Hematological changes in *Tinca tinca* after exposure to lethal and sublethal doses of Mercury, Cadmium and Lead

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Abstract

Tench, *Tinca tinca,* were exposed to three treatments (one acute lethal; 96 hrs Lc_{50}/48 hrs and two chronic sublethal10 and 25% Lc_{50}/504 hrs) each of Mercury, Cadmium and Lead and its behaviour as an indicator of impaired hematology was studied. It was observed that impairments in hematological parameters (increased/decreased Hct, Hb, RBC, WBC and Lct) were reflected in behaviour of fish in the form of hyperactivity, increased breathing, accelerated ventilation with rapid arrhythmic opercular and mouth movement, frequent surfacing and sinking, erratic swimming, gradual onset of inactivity, lethargy, loss of equilibrium, revolving and convulsion on exposure to heavy metal treatments. It was concluded that possibly four physiological systems; (i) faulty gaseous exchange at gills, (ii) stress mediated hormonal imbalance, (iii) impaired osmoregulation and (iv) disturbed metabolism were involved in hematological impairments and their reflection in the behaviour of fish.

Keywords: Hematology, *Tinca tinca*, Heavy metals, Mercury, Cadmium, Lead

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Introduction

The study of freshwater eco-toxicity has immense importance because of multiple use of water. Fish is a key unit in many natural food webs. Tench, *Tinca tinca,* is one of the main species of commercial interest in some countries and is considered a good test organism for heavy metal contamination because of its bottom feeding habit and behaviour (Grosch et al., 2000). Heavy metals have been reported to exert a wide range of metabolic, physiological, ecological and behavioural effects on fish (Soengas et al., 1996). Mercury, cadmium and lead are non-biodegradable and non-beneficial heavy metals and their role in the cell is not known (Bailey et al., 1999). Hematology provides an index of physiological status of fish and the use of blood picture of fish is an effective tool for detection of alterations in functional state of organism (Rambhaskar and Rao, 1987). Behavioural changes in animal are indicative of internal disturbances in the body functions, such as disturbances in metabolic pathways and ionic imbalance in blood serum (Lewis and Lewis, 1971; Shah, 2002). The response criteria in animals vary from detailed physiological measurements to whole animal response, i.e., behaviour (Hartwell, 1989).

The present study was conducted to investigate whether impaired hematology of tench on exposure to mercury, cadmium and lead treatments can be reflected in its behaviour, with the assumption that in open waters, impairments in physiological systems of fish on exposure to heavy metals pollution can be known from its behavioural activities , without carrying out detailed physiological studies in laboratories.

Materials and methods

The study was conducted at fish laboratory of Department of Biology, Ankara University, Ankara, Turkey. Tench, *Tinca tinca* were collected from Mogan Lake near Ankara, with cast nets and transported to experimental laboratory. Fifty litre capacity water tanks supported with air pumps were used for transportation of fish. The fish were allowed two weeks to acclimatize in laboratory conditions. Fish were fed commercial pellet food purchased from local market twice a day, maintained at 12 D/ 12 L photoperiodicity and water replaced twice a week with stored dechlorinated water. Physio-chemical parameters of laboratory water were dissolved oxygen 7.68±0.13 mg/L, water temperature 20.67±0.49 ^{o}C, pH 7.49±0.9, EC 0.29±0.02 mS/cm, bicarbonates 97.6 mg/L, total alkalinity 80 mg/L, chlorine 10.3 mg/L, sulphates 26.1 mg/L, calcium 29.0 mg/L, magnesium 1.2 mg/L and mercury, cadmium and lead <0.005 mg/L.

Three concentrations each of mercury, cadmium and lead (1-acute lethal and 2-chronic sublethal) were tested. Lethal treatments (96-hrs LC_{50} concentrations, i.e., Hg 1.0 ppm, Cd 6.5 ppm and Pb 300.0 ppm) lasted for 48 hours and sublethal treatments (10 and 25% of LC_{50}) lasted for 504 hours. A total of 72 fish were studied and the same number was served as control. All experiments were conducted in 75 liter glass aquaria, each comprising 8 fish (average length 21.89±0.66 cm and average weight 209.31±17.44 g). Mercury

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was used as mercuric chloride ($HgCl_2$), cadmium as cadmium chloride ($CdCl_2$) and lead as lead nitrate ($PbNO_3$) obtained from Department of Chemistry, Ankara University. An untreated group of 8 fish was maintained in separate tanks for each metal exposure and served as control group. Behavioural parameters of fish such as breathing, opercular movement, surfacing, general activity, restlessness, loss of equilibrium, lethargy, unconsciousness, burst of erratic swimming, vertical position, bottom dwelling, ventral lay down, feeding, death mimicry and death observed carefully through direct contact, and blood parameters such as hematocrit, hemoglobin, red blood cell count, white blood cell count and leucocrit were studied through routine methods.

Blood was collected within 35-40 seconds through cardiac puncture in 2 ml disposable heparinized syringes with 21-gauge needle and stored at 4°C. For hematocrit (Hct) determination, a three fourth of microhematocrit capillaries (75 mm L ×1.1 mm ID, Superior Germany) were filled with blood, sealed at one side by capillary sealer (Marion Feld, Germany) and centrifuged at 11000 rpm for 6 minutes in microhematocrit centrifuge (Hawksley and Sons, Co Sussex, England). The hematocrit (%) was determined by a microhematocrit reader (Blaxhall and Daisley, 1973). Hemoglobin (Hb) was determined with a hemoglobin test kit (No.124729, Roach GmbH Mannheim, Germany) using the cyanmethemoglobin method. A 0.02 ml aliquot of blood was mixed with 5 ml of test reagent (potassium hexacynoferrate 0.6 mmol/L and potassium cyanide 0.75 mmol/L), incubated at room temperature for 10-20 min, and absorbance was read at 546 nm using Shimadzu spectrophotometer (UV-120 IV, Shimadzu Cooperation, Japan). Absorbance values were converted to hemoglobin measurements (g/dl) based on standards included with the test kit. Total red blood cells (RBCs) were counted using an improved Neubaur hemocytometer (Clay, Adams, NY). Blood was diluted 1: 200 with Hayem`s fluid and erythrocytes were counted in the loaded hemocytometer chamber. Total numbers were as 10^6 cells/ mm^3. Total white blood cells (WBC) were counted by using an improved neubaur hemocytometer (Clay Adams, NY). Blood was diluted 1:20 with Turk's diluting fluid and four large (1 sq mm) corner squares of the hemocytometer were counted on microscope (Olympus CHK Optical Co. Ltd) at 640 ×. The total number of WBC calculated in $mm^3 \times 10^3$ (Wintrobe, 1967). Leucocrit was determined from the same microhematocrit capillaries following McLeay and Gordon (1977) and values in percent were read on microscope (Olympus CHK Optical Co. Ltd) at 640 × with the aid of ocular micrometer and calculated as; the height of grayish-white buffy layer/height of total blood volume × 100. Hematological alterations resulting from physiological impairments were observed from fish behaviour and any relation between the parameters was noted carefully. Data was analyzed statistically using student's t-test and significance was established at $P<0.05$.

Results

The impairments in hematological parameters of tench were reflected in its

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behavioural abnormalities as: in 1.0/48 treatment of mercury, with the decrease in hematocrit (Ht), hemoglobin (Hb), red blood cells (Rc) and white blood cells (Wc) (27, 29, 37 and 48%, respectively) and 49% increase in leucocrit (Lt). The breathing and surfacing were increased and fish became lethargic and unconscious and lost equilibrium and showed burst of erratic swimming and some fish also died. In 0.1/504 treatment of mercury, Ht, Hb and Rc were decreased (24, 28, and 30%, respectively) while Wc and Lt were increased (119 and 73% respectively, subsequently). Breathing, surfacing and general activity were also increased but feeding was decreased. In 0.25/504 treatment of mercury, Ht, Hb and Rc were decreased (18, 08 and 26% respectively) and Wc and Lt were increased (85 and 36% respectively), subsequently, surfacing and vertical position were increased and general activity and feeding were decreased. Restlessness first was increased and then decreased.

In 6.5/48 treatment of cadmium, there was an increase in Ht, Hb and Lt (21, 23 and 19%, respectively) and a decrease in Rc and Wc (15 and 46% respectively). Surfacing, restlessness, lost of equilibrium and lethargy were also increased. In some fish, bottom dwelling, vertical lay down, death mimicry and some death were also observed. General activities were first increased and then decreased. In 0.65/504 treatment of cadmium, there was decrease in Ht, Hb, Rc and Lt (18, 16, 29 and 6%, respectively) and increase in Wc (92%). The surfacing, general activity, restlessness, lethargy, unconsciousness, burst of erratic swimming, bottom dwelling and ventral lay down were increased. Feeding was decreased. In 1.265/504 treatment of cadmium, there was decrease in Hb, Rc, Wc and Lt (20, 28, 23, and 16% respectively) and increase in Ht (10%). The increased breathing and surfacing, restlessness, lethargy, unconsciousness, burst of erratic swimming, vertical position and bottom dwelling were observed. Feeding was decreased and general activity first was increased and then decreased.

In 300/48 treatment of lead, there was increase in Ht and Wc (21 and 34%, respectively) and decrease in Hb, Rc and Lt (17, 32, and 30%, respectively). The surfacing, bottom dwelling and vertical lay down were increased and general activity first increased and then decreased. Some deaths were also recorded. The 30/504 treatment of lead, was with the decrease in Hb, Rc and Wc (53, 24 and 3%, respectively) and increase in Ht and Lt (2 and 1%, respectively). The bottom dwelling and feeding were increased and general activity first was increased and then decreased. The 75/504 treatment of lead, was with a decrease in Ht, Hb, Rc and Lt (32, 25, 45 and 4% respectively) and an increase in Wc (99%), the breathing, opercular movement, bottom dwelling and feeding increased and general activity first increased and then decreased (Table 1).

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Table 1: Behavioural parameters as indicators of hematological impairments in Tench, *Tinca tinca* on exposure to lethal and sublethal mercury, cadmium and lead treatments

Heavy Metals	Hg (ppm)										Cd (ppm)										Pb (ppm)									
	1.0/48 hrs					0.1/504 hrs					0.25/504 hrs					6.5/48 hrs					0.65/504 hrs					1.265/504 hrs					300.0/48 hrs					30.0/504 hrs					75.0/504 hrs				
	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt	Ht	Hb	Rc	Wc	Lt
Hematological parameters	↓	↓	↓	↓	↑	↓	↓	↓	↑	↑	↓	↓	↓	↑	↑	↑	↑	↓	↓	↑	↓	↓	↓	↑	↓	↑	↓	↓	↓	↓	↑	↓	↓	↓	↑	↑	↓	↓	↓	↓	↑	↓	↓	↓	↑	↓
	27	29	37	48	49	24	28	30	119	73	18	8	26	85	36	21	23	15	46	19	18	16	29	92	6	10	20	28	23	16	21	17	32	34	30	2	.53	24	3	1	32	25	45	99	4
Behavioural Parameters																																													
Breathing	+					+																				+															+				
Opercular movement																																									+				
Surfacing	+					+					+					+					+					+					+													
General activity						+					-					+-					+					+-					+-					+-					+-				
Restlessness											+-					+					+					+																			
Loss of equilibrium	+															+																														
Lethargy	+															+					+					+																			
Unconsciousness	+																				+					+																			
Burst of erratic swimming	+																				+					+																			
Vertical position											+															+																			
Bottom dwelling																+					+					+					+					+					+				
Ventral lay down																+					+										+													
Feeding						-					-										-					-										+					+				
Death mimicry																+																														
Death	+															+															+														

	%Percent Change	Ht	Hematocrit%,
↓	Decrease in Hematological Parameters	Hb	Hemoglobin%
-	Decrease in Behavioural Parameters	Rc	Red blood cells%
↑	Increase in Hematological Parameters	Wc	White blood cells%
+	Increase in Behavioural Parameters	Lt	Leucocrit%
+ -	First Increase, then Decrease and Vice Versa		

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Discussion

The relevant previous work on the topic is very scanty. Dheer (1988) has reported behavioural changes, such as fast swimming, erratic movement and greater surfacing accompanied by loss of equilibrium and turning the body upside down, biochemical changes, such as decrease in blood glucose and muscle and liver glycogen and hematological changes, such as decrease in leucocytes and increase in Rbc, Hb, Hct, MCV, MCH and MCHC in Channa on exposure to thermal stress not to heavy metals, however, he did not study any relationship among these parameters.

Behavioral changes, such as increased locomotion and breathing, frequent surfacing and sinking, accelerated ventilation with rapid arrhythmic opercular and mouth movement, erratic swimming, gradual onset of inactivity, lethargy, loss of equilibrium, revolving, convulsion in various fish species on Cd, Pb, Hg and other heavy metals exposure have been reported, and attributed to inhibition of enzyme function causing paralysis of respiratory muscles and depression of respiratory centers, impairments in neural transmission due to blockage between nervous system and effecter sites, disturbances in metabolic pathways resulting in depletion of energy, muscle spasm due to drop in salt concentration in blood weakening fish, hormonal imbalance and faulty gaseous exchange at gills (Cearley, 1971; Macleod and Pessah, 1973; Holecombe *et al*., 1976; Koyama and Tlazawa, 1977; Das and Banerjee, 1980; Ellgaard and Guillot, 1988; Ghattak and Konar, 1990; Veena *et al*., 1997; Shah, 2002; Shah and Altindag, 2004).

Similarly, impairments in hematological parameters, such as drop and/or elevation in Hct, Hb, Rc, Wc and Lt in different fish species on heavy metals exposure have been reported and attributed to hemodilution, hemoconcentration, hemolysis and hemorraging, damage to hematopoietic tissues, accelerated erythroclasia, increased mechanical fragility, impaired osmoregulation, enzyme dysfunction, faulty gaseous exchange at gills leading to hypoxia and stress mediated hormonal imbalance (Tort et al., 1987; Yamamoto, 1988; Gill and Epple, 1993; Dethloff et al., 2001; Shah and Altindag, 2004).

In all behavioural and hematological impairments observed in the present study, four physiological systems of fish such as; (i) faulty gaseous exchange at gills, (ii) stress mediated hormonal imbalance, (iii) impaired osmoregulation and (iv) disturbed metabolism were found possibly involved and responsible for hematological impairments in tench and their reflection in its behaviour.

The mucus accumulation at gills increases diffusion distance between water and blood impairing gaseous exchange. Similarly, any damage to gill lamellae can result in low intake of O_2. Direct action of heavy metals on respiratory enzymes also causes paralysis of muscles of respiration and depression of the respiratory center at central nervous system. These factors can cause hypoxic conditions and paralysis of muscles of respiration system in the body of fish. Hypoxia triggers erythropoiesis to compensate oxygen deficiency in the body, resulting in increased number of erythrocytes and relevant indices and

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simultaneously accelerated ventilation, rapid opercular and mouth movements, frequent surfacing and sinking and increased breathing. The paralysis of muscles of respiration may result in hypoxia causing erythrocyte increase or decrease in hemoglobin binding capacity with O_2, and simultaneously cause uncontrolled swimming movements, convulsion, loss of equilibrium and apparent coma (Cearley, 1971; Ellgaard and Guillot, 1988; Veena et al., 1997; Witeska et al., 2006).

Impaired osmoregulation has been reported to cause hemodilution and hemoconcentration in fish resulting in increased and/or decreased erythrocytes and related indices. Similarly, erratic and violent swimming and tetany due to muscle spasm because of low calcium and high phosphorus in plasma have been reported (Koyama and Tlazawa, 1977; Tort et al., 1987).

In stress conditions, the release of corticosteroids is a common phenomenon and is a non-specific response to any environmental stress (Wepener et al., 1992). The increased level of stress hormones causes drop in erythrocytes and leucocytes in fish. Further, the spleen of fish sequesters and stores blood cells under resting conditions and releases them into circulating blood during contraction in stress conditions and plays important role in impairing blood parameters (Yamamoto, 1988). However, the process is whether hormonal or otherwise is not known. In stress condition, the energy demand of animal to cope with stress increases and metabolic reactions also disturbs resulting in depletion of energy causing animal lethargic and lost equilibrium and lower locomotor activity. Mobilization of energy reserves via hormones of pituitary-adernal axis and disturbed carbohydrate metabolism due to impaired hormonal control have been reported (Mazeaud and Mazeaud, 1981; Andersson et al., 1988).

Disturbed metabolism in animals has been reported to suppress their immune system thus, impairing blood cells (Witeska et al., 2006). The animals with higher metabolic activity could require higher level of oxygen and thus would have higher respiration or breathing rate (Canli and Kargin, 1995), and depression in metabolism reduce animal activity resulting in impaired swimming ability and other depletory symptoms.

The present study reveals that the relationship between both hematological and behavioural parameters may result in two possibilities. The target organs or systems responsible separately each for physiology and behaviour of fish were affected simultaneously by the Hg, Cd and Pb at different levels and resulted in impaired physiological system in the farm of altered hematological parameters and abnormal fish behavior simultaneously, or the organs/systems responsible directly or indirectly for maintenance of hematology of fish were affected by metals which resulted in impaired hematological parameters of fish and this in turn triggered some other organs/systems responsible for behaviour of fish and this reflected in the form of abnormal behaviour of animal. Such studies may help in knowing the impaired physiology of fish in the form of altered hematological

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parameters from its behavioural activities in open waters without conducting detailed laboratory work.

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