from Vrishbhavati channel	Decline in RBC count, Hb, leukocytosis, Hyperglycemia	[11]
Cu, Ni, Fe, Co, Mn, Cr, Zn	*Mastacembelus armatus* and *Channa punctatus*	Harduaganj Reservoir/ nearby rivulet	Thermal power plant wastewater	In both fishes decrease in RBC, Hb while WBC increased. Oxygen carrying capacity also reduced. In *M.armatus* rise in blood glucose and fall in liver and muscle glycogen reserves. Whereas, *C.punctatus* showed reduction in glucose and glycogen content.	[77, 78, 80, 87, 88]
Cu, Ni, Fe, Co, Mn, Cr, Zn	*Channa punctatus*	Sewage fed pond	Domestic sewage, agricultural run off, wastewater from mechanical shops, ice factory, residues of dairy factory	Decrease in RBC, Hb while WBC increased	[89]
Cu, Ni, Fe, Co, Mn, Cr, Zn	*Channa punctatus*	Sugar mill effluent dominated canal	Sugar mill wastewater	Decrease in RBC, Hb while WBC increased, Oxygen carrying capacity also decline.	[90]
Fe, Mn, Zn, Cu, Pb, Cd	*Clarias gariepinus*	El-Rahawy delta of River Nile	Industrial, domestic and agricultural waste	Much higher count of WBC	[81]
Cd, Zn, Cu	*Perca flavescens*	Lake Dufault, Lake Osisko	Smelter waste, run off from mining	Hyperglycemia, decline in liver glycogen	[82]
Cd, Zn, Cu, Ni, Pb	*Perca flavescens*	20 lakes	Smelter waste, run off from mining	Mobilization of glycogen from liver which leads to decline in glycogen content	[10]
As, Hg, Pb, Cd, Zn	*Liza subviridus*,*Johnius belengerii*, *Cyprius carpio* and *Pomodasy argyrew*	Island Manora and Halegy Lake	Agricultural runoff, industrial, petroleum	Lower glycogen in marine fish muscle than freshwater fish muscle	[83]

RBC total red blood cell, *Hb* hemoglobin, *WBC* white blood cell

Conclusion

One of the key approaches to address the issue of contaminated sites is to prevent their occurrence in the future. The contamination of water resources particularly by heavy metals besides degrading the quality of water also influences the quality of food in the form of fish protein. The heavy metals accumulate in tissues of fish due to which it comes under stress, which is evident from the hematological and glycogen profile of fishes. These heavy metals will enter the food web through water and food, to cause the adverse health effects like that in indicator

organisms. No doubt industries are necessary for development but on the other hand they are also creating heavy loss to the livelihood of humans. Therefore for maintaining the ecological balance it is advisable that the industrialist should dispose their waste only after prior treatment. To completely solve the burden of many years of industrialized activity, it is suggested that flow of metals from the point sources is controlled by reducing the use of products and processes utilizing heavy metals. If the pollution from the point sources is controlled it will ultimately lead to reduction of heavy metals in fish as their environmental level goes down. Therefore, there is a need that such techniques or measures are designed which provide solution to this question. It is thus imperative that one should act now to reduce future emissions and release of heavy metals to the maximum extent possible in order to stop contamination of global aquatic ecosystem and prevent toxic effects on fish and humans.

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