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The Effects of Pollution on Haematological Parameters of Black Goby (*Gobius niger* L., 1758) in Foça and Aliağa Bays*

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Özet: ***Foça ve Aliağa Körfezi'ndeki kirliliğin kömürcü kaya balığı (*Gobius niger *L., 1758)'nin hematolojik parametreleri üzerine etkileri.*** Bu çalışmada, petrol rafinerisinin bulunduğu Aliağa Körfezi'nin ve Foça'nın çeşitli yerlerinden seçilmiş istasyonlardan toplanan kömürcü kaya balıklarından (*G. niger*) alınan kan örneklerinin kan parametreleri, kırmızı kan hücreleri (eritrosit), hemoglobin, hematokrit ve ortalama hücresel hacim, ortalama hücresel hemoglobin miktarı ortalama hücresel hemoglobin konsantrasyonu ve trombosit ölçülmüştür. Yapılan mikroskobik çalışmalar sonucu histolojik değişiklikler ortaya çıkarılmıştır. Normal olarak oval ve yassı şekilli nukleuslar değişikliğe uğrayarak fusiform ve küresel şekil almıştır ve hücre zarı dikensi karakter şekline bozulmuştur. Araştırma sırasında suyun kimyasal özelliklerini tespit etmek için su örnekleri de Foça ve Aliağa Körfezleri'nden alınmış ve çözünmüş oksijen, pH, sıcaklık, tuzluluk, ve turbidite gibi fiziko kimyasal parametreler ölçülmüştür. Bu çalışma sonucunda deniz balıklarının kan parametrelerinin çok çeşitli çevresel kirleticiye karşı fizyolojik bir yanıt olarak kullanılabileceği ortaya koyulmaktadır.

Anahtar Kelimeler: Hematolojik parametreler, *Gobius niger,* kirlilik, eritrosit, ağır metal

Abstract: In this study, the haematological parameters (Red blood cells, haemoglobin, mean celllular haemoglobin content, mean cellular volume, mean cellular haemoglobin concentration, platelet) of *Gobius niger* collected from several selected stations in Foça and Aliağa Bays, where a petroleum refinery is located, have been measured. The statistically important differences were found between the measured red blood cells haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, and mean cellular haemoglobin concentrations obtained from different stations. In the microscopic studies, some histological changes were determined due to environmental pollution. Ovoid shape of the nuclei seen in normal red blood cells hasbeen changed to fusiform and spherical shape and the cell membrane became echinoid. Physico-chemical parameters such as dissolved oxygen, pH, temperature, salinity and turbidity of the water samples taken from Foça and Aliağa Bays were determined. Considering the results obtained in this study of this study, it can be concluded that the changes in the blood parameters of marine fish may be used as a physiological answer to environmental pollution.

Key Words: Haematological parameters, *Gobius niger,* pollution, erythrocyte, heavy metal

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Introduction

It is well known that industrial wastes in aquatic environments have a toxic effect on organisms, especially on fish (Sjöbeck *et al*., 1984). The stress caused by environmental pollution changes the structure of red and white blood cells (Larsson *et al*., 1984). Haematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson *et al*., 1985). Thus, blood parameters such as RBC (Red Blood Cells), HGB (Haemoglobin), HCT (Haematocrit), MCV (Mean Cellular Volume), MCH (Mean Cellular Haemoglobin Concentration), PLT (Trombocytes) are the most common criteria used in the toxicity studies on fish. As an indicator of pollution, blood parameters are used in order to diagnose and describe the general health condition of some fish. Besides, this type of index reflects certain ecological changes in the environment (Roche and Boge, 1996).

Aliağa Bay located on the Aegean Coast is an area affected by domestic and dense industrial pollution especially from petroleum. The industrial plants in Aliağa are the major cause of marine pollution. Although these plants have waste treatment systems and are supposed to release the treated waste water into the sea, even a small amount of untreated waste material leaking into the sea is enough to change the natural balance of the sea. Crude oil products and lubricating oil are produced in Tüpraş which is the second biggest state-run refinery in the country. This refinery has an annual capasity of producing 10 million tonnes crude oil petroleum refining is the main cause of environmental pollution in Aliağa Bay assosiated with industrial activities (Anonim, 1992).

The aim of this study is to determine the sub lethal effects of the pollution in Foça ve Aliağa Bays investing the haematological parameters of *Gobius niger,* 1758 as an indicator species.

Material and Method

In this study, *Gobius niger* known as an indicator species in the polluted areas in the Mediterranean was chosen as study material (Kaya and Mater, 1994). Fifty indivudials with a mean lenght of (10.3±1.65cm) and a mean weight of (13.51 ±6.98g) were investigated during the course of the study.

G. niger was caught from sampling stations in Foça and Aliağa Bays using a fishing line and evaluated for haematological parameters between September 1999 and September 2001 (Figure 1).

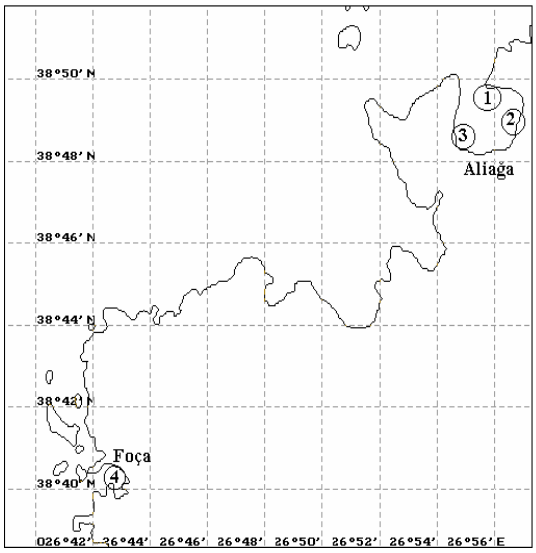

Figure 1. Sampling stations map.

The sampling stations were chosen from the polluted areas in Aliağa Bay and from Foça Bay, which has connection to the open sea and is considered to be clean.

After the individuals were given some time to get rid of their stress, 0.5 ml blood was taken from caudal veine of the by using heparinized hypodermic syringe and transferred to special tubes containing potassium EDTA as an anticoagulant agent.

Haematological parameters were measured maximum 4 hours after blood samples were taken. The Coulter Counter (CELL DN 1700) was used to count RBC (Red Blood Cells), HGB (Haemoglobin), HCT (Haematocrit), MCV (Mean Cellular Volume), MCH (Mean Celllular Haemoglobin Concentration), PLT (Trombocyte). The standart methods to examine the histological structure of blood were used and overspread smear samples were dyed by Giemsa for light microscope (Blaxal and Daisly, 1973).

In this study leucocyte countings were ignored since fish erythrocytes have nuclei and because of the concern that they can be counted at the same time with erythrocytes. Statistical differences between the haemathological parameters of the sampling stations were determined by using One-way ANOVA with the Multiple Range Test.

During the analysis of the samples taken from the sampling stations in Aliağa and Foça Bays, water temperature, salinity, pH, dissolved oxygen, turbidity were performed using Water Quality Checker.

Results

As a results of the analyses, the difference between the data including red blood cells, haemoglobin, haematocrit, mean cell volume, and mean cell haemoglobine concentration from Foça ve Aliağa Bays were found to be statistically important (Table 1).

The highest erythrocyte values were found in the samplings from station no.3 (Aliağa Refinery) ($1.3\pm0.2x10^6$ µlt). Similarly, the highest haemoglobin concentration (5.8. ±0.3g/dl.) was found in the same station (Station no. 3). However, the lowest haemoglobin (3.3±0.8 g/dl.) concentration was found at station no.1 (Aliağa Water Harbor). Inaddition, fish samples from Aliağa Refinery had the highest haematocrit concentration (%17.6±3.4) and mean cell volume (129.1± 0.2 fL) compared to the other stations.

Values of mean cellular haemoglobin concentration (MCHC) were found to be higher in the fish caught from the stations in Foça Bay compared to those in Aliağa Bay (45.4±4.2pg) ($p<0.01$). However, no statistical differences between values of mean cellular haemoglobin content (MCH) and trombocytes were seen between the sampling stations (Figure 2).

During the microscopic studies of the samples on a blood smear obtained from the peripheral blood samples, some histological differences were observed. In the blood samples taken from all sampling stations, erythrocytes and nucleous were deformed and had become fusiform and shuttle-shaped. (Figure 3 A, B, C, D) In the samples from Aliağa Water Harbor and Refinery stations hypochromia (loss of color) was also observed (Figure 3 A, C). The structure of the erytrocyte cell membrane in the samples taken from Aliağa Inner Harbor station became spiny. Besides, erytrocytes with vacuola in their cytoplasm were determined in the station at Aliağa Refinery.

Discussion

It is well known that fish have the ability to concentrate heavy metals and different pollutants in their muscles and different organs. Recently, haematological techniques have been used to determine the sub lethal effects of these pollutants during the clinic diagnosis of fish physiology (Bhagwant and Bhıkajee 2000).

Previous studies have shown that erythrocytes and white blood cells are the most important indicators in examining physiologic changes (Larsson *et al*., 1985).

Related to red blood cells, mean erythrocyte number, mean haemoglobin amount, haematocrit number and mean cellular volume values were found to be higher in Aliağa Refinery station as compared to the other stations. However, the same parameters were found to be less in the samples of Aliağa water harbor and Aliağa Inner harbor. The highest mean cellular hemoglobin concentration (MCHC) was found in the fish samples from Foça station.

A significant increase in the number of RBC stemming from toxic effects was mentioned in an earlier study (Larsson et al., 1984). Similarly, the erythrocyte number of another fish species, *Scyliorchinus canicula,* was increased when the fish was exposed to 15 mg lt^{-1} Cd for a period of 24-96 hours (Tort and Torres 1988). In the erythrocytes of trout apoptotic reaction was observed when it was exposed to the tributyltin toxicity caused by water polution (Tiano *et al*., 2003).

Significant increases in the haemoglobin concentration and the number of the haematocrit were found in *Carassius*

auratus gibelio and they were attributed to the toxic effects of textile dyes (Al-Sabti, 2000). The haematocrite values of *Cyprinus carpio,* which was exposed to different cadmium concentrations, showed differences (Koyoma and Ozaki 1984). MCV and MCHC levels increased in tilapia *Orecohromis mosambicus* when it was exposed to cadmium (Ruperalia *et al*., 1990). Our results agree with those obtained in previous studies.

Table1. Blood parameters values of *Gobius niger* taken from the sampling stations in Foça and Aliağa Bay.

Blood Parameters	Aliağa-water harbor (1.station)	Aliağa-inner harbor (2.station)	Aliağa-Rafinery (3.station)	Foça (4.station)
RBC (x10^6 / μL)	0.7±0.2	1.0 ± 0.3	1.3 ± 0.2	0.9 ± 0.1
Haemoglobin (g/dl)	3.3 ± 0.8	5.3 ± 3.1	5.8 ± 0.3	4.7 ± 0.2
Haematocrit (% V)	6.2 ± 6.6	5.1 ± 5.7	17.6 ± 3.4	10.2 ± 0.7
MCV (fL)	75.9 ± 62.7	87.5 ± 53.2	129.1 ± 10.2	49.7 ± 25.2
MCH (Pg)	46.5 ± 2.5	46.1 ± 6.6	46.8 ± 6.2	49.7 ± 4.9
MCHC (Pg)	37.9 ± 6.8	37.3 ± 5.2	37.3 ± 7.4	45.4 ± 4.2
PLT 10^3 /μl	177.6 ± 184.3	123.4 ± 105	123 ± 64.9	110.6 ± 54.4

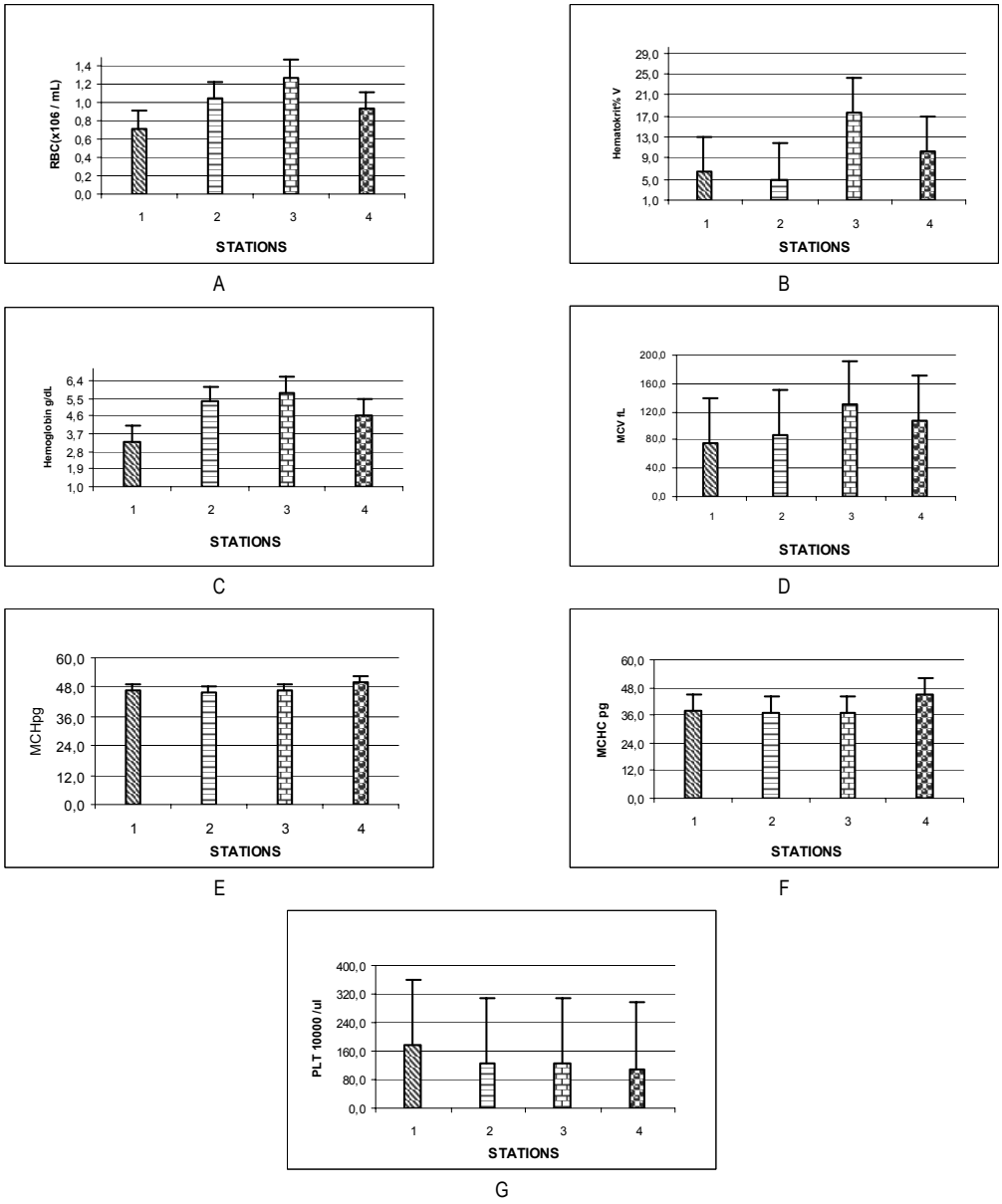

Figure 2. In the samples of *G. niger* catched at the stations of Foça and Aliağa Bays A- Erythrocyte number; B-Haematocrite; C- Haemoglobin concetration; D-MCV concentration; E- MCH concentration; F- MCHC concentration; G- PLT number.

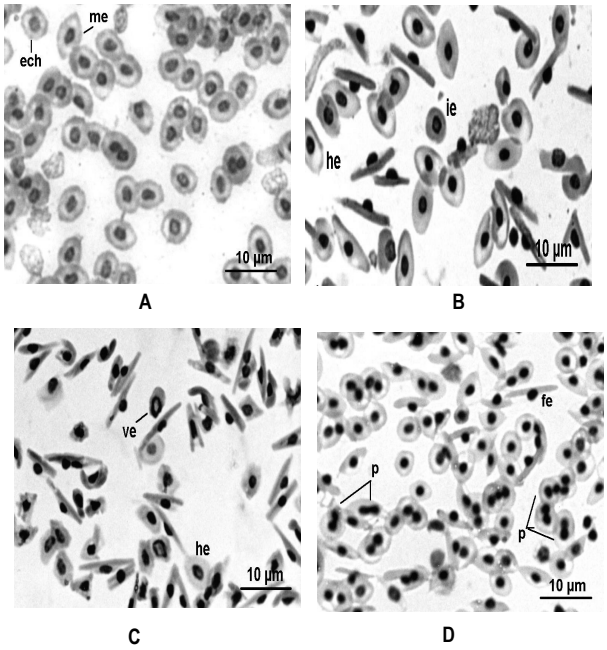

Figure 3. A-Explanations of peripheral blood photographs of *G. niger* catched from Aliağa water harbor coastal area he : hypocromic erytrocyte, ie: immature erythrocyte, fe: fusiform erytrocyte; B- Pictures of peripheral blood of *G.niger* catched from Aliağa Inner harbor coastal arae Ech: echinocyte character, me: erythrocyte with micronuclei; C- Pictures of peripheral blood of *G. niger* catched from Aliağa Rafinery coastal area (fe: erythrocyte with fusiform structure, ve: erythrocyte has vacuola in its cytoplasma he: hypocromic erytrocyte). D- Pictures of peripheral blood of *G.niger* catched from Foça coastal area fe: erythrocyte with fusiform structure. Moreover, in some erythrocytes beginning of prolipheration was observed. P: prolipheration (x 252 and scale is equal to 10µm).

It is known that eryhtrocyte numbers and haemoglobin concentrations show differences depending on the fish species. These differences can even be seasonal. Especially, changes in temperature and oxygen saturation are effective on blood parameters (Lochmiller *et al*., 1989). For this reason, it is really hard to decide if the difference stems from pollution or environmental factors in question. In this study, water quality parameters were determined to show if there are significant differences that can affect the blood parameters. 16.4- 25.3°C (temperature), 37.5$^{0}/_{00}$-39$^{0}/_{00}$ (salinity), 6.91-8.50 (pH), 5.5-10.8 mglt^{-1} (dissolved oxygen), 249-331 mg lt^{-1} (turbidity) were also recorded in Aliağa stations. These results were found to be 18.5-23°C (temperature), 37.5$^{0}/_{00}$–38$^{0}/_{00}$ (salinity), 8.02-8.05 (pH), 10.8-10 mg lt^{-1} (dissolved oxygen), 310-311mg lt $^{-1}$ (turbidity) in Foça station. The data showed no significant differences among the sampling stations. Thus, performing histological observation studies on the peripheral blood tissue has gained utmost importance in determining the effects of environmental pollution.

It was observed that the structure of red blood cells of fish exposed to toxic elements and environmental pollution had deformed and the erythrocytes increased in number (Pacheco and Santos 2002). Due to toxicity, the morphology of red blood cells changed and generally elongated horizontally (Jeney *et al*., 1996). The cell membrane deformations, vacuole occurrence in the cytoplasm of erythrocytes, changes in the nucleus, as well as deformation in nuclei and cytoplasma membrane of *Barbus conchonius* were observed when the fish was exposed to mercuric cloride at regular times (acute treatment) during at a dose of 96h LC_{50}. But, when the fish were left in the mercuric cloride (cronical treatment) poikilocytosis in the erythrocytes, hypochromia, breakdowns in the cytoplasmic membrane and disorders in the nucleous were observed (Tejendra and Jaglish 1985). Similar results were also seen in the samples from different Aliağa Bay stations where the water is polluted due to domestic and industrial factors (Figure 3A, B,C, D).

In the samples from Aliağa Water Harbor and Aliağa Refinery stations hypochromia (loss of color) were observed (Figure 3A, C). In the samples from Aliağa Inner Harbor Station, the cell membrane became spiny. Especially in the samples taken from Aliağa Refinery station where the industrial pollution is the densest, vacuole were detected in the cytoplasm of the erytrocytes. The number of fusiform and spherical erytrocytes increased and cells took a spiny-shape the membrane (Figure 3C). Moreover, in the peripheral blood of the fish samples taken from Foça station, a fusiform shape and a tendency towards in erythrocytes prolipheration was observed in some erythrocytes (Figure 3D). Although no industrial activity was observed in the vicinity of Foça station, it is concluded that the pollution in question may have been assosiated with domestic waste materials containing pesticides and detergent pollution. In a previous study, it has been shown that various organic pollutants directly affect the erythrocyte membrane and change the cell membrane and as a result, the cell membrane become spiny or takes an echinocyte (erythrocyte with spicules) body structure (Nikinmaa, 1992).

Conclusion

Aquatic ecosystems should be protected against all kinds of adverse activities, which may lead to dramatic changes. Studies aimed at determining the effects of various pollutants on the organisms take a long time and require expensive analytic operations. More practical biological tests have many advantages such as saving time and money, and obtaining reliable results and conclusions. As well as the other aquatic organisms, a fish is a very suitable experimental organism for bio tests and toxicity assays.

It is believed that measuring the haematological parameters and especially performing the histological studies of red blood cells, which are used in this study, have both provided valuable information. It may show clearly that how marine fish respond to some changes in water quality parameters in its environment. We also believe that further researches is needed to protect the aquatic ecosystem in Aliağa Bay by means of biological methods.

References

Al-Sabti, K.2000. Chlorotriazine reactive azo red 120 textile dye induces micronuclei in fish: Ecotoxicol Environ Saf., 47: 149-155.

Anonim, 1992. Aliağa Termik Santrali Çevresel Etki Değerlendirmesi ODTÜ Çevresel Etki Değerlendirme Raporu., 160s.

Bhagwant, S., M. Bhıkajee, 2000. Induction of Hypochromic macrocytic Anemia in *Oreochromis hybrid* (Cichlidae) Exposed to 100mg/L (Sublethal dose) of Aliminium . Science and Technology—Research Journal., 5: 9-20.

Blaxall, P. C., K. W. Daisley, 1973. Routine Haematological Methods for Use with fish blood J.Fish Biol., 5: 771-778.

Jeney, Z., E. Valtonen., G. Jeney, E. I. Jokinen, 1996. Effects of Pulp and Paper Mill Effluent (BKME) on Physiology and Biochemistry of the of the Roach (*Rutilus rutilus L*.) Arch. Environ. Contam. Toxicol., 30: 523-529.

Kaya, M., S. Mater, 1994. Invastigation on possible effects of inner harbor mud on Benthic Fish Fauna in İzmir Bay, (in Turkish). E:Ü Fen Fak.. Dergisi Seri B; 16: 367-374.

Koyama, J., H. Ozaki, 1984. Haematological Changes of Fish Exposed to Low Concentrations of Cadmium in the water. Bulletin of the Japanese Society of Scientific Fisheries., 50: 199-203.

Larsson, A., C. Haux, M. L. Sjöbeck, G. Lithner, 1984. Physiological Effects of an Additional Stressor on Fish exposed to a Simulated Heavy-Metal-Containing effluent from sulfice ore smeltery. Ecotoxicol. Environ Safety , 9: 118-128.

Larsson, A., C. Haux, M. L. Sjöbeck, 1985. Fish physiology and metal pollution: results and experiences from laboratory and field studies. Ecotoxicol. Environ Safety., 9: 250-281.

Lochmiller, R. L., J. D. Weichman, A. V. Zale, 1989. Haematological Assesment of temperature and oxygen stress in reservoir population of Striped Bass (*Morone saxasitilis*) Comp. Biochem. Physiol., 93: 535-541.

Nikinmaa, M., 1992. How does environmental pollution effect red cell function in fish? Aquatic Toxicology; 22: 227-238.

Pacheco, M., M. A. Santos, 2002. Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel *(Anguilla anguilla*L .) Ecotoxicol Environ Saf., 53: 331-347.

Roche, H., G. Boge, 1996. Fish Blood Parameters as a Patential tool for identification of stress caused by environmental factors and chemical intoxication. Mar. Environ. Res. 41: 27-43.

Ruparelia, S. G., Y. Verma, S. R. Saiyed, U. M., Rawal, 1990. Effect of Cadmium on Blood of Tilapia, *Oreochromis mosambicus* (Peters), During Prolonged Exposure. Bull. Environment Contam. Tox., 45: 305-312.

Sjöbeck, M. L., C. Haux, A. Larsson, G. Lithner. 1984. Biochemical and Haematological Studies on Perch, *Perca fluviatilis,* from the Cadmium-Contaminated River Eman. Ecotoxicol. Environ. Safe. 8: 303-312.

Tejendra, S. G., C. P Jaglish, 1985. Mercury-Induced Blood Anomalies in the Freshwater Teleost. Water Air and Soil Poll. 24: 165-171.

Tiano, L., D. Fedeli, G. Santoni, I. Davies, G. Falcioni, 2003. Effect of tributyltin on trout blood cells: changes in mitochondrial morphology and functionality Biochim Biophys Acta., 1640: 105-112.

Tort, L., P. Torres, 1988. The Effects of Sub lethal Concentrations of Cadmium on Haematological Parameters in the Dog fish. *Scyliorhinus canicula*. J. Fish Biol., 32: 277-282.