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Research Article

Alterations in The Immunological Parameters of Tench (*Tinca tinca* L. 1758) After Acute and Chronic Exposure To Lethal and Sublethal Treatments With Mercury, Cadmium and Lead

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Abstract: *Tinca tinca* were exposed to lethal and sublethal treatments with mercury, cadmium and lead for acute and chronic periods to study alterations in immunological parameters. Acute lethal exposure (Lc_{50}/48 h ; 96-h Lc_{50} of Hg 1.0, Cd 6.5, Pb 300 ppm) caused a significant increase in Lct in the Hg treatment and a significant decrease in the Pb treatment, and a significant decrease in total WBC count in the Hg and Cd treatments. Lower acute sublethal exposures (10% Lc_{50}/24 h), caused a significant increase in Lct in the Hg and Pb, treatments and a significant increase in total WBC count in the Hg treatment. The same concentration after 96-h exposure resulted in a significant increase in Lct in all 3 treatments and a significant decrease in total WBC count in the Cd treatment, and after chronic exposure caused a significant increase in Lct in the Hg treatment and a significant increase in total WBC count in the Hg and Cd treatments. Higher acute sublethal exposures (25% Lc_{50}/24 h) caused a significant increase in Lct in the Hg and Cd treatments and a significant increase in total WBC count in all 3 treatments. The same concentration after 96-h exposure caused a significant increase in both Lct and total WBC count in all 3 treatments, and after 3 weeks exposure resulted in a significant increase in Lct in the Hg treatment and a significant increase in total WBC count in the Hg and Pb treatments and a significant decrease in the Cd treatment.

The hypothesized greater resistance of tench to mercury, cadmium and lead toxicity compared to other fish species did not prove true; however, it was evident from the data that both acute and chronic metal exposure caused immunological impairment in tench, which suggests that the metals may weaken the immune system, resulting in increased susceptibility to infections. Further, the toxicity order of metals for the hematological parameters of tench was Hg > Cd > Pb.

Key Words: Leucocrit, total white blood cells count, mercury, cadmium, lead, *Tinca tinca*.

Civa, Kadmiyum ve Kurşunun Letal ve Subletal Konsantrasyonlarının Akut ve Kronik Uygulamalarından Sonra Kadife Balıklarının (*Tinca tinca* L. 1758) İmmünolojik Parametrelerindeki Değişimler

Özet: Civa, kadmiyum ve kurşunun letal ve subletal konsantrasyonlarına maruz bırakılan *Tinca tinca*'nın akut ve kronik periyotları için immünolojik parametrelerindeki değişimler çalışılmıştır. Akut letal uygulamalar (48 saat / Lc_{50} konsantrasyonları; 96 saat / Lc_{50} değeri Hg 1,0, Cd 6,5, Pb 300 ppm) Hg'da Lct'de önemli derecede artışa ve Pb'da önemli derecede azalışa, Hg ve Cd konsantrasyonlarında toplam WBC sayımında önemli derecede azalışa neden olmuştur. Daha düşük akut subletal uygulamaları (24 saat / Lc_{50} konsantrasyonunun % 10'u), Hg ve Pb'da Lct'de önemli derecede artışa ve Hg konsantrasyonlarında toplam WBC sayımında önemli derecede artışa neden olmuştur. Aynı konsantrasyon 96 saat uygulandıktan sonra, tüm Hg, Cd ve Pb konsantrasyonlarında, Lct'de önemli derecede artışlara ve Cd konsantrasyonunda toplam WBC sayımında önemli derecede azalışla sonuçlanmıştır. Kronik uygulamadan (3 hafta) sonra, Hg'da Lct'de önemli derecede artışa, Hg ve Cd konsantrasyonlarında toplam WBS sayımında önemli derecede artışa neden olmuştur. Daha yüksek akut subletal uygulamalar (24 saat / Lc_{50} konsantrasyonunun % 25'i) Hg ve Cd konsantrasyonlarında Lct'de önemli derecede artışa ve tüm Hg, Cd ve Pb konsantrasyonlarında, toplam WBC sayımında önemli derecede artışa neden olmuştur. Aynı konsantrasyonlara 96 saat uygulamadan sonra tüm Hg, Cd ve Pb konsantrasyonlarında, toplam WBC sayımında ve Lct'de önemli derecede artışa neden olmuştur. Üç haftalık uygulamadan sonra Hg'da Lct'de önemli derecede artış, Hg ve Pb'da toplam WBC sayımında önemli derecede artış ve Cd konsantrasyonlarında önemli derecede azalışla sonuçlanmıştır.

Diğer balık türleri ile kıyaslandığında kadife balığının Hg, Cd ve Pb toksisitesine daha dirençli olduğu iddia edilmesine rağmen, bu çalışmada bu görüş desteklenmemektedir. Ancak bu çalışmada elde edilen veriler, metallere hem akut hem de kronik maruz bırakmanın, kadife balığında immün sistemin bozulmasına yol açtığını desteklemektedir. Bu durum balığın immün sisteminin zayıflamasına ve hastalıklara karşı daha duyarlı hale gelmesine neden olabilir. Ayrıca, kadife balığının hematolojik parametreleri dikkate alındığında ağır metal toksisite sırası; Hg > Cd > Pb olarak bulunmuştur.

Anahtar Sözcükler: Lökokrit, toplam beyaz kan hücresi sayımı, civa, kadmiyum, kurşun, *Tinca tinca*.

Introduction

Heavy metals are serious pollutants of the aquatic environment because of their environmental persistence and ability to be concentrated by aquatic organisms (1). They cause serious impairments in metabolic, physiological and structural systems, resulting in various diseases and disorders (2). Mercury, cadmium and lead are non-biodegradable and non-beneficial heavy metals and their role in the cell is not known (3). The use of immune system parameters to assess alterations in fish experiencing heavy metal exposure and interest in defense mechanisms stem from the need to develop health management tools to support a rapidly growing aquaculture industry (4). Stressed fish show fragile health and become more susceptible to pathogens. Total leucocyte (WBC) count reflects the presence and possible type of infectious or organic disease and level of resistance of fish to diseases (5). Leucocrit offers a robust measure to assess the effects of metals on fish (6). Heavy metals may directly affect leucocytes or indirectly exhaust the defense system of fish to a degree that they become unable to cope with added stress and reduce the survival potential of the population. Tench is considered a good test organism for heavy metal contamination because of its bottom feeding habit and behavior (7).

The present study was conducted based on the hypothesis that the tench is a more resistant fish and can survive in the environment, wherein the other fish are eliminated, and therefore it may show more resistance demonstrated here with hematological parameters to mercury, cadmium and lead toxicity, which proved untrue as it was affected by metals like other fish species.

Materials and Methods

Tench (*Tinca tinca* L., 1758) were collected from Mogan lake near the city of Ankara with cast nets and transported to the fish laboratory of the Department of Biology, Ankara University, Ankara. Fifty liter capacity water tanks supported with air pumps were used to transport the fish. The fish were allowed 2 weeks to acclimatize in laboratory conditions. They were fed commercial pellet food twice a day, and the water replaced twice a week with stored dechlorinated water.

The physico-chemical parameters of the laboratory water were as follows: dissolved oxygen 7.68 ± 0.13 mg/l, water temperature 20.67 ± 0.49 °C, pH 7.49 ± 0.9, EC 0.29 ± 0.02 mS/cm, photoperiodicity 12D:12L, bicarbonates 97.6 mg/l, total alkalinity 80 mg/l, chlorine 10.3 mg/l, sulfates 26.1mg/l, calcium 29.0 mg/l, magnesium 1.2 mg/l, and mercury, cadmium and lead < 0.005 mg/l each. Water temperature and dissolved oxygen (YSI 51B, USA oxygen meter, sensitivity 0.1), pH (WTW Weilheim, Germany pH meter, sensitivity ±0.01), EC (WTW Weilheim, Germany EC meter, sensitivity 0-199.999 µS/cm) and photoperiodicity (with fluorescent tubes) were measured daily and the other parameters were determined at the DSİ, TAKK, Chemical Laboratories, Ankara.

Three concentrations each of mercury (0.1, 0.25, 1.0 ppm), cadmium (0.65, 1.625, 6.5 ppm) and lead (30, 75, 300 ppm) with 7 exposures (1 acute lethal, 4 acute sublethal and 2 chronic sublethal) each were tested on fish in 120-l capacity glass aquaria. The acute lethal treatment (96-h Lc_{50}, Hg 1.0, Cd 6.5, Pb 300 ppm) calculated from percentage mortalities of fish as described by Veena et al. (1) lasted 48 h, and the acute and sublethal treatments (10% and 25% of Lc_{50}) lasted 24 and 96 h and the chronic treatment lasted 3 weeks. Heavy metals were used in the form of mercuric chloride ($HgCl_2$), cadmium chloride ($CdCl_2$) and lead nitrate ($PbNO_3$) in concentrations of ppm from the stock solution (8). Circulation of metals in the aquaria was ensured by 2 air pumps in each one. A group of 8 healthy fish was used for each of the 7 exposures (9), and for each treatment, and the same number for control with each exposure (approx. age group II-III, average total length 25.23 ± 0.33 cm and 197.29 ± 8.05 g). Age was determined

from the scales. The experiments were not repeated because of controlled conditions developed from experience.

Blood was collected within 35-40 s through cardiac puncture into 2 ml disposable heparinized syringes with 21-gauge needles after stunning the fish by a blow to the head. Syringes were kept at 4 °C up to the completion of study. For leucocrit determination, three-fourths 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 11,000 rpm for 6 min in a microhematocrit centrifuge (Hawksley and Sons, Sussex, England). Values in percent were read under a microscope (Olympus CHK Optical Co. Ltd) at 640 × with the aid of an ocular micrometer and calculated as the height of the grayish-white buffy layer/height of total blood volume × 100 (6). Total white blood cells (WBC) were counted using an improved Neubauer hemocytometer (Clay Adams, NY, USA). Blood was diluted 1:20 with Turk's diluting fluid and 4 large (1 sq mm) corner squares of the hemocytometer were counted under the microscope (Olympus CHK Optical Co. Ltd) at 640 ×. The total number of WBC was calculated in $mm^3 \times 10^3$ (10). The data were analyzed statistically using Student's t-test and a significant difference was established at 0.05 level.

Results

Leucocrit (%)

All lethal and sublethal treatments with mercury caused a significant increase in leucocrit. A 49.20% increase was observed in the acute lethal (Lc_{50} for 48 h) treatment and 45.76%, 55.88% and 73.53% increases in the lower sublethal (10% of Lc_{50}) treatments after 24- and 96-h and 3-week exposures, respectively. Higher sublethal (25% of Lc_{50}) treatments caused 30.66%, 66.15% and 35.82% increases in leucocrit after 24- and 96-h and 3-week exposures, respectively. Cadmium caused significant increase in leucocrit in the 3 acute sublethal treatments only. A 57.35% increase was observed in the lower sublethal treatment after 96-h exposure and 36.0% and 40.0% increases in the higher sublethal treatments after 24- and 96-h exposures, respectively. In the other cadmium treatments, a drop was observed in leucocrit; however, it was non-significant when compared with the control. Lead caused a significant change in leucocrit in the acute lethal and sublethal treatments. A 30.15% decrease was observed in the acute lethal treatment and 28.81%, 42.64% and 32.30% increases in the lower sublethal treatments after 24- and 96-h exposures and in the higher sublethal treatment after 96-h exposure, respectively. A non-significant drop in leucocrit was observed in the higher acute and chronic sublethal treatments (Table).

Total WBC count ($mm^3 \times 10^3$)

All treatments with mercury, except for the lower sublethal treatment, after 96-h exposure, caused a significant change in total WBC count. A 48.66% drop was observed in the acute lethal treatment and 48.07% and 119.26% increases in the lower sublethal treatments after acute (24 h) and chronic exposures, respectively. Furthermore, 107.65%, 81.50% and 85.43% increases were recorded in the higher sublethal treatments after 24- and 96-h and 3-week exposures, respectively. Similarly, all treatments with cadmium, except for the acute (24 h) lower sublethal treatment, caused significant changes in total WBC count; 46.47%, 38.74% and 23.17% decreases were observed in the acute lethal, acute (96 h) lower sublethal and chronic higher sublethal treatments, respectively. Increases of 92.09%, 182.27% and 79.35% in total WBC count were recorded in the lower chronic sublethal, acute (24 h) and chronic higher sublethal treatments, respectively. Lead caused a significant change in total WBC count in the 3 higher sublethal treatments only. Increases of, 74.83%, 70.53% and 99.33% were observed in the higher sublethal treatments after 24- and 96-h and 3-week exposures, respectively. A non-significant increase in the acute lethal and a drop in the acute and chronic lower sublethal treatments were also recorded (Table).

Discussion

The immune system of different fish species including tench has been studied by various researchers (8,11,12); however, studies on the effect of heavy metals on immunological parameters are limited. Generally, both increases and decreases in leucocrit and WBC counts are caused by increases and decreases in the number of lymphocytes, eosinophils and thrombocytes. The leucopenia in *Colisa fasciatus* after zinc (13) and in

Table. Leucocrit (Lct %) and total WBC count ($mm^3 \times 10^3$) of tench after acute and chronic exposure to lethal and sublethal treatments of mercury, cadmium and lead. n = 8, ± SE, ( ) range, + % increase, - % decrease, $P < 0.05$.

Exposure	Time	Control	Leucocrit			Control	WBC Count		
			Hg	Cd	Pb		Hg	Cd	Pb
96-h Lc_{50}	48 h	0.63 ± 0.04 (0.45-0.82)	* 0.94 ± 0.04 (0.78-1.10) 49.2	0.75 ± 0.06 (0.46-1.0) 19.04	*0.44 ± 0.06 (0.16-0.71) -30.15	41.1 ± 6.53 (15.2-72.0)	*21.1 ± 2.11 (12.8-29.6) -48.66	*22.0 ± 4.72 (8.80-44.8) -46.47	55.1 ± 3.69 (39.2-72.8) 34.06
10% of Lc_{50}	24 h	0.59 ± 0.02 (0.48-0.69)	* 0.86 ± 0.05 (0.73-1.07) 45.76	0.56 ± 0.05 (0.42-0.86) -5.08	*0.76 ± 0.05 (0.54-0.95) 28.81	46.80 ± 3.84 (24.0-61.6)	*69.30 ± 4.30 (47.2-87.2) 48.07	46.50 ± 3.85 (30.4-64.8) -0.64	55.8 ± 4.69 (40.0-78.4) 19.23
10% of Lc_{50}	96 h	0.68 ± 0.04 (0.48-0.85)	*1.06 ± 0.1 (0.77-1.69) 55.88	*1.07 ± 0.13 (0.77-1.67) 57.35	*0.97 ± 0.08 (0.59-1.28) 42.64	51.10 ± 3.94 (31.2-64.8)	62.0 ± 4.36 (44.8-87.2) 21.33	*31.30 ± 6.59 (14.4-71.2) -38.74	43.5 ± 5.72 (20.0-66.4) -14.87
10% of Lc_{50}	3 weeks	0.68 ± 0.05 (0.46-0.89)	*1.18 ± 0.1 (0.74-1.65) 73.53	0.64 ± 0.02 (0.54-0.74) -5.88	0.69 ± 0.08 (0.45-1.23) 1.47	40.5 ± 1.79 (32.0-48.0)	*88.8 ± 11.81 (35.2-136.0) 119.26	*77.8 ± 2.06 (68.0-88.0) 92.09	39.4 ± 2.95 (27.2-55.2) -2.71
25% of Lc_{50}	24 h	0.75 ± 0.03 (0.59-0.90)	*0.98 ± 0.06 (0.78-1.32) 30.66	*1.02 ± 0.08 (0.81-1.38) 36	0.68 ± 0.06 (0.46-0.92) -9.33	45.70 ± 5.99 (24.0-71.2)	*94.90 ± 14.67 (44.80-164.8) 107.65	*129.0 ± 12.05 (75.2-175.2) 182.27	*79.9 ± 8.28 (52.0-132) 74.83
25% of Lc_{50}	96 h	0.65 ± 0.04 (0.44-0.82)	*1.08 ± 0.08 (0.80-1.50) 66.15	*0.91 ± 0.05 (0.79-1.27) 40	*0.86 ± 0.07 (0.63-1.28) 32.3	46.5 ± 4.61 (24.0-64.0)	*84.40 ± 4.04 (68.0-98.4) 81.5	*83.4 ± 7.24 (52.8-118.4) 79.35	*79.3 ± 3.79 (62.4-97.6) 70.53
25% of Lc_{50}	3 weeks	0.67 ± 0.04 (0.45-0.83)	*0.91 ± 0.04 (0.74-1.16) 35.82	0.56 ± 0.04 (0.33-0.67) -16.41	0.64 ± 0.04 (0.46-0.83) -4.47	30.20 ± 0.63 (27.2-32.8)	*56.0 ± 6.47 (23.2-71.2) 85.43	*23.20 ± 2.18 (14.4-32.0) -23.17	*60.2 ± 2.62 (48.8-72.0) 99.33

Oncorhynchus mykiss after As, Cd and Hg exposure (14) has been attributed to the reduced number of lymphocytes. Similarly, decreased leucocrit in *Scyliorhinus canicula* after copper and cadmium exposures due to a decreased number of lymphocytes has been reported (2). In contrast, increased WBC (leucocytes) counts in *Heteropneustes fossilis* after copper exposure (15), in *Anguilla anguilla* after lead exposure (16) and in *Oreochromis aureus* after mercury exposure (8) have been reported. An increase in WBC count has been attributed to the increased number of eosinophils (17) and thrombocytes (8). Thrombocytosis in *Oreochromis mossambicus* and a reduction in WBC count, because of a decreased number of thrombocytes along with lymphocytes in *Carassius auratus* after cadmium exposure, have been reported (18). The lymphopenia may be attributed to decreased mean cellular life span and impaired proliferative capacity of cells (19), necrosis of leucopoietic tissues (19), impairment in hematopoetic cells in the kidney (14) and accumulation of lymphocytes in lymphoid tissues or destruction by corticosteroid hormones (20). The spleen has been reported to be a potent blood storage organ in some teleosts (21). The immunosuppressive effects of corticosteroids via protein synthesis on lymphocytosis have been reported (22). In addition lymphocytes may also be destroyed by the direct action of heavy metals. Prominent changes in lymphocytes such as vacuolation, cellular and nuclear hypertrophy and the appearance of cytoplasmic outgrowths in *Barbus conchonius* on mercury exposure have been reported (23). Decreased leucocrit because of hemodilution has also been reported (2). In contrast, the lymphocytosis may be attributed to injury in hematopoietic tissues, acceleration of lymphopoiesis or efflux of lymphocytes from lymphopoietic loci (24), direct stimulation of immune defense by heavy metals as foreign

substances (25) and glucocorticoid mediated mobilization of the defense mechanism (26). Cadmium has been reported to interfere with the "adrenocortical axis" of teleosts (26). An increase in lymphocyte number may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes. The decrease in thrombocyte number may be attributed to insufficient thrombopoiesis correlated to pathological lesions in tissues responsible for this process and/or excessive destruction of thrombocytes (20). Thrombocytosis may be glucocorticoid mediated (24) and may be due to tissue damage and hemorrhage.

An initial increase and subsequent decrease and vice versa in both leucocrit and WBC count may probably be attributed to the activity of the spleen, which sequesters and store blood cells under resting conditions and releases them into circulating blood during contraction associated with various states of stress (21), and to the gradual repairment of damaged hemopoietic tissues. Gill and Pant (24) have reported that first the stimulation of the immune system causes an increase in lymphocytes by an injury or tissue damage, but a prolonged or continuous stimulus may cause the suppression or exhaustion of this capacity, resulting in a decrease in lymphocytes and so in total WBC count. The hypothesized greater resistance to mercury, cadmium and lead toxicity studied hematologically in tench, compared to other fish species, did not prove true. This notion, however, needs to be demonstrated more conclusively with more detailed studies. However, from the data the toxicity order of metals for the hematological parameters of tench was Hg > Cd > Pb. Further, it is evident from the data that both acute and chronic metal exposure caused immunological impairments in tench, which suggests that the metals may weaken the immune system, resulting in increased susceptibility to infections.

References

1. Veena, B., Radhakrishnan, C.K., Chacko, J.: Heavy metal induced biochemical effects in an estuarine teleost. Indian J. Marine Sci., 1997; 26: 74-78.
2. Tort, L., Torres, P., Flos, R.: Effects on dogfish haematology and liver composition after acute copper exposure. Comp. Biochem. Physiol., 1987; 87C: 349-353.
3. Bailey, S.E., Olin, T.J., Bricka, R.M., Adrian, D.D.: A review of potentially low-cost sorbents for heavy metals. Water Res., 1999; 33: 2469-2479.
4. Jones, S.R.M.: The occurrence and mechanisms of innate immunity against parasites in fish. Dev. Comp. Immunol., 2001; 25: 841-852.
5. Blaxhall, P.C., Daisley, K.W.: Routine hematological methods for use with fish blood. J. Fish Biol., 1973; 5: 771-781.
6. McLeay, D.J., Gordon, M.R.: Leucocrit: a simple haematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulp mill effluent. J. Fish. Res. Board Can., 1977; 34: 2164-2175.
7. Guerrin, F., Burgat-Sacaze, V., Saqui-Sannes, P.: Level of heavy metals and organochlorine pesticides of cyprinid fish reared four years in wastewater treatment pond. Bull. Environ. Contam. Toxicol., 1990; 44: 461-467.
8. Allen, P.: Changes in the hematological profile of the cichlid, *Oreochromis aureus* (Steindachner) during acute inorganic mercury intoxication. Comp. Biochem. Physiol., 1994; 108 C: 117-121.
9. Shah, S.L., Altindag, A.: Hematological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull. Environ. Contam. Toxicol., 2004; 73: 911-918.
10. Wintrobe, M.M.: Clinical haematology. 10th ed. Lea and Febiger. Philadelphia. Library of Congress, Print. USA, 1967; 2680 p.
11. Ellis, A.E.: Innate host defense mechanisms of fish against viruses and bacteria. Dev. Comp. Immunol., 2001; 25: 827-839.
12. Svobodova, Z., Flajshans, M., Kolarova, J., Modra, H., Svoboda, M., Vajcova, V.: Leukocyte profiles of diploid and triploid tench, *Tinca tinca* L. Aquaculture, 2001; 198: 159-168.
13. Mishra, S., Srivastava, A.K.: Haematology as index of sublethal toxicity of zinc in fresh water teleost. Bull. Environ. Contam. Toxicol., 1979; 22: 695-698.
14. Kotsanis, N., Georgudaki, J.L., Zoumbos, K.K.: Changes in selected hematological parameters at early stages of the rainbow trout, *Oncorhynchus mykiss*, subjected to metal toxicants: arsenic, cadmium and mercury. J. Appl. Ichthyol., 2000; 16: 276-278.
15. Singh, H.S., Reddy, T.V.: Effect of copper sulfate on hematology, blood chemistry and hepato-somatic index of an Indian catfish, *Heteropneustes fossilis* (Bloch) and its recovery. Ecotoxicol. Environ. Saf., 1990; 20: 30-35.
16. Santos, M.A., Hall, A.: Influence of inorganic lead on the biochemical blood composition of the eel, *Anguilla anguilla* L. Ecotoxicol. Environ. Saf., 1990; 20: 7-9.
17. Hilmy, A.M., Shabana, M.B., Said, M.M.: Haematological responses to mercury toxicity in the marine teleost, *Aphanius dispar* (Rupp). Comp. Biochem. Physiol., 1980; 67C: 147-158.
18. Murad, A., Houstan, A.H.: Leucocytes and leucopoietic capacity in goldfish, *Carassius auratus*, exposed to sublethal levels of cadmium. Aquatic Toxicol., 1988; 13: 141-154.

19. Wepener, V., VanVuren, J.H.J., DuPreez, H.H.: Effect of manganese and iron at a neutral and acidic pH on the haematology of the banded tilapia, *Tilapia sparrmanii*. Bull. Environ. Contam. Toxicol., 1992; 49: 613-619.

20. Angelidis, P., Laurencin, F.B., Youinou, P.: Stress in rainbow trout, *Salmo gairdneri*: effects upon phagocyte chemiluminescence, circulating leucocytes and susceptibility to *Aeromonas salmonicida*. J. Fish Biol., 1987; 31A: 113-122.

21. Yamamoto, K.I.: Contraction of spleen in exercised freshwater teleost. Comp. Biochem. Physiol., 1988; 89A: 65-66.

22. Dale, M.M.: Corticosteroids-1. Corticosteroids and their anti-inflammatory effects. In: Textbook of Immunopharmacology (MM Dale & JC Foreman eds) Blackwell Scientific Publications, London, 1984. pp. 283-288.

23. Gill, T.S., Pant, J.C.: Mercury induced blood anomalies in the freshwater teleost, *Barbus conchonius*. Water Air Soil Pollut., 1985; 24: 165-171.

24. Gill, T.S., Pant, J.C.: Erythrocytic and leukocytic responses to cadmium poisoning in freshwater fish, *Puntius conchonius* Ham. Environ. Res., 1985; 30: 372-373.

25. Sjobeck, M.L., Haux, C., Larsson, A., Lithner, G.: Biochemical and hematological studies on perch, *Perca fluviatilis*, from the cadmium-contaminated river Eman. Ecotoxicol. Environ. Saf., 1984; 8: 303-312.

26. Gill, T.S., Epple, A.: Stress-related changes in the hematological profile of the American eel, *Anguilla rostrata*. Ecotoxicol. Environ. Saf., 1993; 25: 227-235.