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Bioaccumulation of Lead and Cadmium and its Impact on fresh water catfish, *Heteropneustes fossilis*

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Abstract: Heavy metals are key components to cause environmental pollution and heightened level of concentration is toxic to the living organisms. In generally food is the main source of accumulation of toxic metals day by day from various source, they include through soil, water and atmospheric dust. Many disorders like cancer, gene mutation, physiological malformation or physical deformations in pregnant women or very young children. May be one of the reason of consumption of indigenous food. Presently people are fascinate to have fish is the one of the most important ingredients in the daily diet due to this rapid growth of consumption and different practices to produce the aqua products based on the demand. Hence forth the present study focused on intoxication studies on the consumption of freshwater catfish namely *Heteropneustes fossilis*, collected from different aqua farming cultures. Aim of the work is to predict the different adverse effects of LC_{50} of lead and cadmium and mortality, behavioural and haematological studies.

Key words: Food Chain, Toxicity, Catfish, $LC_{50,}$ Haematological, Lead, Cadmium, Mortality, Behavioural.

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Introduction: Pollution of aquatic environments with heavy metals has seriously increased worldwide and under certain environmental conditions fish may concentrate large amounts of some metals from the water in their tissues (Vhahangwele Masindi and Khathutshelo L. Muedi (2018)). The heavy metal contamination of aquatic ecosystem has attracted the attention of researchers all over the world owing to their toxicity at very low levels, persistence in the environment and ability to get incorporated in the tissues of organisms (Rai, Prabhat. (2008)). All these factors make the toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic products as source of food (Paul *et al*., 2014). Heavy metals can accumulate in the tissues of aquatic animals and as such tissue concentrations of heavy metals are of public health concern to both animals and humans (Tembo Rostern (2017)).

Bioaccumulation of non-essential metals in tissues leads to intoxication, decreased fertility, tissue damage and dysfunction of a variety of organs (Nitasha Khatri and Sanjiv Tyagi (2015)). Heavy metals cause adverse biological effects (Golovanova, Irina. (2008)). It was reported that metals are taken up through different organs of the fish and induce morphological, histological and biochemical alterations in the tissues which may critically influence fish quality (Mehjbeen Javed1 and Nazura Usmani (2019)). Common sub-lethal effects are behavioral (e.g. swimming, feeding, attraction-avoidance, and prey-predator interactions), physiological (e.g. growth, reproduction and development), biochemical (e.g. blood, enzyme levels), and histological changes (Sanaa Abdulaziz Mustafa (2020)).

Once heavy metals are accumulated by aquatic organisms, they can be transferred to upper class of food chain. Heavy metals generally do not degrade and tend to biomagnify in man through food chain

(Hadeel M Huseen and Ahmed J Mohammed (2019)). Thus human health eventually is threatened by the consumption of such food. There are two ways of penetration of heavy metals into the organism either by direct water absorption or by consuming fish as food (Michael A Clark *et al*., 2020). Reported larger metal loads in the tissues of predatory fish species. The ecological specificity of metal pollutant is that there are practically no self-cleaning mechanism known for them when present in water, they pass through the trophic chain of aquatic communities (Elisabet Lindgren *et al*., 2018).

Heavy metals affect specific vital organs such as liver, gill and kidney. Liver contains highest metal concentration because it is an organ of storage and detoxification of metal. Heavy metals have the ability to bioaccumulate in the liver and kidney, the target organs of heavy metal pollution and also body's detoxification organs. Changes in histological structure of specific vital organs due to exposure of sub-lethal concentration of metal in various fishes have been reported by many workers (Gandhewar and Zade (2019)). The body constantly tries to eliminate heavy metals *via* the available exit routes: the liver, kidney and skin. Detoxification mechanism includes acetylation, sulfonation, oxidation, etc. Liver is a detoxification organ and essential for both the metabolism and excretion of toxic substances. Liver has the ability to degrade the toxic compounds but its regulating mechanism can be overwhelmed by elevated concentration of these compounds and could subsequently result in structural damage. Here most of the products are expelled through the bile into the small intestine and should leave the body *via* the digestive tract. Histological analysis is crucial in determining cellular changes that may occur in target organs, such as gill, liver and kidney (Ting Liu *et al.*, 2020).

Heavy metals can cause genetic mutation. They disrupt the metabolic process. Heavy metals alter prooxidant-antioxidant balance and bind to free sulphydryl groups resulting in inhalation of glutathione, metabolism, numerous enzyme and hormone functions. Chemically reactive pollutants such as electrophiles react with different nucleophilic biological molecules. Depending on its electrophilicity, an electrophilic pollutant reacts with soft nucleophile, such as thiol groups in protein and peptides or harder nucleophiles, such as nucleotides in DNA. They also pointed out that reaction with peptides and proteins interfere with the cellular reducing capacity through conjugation with glutathione or interfere with the enzyme activity, while DNA damage leads to mutation (Richard et al., 2019).

Lead is the nonessential and most toxic metal which is widely distributed in the aquatic environment and earth's crust. Heavy metals such as lead, mercury, and cadmium are considered to cause public health hazards. Nonessential components of lead may cause nephrotoxicity, neurotoxicity, decrease growth rate, survival, metabolisms and development, and several adverse health effects. Consumption of such metal-contaminated fishes by a human can cause serious health issue. Metals deteriorate the ecological balance of the aquatic environment because fish are at the end of the aquatic tropic level and they have a higher tendency to accumulate metals in their body (Richard *et al*., 2014). The aim of the present study was to investigate heavy metal bioaccumulation and alteration in hematological indices and red blood cell and nucleus morphology and in different organs like gills and muscles of grass carp exposed with different concentrations of heavy metals.

MATERIALS AND METHODS

Animal: (8.5±5.5cm; 9.5±6.5g) were transported in oxygenated bags (50 fish per bag) from carp hatchery of Mardan and Peshawar to the lab. The fish were treated with 0.2% $KMnO_4$ solution for two minutes to remove any external infection.

Acclimatization: Fishes were acclimatized to the laboratory conditions in large fiber glass tanks with unchlorinated ground water for 3 to 4 weeks at a room temperature of 28 ± 2°C. As these catfishes are benthic in nature, overcrowding was avoided by keeping small numbers of fishes in each tank. Water was changed on alternate days. Tanks were covered with fish netting to prevent the escape of fishes.

Selection of sub-lethal concentrations: In the present study $1/10^{th}$ of the 96h LC_{50} value was taken as sub-lethal concentration (A). The two other doses, B & C, used were a reduction in concentration of the sub-lethal concentration (A) in a graded manner. The half concentration of the sub-lethal concentration a (50% reduction) was used as the second dose (B) while the third dose (C) was 50% reduction in concentration of the second dose B (Kayode *et al.*, 2016).

Haematological studies: After determining 96 h LC50 value, 3 sub-lethal concentrations (A, B, C) of Cadmium chloride were taken and 10 fishes were introduced in each concentration. For each sub-lethal exposure, five replicates were maintained. The water was changed every day in the control and renewed in the treatment group, so that the concentration of cadmium chloride remained the same during the experimental period. *Heteropneustes fossilis* was exposed to sub-lethal concentration of Cadmium for 21 days. At the end of 7th, 14thand 21st day sampling was done. At the end of the exposure period, blood was

taken by the following method. The fish were caught very gently using a small dip net, one at a time with least disturbance. Each fish was held and wrapped with a clean, dry towel and the posterior half of its body was blotted with a clean coarse filter paper. Blood from the Control and Cadmium chloride treated fishes were obtained by severance of caudal peduncle and collected in Eppendorf tubes containing 1% of Ethylene diamine tetra acetic acid (EDTA) as anticoagulant (Mgbenka *et al.,* 2003). Haematological parameters were estimated by standard methods as described by Hesser (1960) and Blaxhall and Daisley (1973).

Tissue Digestion for Accumulation: Estimation of heavy metals was carried out by following the tissue digestion. Tissue samples were thawed, rinsed in distilled water, and blotted with blotting paper. After blotting, the samples were transferred to 100 ml volumetric flasks. The entire flask was washed properly and rinsed with distilled water, before transferring the tissue samples. Then, the known weights of each tissue were transferred to these volumetric flasks. Samples digestion was carried out according to the methods presented. A slight modification was made in the procedure; instead of putting 10 ml nitric acid (60%) and 5 ml per chloric acid (70%) at the time of digestion, 5 ml nitric acid (60%) and 1 ml per chloric acid (70%) were added to each flask and the flasks were then kept overnight. The next day, a second dose of 5 ml nitric acid (60%) and 4 ml per chloric acid (70%) was added to each flask. The flasks were kept on a hot plate, covered with Pyrex glass cover, and allowed to digest at 200 to 250°C until a clear transparent solution was observed. Initially, dark brown fumes appeared followed by white fumes. The dense white fumes from the flask, after brown fumes, were an intimation of completion of the digestion process. By this method, digestion was accomplished in about 30 minutes instead of 3 to 4 hours as described in. After digestion, the samples were cooled, filtered through Whatman 42 filter paper and diluted to 100 ml with distilled water by proper rinsing of the digestion beakers.

Histological Studies: After the fish dissection, portions of tissues (gills and muscles) were preserved in 10% formalin for histological studies. The preserved tissues were processed in various grades of ethanol, cleared in xylene, and impregnated with wax (mp; 58°C). Five-micron-thick sections were cut using a rotary microtome (Leica RM 2165) at 100x. Tissue sections were stained with hematoxylin and eosin (H&E). Stained slides were observed and photographed under a high-resolution microscope (Lecia, Japan) fitted with a digital camera.

Statistical Analysis*:* the statistical analysis was performed using IBM SPSS (version 20).

RESULT AND DISCUSSION

Mortality and probit mortality of Lead and Cadmium (Toxicological Studies): Toxicological studies were conducted to evaluate the rate of mortality and probed mortality of *H. fossilis* under various known concentrations of cadmium in the aquaria. In the Aquarium or ecological pond (static and continuous flow) gradual increment of cadmium, Lead concentrations with time intervals proportional increment had been observed rate of mortality and probit mortality and plotted to determine the LC_{50} value and vulnerability rate of the fish (Table 4.4 and 4.5). Acute toxicity was found at 96 h LC_{50} of cadmium, lead in static was 20.68619 ppm; 65.00476 ppm and continuous flow was 16.66384 ppm 58.42679 ppm and lethal concentration (LC_{50}). Rate of mortality at 95% confidential levels includes both the cases of lower and upper at 96hours of exposure was observed good mortality rate at lower concentration for longer periods of time was more toxic and caused complete death resulted in both static and continuous flow system (static lower, upper 18.55514; 24.44486; continuous lower and upper 14.55514; 20.44486 ppm. Similar reports were observed in the case of salmonids, *Oncorhynchus mykiss, Salvelinus confluentus* and *Oncorhynchus tshawytscha* (Finalayson and Verrue, 1982; Hansen *et al*., 2002), guppy, *Poecilia reticulate (*Yilmaz *et al*., 2004), Cyprinus *carpio (*Muley *et al*., 2000; Dardenne *et al.,* 2007), Nile tilapia, *Oreochromis niloticus* (Mahnaz Sadat Sadeghi and Sadegh Peery(2018),Garcia *et al*., 2006) and Rohu, *Labeo rohita* (Dutta and Kaviraj, 2001). It also concurs the Canadian Environmental Protect Act, 1994 report in which it has been suggested that toxicity of cadmium in fish varies from species to species. Cadmium has shown toxic effects on the *H. fossilis* (Nilalohit *et al.,* 1981; Henary and Atchison, 1990; Brown *et al.,* 1994; Maruthayanagam *et al.,* 2002; Sobha *et al.,* 2007; Kasherwani *et al.,* 2009).The mortality due to the absorption and bio-accumulation of cadmium. The variations observed in the 24, 48, 72 and 96 h LC_{50} values between *H. fossilis* and other fishes may be attributed to the fact that metal induced changes in physiology and survival of aquatic organisms under metallic stress differ from metal to metal, species to species and from one experimental condition to other. The exact causes of death due to heavy metal poisoning are multiple and depend mainly on time-concentration combination. The 96 h LC_{50} values for cadmium were recorded as

5.36 mg L^{-1}. Sobha *et al.* (2007) reported 96 h LC_{50} of *Catla Catla* for Cd, Lead as 4.53 mg L^{-1}. El-Moselhy (2001) reported decrease in the *Heteropneustes fossilis.*

Table 4.1.2 : The LC_{50} values of cadmium exposed to *H. fossilis* for 24, 48, 72 and 96 h in Static system

S.No	Exposure period	Conc. (ppm)	LogConc.	No. of fish exposed	No of fish alive	No of fish dead	Percent Mortality	Probit Mortality	LC50
1	24 h	27.5	1.439333	10	9	1	10	3.7184	32.68056
2		29.0	1.462398	10	8	2	20	4.1584	
3		30.5	1.484300	10	7	3	30	4.4756	
4		32.0	1.505150	10	6	4	40	4.7467	
5		33.5	1.525045	10	4	6	60	5.2533	
6	48 h	24.5	1.389166	10	9	1	10	3.7184	28.14915
7		26.0	1.414973	10	8	2	20	4.1584	
8		27.5	1.439333	10	6	4	40	4.7467	
9		29.0	1.462398	10	4	6	60	5.2533	
10		30.5	1.484300	10	3	7	80	5.8416	
11	72 h	21.5	1.332438	10	8	2	20	4.1584	23.69800
12		23.0	1.361728	10	6	4	40	4.7467	
13		24.5	1.389166	10	4	6	60	5.2533	
14		26.0	1.414973	10	3	7	80	5.8416	
15		27.5	1.439333	10	2	8	90	6.2816	
16	96 h	18.5	1.267172	10	8	2	20	4.1584	20.68619
17		20.0	1.301030	10	6	4	40	4.7467	
18		21.5	1.332438	10	4	6	60	5.2533	
19		23.0	1.361728	10	2	8	80	5.8416	
20		24.5	1.389166	10	1	9	90	6.2816	

Table 4.1.3: The LC_{50} values of cadmium exposed to *H. fossilis* for 24, 48, 72 and 96 h in Continuous flow through system

S.No	Exposure period	Conc. (ppm)	Log Conc.	No. of fish exposed	No. of fish alive	No of fish dead	Percent Mortality	Probit Mortality	LC_{50}
1	24 h	23.5	1.371068	10	9	1	10	3.7184	28.68277
2		25.0	1.397940	10	8	2	20	4.1584	
3		26.5	1.423246	10	7	3	30	4.4756	
4		28.0	1.447158	10	6	4	40	4.7467	
5		29.5	1.469822	10	4	6	60	5.2533	
6	48 h	20.5	1.311754	10	9	1	10	3.7184	24.13725
7		22.0	1.342423	10	8	2	20	4.1584	
8		23.5	1.371068	10	6	4	40	4.7467	
9		25.0	1.397940	10	4	6	60	5.2533	
10		26.5	1.423246	10	3	7	80	5.8416	
11	72 h	17.5	1.243038	10	8	2	20	4.1584	19.68146
12		19.0	1.278754	10	6	4	40	4.7467	
13		20.5	1.311754	10	4	6	60	5.2533	
14		22.0	1.342423	10	3	7	80	5.8416	
15		23.5	1.371068	10	2	8	90	6.2816	
16	96 h	14.5	1.161368	10	8	2	20	4.1584	16.66384
17		16.0	1.204120	10	6	4	40	4.7467	
18		17.5	1.243038	10	4	6	60	5.2533	
19		19.0	1.278754	10	2	8	80	5.8416	
20		20.5	1.311754	10	1	9	90	6.2816	

Table 4.1.12: 95% Confidence levels of Cadmium exposed to H. *fossilis* for 24, 48, 72 and 96 h in static and continuous flow through methods

S.No	Exposure period	95% Confidence levels			
		Static Method		Continuous flow through Method	
		Lower	Upper	Lower	Upper
1	24 h	27.55514	33.44486	23.55514	29.44486
2	48 h	24.55514	30.44486	20.55514	26.44486
3	72 h	21.55514	27.44486	17.55514	23.44486
4	96 h	18.55514	24.44486	14.55514	20.44486

Table 4.1.13. The LC_{50} values of Lead exposed to *H. fossilis* for 24, 48, 72 and 96 h in Static system

S.No	Exposure period	Conc. in ppm	Log Conc.	No. of fish exposed	No. of fish alive	No. of fish dead	Percent Mortality	Probit Mortality	LC_{50}
1	24 h	72.0	1.857332	10	9	1	10	3.7184	82.35547
2		75.0	1.875061	10	8	2	20	4.1584	
3		78.0	1.892095	10	7	3	30	4.4756	
4		81.0	1.908485	10	6	4	40	4.7467	
5		84.0	1.924279	10	4	6	60	5.2533	
6	48 h	68.0	1.832509	10	9	1	10	3.7184	75.33431
7		71.0	1.851258	10	8	2	20	4.1584	
8		74.0	1.869232	10	6	4	40	4.7467	
9		77.0	1.886491	10	4	6	60	5.2533	
10		80.0	1.903090	10	3	7	80	5.8416	
11	72 h	64.0	1.806180	10	8	2	20	4.1584	68.44619
12		67.0	1.826075	10	6	4	40	4.7467	
13		70.0	1.845098	10	4	6	60	5.2533	
14		73.0	1.863323	10	3	7	80	5.8416	
15		76.0	1.880814	10	2	8	90	6.2816	
16	96 h	61.0	1.785330	10	8	2	20	4.1584	65.00476
17		64.0	1.806180	10	6	4	40	4.7467	
18		66.0	1.819544	10	4	6	60	5.2533	
19		69.0	1.838849	10	2	8	80	5.8416	
20		72.0	1.857332	10	1	9	90	6.2816	

Table 4.1.14. The LC_{50} values of Lead exposed to *H. fossilis* for 24, 48, 72 and 96 h in Continuous flow through system

S.No	Exposure period	Conc. in ppm	Log Conc.	No .of fish exposed	No. of fish alive	No. of fish dead	Percent Mortality	Probit Mortality	LC_{50}
1	24 h	66.0	1.819544	10	9	1	10	3.7184	76.35705
2		69.0	1.838849	10	8	2	20	4.1584	
3		72.0	1.857332	10	7	3	30	4.4756	
4		75.0	1.875061	10	6	4	40	4.7467	
5		78.0	1.892095	10	4	6	60	5.2533	
6	48 h	62.0	1.792392	10	9	1	10	3.7184	69.32508
7		65.0	1.812913	10	8	2	20	4.1584	
8		68.0	1.832509	10	6	4	40	4.7467	
9		71.0	1.851258	10	4	6	60	5.2533	
10		74.0	1.869232	10	3	7	80	5.8416	
11	72 h	58.0	1.763428	10	8	2	20	4.1584	62.43528
12		61.0	1.78533	10	6	4	40	4.7467	
13		64.0	1.80618	10	4	6	60	5.2533	
14		67.0	1.826075	10	3	7	80	5.8416	
15		70.0	1.845098	10	2	8	90	6.2816	
16	96 h	54.0	1.732394	10	8	2	20	4.1584	58.42679
17		57.0	1.755875	10	6	4	40	4.7467	
18		60.0	1.778151	10	4	6	60	5.2533	
19		63.0	1.799341	10	2	8	80	5.8416	
20		66.0	1.819544	10	1	9	90	6.2816	

Table 4.1.23. 95% confidence Levels of Cadmium exposed to H. *fossilis for* 24, 48, 72 and 96 h in static and continuous flow through methods

S.No	Exposure period	95% Confidence levels			
		Static Method		Continuous flow-through Method	
		Lower	Upper	Lower	Upper
1	24 h	72.11027	83.88973	66.11027	77.88973
2	48 h	68.11027	79.88973	62.11027	73.88973
3	72 h	64.11027	75.88973	58.11027	69.88973
4	96 h	61.08835	71.71165	54.11027	65.88973

Behavioural Studies (Gill and Tissue): Various sub-lethal concentrations of cadmium and lead shown adverse effects on behaviour of fish. In the both cases of stagnant and continuous flow culture. Observable dysfunctions were found which includes sluggish movement due to this condition fish slowly moved to the bottom of the aquarium. Swim independently and trying to jump out of the water in the 7, 14 and 21 day. This kind of behaviour is taking as index to measure the physiological and biochemical alterations of an organism. Inability to take feed or reduced quantity consumption by the animal, position and defend nature was altered ((Prashanth *et al.*, 2011)). In the second day onwards animal secretes mucous all over the body, more in gill region. Physiological responses were measured based on the moderate and mild gulping of air and moderate opercular movement was observed. The animal, *H. fossilis* showed various behavioral changes at different cadmium and lead concentrations. The type, rate and duration of the behavioral changes increased with increase in concentrations. Animal is Hyperactive and attempted to escape from the tank during the first hours of all treatments. The behavioural disorders included loss of balance, respiratory difficulty, slowness of motion; frequent surfacing activity and increased mucus secretion were observed after 48 h of exposure. These toxic effects increased as the dose increased. After 72 h of exposure in higher concentration, the secretion of mucus increased and the fish turns upside down in the water, became motionless, sideways swimming and loss of balance were observed concurrent with the reports of Puvaneswari and Karuppasamy (2007), Asim Ullah et al., (2016). The anal fin, the anus and the area around the eyes were bloody. The fish behaviour in laboratory can be a sensate marker of toxicant-induced stress (Smita Srivastava *et al*., 2007). The target organ most frequently involved in systemic toxicity is the CNS (brain and spinal cord) (Klaassen, 2008), resulting in loss of coordination and locomotion, instability followed by hyper excitability, tremors and convulsions (Wouters and Vanden Brecken, 1978).

Table 4.2.1.Behavioural responses of *H. fossilis* exposed to various sub-lethal concentrations of Cadmium at different periods of exposure

Exposure Periods	Sub-lethal conc.	Behavioural Responses					
		Surface visit	Jumping	Fast swimming	Mucous secretion	Air gulping	Opercular movement
7th Day	Control	++	++	++	-	++	++
	A	++	++	++	-	++	++
	B	++	++	++	-	++	++
	C	++	++	++	-	++	++
14th Day	Control	++	++	++	-	++	++
	A	+	+	-	+	+	++
	B	++	+	+	-	++	++
	C	++	++	++	-	++	++
21st Day	Control	++	++	++	-	++	++
	A	+	-	-	+	+	++
	B	+	-	-	+	+	++
	C	++	+	+	-	++	++

A = Sub-lethal conc. (2.068 ppm)**; B** = 50% SL of A (1.034 ppm)**; C** = 50% SL of B (0.517 ppm) **Notes:** - none, + mild and ++ moderate.

Table 4.2.2. Behavioural responses of *H. fossilis* exposed to various sub-lethal concentrations of Lead at different periods of exposure

Exposure Periods	Sub-lethal conc.	Behavioural Responses					
		Surface visit	Jumping	Fast swimming	Mucous secretion	Air gulping	Opercular movement
7th Day	Control	++	++	++	-	++	++
	A	++	++	++	-	++	++
	B	++	++	++	-	++	++
	C	++	++	++	-	++	++
14th Day	Control	++	++	++	-	++	++
	A	+	+	-	+	+	++
	B	++	+	+	-	++	++
	C	++	++	++	-	++	++
21st Day	Control	++	++	++	-	++	++
	A	+	-	-	+	+	++
	B	+	+	+	+	++	++
	C	++	+	+	-	++	++

A = Sub-lethal conc. (6.50 ppm)**; B** = 50% SL of A (3.25 ppm)**; C** = 50% SL of B (1.625 ppm)**Notes:** - none, + mild and ++ moderate.

Haematological Studies: Haematogram was carried out to determine the erythrocytes (RBC), leucocytes (WBC) and thrombocytes. Total 82 animals belong to the H. *Fossilis* were used for this study. The animal weight was approximately 54±4g. RBC was immature at low temperature count of the RBC found to be 3.26±0.63 millions/mm^3 for males and 3.24±0.76 millions/mm^3 for females and leucocytes (WBC) were found to be 3.74±2.25 millions/mm^3 for males and 3.76±2.16 millions /mm^3 for females. Hemoglobin (Hb) 10.52±1.62 g/dl in males and 10.66 ±1.72 g/dl in females were predicted, during the breeding season and the minimum when the gonads were immature. All blood cells were found highest value where gonads were maturing at highest temperature in summer months. Variations found in the haematological studies

depend on the body weight, physiological condition of the animal, temperature and season. Haematological parameters more quickly reflect the health status of fish than any other commonly measured parameters (Arun Thomas *et al.*, 2017; Atkinson and Judd, 1978).

Table 4.3.1. Haematological profile of *H. fossilis* (Male and Female)

	RBC (millions)	Hb (g/dl)	PCV (%)	WBC (millions)	MCV (μm^3)	MCH (pg)	MCHC (%)
Male (N=42)							
Mean±SD	3.26±0.63	10.52±1.62	39.16±3.28	3.74±2.25	120.12±2.57	32.26±2.25	26.86±4.93
Range	2.52 – 4.36	7.12 – 14.98	32.12 – 46.56	3.56 -3.86	106.78- 127.46	28.25 - 34.35	22.1 – 32.17
Female (N=40)							
Mean±SD	3.24±0.76	10.66 ±1.72	39.34±2.16	3.76±2.16	121.41±2.26	32.90±2.66	27.08±3.98
Range	2.38 – 4.22	7.16 – 15.26	32.14 – 48.12	3.58 – 3.85	114.02 – 135.04	30.08 – 36.16	22.27 – 31.71

Haematologically significant changes were seen when subjected with various concentrations of Cadmium and Lead exposure of *H. fossilis* for 7, 14 and 21 days has shown significant decrease in haematocrit and haemoglobin concentration, Red blood cell counts with increased count of white blood cell (Table 4.3.2). Where decline stat of MCHC found when compared to control. But opposite levels of increment in the case of both MCV and MCH values respectively. . Increase in erythrocyte number during spawning season has been reported by Ezzat *et al*. (1973); Yazan et al., (2014) for *Tilapia zilli* and Fourie and Hattingh (1976) for carp. More number of erythrocytes is needed for the high energy demands associated with gonadial maturation. Cameron (1970) has shown that changes in RBC counts in pin fish are of some importance in meeting seasonal increase in respiratory demands (Srivastava and Sanjeev Choudhary (2011)).

Table 4.3.2. Cadmium induced changes in Hematological parameters of *H. Fossilis*

Treatments	Exposure period	Experiment						
		RBC (millions)	Hb (g/dl)	PCV (%)	WBC (millions)	MCV (μm^3)	MCH (pg)	MCHC (%)
Control	7th Day	3.10±0.112	10.52±0.16	38.94±1.16	3.72±0.160	119.80±3.26	33.93±1.22	27.01±0.34
	14th Day	3.11±0.342	10.64±0.68	38.98±1.18	3.78±0.226	121.15±4.12	34.21±1.02	27.29±0.64
	21th Day	3.11±0.645	10.72±0.12	38.98±0.96	3.78±0.468	121.92±2.62	34.46±1.46	27.50±0.46
A	7th Day	2.08±0.342	8.28±0.34	37.18±0.84	3.98±0.234	199.42±2.88	39.80±1.20	22.27±0.48
	14th Day	1.66±0.112	7.02±0.48	35.06±0.82	4.09±0.146	260.60±3.12	42.28±1.16	20.02±0.74
	21th Day	1.22±0.126	5.72±0.34	32.88±0.68	4.24±0.248	367.86±3.78	46.88±1.28	17.39±0.66
B	7th Day	2.42±0.246	9.64±0.28	38.18±0.66	3.90±0.106	164.38±4.12	39.83±0.86	25.27±0.78
	14th Day	1.98±0.242	8.16±0.46	36.68±0.96	3.98±0.426	210.50±3.46	41.21±0.96	22.24±0.84
	21th Day	1.78±0.422	7.20±0.68	35.26±0.88	4.08±0.346	240.78±2.86	42.69±1.08	20.41±0.56
C	7th Day	2.76±0.116	9.82±0.24	38.85±0.86	3.82±0.420	140.76±2.68	35.57±1.28	25.27±0.86
	14th Day	2.48±0.112	9.10±0.26	37.86±0.68	3.88±0.268	160.72±2.78	36.69±1.02	24.03±0.66
	21th Day	2.04±0.246	8.46±0.42	36.24±0.84	3.96±0.262	202.15±3.26	41.47±0.98	23.34±0.82

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test)
A = Sub-lethal conc. (2.068 ppm)**; B** = 50% SL of A (1.034 ppm)**; C** = 50% SL of B (0.517 ppm)

Table 4.3.10. Lead induced changes in Haematological parameters of *H. fossilis*

Treatments	Exposure period	Experiment						
		RBC (millions)	Hb (g/dl)	PCV (%)	WBC (millions)	MCV (μm^3)	MCH (g)	MCHC (%)
Control	7th Day	3.10±0.112	10.52±0.16	38.94±1.16	3.72±0.160	119.81±1.88	33.94±0.46	27.01±0.34
	14th Day	3.11±0.342	10.64±0.68	38.98±1.18	3.78±0.226	121.16±2.12	34.21±0.86	27.29±0.64
	21th Day	3.11±0.645	10.72±0.12	38.98±0.96	3.78±0.468	121.93±2.22	34.47±0.78	27.50±0.46
A	7th Day	2.16±0.348	8.78±0.14	37.88±0.84	3.94±0.342	185.06±2.08	40.50±0.82	23.17±0.46
	14th Day	1.78±0.124	7.32±0.82	35.86±0.82	4.05±0.416	235.28±2.26	41.12±0.68	20.41±0.36
	21th Day	1.29±0.142	5.98±0.42	33.68±0.68	4.18±0.242	340.16±2.32	46.36±0.62	17.75±0.28
B	7th Day	2.56±0.222	9.84±0.18	38.68±0.66	3.86±0.222	152.27±1.68	38.44±0.78	25.24±0.52
	14th Day	2.03±0.242	8.66±0.62	36.98±0.96	3.94±0.260	201.38±1.98	42.66±0.88	23.95±0.46
	21th Day	1.84±0.226	7.52±0.68	35.86±0.88	4.04±0.412	228.04±2.12	43.40±0.58	20.97±0.54
C	7th Day	2.82±0.142	10.08±0.40	38.80±0.86	3.80±0.280	137.06±1.86	35.74±0.82	25.98±0.46
	14th Day	2.56±0.136	9.58±0.16	37.92±0.68	3.86±0.408	154.14±1.68	37.42±0.38	25.26±0.44
	21th Day	2.12±0.264	8.86±0.24	36.48 ±0.84	3.94±0.320	192.17±1.22	41.79±0.88	24.28±0.64

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test)
A = Sub-lethal conc. (6.50 ppm)**; B** = 50% SL of A (3.25 ppm)**; C** = 50% SL of B (1.625 ppm).

Conclusion: The present study implies that the cadmium and Lead are at increased dosage subjected, toxicities, initiation of inflammation and redness. Prolonged exposure causes acute physiological and anatomical changes could result. Henceforth the present investigation depicts that with accordance of pollution rate and toxicity, enhanced in the fish. So that degree of harmfulness increased day bay day leads to cause many alternations by consuming such kind of food regularly, to avoid the toxicity of the above said metal free or required quantity aquarium or ecological cultivation must be maintained and recommended to practise to give or supply the vital with good protienacious food.

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References:

1. Elisabet Lindgren, Francesca Harris, Alan D. Dangour, Alexandros Gasparatos, Michikazu Hiramatsu, Firouzeh Javadi, Brent Loken, Takahiro Murakami, Pauline Scheelbeek & Andy Haines (2018). Sustainable food systems—a health perspective, Sustainability Science Vol (13):1505–1517.
2. Gandhewar S. S. and Zade S. B. (2019).Bioaccumulation Of Some Heavy Metals In The Fish,Clarias Batrachus (Linn.) RJLBPCS.
3. Golovanova, Irina. (2008). Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. Inland Water Biology. Vol(1): 93-101.
4. Hadeel M Huseen and Ahmed J Mohammed (2019). Heavy Metals Causing Toxicity J. Phys.: Conf. Ser. 1294.
5. Mehjbeen Javed1 and Nazura Usmani (2019). An Overview of the Adverse Effects of Heavy Metal Contamination on Fish Health, Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. Vol 89(2):389–403.
6. Michael A Clark, Marco Springmann, Jason Hill, and David Tilman, (2019). Multiple health and environmental impacts of foods, Vol 116 (46) 23357-23362.
7. Nitasha Khatri and Sanjiv Tyagi (2015). Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas, Publication Cover, Frontiers in Life Science, Vol 8(1):23-39.
8. Paul B Tchounwou, Clement G Yedjou, Anita K Patlolla, and Dwayne J Sutton (2014). Heavy Metals Toxicity and the Environment, EXS; Vol 101: 133–164

9. Rai, Prabhat. (2008). Heavy Metal Pollution in Aquatic Ecosystems and Its Phytoremediation Using Wetland Plants: An Ecosustainable Approach. International journal of phytoremediation. Vol 10: 131-58.
10. Richard M. LoPachin, Brian C. Geohagen, B.S., and Lars U Nordstroem (2019). Mechanisms of Soft and Hard Electrophile Toxicities, Toxicology.Vol 418: 62–69.
11. Sanaa Abdulaziz Mustafa (2020). Histopathology and heavy metal bioaccumulation in some tissues of *Luciobarbus xanthopterus* collected from Tigris River of Baghdad, Iraq, Egyptian Journal of Aquatic Research journal homepage, Vol 46, 123-129.
12. Tembo Rostern N (2017) The Effects of Some Metals in Acidified Waters on Aquatic Organisms, Fish & Ocean Opj 4(4).
13. Ting Liu, Xue Liang, Chao Lei, Qinhong Huang, Weiqi Song, Rong Fang, Chen Li, Xiaomei Li, Hui Mo, Ning Sun, Haoran Lv and Zhihua Liu (2020).High-Fat Diet Affects Heavy Metal Accumulation and Toxicity to Mice Liver and Kidney Probably via Gut Microbiota, Front. Microbiol.
14. Vhahangwele Masindi and Khathutshelo L. Muedi (2018). Environmental Contamination by Heavy Metals, intechopen.
15. Arun Thomas, E.T. Bhagya, S. and Abdul Majeed, (2017). Clinical Utility of Blood Cell Histogram Interpretation, J Clin Diagn Res; Vol 11(9): OE01–OE04.
16. Asim Ullah, Hameed Ur Rehman, Waqar Saeed, Muhammad Faheem Quraish, Zakir Ullah, Haroon, Riaz Ur Rehman, Sajid Awais, Raqeebullah, Waqar Ahmad and Kausar Saeed (2016). Determination of 96-hr LC50 value of cadmium for a fish, Labeo rohita, Journal of Entomology and Zoology Studies; Vol 4(5): 380-382.
17. Srivastava S. and Sanjeev K. Choudhary (2011). Haematological and Biochemical Reference Intervals for *Heteropneustes fossilis,* Asian Fisheries Science 24 (2011):314-320.
18. Mahnaz Sadat Sadeghi and Sadegh Peery(2018). Evaluation of toxicity and lethal concentration (LC50) of silver and selenium nanoparticle in different life stages of the fish Tenualosa ilish (Hamilton 1822); Oceanography Fish Open Access J, Vol 7(5).
19. Yazan M. Alomari, Siti Norul Huda Sheikh Abdullah, Raja Zaharatul Azma, and Khairuddin Omar (2014). Automatic Detection and Quantification of WBCs and RBCs Using Iterative Structured Circle Detection Algorithm, Vol 2014:1-17.