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A REVIEW ON SOME HEAVY METALS TOXICITY ON HAEMATOLOGICAL PARAMETERS IN FRESH WATER FISHES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author SUR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MU managed the analyses of the study. Both authors read and approved the final manuscript.

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Review Article

ABSTRACT

Heavy metals contamination could be a significant subject within the aquatic ecosystem. The toxic levels of heavy metals are also of agricultural, industrial, and mining activities. This may cause pollution and changes within the physicochemical characteristics of the aquatic environment. This pollution has high toxicant effects on fishes, fish products as well as fish environment and human health. The Aim of this study was to detect the contamination levels and the health status of fishes, and study the effect of various heavy metals like Pb, Hg, Fe, Cr, Cu, and Zn etc. on several blood parameters like RBC, WBC, Hb, PCV, MCH, MCV, and MCHC. The obtained results the contamination of the aquatic systems with heavy metals from natural anthropogenic sources has become a worldwide problem which poses threats to ecosystems and natural communities. Hence, this study reviews the consequences of heavy metals in freshwater fishes. Fishes bioaccumulate heavy metals (including cadmium, zinc, lead, and copper) through various organs like gills, liver, stomach and intestine. The consequences of those heavy metals are highlight and compared with their permissible limit advised by the Food and Agriculture Organization (FAO) of the United Nations, 1983. For all the above reasons, this review aimed to check the contributed of such heavy metals within the environment, toxic mechanism, and toxic effects on fish.

Keywords: Heavy metals; toxic; pollution; haematological; fish.

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1. INTRODUCTION OF HEAVY METALS

Nazish Iftikhar and Imran Hashmi [1] reported to effect of heavy metals in fish tissues (gills, liver, gut, muscle and ovary) through the biochemical, haematological, and immunological test. Haematology as the study of the physiology and pathology of the blood cells. In this study, the toxic effect of heavy metals on freshwater fishes health through estimating hematological parameter. Hematological parameters, like RBCs, WBCs, Haemocrit, Haemoglobin, Meancorpuscular haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration in fish. An aquaculture system is highly polluted from domestic waste, land runoff, improper disposal, Agrochemical waste, and industrial effluents contains heavy metals these are highly affected by the fish health [2,3]. Blood is the most important and abundant body fluid and fast mobilizing defense against trauma and health illness [4].

Heavy metals are presents in the aquatic ecosystem, and then reread affected fish are polluted with the heavy metals and accumulates it. Metal occur in varying parts of the body depends upon water solubility, feeding behavior, ecology, and fish physiology including species, age, size, reproductive state, fish health, bioavailability, and different habitats [5,6]. Mono-species or multispecies have been reported to enhance fish health particularly enhancement of antibody levels, as well as proinflammatory cytokine [7,8] as well as hematological parameters in fish [9]. Variations in blood parameters in fish are highly sensitive to environment changes. Quality of oxygen, temperature and salinity are directly affected in blood of fish [10] as well as ecological factors such as feeding habitat, and stocking density [11]. Microbial production reduces dissolved oxygen level in the aquatic system, so increasing the mortality of Fishes and another aquatic organism. The status of the above haematological parameters in fish depend on many factors, like the level of contaminants temperature, nutrition, and fish species, etc. Therefore, changes in haematological parameters are considered as a diagnostic tool for the disturbance in homeostatic defense mechanism [12]. Progress in establishing normal range values for blood parameters of diverse fish species [13]. The biological and environmental factors including age, sex, food, pathogens like bacteria, virus, fungi, and water quality parameters affected by the fish blood parameters [14]. The purpose of this review is to reveal the toxic effects of heavy metals on fish blood profile.

2. MATERIALS AND METHODS

2.1 Blood Sample Collection

Fishes collected from heavy metal contaminated water system from Tenkasi district, Tamilnadu. Fish ranges approximately 250g and 6-10 fishes collected from tenkasi district [15]. The blood was collected from a cardiac puncture, 5 milliliter heparinized syringe. The blood was collected in sterile tubing containing EDTA solution. This EDTA using for an anticoagulant [16]. The blood in the EDTA centrifuge at a high-speed of regarding 17,000 rates for 10 minutes. After the 10 minutes plasma settled during a bottom of the tube. Fish blood includes 60–80% plasma, 20–40% RBCs, and 0.5–2.0% WBCs [17].

2.2 Automatic Haematological Analysis

The medicine profile determined now when blood samples victimization an automated medical specialty analyzer. This equipment uses an analysis system that was already used and valid by comparative manual tests within the veterinary field to analyze medicine profiles in various fish species [18,19,20]. The centrifuge blood was subjected to an automatic machine of KX 21 N an analyzer. Red Blood Cell (RBC) associated White Blood Cell (WBC) counts were counted below microscope with improved Neubauer haemocytometer [21,22] Red blood cells (RBCs), White blood cells (WBCs), Hb count, and Platelets (PLT) were measured victimisation Sysmex blood analyzer XP-100. The microhaematocrit technique of [23] was accustomed to verifying the Haematocrit (PVC). Hb concentration was measured with haemoglobin check kit victimization in the cyanmethemoglobin technique [24]. The derived haematologic indices of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated using standard formulae as described by [25]. Data collected from the experiment using statistical analysis of variance (ANOVA). Variance (SD) and Pearson correlation were calculated. Significance value was set at P = 0.01 and 0.05. All analysis was performed using SPSS (statistical package for the Social Science) software package (version 13.0).

2.2.1 Estimation of total red blood cell (RBC)

The total red blood cells (RBCs) were counted using a Neubaur haemocytometer [26]. Blood was diluted using Hayem's fluid with 1:200 [27] Erythrocytes were counted in the loaded haemocytometer chamber.

Under compound microscope total erythrocyte count showed alterations concentrations of heavy metals. The RBC count was 2.98 million/cu.mm in the blood of control fish, and this count was decreased to ~ 1.32 million/cu.mm [28].

2.2.2 Estimation total white blood cell (WBC)

WBC was counted using a Neubaur haemocytometer [29,30]. Blood was diluted with Turk's diluting fluid concentration of 1:20 and placed in haemocytometer. Under compound microscope the WBC count altered specifically, and the WBC count exhibited a different direction when compared to the RBC count. It exhibited a slight increasing direction as the increasing number of heavy metal concentration [31].

2.2.3 Estimation of haemoglobin

Haemoglobin (Hb) was determined with a haemoglobin test kit (DIAGNOVA, Ranbaxy, India) using the cyanmethemoglobin method [32]. Hb is converted into cyanmethaemoglobin by the addition of potassium ferricyanide (KCN) and the colour was read in a spectrophotometer at 540 nm against a reagent blank. The haemoglobin content of blood fish was 7.06±0.64 in control fish. The Hb content decreased in test fish when exposed heavy metals concentrations. The decreasing in Hb content is comparable to that of RBC count in the blood of fish [33].

2.2.4 Determination of packed cell volume (PCV)

Packed cell volume was examined by micro haemocrit method of [3 cell volume regarding percentage of total blood column 4]. The heparinised blood was filled up to the mark 100 of the haemocrit tubes with the help of Pasteur pipette and centrifuged at 3000rpm for 30min. The height volume of the RBC's packed at the bottom of the haemocrit tube was recorded as packed taken in the haemocrit tube.The trend of PCV value was like that of RBC and Hb Concentration during the life span of fish decrease was up to (56.07%).

2.2.5 Determination of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)

MCV values showed vast variations during different seasons of the year. The reduced MCV value was usually found during gonadial maturation as erythropoiesis happened at this time discharge small immature cells into the blood stream.

The highest MCH and MCHC were found on high toxic content in the aquatic system. These high values are a reflection Hb values during this time, as MCH and MCHC are derivatives from Hb. MCH and MCHC were found to be directly related to Hb in both sexes. The MCH and MCHC values reported by [34]. The food availability, feeding behaviour, the nature of the habitat, the method of Hb and PCV determination all these affects the haematological values. MCV indicatesthe average size of the RBC in a fish blood. MCV value calculated based on of PCV and RBC values. Elevated values of MCV were recorded over control (62.19±0.22) at heavy metals concentrations.

MCV = Haematocrit (%) × 10 / RBC count femtoliter (fL).

MCH represents the average content of the Hb in each red blood cell. MCH exhibited increase in heavy metals concentration.MCH exhibited increase in heavy metals concentration. Maximum increase was observed (25.70%).

MCH = Haemoglobin (g/ dL) × 10 / RBC count Picogram(Pg).

MCHC reflects the average concentration of the haemoglobin in the red blood cells in given volume of blood. The MCHC of blood of fish was 28.32±0.46 in control fish. The MCHC value decreased heavy metals concentrations. The decrease was 0.56%. Maximum increment was observed (11.22%) and minimum was (7.02%) over control [35].

MCHC = Haemoglobin (g/dL) ×100/ Haemocrit (%) gram percentage [36].

The effect of heavy metal in fresh water fishes and human are shown in Table 1.

The heavy metal in different tissues of fishes leads to several harmful effects. It may have mutagenic, immunological cytotoxic and genotoxic effects. Abnormalities in fish reproduction; human consumption such as polluted fish [45,46].

2.3 The Effect of Heavy Metals in Fishes

2.3.1 A heavy metal population index

The total population was calculated to determine a total accumulation of the metals in different tissues of fishes. The MPI was calculated the following equation [47].

Table 1. effect of heavy metal in fresh water fishes and human

Heavy metals	Fish	Human	References
Cadmium	Myocardial infection	Diarrhea,Vomiting ,Fractures in bone. Damage to DNA,Cause damage to nervous system,Damage to immune system, Cause cancer Failure in reproduction.	[37]
Zinc	Tissue respiration Affect growth metabolism	Damages the pancreas Disturb the protein metabolism, and Cause arteriosclerosis Chloride can cause respiratory disorders.	[38]
Copper	Necrosis in liver Decrease egg production Low fertility	Higher dose damage liver and kidney. Wilson's disease.	[39]
Chromium	Anemia, eosinophilia and lymphocytosis, bronchial and renal lesions. higher concentrations of Cr damage the gills	Faded immune system Cause ulcer and upset stomach, Respiratory track problem, Alteration in genetic material,Lung cancer, Liver and kidney damage, Death.	[40]
Nickel	Respiratory Disease Hyperglycemia	upset stomach, damage liver and kidney	[41]
Lead	Damage to liver, kidneys, brain, nerves, and other organs. reproductive disorders osteoporosis (brittle bone disease)	Heart disease, High blood pressure Anemia Mental retardation	[42]
Iron	Disruption in Respiration damage Respiratory epithelium and death due to suffocation	High blood pressure Anemia Headache	[43]
Mercury	Olfactory epithelium Damage Liver, gills, Nervous system and blood parameters damage.	Gingivitis, Congenital Malformation Damage CNS and brain Damage**.**	[44]
Arsenic	Fish death , Increase mucous secretion, defect in gill epithelium.	Stomach and Lungs irritation Decrease RBC and WBC	[44]

Table 2. Maximum permissible limits

No	Heavy metal	Maximum permissible limit (MPL) in ppm	Reference
1	Cadmium	0.05	FAO, 1983 [51]
2	Zinc	30	FAO, 1983
3	Copper	30	FAO, 1983
4	Chromium	1	FAO, 1983
5	Nickel	0.05-5.5	FAO, 1983
6	Nickel	0.5	FAO, 1983
7	Iron	100	WHO, 1989 [52]
8	Mercury	0.5	WHO, 1989
9	Arsenic	1.4	WHO, 1989

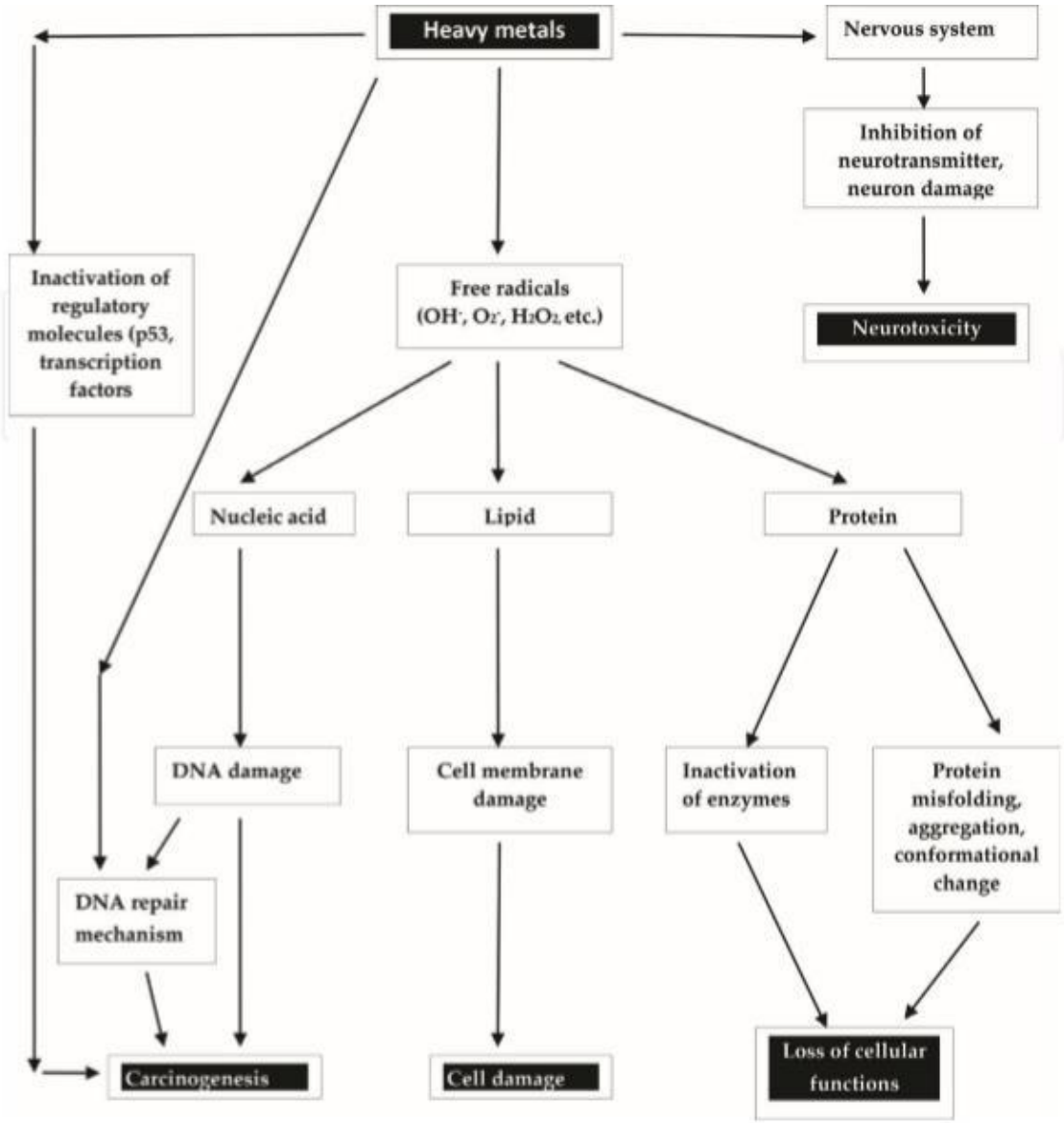

Fig. 1. Pathway of heavy metals in fish body [53]

Table 3. The effect of heavy metals in haematological parameters of freshwater fishes

Heavy metals	Alteration in hematology	Reference
Cadmium	• Increase RBC, Hb but decrease % Hct. WBC in *Scyliorhinus canicula* • Increase WBC and Neutrophil in *Oreochromis mossambicus* • No effect on % Hct and tRBC, increased % Hct and TEC in *Cyprinus carpio* • *Catla catla* Hyperglycemia • *Tilapia zilli* Hyperglycemia • *Channa punctatus* Erythrocytopenia • *Cyprinus carpio* Hyperglycemia • *Oreochromis niloticus* Hyperglycemia	[54,55,56,57,58,59]
Zinc	• *Clarias lazera* and *Tilapia zilli* increase in RBC, Hb, Hct and PCV	[60,61,62]

Heavy metals	Alteration in hematology	Reference
	• Reduction in RBC, Hb, MCV, MCH, WBC. Increase in MCHC and Hct at low and medium concentrations in *Oreochromis mossambicus* • Increase RBC, Hb and no change in WBC in *Cyprinus carpio* • *Oncorhynchus mykiss* Hyperglycemia	
Copper	• Increase in RBC, Hb, Hct, thrombocytosis and decrease in large and small lymphocytes in *Colisa fasciatus* • Hemolysis and anemia in *Clarias lazera* • Acute exposure cause hyperglycemia in *Cyprinus carpio* • Decrease in Hb, RBC and PCV whereas WBC, CT, ESR, MCV, MCH, MCHC, DLC increased in *Channa punctatus* • Increase in TEC, Hb, and Hct decline in WBC in both fishes in *Cyprinus carpio* and *Oncorhynchus mykiss*	[63,64,65]
Chromium	• Elevation in MCHC in *Tilapia sparmanii* • Reduction in RBC, Hb, PCV, Anemia in *Saccobranchus fossilis* • Decrease in RBC, Hb and WBC increased in *Labeo rohita* • Low dose for lon- term increase RBC, Hb and Hct levels in *Salmo gairdneri* • Low dose for long-term increase RBC, Hb and Hct levels in *Barbus conchonius* • Decline in RBC, Hb content but WBC increased in *Cyprinus carpio* • Significant reduction RBC, Hb, WBC, Hct, MCH, MCHC whereas, lymphocytes, neutrophils and MCV were insignificant in *Cyprinus carpio*	[66,67,68,69]
Nickel	• *Colisa fasciatus Polycythemia* with concomitant increase in Hb, Hct, leucopenia, • Elevation in RBC, Hb, PCV along with leucopenia and lymphopenia. Whereas, hyperglycemia in *Tilapia nilotica* • Reduction in RBC, Hb, Hct, WBC, MCV, MCH and MCHC in *Clarias gariepinus* • *Cyprinus carpio* high concentrations heavy metals decrease RBC, Hct, Hb, WBC, MCV, MCH, MCHC	[70,71,72,73]
Lead	• Elevation in RBC, Hb, Hct and MCHC in *Oreochromis mossambicus* and *Oreochromis niloticus*. • Low concentrations (0.06 ppm) increase Hct while 0.12 ppm had no effect in *Anguilla Anguilla*	[74,75,76]
Mercury	• Leukocytosis in *Oreochromis mossambicus* • Lowering in RBC and Hb in *Channa punctatus* • Decrease in RBC and Hb content in *Oreochromis niloticus* • Decline in RBC, Hb and PCV while WBC increased in *Clarias batrachus* • Variation in DLC, lymphocytosis, neutrophilia, monocytosis, eosinophilia, and thrombocytopenia in *Anabas testudineus* • *Tinca tinca* cause an increase in WBC • Considerable rise in Hb, Hct and monocyte count while WBC, lymphocyte and eosinophil decrease in *Acanthopagrus latus* • Drastic reduction RBC, Hb and Hct while WBC increased in *Clarias gariepinus*	[77,78,79,80,81,82,83]

2.3.1.1 Classification of heavy metals

1. Essential heavy metals

Copper, chromium, zinc-nickel, cobalt, and iron are essential metals required for all vital processes inside the body with an optimum level. Otherwise, inadequate amount causes deficiency and high-a level causes toxicity [48,49].

2. Non-essential heavy metals

Those haven't biological roles, and also called xenobiotics. When they are increased in, concentrations will cause toxopathic effects in different kind of tissue; those involve Aluminum, Mercury, Lead, Cadmium, and others [50].

2.4 Maximum Permissible Limit (MPL)

Maximum permissible limit (MPL) in a ppm (parts per million) of heavy metal in freshwater fishes according to International standards (Table 2). The pathway of heavy metals in fish body are shown in Fig. 1. Besides, the effect of heavy metals in haematological parameters of freshwater fishes are shown in Table 3.

3. CONCLUSION

Heavy metal's contamination could be a serious problem within the aquatic ecosystem. The harmful levels of significant metals are also of agricultural, industrial and mining activities. This can cause pollution and changes within the physicochemical characteristics of the aquatic ecosystem. More investigation has to be done on different aquatic organisms to confirm the impact of the heavy metals on the freshwater ecosystem. This pollution has high harmful effects on fish, and conjointly impact on human health. To evaluation of blood parameters may be the quickest way to study the toxicological impact on fish. To conclude of this study was to analyze the contamination level and health standing of fish to gauge the contamination level, concentrations of various heavy metals (Pb, Mn, Fe, Cr, Cu, Zn) etc in numerous blood parameters like RBC, WBC, Hb, PCV, MCH, MCV, MCHC were analyzed. For all higher than reasons, this review was written to subsidize to significant metals role within the setting, harmful mechanism and harmful effects on fish.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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