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RESEARCH ARTICLE

ALTERATION IN HAEMATOLOGICAL INDICES OF *HETEROPNEUSTIS FOSSILIS* UNDER STRESS OF HEAVY METALS POLLUTION IN THE KALI RIVER, UTTAR PRADESH, INDIA

Malik D. S., *Pradip Kumar Maurya and Hemant Kumar

Department of Zoology and Environmental Science, Gurukul Kangri University, Haridwar, Uttarakhand, India

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ABSTRACT

The present investigations confirm that stress due to various pollution specially heavy metals in fish *H. fossilis,* collected from Kali river. Haematological (Hb, RBC, WBC, MCV, MCH, MCHC, platelets count, HCt, neutrophile, lymphocyte, eoinophil, and monocryte), distervence does create physiological stress like, swimming imbalance, breeding, sexual maturity (Haemolysis) and leucocytosis in fish population affecting the immune system and making the fish vulnerable to stress. The results of correlation analysis of haematological indices show highly, medium, positive and negative correlation. Concentration of heavy metals (manganese (Mn), cadmium (Cd), chromium (Cr), lead (Pb), Cupper (Cu)) was determined in the gills, liver and muscles of *Heteropneustis fossilis* from Kali river located at Muzaffarnagar. The pattern of metal accumulation in studied selected organs was in the order: Mn> Cu> Cr> Cd> and Pb. All the metals concentration in the liver showed greater but Cu and Cr was maximum compare than other metals.

INTRODUCTION

Water pollution from man-made sources can easily create local conditions of elevated metal presence, which could lead to disastrous effects on animals and humans (Naja and Volesky 2009). Heavy metals are one of the important pollutants in river ecosystem. Some of the metals for which no biological function has been identified are considered as xenobiotic (like Cd, Cr, Co and Pb), others are essential for growth and development like Cu, Zn, and Mn (Sigel *et al*., 2009). Anthropogenic activities caused an increased discharge of various concentrations of both essential and nonessential metals into aquatic ecosystems (Mekkawy *et al*., 2011). Chromium (Cr) is a metallic element which is introduced by the Environmental Protection Agency as one of the 129 priority pollutants (Keith and Telliard 1979). Chromium, an important environmental pollutant, is present in the aquatic environment as a result of plating and electroplating factories, textile manufacturing facilities, steel producing factories, rinse waters, leather tanneries, dyeing, sanitary land fill leaching, the combustion of coal and oil, welding, bricks of furnaces, and wood-preserving industry (anthropogenic sources) (Palanippan and Karthikeyan 2009; Monterio *et al*. 2002; European Chemicals Bureau ECB 2005; ATSDR 2013).

****Corresponding author: Pradip Kumar Maurya***
Department of Zoology and Environmental Science, Gurukul KangriUniversity, Haridwar, Uttarakhand, India.

Aquatic pollution undoubtedly has direct effects on fish health and survival. Heavy metals are regarded as serious pollutants of the aquatic environment because of their persistence and tendency to be concentrated in aquatic organisms (Veena *et al*, 1997). Most heavy metals released into the environment by various anthropogenic processes, atmospheric deposition and erosion due to rainwater (Kalay and Canli, 2000). Fish are useful bioindicator to evidence environmental degradation (Fausch *et al*., 1990). Fish haematology provides an important tool in the evaluation of its physiological status, reflecting the relative health of the aquatic ecosystem. Therefore, it is necessary to know the normal range of the blood parameters and their changing variation as per existing aquatic ecosystem to use them as biomarkers (Luskova, 1995). The haematological parameters in fish may be influenced by constitutional factors such as sex, reproductive stage, age, size and health (Joshi, 1982; Ranzani paiva and Godinho, 1985; Halavova, 1993; Luskova *et al*., 1995; Nespolo and Rosenmmann, 2002). They are also affected by external factors like seasonal variation of water quality, Water temperature, environmental quality, food, stress, etc. (Mahajan and Dheer, 1979; Tisa *et al*., 1983; Rios *et al*., 2002). Fish food characteristics change in response to environmental condition. Thus the variation of haematological features could serve as a biomarker of sub lethal environmental stress. Haematological values have been widely used as indices for determining the health status of fish and as diagnostic tools for diagnosing diseases and stress induced conditions in fish. The study of fish haematology has contributed significantly to our understanding of the comparative physiology, phylogenetic relationships, habit, habitat, food selection, and other significant ecological parameters of fishes (Bhaskar and Rao 1990; Ayotunde´ 1998; Ayotunde and Ochang 2004, 2006). The use of haematological indices in commercial fish cultivation may enhance production by facilitating the early detection of situations of stress and/or diseases (Rehulka *et al*., 2004; Tavares-Dias and Barcellos 2005). A number of haematological indices are used to assess the functional status and oxygen-carrying capacity of the blood stream (Shah and Attendag 2005). The haemoglobin (Hb) content and erythrocyte count are directly related to environmental factors such as temperature and salinity (Graham 1997), while immune system parameters are used to assess any alterations in the defence mechanism of fish (Jones 2001). As such, an analysis of the normal haematological profile of a fish enables an expert to identify external and internal stress conditions and to predict the chances of disease. The changes that occur in the blood characteristics of fishes with changing environmental variables are species-specific. The haematological make-up of fish is known to be affected by environmental factors, with the variations also significantly influenced by physical factors, including nutritional status, feeding conditions, season, spawning, migration, age, sex, genetic variation, activity of species, environmental stress conditions, among others. Environmental variables exert a profound effect on the survival and oxidative metabolism of fish and increase plasma volume, resulting in a decrease in haematocrit concentration.

Any deterioration in the water quality caused by toxicants may alter physiology of the fish which ultimately get reflected in their haematological parameters. Hence haematological profiles of fishes are widely being used to monitor the aquatic pollution.

Among the haematological parameter, total erythrocytes count (TLC), haemoglobin (Hb), haematocrit (Ht), total leucocytes count (TLC), and other haematological indices like mean corpuscular haemoglobin (MCH), mean corpuscular haematoglobin concentration (MCHC), and mean corpuscular volume (MCV) are being used to assess the health status of the affected fishes (Nussey *et al*., 1995).

MATERIALS AND METHODS

Study area

Kali River that flow in western Uttar Pradesh (India) is a small perennial river having a basin area of about 150 km^2, and lie between latitude 29^{0}23' - 29^{0}21' N and longitude 77^{0}43' - 77^{0}39' E in the Muzaffarnagar district. Fish were taken randomly from Dhobighat at Muzaffarnagar (Fig. 1) seasonally in the year 2013 to 2014. The river kali is receiving several types of untreated municipal, industrial and agriculture wastes from different point and non-point sources.

Sample collection

Fish species (*Heteropneustis fossilis*) were collected from the Kali river from Muzaffarnagar near dhobighat. This site has been highly polluted by industrial and agriculture wastes (Malik and Maurya, 2015). Approximately five fish with a body weight varying from 200 to 700 gram were collected randomly near dhobighat with upstream to downstream. The fish are collected by fishing net and transported to the laboratory within 4 h after capture. They were immediately transported in laboratory for anesthetized using MS-222 at 100 mg/l. The fish was washed with tap water and dried using blotting paper before collecting the blood sample.

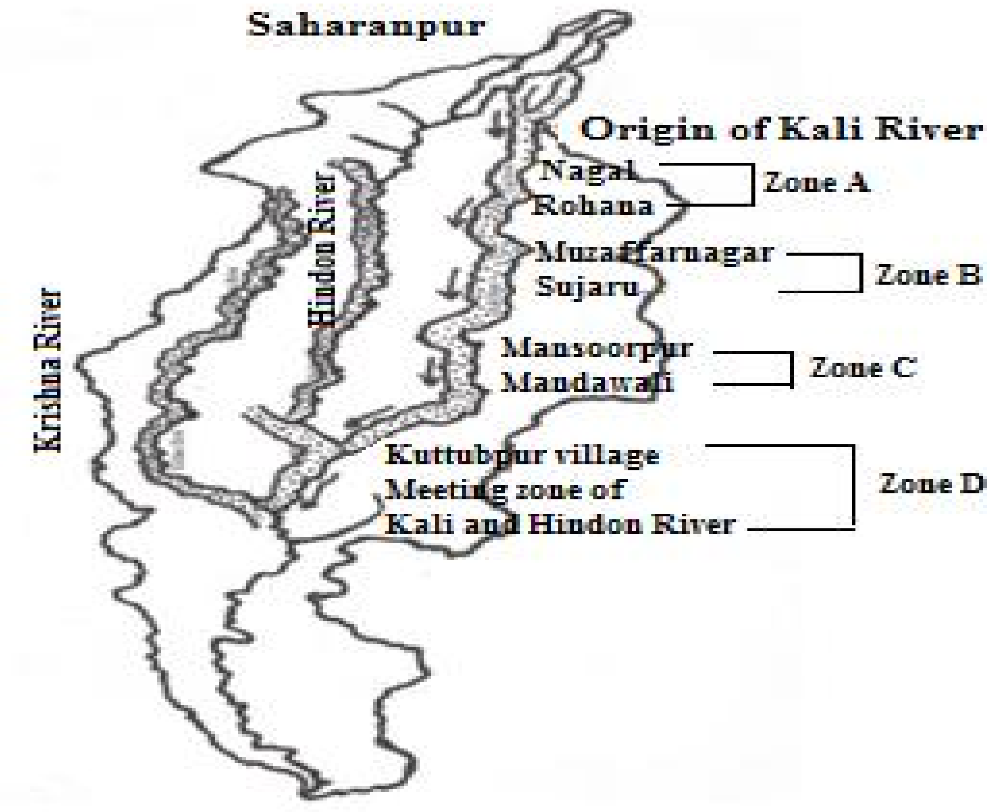

Fig.1. Map of Kali river showing the sampling zones

Collection of blood

Blood of fish was collected from the cardiac puncture using 3-cm3 syringes region by puncturing the heart using a plastic disposable syringe fitted with a needle, moisturised with heparin. The Blood was obtained by cardiac puncture and 18 gauge hypodermic needles and immediately transferred to 2-ml glass vials containing 1% EDTA solution. Haematological parameters for analysis like, red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), and Hb. The methods employed for determination of various haematological parameters were RBC and WBC, counted by Neubauer's improved haemocytometers using Hayem's and Tuerk's solution as a diluting fluid, respectively (Samue, 1986), the packed cell volume (PCV) or hematocrit values (measured by Wintrobe's method), and Hb (treated by N/10 HCl and the colour of the acid haematin was matched with given standards using Sahli's hemoglobinometer). Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were calculated by the following standard formulae (Dacie and Lewis, 1991).

RESULTS AND DISCUSSION

Metal accumulation in fish organs provides evidences of exposure to contaminated aquatic environment (Kotze 1999). Fish organs show significant variation in metals accumulation, which is related to differences pathway like, absorption, storage, regulation, and excretion abilities of studies fish species (Kotze 1999). In the present study indicated that the concentrations of heavy metals in the summer season were found greater, compare than other season like winter and monsoon while the volume of water is reduced for the evaporation shown in (Table 1). The current data indicated that the concentration of heavy metals in bottom dwelling fish *H. fossilis* liver was high especially Cr (1.93µg/kg), Cd (0.0.62µg/kg) and Cu (2.90 µg/kg) shown in (Table 1 & Fig. 3), compare than gills and muscles, these concentration was caused by fish mortality, growth retardation and destroys gills epithelium causing hypoxia. Metals get to fish organs by different path ways as directly from water by gills and skin or by alimentary tract (with food).

Table 1. Concentration of heavy metals in the fish (*Heteropneustis fossilis*) organs

S.No	Season	Fish Organs	Heavy Metals (µg/kg) in dry weight				
			Cd	Cr	Pb	Cu	Mn
1	Summer	Gills	0.60	1.87	0.04	2.41	3.21
		Liver	0.62	1.93	0.08	2.90	3.39
		Muscles	0.53	1.37	BDL	1.67	3.28
		Mean±sd	0.58±0.47	1.72±0.30	0.06±0.02	2.32±0.61	3.29±0.09
2	Winter	Gills	0.50	1.62	BDL	2.90	3.05
		Liver	0.45	1.72	0.04	2.92	2.81
		Muscles	0.54	0.90	BDL	1.39	2.70
		Mean±sd	0.49±0.04	1.41±0.44	0.04±0.0	2.40±0.87	2.85±0.17
3	Monsoon	Gills	0.50	1.29	BDL	1.81	2.27
		Liver	0.38	1.32	0.05	2.70	3.40
		Muscles	0.48	1.21	BDL	1.45	2.50
		Mean±sd	0.45±0.06	1.27±0.05	0.05±0.0	1,98±0.64	2.78±0.59
4	Detection limit (µg/kg) WHO		ND	0.15	0.2	3.0	150

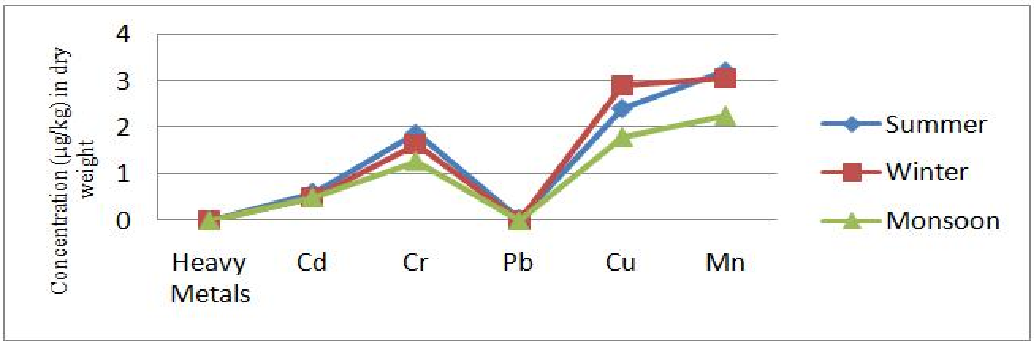

Fig. 2. Seasonal variation of heavy metals in the gills of *H. fossilis*

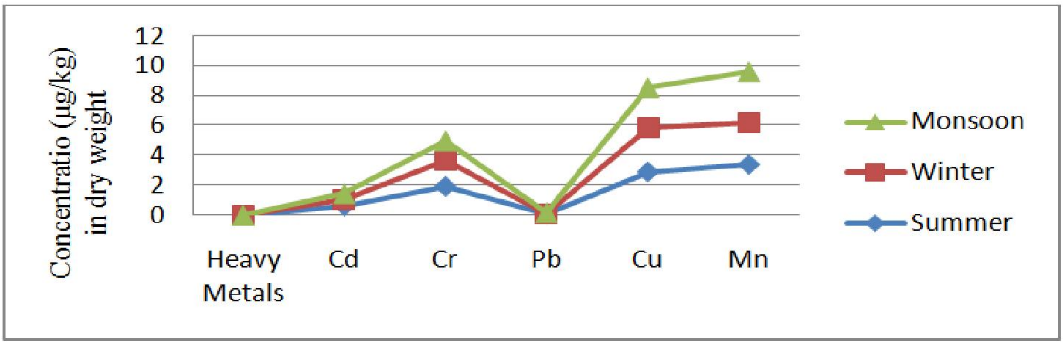

Fig. 3. Seasonal variation of heavy metals in the liver of *H. fossilis*

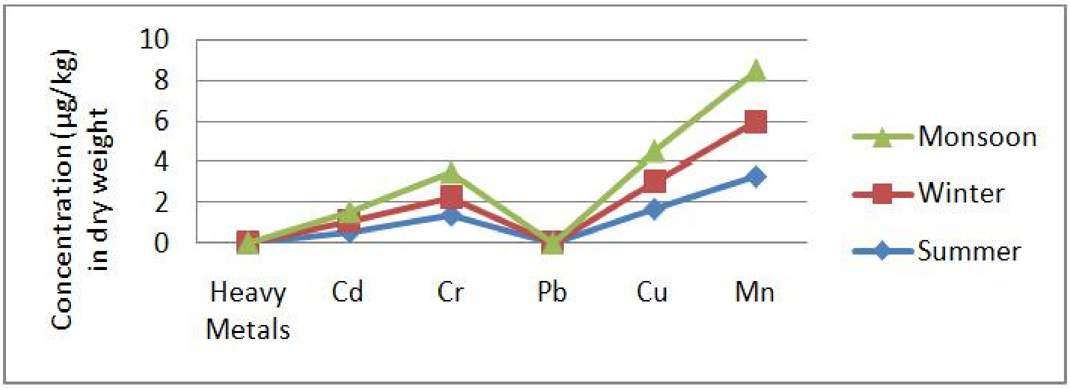

Fig. 4. Seasonal variation of heavy metals in the muscles of fish *H. fossilis*

Table 2. Haematological indices in the fish (*Heteropneustis fossilis*)

S.N	Blood Indices	Unit	Summer	Monsoon	Winter	Mean±sd
1	Hb	Gm/dl	8.8	3.9	8.9	7.2±2.33
2	RBC	Millions/cmm	1.72	0.78	1.82	1.44±0.57
3	WBC	g/cumm	11330	130000	229500	123610±109225.3
4	MCV	fL	153.3	116.7	133.5	134.5±18.32
5	MCH	Pg	51.2	50.0	48.9	50.03±1.1503
6	MCHC	g/DL	33.4	42.9	36.6	37.63±4.83
7	Platelets count	x 10^3/µL	3	103	6	37.33±56.88
8	HCt	Millions/cmm	26.4	9.1	24.3	19.93±9.44
9	Neutrophile	%	18	2	1	7±9.53
10	Lymphocyte	%	8	96	98	67.33±51.39
11	Eoinophil	%	73	3	1	25.33±41.28
12	Monocryte	%	1	0	0	0.33±0.57

Table 3. Correlation analysis of haematological parameters by season in *Heteropneustis fossilis*

Parameters	Hb	RBC	WBC	MCV	MCH	MCHC	Platelets count	HCt	Neutrophil	Lymphocyte	Eoinophil	Monocryte
Hb	1											
RBC	0.997	1										
WBC	-0.033	0.0365	1									
MCV	0.831	0.791	-0.582	1								
MCH	0.007	0.791	-0.999	0.561	1							
MCHC	-0.937	-0.911	0.378	-0.972	-0.354	1						
Plateletes count	-0.999	-0.993	0.076	-0.855	-0.051	0.952	1					
HCt	0.991	0.980	-0.161	0.896	0.136	-0.974	-0.996	1				
Neutrophil	0.438	0.374	-0.912	0.863	0.902	-0.723	-0.477	0.550	1			
Lymphocyte	-0.467	-0.404	0.898	-0.879	-0.887	0.7456	0.505	-0.577	-0.999	1		
Eoinophil	0.474	0.411	-0.895	0.883	0.884	-0.750	-0.512	0.583	0.999	-0.999	1	
Monocryte	0.484	0.422	-0.890	0.888	0.878	0.952	-0.522	0.593	0.998	-0.999	0.999	1

Most of heavy metals absorbed by fish organs, transported within the body through blood (Pelgrom *et al*. 1995; Akahori *et al*., 1999; Bonda *et al*., 2007). The largest quantities of cadmium and copper are accumulated in metabolically active tissues (e.g., liver, gills, alimentary tract, spleen), where they are bound to metallothioneins (Kito *et al*. 1982; Castano *et al*., 1998; Hermesz *et al*., 2001; Calta and Canpolat 2006; Rose *et al*., 2006; Panchanathan and Vattapparumbil 2006; Wu *et al*., 2007; Asagba *et al*., 2008; Dang *et al*., 2009; Kovarova *et al*., 2009). The obtained results show that initial concentrations of copper in fish tissues were higher than the levels of cadmium, chromium and lead (Table 1). According to various authors, cadmium concentration in freshwater fish tissues (intestine, muscles, kidney, gill, skin, spleen, brain, liver) usually does not exceed 2.90 mg/g (Kraal *et al*. 1995; Hollis *et al*., 1999; Panchanathan and Vattapparumbil 2006; Wu *et al*., 2007; Dang *et al*., 2009). Higher natural level of copper in fish in comparison with cadmium obviously results from the fact that Cu is a microelement and thus the natural component of body involved in metabolic processes, while cadmium is a xenobiotic (Ghedira *et al*., 2010). According to Kraemer, (2005). Copper and cadmium first show affinity to gill which is main uptake site of waterborne elements, and then they are transported via blood to liver and kidney. Metal ions usually accumulated less in gills since they are a temporary target organ of accumulation, and then Cd is transferred to other organs (Wu *et al*., 2007). Various authors (e.g., Kraal *et al*., 1995; Jacobson and Reimschuessel 1998; McGeer *et al*., 2000; Celechovska *et al*., 2007; Ghedira *et al*. 2010; Radhakrishnan 2010; Shao *et al*., 2010) showed that the highest concentrations of copper were noted in fish liver. Liver being main detoxification site in organism is also an organ that bioaccumulates toxic substances, and thus, it usually shows higher concentrations of metals than another tissues (Allen 1995; Olowoyo *et al*., 2011). Large fraction of copper, cadmium, chromium and lead absorbed by fish organism is transported within the body by blood plasma bound to albumin, histidine, threonine and glutathione (Bettger *et al*., 1987;

Pelgrom *et al*., 1995) and then is deposited in liver. This gland plays essential role in copper metabolism. In liver, copper is attached to ceruloplasmin. Also metallothioneins (MT) play important role in binding this metal in vertebrates. They protect against toxic action of metals by reducing the concentration of free metal ions to physiological values in the tissues (Roesijadi *et al*., 1996). After absorption, ions of toxic cadmium in circulating blood are mainly absorbed by erythrocytes (they bind to proteins of cells membrane or to hemoglobin). Only a small quantity of Cd in blood is transported bound to albumin, cysteine or glutathione (Bonda *et al*., 2007). Upon entry into blood plasma, it is distributed throughout the body, with the greatest burdens in the liver and kidneys. At this time, organism activates mechanisms of detoxification (Jonsson and Part 1998). In the liver, Cd not bound to MT induces synthesis of new MT (Pelgrom *et al*., 1995; De Conto Cinier *et al*., 1997; Rose *et al*. 2006; Huang *et al*., 2007; Wu *et al*., 2007; Dang *et al*., 2009; Kovarova *et al*. 2009; Bozhkov *et al*., 2010). Riggio *et al*. (2003). Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Li *et al*., 2010). In the present study, exposure of fishes to sub- lethal concentration of chromium, cadmium and cupper showed remarkable alteration in haematological and physiological stress markers.

The significant decrease in total erythrocyte count, haemoglobin concentration and haematocrite value was observed in fishes exposed to a sub-lethal concentration of Cd, Cr and Cu. Decrease in haemoglobin concentration TEC and haematocrit may be the indicator of anaemia. These criteria should receive enough attention in assessing the health of the fish with regard to aquatic pollution and has been accepted by many workers such as McCarthy *et al.* (1973) and Christensen *et al.* (1978). The present research reported shown that alterations in PCV, MCV, and MCH values due to exposure of fish to effluents from sources of pollution. While evaluating the total effect of Cd, Cr, Cu and Pb on the haematological indices of *H. fossilis*, a synergetic effect of these metals was found on the erythrocyte count, concentration of hemoglobin, and the present leucocytes (Vosyliene, 1999). Similar changes have been reported in the present study on the polluted river highly contaminated in summer season from different sources of pollution as well as heavy metal.

The correlation analysis of haematological parameters, used to determine the relationship between two or more variables. The current results of correlation between WBC and Hb, Hb and RBC, HCt and Hb, neutrophil and MCH, eoinophil and neutrophil, monocryte and neutrophil, was indicated that high positive correlation but another site MCH and WBC, MCHC and Hb, platelets and Hb, platelets and RBC, nutrophil and WBC, monocyte and lymphocyte, eoinophil and lymphocyte was indicated that high negative correlation shown in (Table 3). This study on haematological changes in fish serves as an effective tool in the diagnosis of the extent of environmental pollution and also the abiotic fish diseases. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. Decreased availability of oxygen generally causes increased synthesis of haemoglobin, release of blood cells from storage sites, and enhanced erythropoiesis. This condition is clearly evident in the initial decrease and then increase in the PCV content of our present study which could be attributed to swelling of RBCs as a result of disturbed ion regulatory mechanisms caused by the effluent (Vosyliene, 1999).

Conclusion

The present studies indicated that the concentrations of Cd, Cr, Pb, Cu, Mn in fish *H. fissilis* tissue from exposed site clearly major variation in the detection limits proposed by WHO (1993). The results indicate that the concentrations of metals are highly toxic and may pose hazard. The increase accumulation tendency of heavy metals in fish tissue indicates that the constant monitoring of this reverie system is needed before the level cross its threshold and become toxic to fish including to human. The chromium and lead show the higher concentration than other metals by the different point and non point sources of Kali river fishes (*H. fossilis*). The statistical analysis of haematological indices indicated by correlation, WBC between Hb, Hb between RBC, HCt between Hb, neutrophil between MCH, eoinophil between neutrophil, monocryte between neutrophil, was indicated that high positive correlation but another site MCH between WBC, MCHC between Hb, platelets between Hb, platelets between RBC, nutrophil between WBC, monocyte between lymphocyte, eoinophil between lymphocyte was indicated that high negative correlation. The information obtain from this study, fish could not use direct for food, for without monitoring for all river aquatic system and finally to management of human health practices.

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