Analysis of Influence of Pollution on Haematology of Fresh Water Fishes in Varahanadhi River

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Abstract:
Water is the universal solvent which is of prime importance to both plants and animals. Being a natural resource, river water is being contaminated by the industrial discharge, effluents from factories and by anthropogenic sources. Heavy metal pollution is the major factor in affecting the health of living beings. Downstream site of Varahanadhi river is polluted with heavy metals like chromium, cadmium, lead and arsenic.

Haematological parameters are considered as an indicator of physiological and pathological changes in fishes.Blood was collected from the caudal vein of fish species Rohu (*Labeorohita*) andTilapia (*Oreochromis niloticus*)collected from upstream, midstream and downstream sites of Varahanadhi river and from control.RBC, Hb, PCV, MCH, MCHC levels were found to be significantly reduced in all fish species collected from downstream site of Varahanadhi river compared to upstream, midstream and control fishes.The level of WBC was found to be slightly increased in fishes of downstream site compared to other sites and control. These altered levels of hematological parameters indicated the effect of pollutants to the life of piscine.

Key words: Rohu, Tilapia, Haemotology, Heavy metals, Varahanadhi river.
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Introduction

Water is the foremost necessity of all living beings. The components which are dissolved in water determine the quality of water for the growth of plants and animals. The requirement of adequate nutrients, pleasant and healthy environment is necessary for the growth of aquatic organisms. The productivity of the organisms present in water depends on the physicochemical characters. Water quality of Varahanadhi river was assessed in the study at different locations for a period of one year by measuring the various physico chemical parameters with the standard certified methods (Jasmin Lena and Maneemegalai, 2015).

Varahanadhi river in TamilNadu, India is relied by people for their agricultural and domestic uses. The magnitude of pollution in the upstream, midstream and downstream region of the river is assessed by the measurement of heavy metals content. The impact of heavy metals on the livelihood of water borne living beings was assessed by analysing the haematological parameters of fishes.

Hematology deals with the study of blood parameters. Aquatic organisms like fish have been used as indicator species to assess pollution in the environment.These studies are used to detect the changes in fish during stress condition upon exposure to contaminants. The present study revealed that the water from the river has been polluted by heavy metals; therefore by performing hematological studies, the effects of pollutants on aquatic organisms can be studied.

The aquatic systems physical, chemical and biological characters were greatly affected by the disposal of pollutants, as a result ecological balance is altered. The survival of plants and animals in water bodies are threatened by the release of effluents from industries. Accumulating pollutants exerts a high degree of impact and stress on aquatic living beings (Censiet al, 2006). Pollution causes a gradual decrease in aquatic flora and fauna. Wedemeyeret al, (1984) reported that the lives of fishes are entirely dependent on the aquatic environment and they are more susceptible to the stresses of pollutants. Analyses of hematological parameters are the sensitive indicators to assess the changes in fishes exposed to stressors such as pollutants and microorganisms in aquatic environment (Blaxhall and Daisely, 1973; Chekrabarty and Benerjee, 1988; Houston and Carlile, 1997). The life of fishes are affected at biochemical and molecular level by the absorption of pollutants or toxicants via food chain or through body surface and they cause quick changes in hematological parameters (Johansen et al, 1994;Rizkallaet al, 1999).

Materials and Methods

Varahanadhi River is located in parts of Villupuram District in Tamil Nadu, India. It lays 12.04˚ N latitude and 79.3˚ 4 E longitude. It covers a total area of 798 ha and covers within the survey of India, with a total catchment area of about 21 Km. Water drains into the Varahanadhi River from areas ofVillupuram, Thiruvannamalai, Kancheepuram and Cuddalore districts of TamilNadu and Pondicherry and finally confluences with the ocean Bay of Bengal. Ongur and Nallavur sub basins are the main constituents of Varahanadhi basin which was surrounded Bay of Bengal in the east, Palar basin and Nallavur sub basin in the north and Ponnaiyar basin in the south and west. The sampling point includes the upstream (US), midstream (MS) and downstream (DS) regions.Site A (upstream) which is situated in Gingee town and has its source at the hills of Melmalayanur in the South Arcot District of Tamil Nadu, site B (midstream) is the branch of the river that flows through VillupuramDistrict and site C (downstream) part of the river called Sankaraparani that drains into Bay of Bengal.

Water was collected during pre-monsoon, monsoon and post monsoon seasons. Approximately 2 litre of water sample was collected in a polythene can from a depth of 0.5 m up to the top with the mouth facing slightly upward in the direction of the current. Properly labeled container with sample were kept in an icebox and transported to the laboratory to avoid alterations in the biological and physico chemical characteristics. Samples were analyzed by the method prescribed by WHO (1999).

Heavy Metals Quantification in Water

Digestion of heavy metals in water:

Metal digestion was done using the Milestone Acid digestion method.

5 ml of water sample was pipetted into 20ml teflon tube. 6ml of nitric acid (HNO_3, 65%), 3ml of hydrochloric acid (HCl, 37%) and 0.25ml hydrogen peroxide (H_2O_2) were added to each sample. 6ml HNO3 (65%), 3ml of HCl (37%) and 0.25ml H_2O_2 served as the blank. The samples were subjected to microwave digestion for thirty minutes. The samples were allowed to cool to room temperature after digestion and the solutions were diluted to 20 ml using distilled water. The liquid extract was then used for the determination of arsenic, chromium, cadmium and lead.

Heavy metals like chromium (Cr), lead (Pb), cadmium (Cd), and arsenic (As) in acidified water samples were analyzed using Inductively coupled plasma-atomic emission spectroscopy (ICP-AES). For the determination of total heavy metals, the extraction was carried out in Teflon containers provided with screw stoppers, using strong acid mixtures, as described by Tessier et al, (1979). All strong acid mixtures were prepared just before the analysis of total heavy metal contents. All the reagents and chemicals used were of analytical reagent grade suitable for ultra-trace analysis.

Hematological parameters (Blood) of the fish acts as an indicator of physiological and pathological changes in fishes. Collection of blood and analysis was carried out by following the standard methods of WHO (1999) in fresh water fishesRohu (*Labeorohita*)and Tilapia (*Oreochromis niloticus*) authenticated by a zoologist. The fishes that were maintained in the laboratory were used as control (C). Collection site includes;upstream (US), midstream(MS) and downstream(DS) in Varahanadhi river located in Villupuram district.

Five fishes of each species were collected from the upstream, midstream and downstream site and transported in polythene bags, half filled with water, without any disturbance. About five fishes were put in each bag with water, the bags were aerated using pressurized air flow from a cylinder. This mode of transport was successful, since there was no mortality in all consignments throughout the course of this study. The samples were brought to the laboratory on the same day. Fish samples were measured and weighed (15 ± 5cm and 150-200g).

Collection of Blood

Blood was drawn directly from the caudal vein using 1ml plastic insulin syringe containing 0.2% EDTA as anticoagulant. Determination of red blood corpuscles (RBC) count (Shaperclaus, 1979), White Blood Corpuscles (WBC) Count (Bomfordet al,1975), Packed Cell Volume (PCV) or Hematocrit Value (Schalm,1975), Hemoglobin (Cyanmethaemoglobin method) was estimated by the method of Blaxhall and Daisley(1973), Mean Corpuscular Hemoglobin (MCH) (Hurkat and Mathur,1976) and Mean Corpuscular hemoglobin concentration (MCHC) (Hurkat and Mathur,1976) were carried out.

Statistics

All the experiments were conducted using at least five animals in each group and the values were expressed as mean ± SD. The student’s *t* test was used to compare the means of two groups.

Results and Discussion

Table 1 presented the heavy metals chromium, lead, cadmium and arsenic in water samples of pre monsoon, monsoon and post monsoon seasons, collected at different sites (upstream, midstream and downstream) of Varahanadhi river. A significant rise in the level of all heavy metals in downstream site was observed in all seasons. Notable variations wasalso observed in the heavy metal contents between the upstream and downstream regions of all seasons. Compared to the standard WHO values of heavy metals in water, significantly high level of heavy metals were observed in the downstream site of all seasons.

Table1: Heavy metal content analysis in the Varahanadhi river water sample Values are expressed as mean values of samples taken in triplicates.

Parameters (WHO value)	Site	Pre Monsoon	Monsoon	Post Monsoon
Chromium (0.05mg/dl)	Upstream	0.06	0.05	0.04
	Midstream	0.06	0.04	0.07
	Downstream	0.10	0.11	0.13
Lead (0.05 mg/dl)	Upstream	0.03	0.04	0.06
	Midstream	0.04	0.02	0.05
	Downstream	0.13	0.12	0.15
Cadmium (0.005 mg/l)	Upstream	0.03	0.02	0.04
	Midstream	0.06	0.05	0.05
	Downstream	0.13	0.16	0.12
Arsenic (0.05 mg/l)	Upstream	0.06	0.02	0.04
	Midstream	0.03	0.03	0.06
	Downstream	0.15	0.13	0.14

Arsenic may be found in ground water naturally and in surface water as an industrial pollutant or as a product of agricultural runoff from previously used pesticides. The high toxicity of arsenic is cumulative in the body with symptoms ranging from fatigue to coma and death.Cadmium is primarily found in surface water as a pollutant from industries such as electroplating.Potential damage from cadmium may take the form of anemia, retarded growth and increased hypertension.Lead is normally found in surface water from industrial pollution, but some groundwater naturally contains elevated levels. The symptoms range from gastrointestinal disturbances to inflammation of the brain and spinal cord. Brain damage is common among children exposed to high levels of lead. Chromium may be found in ground water as a natural occurrence and in surface water as an industrial pollutant commonly from the plating industry. Large amount is consumed to manufacture pigments for use in paints and inks. Other applications include leather tanning, metal corrosion inhibition, drilling muds, textile dyes, catalysts, wood and water treatment. Chromium can be toxic to humans and produce skin irritations when externally exposed. Liver and kidney damage may result from internal exposure.

Their accumulation and distribution in soil, water and environment are increasing at an alarming rate causing deposition and sedimentation in water reservoirs and affects aquatic organisms as well (Hobbelenet al, 2004; Okafor and Opuene, 2007;Mohiuddinet al, 2010). Heavy metals like chromium,lead, cadmium, arsenic, etc. exhibit extreme toxicity even at trace levels. Rivers are a dominant pathway for metaltransportand heavy metals become significant pollutants of many riverine systems. The behavior of metals in natural waters is a function of the substrate sediment composition, the suspended sediment composition, and the water chemistry.

During their transport, the heavy metals undergo numerous changes in their speciation due to dissolution, precipitation, absorption and complexation phenomena (Abdel- Ghani and Elchaghaby, 2007) which affect their behavior and bioavailability (Nouri et al, 2011). Hence, heavy metals are sensitive indicators for monitoring changes in the water environment. Geochemical distribution results have also been used as an aid in predicting potential contaminant mobility and bioavailability (Pueyoet al, 2003).

The chromium concentration was much higher than the standard level for drinking water (0.05 µg/ml) proposed through Environment conservation rules (ECR, 1997). However, seasonal industrial discharges may also have direct effect on these variations, as some metals in water are higher in summer and some others in winter.

Considering the Toxicity reference values (TRV), almost all the heavy metals greatly exceeds the limit for safe fresh water and for Cr, Pb, Cd and Cu the values exceed ~100 times of TRV. Heavy metals like Cd, Pb, Cr and Zn were extremely enrichedin the Buriganga river sediments. The condition factor changes may reflect fairly and faithfully the changes in body protein and lipid content (Weathereyand Gill, 1983). The heavy metal overload of such fish can cause severe health problems in man especially kidney and liver.

Human activity has drastically changed the biogeochemical cycles and balance of some heavy metals in the environment. Therefore, a tendency towards their accumulation in the soil, seawater, freshwater and sediments was observed. Various field observations indicated a significant increase of heavy metal concentration in agricultural and forest soils as well as in marine and inland water sediments (Bielickaet al, 2002).

Red blood cells count in Rohu (*Labeorohita*) and Tilapia (*Oreochromis niloticus*)collected from different sites of Varahanadhi river along with control was represented in table 2. RBC Count in blood sample of fishes collected from downstream site was found to be significantly reduced compared to control. It was observed that there was no significant change in the RBC level in blood sample of fishes collected from upstream and midstream sites of river compared to control.

Table 2: Estimation of RBC Count in fish blood samples (millions/ mm^3)

	Rohu	Tilapia
Control	3.38±0.2	1.46±0.1
Upstream	3.53±0.2	2.60±0.2
Midstream	3.33±0.2	1.71±0.1
Downstream	2.16±0.1*	1.1±0.8*

Values were represented as Mean ±SD of five individual observations
p value: * $p<0.05$;Control vs Fishes collected from different sites

Table 3: Estimation of WBC Count in fish blood samples (cells x $10^3/mm^3$)

	Rohu	Tilapia
Control	6.76±0.6	7.1±0.5
Upstream	6.75±0.6	7.16±0.5
Midstream	6.80±0.6	7.00±0.5
Downstream	7.86±0.7*	7.35±0.7

Values were represented as Mean ±SD of five individual observations.
p value: * $p<0.05$;Control vs Fishes collected from different sites

Table3 presented the level of WBC Count in blood sample of fishes Rohu and Tilapia collected from upstream, midstream and downstream sites of Varahanadhi River. It was observed that Rohu showed ansignificant increase in the level of WBC compared to control fishes.

Hb level in fishes Rohu and Tilapia collected from different sites of Varahanadhi River, along with control was represented in table 4. Hb in blood sample of fishes collected from downstream site was found to be significantly reduced when compared to control. It was observed that there was no significant change in the Hb level in blood sample of fishes collected from upstream and midstream sites of river compared to control.

Table4: Estimation of Hb in fish blood samples (g/dl)

	Rohu	Tilapia
Control	8.2±0.7	7.2±0.6
Upstream	8.3±0.7	7.1±0.6
Midstream	7.8±0.6	7.1±0.6
Downstream	3.4±0.3*	3.8±0.3*

Values were expressed as Mean ±SD of five individual observations
p value: * $p<0.05$;Control vs Fishes collected from different sites

Table 5 presented the level of PCV (Hematocrit) in blood sample of fishes Rohu and Tilapia collected from upstream, midstream and downstream sites of Varahanadhi River. PCV value in blood sample of fishes collected from downstream site was found to be significantly reduced compared to control. It was observed that there was no significant change in the PCV value in blood sample of fishes collected from upstream and midstream sites of river compared to control.

Table 5: Estimation of PCV in fish blood samples (%)

	Rohu	Tilapia
Control	24.50±0.56	12.33±0.09

Upstream	24.00±0.47	11.33±0.10
Midstream	23.83±0.34	13.67±0.29
Downstream	15.50±0.36*	08.30±0.24*

Values were expressed as Mean ±SD of five individual observations
p value: * $p<0.05$;Control vs Fishes collected from different sites

Table 6: Estimation of Mean Cell Hemoglobin (pg)

	Rohu	Tilapia
Control	24.38±1.6	49.42±1.3
Upstream	23.54±1.5	27.57±1.3
Midstream	23.55±1.6	41.75±1.4
Downstream	16.65±1.5*	20.17±1.2*

Values were expressed as Mean ±SD of five individual observations
p value: * $p<0.05$;Control vs Fishes collected from different sites

MCH levels in different fishes collected from different sites of Varahanadhi river along with control was represented in table 6.MCH levels in blood sample of fishes collected from downstream site was found to be significantly reduced compared to control. It was observed that there was no significant change in the MCH level in blood sample of fishes collected from upstream and midstream sites of river compared to control.

Table7 represented the level of MCHC in blood sample of fishes Rohu and Tilapia collected from upstream, midstream and downstream sites of Varahanadhi River. MCHC value in blood sample of fishes collected from downstream site was found to be significantly reduced compared to control. It was observed that there was no significant change in the MCHC value in blood sample of fishes collected from upstream and midstream sites of river compared to control.

Table 7 Estimation of Mean Cell Hemoglobin Concentration (g %)

	Rohu	Tilapia
Control	33.67±2.7	58.79±4.8
Upstream	34.65±2.8	62.81±5.8
Midstream	32.94±2.7	52.06±4.7
Downstream	22.15±1.8*	46.18±4.3*

Values were expressed as Mean±SD of five individual observations,
p value: * $p<0.05$;Control vs Fishes collected from different sites

Many reasons are involved for the variations in hematological parameters of fishes, of all quality of water is considered to be the one of the major factor. Since they are sensitive to slight fluctuation that may occur within their internal milieu (Fernandes et al, 2003). The knowledge of the hematological characteristics is an imperative means that can be used as a significant and effective indicator to study the biochemical and pathological changes taking place in fishes.

RBC Count and Hemoglobin content

The RBC count and Hemoglobin content was significantly decreased in the fishes from downstream region , such decrease may be due to damage and destruction of blood cells (Hardy et al, 1971). Changes in blood parameters are caused by stressors and pollutants (Agarwal and Srivastava,1976). The reduction in RBC Count either by hemolysis or erythropoietic disorders lead to anemic condition in fish. The anaemic condition in fishes is attributed to an inhibition on erythrocyte production or haemodilution (Larson ,1975). Similar results with RBC and hemoglobin content reduction was reported with fishes exposed to different heavy metals (Karuppasamyet al, 2005). Tilapia fish expressed a low RBC count, when equated to other fishes.

As like RBC count, hemoglobin content also showed a vast significant variation in fishes from the downstream region. In particular, Rohu showed a drastic reduction in RBC Count. The anemic response could be as a result of disruption in erythrocyte production (Omoregie, 1995), haemodilution (Sampath et al, 1993) and destruction of intestinal cells involved in the production of vitamin B_{12} used in the production of the hemoglobin portion of red cells (Gardner and Yenich, 1970). Hemoglobin level was found to be reduced due to pollution, caused by heavy metals and lose their oxygen binding property, which resulted in erythrocyte damage (Witeskaand Kosciuk, 2003). Anemic condition may be due to an increased rate of erythrocyte destruction in hemopoietic organs (Jenkins et al, 2003).

WBC Count

WBC count is found to be increased in the Rohu fishes from the downstream region; which is considered as an adaptive mechanism. This may be due to the direct stimulation of the immunological defense mechanism against stressor (Davidsohnand Henry, 1969). Such increase in WBC count may be due to lymphocytosis and immune response (Shah and Altindag, 2005). The increase in number of WBC's may play an important role in immunological defense systems during exposure to toxicants like heavy metals and appears to be associated with circulatory levels of granulocytes, which are known to respond for phagocytosis (Briton,1963, Kori – Siakpere and Ubogu 2008). Increased leucocytes results in damage that might be due to severe physical stress and infection and also adapts to the stressful condition with the raised levels. It has been reported that, alteration in hematological parameters are not only associated with physico-chemical properties of water. But also due to season, reproductive activities and sex (Pandey et al, 1976), photoperiodism (Srivastavaet al, 2010) and availability of food (Dacieet al, 1982). The present study was found to be in concordance with the reports were given by Murugesan and Haniffa (1985) and Venkatachalam and Natarajan(2014) stated that heavy metals increases the WBC count.

Packed Cell Volume (PCV)

A precise low value of PCV (Hematocrit value) in fishes was observed in the downstream region, which are highly significant. Fish exposed to stress was attributed to a reduction in RBC volume caused by osmotic changes (Alwanet al, 2009). Gaafaret al,(2010) has reported that a pollutant edifenphos caused anemia and hypoproteinemia in fishes. Similar reports were given by Kandariand Routhan (2015) in Lacepede for hematologicalparameters in seasonal background.

MCH& MCHC

A decreasing trend in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were seen in fishes from the polluted site when compared to the unpolluted site. The fishes from downstream region showed an exceedingly significant variation, when compared with the control fishes. Karuppasamyet al, (2005), reported similar reduction in MCH values with heavy metal pollution. These hematological disturbances are a hemopoietic or erythrocyte mobilization response to hypoxemia induced by heavy metal stress.

The changes in MCH and MCHC may be either due to the (i) prevalence of large number of small sized immature RBCs in general circulation or even (ii) reduction in cellular blood iron thereby resulting in reduced Hb synthesis. Thus decline or fluctuating pattern of MCH and MCHC definitely appears to be the reflection of marked decline in Hb, which was reported by Raina and Sachar (2014) in chronic toxicity studies in fish exposed to zinc and a carbamate pesticide - sevin. MCH and MCHC calculated indirectly with reference to RBC, Hct and Hb. Therefore, their changes are directly linked with these blood parameters (Fazio et al, 2013).

Conclusion

Water in the down stream site of Varahanadhi river was highly polluted with heavy metals like chromium, lead, cadmium and arsenic. Fishes were collected and analyzed for haematological parameters. Haematological parameters are considered as an indicator of physiological and pathological changes in fishes.A significant reduction in RBC Count, Hemoglobin, PCV and MCH, MCHC values were observed in the downstream site of fishes Rohu (*Labeorohita*) and Tilapia (*Oreochromis niloticus*)when compared to those fishes collected from control, upstream and midstream sites of Varahanadhi River. An increase in WBC Count was observed in the downstream site of both the experimental fishes when compared to the fishes collected from the upstream and midstream site of the Varahandhi river. The changes in the hematological indices might be due to the heavy metals and the other pollutants being deposited in the river.

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References

Abdel-Ghani NT and Elchaghaby GA. Influence of operating conditions on the removal of Cu, Zn, Cd and Pb ions from wastewater by adsorption. International Journal of Environmental Science and Technology 2007; 4: 451-456.

Agarwal U and Srivastava K. Effect of cold-shock on blood cells in a fresh water teleost, *Colisa fasciatus*.Archives D'anatomieMicroscopique et de MorphologieExperimentale1976; 65: 165-174.

Alwan SF, 1-ladi AA ,Shokr AE . Alterations in haematological parameters of fresh water fish *Tilapia zilli* exposed to aluminium. Journal of Science and Its Applications2009; 3: 12-19.

Blaxhall PC and Daisely KW. Routine haematological methods for use with fish blood. Journal of Fish Biology 1973;5: 771-781.

Bielicka K, Kaczorek E, Olszanowski A, Voelkel A. Examination of biodegradation of hydrocarbons in emulsified systems, Polish Journal of Environmental Studies 2002; 11: 11-16.

Bomford R, Mason S, Swash M . In: Hutchinson's clinical methods, The Mc Milan Publishing Company, Inc: NY,1975.

Briton CJ. In: Disorders of the blood, 9th Ed Churchill, London,1963.

Censi P, Spoto SE, Saiano F, Sprovieri M, Mazzola S, Nardone G, Di Geronimo SI, PunturoR ,Ottonello D. Heavy metals in coastal water systems. A case study from the north western Gulf of Thailand. Chemosphere 2006; 64: 1167-1176.

Chekrabarthy P and BenerjeeV . Effects of sublethal toxicity of three organophosphorous pesticide on the peripheral heamogram of the fish, (*Channa punctatus*).Environmental Ecology 1988; 6:151- 158.

Dacie JV and Lewis SM. Blood cell morphology in health and disease In: Practical haematology, 6th edition. Churchill Livingstone, London, UK,1982.

Davidsohn I and Henry JB. Todd-Sanford Clinical Diagnosis by Laboratory Methods, 14th Edition. W. B. Saunders Company, Philadelphia, Pennsylvania, USA,1969.

ECR, (The Environment Conservation Rules). Ministry of Environment and Forest, Government of the People's Republic of Bangladesh, 1997; 205-207.

Fazio F, Marafioti S, Torre A, Sanfilippo M, PanzeraM ,Faggio C. Haematological and serum protein profiles of *Mugil cephalus*: effect of two different habitats. Ichthyological Research 2013; 60: 36-42.

Fernandes MN. Environmental pollution and fish gill morphology.In: Val, A.L. and Kapoor, B.G., Eds., Fish Adaptation, Science Publishers, Enfield, 2003;203-231.

Gaafar AY, El-Manakhly EM, Soliman MK, Soufy H, Mona S, Zaki, Mohamed SG , Hassan SM. Some pathological, biochemical and hematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide. Journal of American Science 2010; 6: 542-551.

Gardner GR and Yenich PP. Histological and haematological responses of an estuarine teleost to cadmium. Journal of the Fisheries

Research Board of Canada 1970; 27: 2185 - 2196.

Hardy HL, Chamberlin RI, Maloof CC, BoylenGW , Howell MC. Lead as an environmental poison. Clinical Pharma Therapy 1971; 12: 982-1002.

Hobbelen PHF, Koolhaas JE , van Gestel CAM. Risk assessment of heavy metal pollution for detritivores in floodplain soils in the Biesbosch, The Netherlands, taking bioavailability into account. Environmental Pollution 2004; 129: 409-419.

Houston JB and Carlile DJ. Incorporation of in vitro drug metabolism data into physiologically-based pharmacokinetic models. Toxicology Invitro1997;11:473- 478.

Hurkat, PC and Mathur PN. Text book of animal physiology. General and Comparative Physiology and Biochemistry .S. Chand and Co (p) Ltd, New Delhi,1976;182-204.

Jasmin Lena P and Maneemegalai S. Studies on the pollution levels of Varahanadhi river. Biomedicine 2015; 35(3): 318 – 322.

Jenkins F, Smith J, Rajanna B, Shameem U, Umadevi K, Sandhya V ,Madhavi R. Effect of sub-lethal concentrations of endosulfan on haematological and serum biochemical parameters in the carp *Cyprinus carpio*.Bulletin of Environmental Contamination and Toxicology2003, 70:0993-0997.

Johansen JA, Kennedy CJ, Sureeting RM, Farrell AP ,Ckeown BA. Sublethal effects of tetra chloroguaiacol on juvenile rainbow trout, Oncorhynchus mykiss, following acute and chronic exposure. Canadian Journalof Fish Aquatic Sciences 1994, 51: 1967-1974.

Kandari M and Rauthan JVS. Hematological changes in fish *Mastacembelusarmatus* (Lacepede) of Song River. Academic Journal of Biosciences 2015; 3: 34-37.

Karuppasamy R, SubathraS ,Puvaneswari S. Haematological responses to exposure to sublethal concentration of cadmium in air breathing fish *Channa punctatus* (Bloch). Journal of Environmental Biology 2005; 26: 123-128.

Kori-Siakpere O and Ubogu EO. Sublethal haematological effects of zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae). African Journal of Biotechnology 2008; 7: 2068-2073.

Larson A. Some biochemical effects of cadmium on fish in sub-lethal effects of toxic chemical on aquatic animals: In: Koeman JH, Strick Amsterdam JJTWA (Ed). The Netherlands Elsevier Scientific Publications 1975 :3-13.

Murugesan AG and Haniffa MA. Effects of textile mill effluent on haematological changes of the obligatory air breathing fish, *Anabas testudineus* (Bloch). Proceedings of the Symposium on Assessment of Environmental Pollution 1985; 121-128.

Mohiuddin KM, Zakir HM, Otomo K, SharminS ,Shikazono N. Geochemical distribution of trace metal pollutants in water and sediments of downstream of an urban river.International Journal of Environmental Science Technology 2010; 7: 17-28.

Nouri J, Lorestani B, Yousefi N, Khorasani N, Hasani AH, Seif F , Cheraghi M. Phytoremediation potential of native plants grown in the vicinity of Ahangaran lead–zinc mine (Hamedan, Iran). Environmental Earth Sciences 2011; 62: 639-644.

Okafor EC and Opuene K. Preliminary assessment of trace metals and polycyclic aromatic hydrocarbons in the sediments. International Journal of Environmental Science Technology 2007; 4:233 -240.

Omoregie E. Effects of petroleum on Nile tilapia and its helminth infection, PhD thesis, University of Jos (Nigeria),1995; 152.

Pandey BN, Pandey PK, Choubey BJ ,Datta munshi JS. Studies on blood components of an air-breathing siluroid fish,*Heteropneustesfossilis* (Bloch) in relation to body weight. Folia lzaematol 1976; 103: 101-116.

Pueyo M, Sastre J, Hernandez E, Vidal M, Lopez-Sanchez JF ,Rauret G. Prediction of trace element mobility in contaminated soils by sequential extraction. Journal of Environmental Quality Abstract – Heavy Metals in the Environment 2003; 32: 2054-2066.

Raina S and Sachar A. Effect of heavy metal, Zinc and carbamate pesticide, sevin on haematological parameters of fish, *Labeoboga*, International Journal of

Innovative Research in Science. Engineering and Technology 2014; 3: 12636-12644.

Rizkalla EH, Abd El -Haleem ME ,Shalaby AME. Haematological changes in*Cyprinuscarpio* L. as a result of short and long term exposure to different combinations of copper, cadmium and zinc. Egyptian Journal of Aquatic Biology and Fish 1999;175-193.

Sampath K, Velamman S, Kennedy L and James R. Haematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sublethal levels of Ekalus. Acta Hydrobiologia 1993; 35: 73 - 83.

Schalm OW, Jain NC and CarrollEJ.VeterinaryHaematology, 3rd Ed. Lea and Febiger, Philadelphia, 1975.

Shah SL and Altindag A. Alterations in the immunological parameter of tench (*Tincatinca* L 1758).After acute and chronic exposure to lethal and sub-lethal treatments with mercury, cadmium and lead. Turkish Journal of Veterinary Animal Science 2005; 29:1163 - 1168.

Shaperclaus W. Fish Diseases, Akademie Verlag, Berlin.1979; 1: 570.

Srivastava AK, Mishra D, Shrivastava S, Srivastav SK , Srivastav AK. Acute toxicity and behavioral responses of *Heteropneustesfossilis* to an organophosphate insecticide, dimethaoate. International Journal of Pharmacology and Biological sciences 2010; 1: 359-363.

Tessier A, Campbell PGC , Bisson M. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 1979; 51: 844-851.

Venkatachalam T and Natarajan AV.Hematological investigation on freshwater teleost *Labeorohita* (Ham.) following aquatic toxicities of Cr (III) and Cr (VI). International Journal of Research Biosciences 2014; 3: 1-14.

Weatherley AH and Gill HS. Relative growth of tissues at different somatic growth rates in rainbow trout *Salmogairdneri* Richardson. Journal of Fish Biology 1983; 22: 43-60.

Wedemeyer O, McleayDJ , Goodyear CP.Assessing the tolerance of fish and fish population to environmental stress.The problems and methods of monitoring.In Contaminant effects on fisheries.Cairns WV, Hudson PV and Nriaagu JO (Ed).John Wiley and Son Inc, New York, 1984; 164- 195.

WHO, World Health Organisation.International standards for drinking water. Geneva, 1999.

Witeska M and Kosciuk B. The changes in common carp blood after short-term zinc exposure. Environmental Science and Pollution Research 2003; 10:284 - 286.