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The Investigation of Heavy Metal Accumulation, Oxidative Status and Hematological Parameters in Common Rudd, *Scardinius erythrophthalmus* (L. 1758) from Bafra Fish Lakes

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Abstract: The use of fish in environmental monitoring has become increasingly important in recent years. In the current study, heavy metal levels (Cd, Co, Ni, Cu, Fe, Se, Zn, Cr, Mn and As) were determined with ICP-OES in some tissues (muscle, liver, gill, gonad and kidney) of *Scardinius erythrophthalmus* L., 1758 from Bafra fish lakes. In addition, antioxidant enzyme activities (CAT, GSH-Px and SOD), Malondialdehyde (MDA) levels and hematological parameters were investigated. Heavy metal results were compared to European Communities, the Turkish Food Codes (TFC) and the literature. As, Cd and Zn levels in some tissues were determined higher than the limits. In conclusion, heavy metal levels in Bafra fish lakes may increase via anthropogenic source in the future. Therefore, heavy metal levels of Bafra fish lakes should be checked and oxidative stress of the fish controlled regularly for food safety and environmental pollutions.

Key words: Heavy metal, oxidative stress, hematology, *Scardinius erythrophthalmus*, Bafra fish lakes

INTRODUCTION

Environmental contaminants can disrupt the normal functioning of a number of fundamental biological processes (Kelly and Janz, 2008). Heavy metals are important sources of environmental pollution and they can form compounds that are toxic even in very low concentrations (Tuzen and Soylak, 2009). Heavy metals from geological and anthropogenic sources are increasingly being released into natural waters. Under certain environmental conditions, heavy metals may accumulate to toxic concentrations and cause ecological damage (Yilmaz *et al*., 2007).

Fish is the final chain of aquatic food web and an important food source for human (Uysal *et al*., 2008). Furthermore, fish is one of the most indicative factors in freshwater systems, for the estimation of trace metals pollution and risk potential of human consumption (Papagiannis *et al*., 2004). For this reason, determination of the chemical quality of aquatic organisms, particularly the contents of trace metals in fish is very extremely important for human health (Dural *et al*., 2007). Metal accumulation can cause an increase in Reactive Oxygen Species (ROS) leading to oxidative stress. Antioxidant defense systems that prevent the formation of ROS and Lipid Peroxidation (LPO) of membrane lipids or the oxidation of polyunsaturated fatty acids have been extensively used as biomarkers of oxidative stress (Ferreira *et al*., 2008).

Bafra fish lakes are located in the lagoons within the delta of Kizilirmak, 20 km away from town center of the South Town of Bafra, province of Samsun in the middle Black Sea region. The maximum depths of the lakes are between 75 cm and 1.5 m. Total surface area in dry seasons is about 2440 ha, rainy seasons is about 9250 ha. These lakes are surrounded by reed. Bafra fish lakes have an economical importance in terms of their aquatic products (Yilmaz and Polat, 2004) (Fig. 1). The main goal of the present study was to evaluate the accumulation of heavy metals and oxidative status in *Scardinius erythrophthalmus* samples. The indicative parameters of oxidative stress Catalase (CAT), Glutathione Peroxidase

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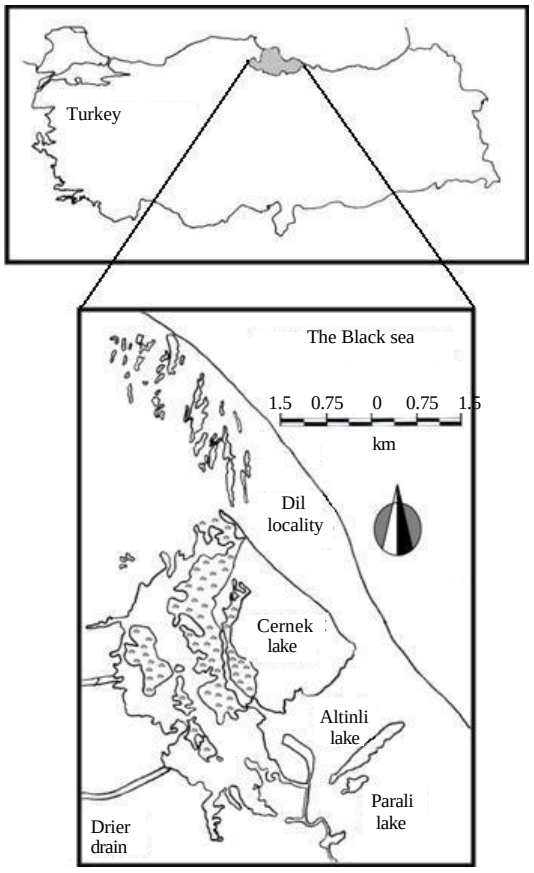

Fig. 1: The map of Bafra fish lakes

(GSH-Px), Superoxide Dismutase (SOD) enzyme activities and Malondialdehyde (MDA) levels were determined. Also, hematological parameters of blood samples were studied.

MATERIALS AND METHODS

The fish samples were collected by fish nets from Bafra fish lakes. Mean body weight and length were 203.83±16.19 g and 23.4±1.02 cm, respectively. Blood was collected from alive fish by tail cutting method after severing of the caudal peduncle (Ezzat *et al*., 1974; Blaxhall and Daisley, 1973) and then fish samples were transported to the laboratory for the dissection. After dissection, tissue samples were weighed and kept at -20°C until experimental assays.

Hematological assay: Blood samples were stored in glass tubes containing anticoagulant (EDTA) and analyses were carried out immediately after sampling. The determination of erythrocytes and thrombocytes (per mm^3) in a blood sample was carried out by pipetting and diluting (1/200) the samples in Hayem solution. One drop of hemolysed blood was transferred onto Thoma lamella and examined under the light microscope (Soif, XZS-107B model) with a magnification of 400x. Leukocyte count was performed by transferring blood samples (diluted in Turck solution) with a leukocyte pipette onto counting lamella and examined as for erythrocytes (Blaxhall and Daisley, 1973; Blaxhall, 1981). The amount of hemoglobin was determined according to the cyan-methemoglobin procedure (Kit 525-A, Sigma Chemical Co.). Non-clotted blood (20 μL) was diluted with 1 mL Drabkin solution and left to stand for 10 min at room temperature. The absorbance of the mixture was read at 540 nm and the amount of hemoglobin was calculated according to hemoglobin standard (Blaxhall and Daisley, 1973). The microhematocrit method was utilized in hematocrit determination (Van Kampen and Zijlstra, 1961). Non-clotted blood was pipetted with a microhematocrit pipette, centrifuged at 12.500 rpm for 5 min and the ratio of blood components in plasma was determined.

Enzyme assays: Tissue for enzyme activity studies was homogenized (PCV Kinematica Status Homogenizator, Littau-Luzern, Switzerland; Bronson sonifier 450, Danburg, CT, USA) in ice-cold phosphate-buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Bronson sonifier 450) by 6 cycles (20 sec sonications and 40 sec pause on ice). The homogenate was centrifuged (15000 g, 10 min, 4°C) and the supernatant was subjected to enzyme assay immediately.

The activities of CAT, GSH-Px and SOD were determined spectrophotometrically. CAT activity was measured at 37°C by following the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm ($\varepsilon 240 = 40\ M^{-1}\ cm^{-1}$) (Luck, 1963). One unit of catalase activity is defined as the amount of enzyme catalysing the degradation of 1 μmol of H_2O_2 per min at 37°C and the specific activity corresponding to transformation of substrate (μmol) (H_2O_2) per min per mg protein. GSH-Px activity was determined in a coupled assay with glutathione reductase by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate (Lawrence and Burk, 1976). The specific activity is given as the amount of NADPH (μmol) disappearing per min per mg protein. SOD activity in the extracts was determined by measuring the inhibition of cytochrome c reduction by 50% using xanthine/xanthine oxidase superoxide generating system at 550 nm (McCord and Fridovich, 1969). One unit of SOD is defined as the amount of protein that inhibits the rate of cytochrome c reduction by 50%.

Lipid peroxidation assay: For lipid peroxidation analysis, tissue was washed three times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The

Table1: The heavy metal concentrations (µg/g wet weight) in tissues of *S. erythrophthalmus* from Bafra fish lakes

Metals	Muscle	Liver	Kidney	Gill	Gonad
Cd	0.092±0.003^{bc} (0.09-0.097)	0.119±0.039^{ab} (0.0825-0.176)	0.128±0.024^{a} (0.0975-0.163)	0.109±0.010^{abc} (0.1-0.125)	0.094±0.004^{bc} (0.09-0.097)
Co	0.177±0.021^{bc} (0.15-0.204)	0.239±0.078^{a} (0.165-0.352)	0.225±0.043^{ab} (0.1725-0.289)	0.161±0.013^{a} (0.142-0.18)	0.187±0.008^{abc} (0.18-0.195)
Ni	0.147±0.058^{a} (0.052-0.21)	0.076± 0.024 b (0.0525-0.112)	0.088 ± 0.017^{b} (0.0675-0.113)	0.056 ± 0.019 b (0.03-0.082)	0.063±0.008^{b} (0.0525-0.073)
Cu	0.365±0.046^{a} (0.3225-0.442)	8.427±2.757^{a} (5.79-12.383)	1.256±0.238^{a} (0.9525-1.598)	0.962± 0.242 c (0.637-1.275)	2.865±0.185^{b} (2.61-3.133)
Fe	4.088±0.599^{a} (3.09-4.59)	14.627±4.787^{b} (10.05-21.495)	12.435±2.362^{b} (9.427-15.821)	15.553±0.270^{b} (15.1-15.8)	5.422±0.888^{a} (4.3725-6.544)
Se	1.552±0.158^{b} (1.373-1.807)	1.635±0.535^{b} (1.125-2.406)	1.492±0.225^{b} (1.17-1.76)	1.016±0.067^{a} (0.95-1.117)	2.344±0.098^{a} (2.243-2.448)
Zn	77.588±18.546^{b} (64.672-109.5)	42.037±13.760^{a} (28.88-61.774)	206.28±39.192^{a} (156.375-262.42)	203.145±21.126^{a} (180.375-235.35)	72.560±7.925^{b} (62.505-83.232)
Cr	0.032±0.009^{b} (0.0225-0.045)	ND	ND	0.083±0.015^{a} (0.067-0.105)	0.044±0.053^{b} (0.0-0.106)
Mn	ND	ND	ND	1.156±0.127^{a} (1.005-1.305)	0.924±0.055^{b} (0.847-1.003)
As	2.071±1.71 (0.0-3.84)	ND	ND	ND	ND

Values in parentheses indicate the minumum and maximum levels. Values with different letters in each line are significantly different at p<0.05 level. ND: Not Detected

homogenates were subjected to lipid peroxidation assay immediately. The analysis of the lipid peroxidation was carried out as described by Buege and Aust (1978) with a minor modification. The reaction mixture was prepared by adding 1 mL homogenate into 4 mL reaction solution (15% trichloroacetic acid: 0.375% thiobarbituric acid: 0.25 N NaOH, 1:1:1, w/v) and heated at 100°C for 15 min. The mixture was cooled to room temperature, centrifuged (10.000 g for 10 min) and the absorbance of the supernatant was recorded at 532 nm. MDA results were expressed as nmol/mg protein in the homogenate.

The protein content of the samples was determined using the colorimetric method of Bradford (1976) using BSA as the standard. All analyses were performed in duplicate.

Heavy metal estimation: For heavy metal determination, wet tissue weight was recorded. After digestion with concentrated nitric and perchloric acid (2:1) samples were brought to a constant volume. The digested samples of tissue were analysed two times for Cd, Co, Ni, Cu, Fe, Se, Zn, Cr, Mn and As against suitable standards in linear range by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP/OES) Optima 2100-DV-Perkin Elmer which is a fast multi-element technique with a dynamic linear range and moderate-low detection limits. Moreover, arsenic measurements were implemented with hydride generation method.

All chemicals and reagents were analytical grade, Merck (Darmstadt, Germany). Standard solutions of metals were prepared by dilution of 1000 ppm certified solution (Inorganic Ventures). Argon gas has been 99.99% purity. The absorption wavelength were 228.802 nm for Cd, 228.616 nm for Co, 231.604 nm for Ni, 327.393 nm for Cu, 238.204 nm for Fe, 196.026 nm for Se, 206.200 nm for Zn, 267.716 nm for Cr, 257.610 nm for Mn, 193.696 nm for As, respectively. The concentrations of heavy metals are expressed as microgram per gram wet weight of tissue.

Statistical analysis: Statistical analysis was carried out using the SPSS 10.0 Statistical Program (SPSS Inc., Chicago, IL, USA). All data were expressed as arithmetic mean±SD. For the analysis of the experimental parameters one-way ANOVA followed by Duncan's multiple range test was used. Value of $p<0.05$ was considered to be statistically significant.

RESULTS

Mean concentrations of heavy metal levels in tissues of *S. erythrophthalmus* from Bafra fish lakes were illustrated in Table 1. Mean concentrations in the muscle, liver, kidney, gill and gonad of were as follows: Zn>Fe>As>Se>Cu>Co>Ni>Cd>Cr; Zn>Fe>Cu>Se> Co>Cd>Ni; Zn>Fe>Se>Cu>Co>Cd>Ni; Zn>Fe>Mn>Se >Cu>Co>Cd>Cr>Ni and Zn>Fe>Cu>Se>Mn> Co>Cd>Ni>Cr, respectively. MDA levels and antioxidant enzyme activities were shown in Table 2. MDA levels in tissues were as gill>muscle>gonad>liver. Mean antioxidant enzyme activities (CAT, GSH-Px and SOD) in tissues of *S. erythrophthalmus* were as follows: gonad>gill>muscle>liver; liver>gill>muscle>gonad and muscle>gill=gonad>liver, respectively. The hematological parameters were given in Table 3.

DISCUSSION

According to EU Commission Regulation (2001) and Turkish Food Codes (TFC, 2002) maximum permissible cadmium, copper and zinc concentrations for fish are 0.1, 20 and 50 mg kg^{-1}, respectively. In all tissues Cu levels, Cd levels in muscle and gonad and Zn levels in liver were determined to be lower than the limits. On the other hand, Cd and Zn levels in kidney and gill were higher than the limits. Moreover, Zn levels in gonad and Cd levels in liver were also determined to be higher than the maximum permissible levels for fish.

The heavy metals accumulate mainly in metabolically active tissues such as liver and kidney (Oymak *et al*., 2009). Moreover, gills are the first organs which come in contact with environmental pollutants (Pandey *et al*., 2008). Metal absorption in fish is carried out via two uptake routes: digestive tract (diet exposure) and gill surface (water exposure). Metals are further transferred via blood to other target organs such as the liver and

Table 2: MDA levels and antioxidant enzyme activities in tissues of *S. erythrophthalmus* from Bafra fish lakes

	Protein			
Tissues	MDA (nmol/mg)	CAT (U/mg)	GSH-Px (U/mg)	SOD (U/mg)
Muscle	1.07±0.19^{b}	1.30±0.43^{c}	4.66±0.53^{c}	3.60±1.05^{a}
Liver	0.50±0.01^{d}	0.76±0.35^{d}	35.24±1.28^{a}	0.41±0.01^{c}
Gonad	0.84±0.02^{c}	5.42±0.14^{a}	0.31±0.01^{d}	1.14±0.17^{b}
Gill	2.21±0.04^{a}	4.29±0.11^{b}	7.49±0.06^{b}	1.40±0.04^{b}

Values with different letters in each column are significantly different at $p<0.05$ level

Table 3: Hematological parameters of *S. erythrophthalmus* from Bafra fish lakes

Parameteres	Concentration
Total leukocyte count (10^3/mm^3)	11.18±0.910
Granulocyte (%)	79.00±8.450
Agranulocyte (%)	21.00±8.450
Erythrocyte count (10^6/mm^3)	0.88±0.200
Hemoglobin (g/dL)	6.98±0.490
Hematocrit (%)	28.00±3.640
Erythrocyte index	
MCV	318.18±1.920
MCH	79.31±0.350
MCHC	24.92±2.065

kidney (Turkmen *et al*., 2009). In the present study, high Cd and Zn levels in metabolically active tissues and high MDA levels in gill are in agreement with the situations afore mentioned.

The permissible arsenic concentration for fish is 1 mg kg^{-1} according to TFC. It is well known that fish muscle is not an active tissue in accumulating heavy metals (Uysal *et al*., 2008). But researchers determined high As levels in muscle. Similar results were obtained in the earlier studies in *Cyprinus carpio* and *Sander lucioperca* from Bafra fish lakes published elsewhere (Kandemir *et al*., 2010; Dogru *et al*., 2011).

Researchers could determine Cr levels in muscle, gill and gonad. There is no record on maximum permissible Cr concentrations in fish tissues in TFC. On the other hand, the maximum permissible Cr levels for fish are reported as 1.0 mg kg^{-1} by Food and Agriculture Organization (FAO, 1983). The Cr levels were under this limit.

There is no record on maximum permissible Co, Ni, Fe, Se and Mn concentrations in fish tissues in TFC. Turkmen *et al*. (2008) reported the literature Co levels in twelve fish species between 0.003-0.67 mg kg^{-1}. Reported Ni levels in literature are in the range of 0.03-0.69, 0.66-1.59, 0.009-0.011 mg kg^{-1} for muscles (Sivaperumal *et al*., 2007; Turkmen *et al*., 2006; Turkmen and Ciminli, 2007) and 0.07-0.10 mg kg^{-1} for livers of fish (Turkmen and Ciminli, 2007). Iron contents in the literature have been reported in the range of 8.87-18.8 mg kg^{-1} (Turkmen *et al*., 2006), 1.49-3.69 mg kg^{-1} (Turkmen and Ciminli, 2007) and 7.16-16.5 mg kg^{-1} (Dural *et al*., 2007) for muscles of fish and 19.5-21.6 mg kg^{-1} (Turkmen and Ciminli, 2007) and 48.1-384 mg kg^{-1} (Dural *et al*., 2007) for livers of fish. Tuzen and Soylak (2007) reported the minimum and maximum Se levels as 0.96-3.64 μg g^{-1}, respectively. Karadede-Akin and Unlu (2007) reported manganese levels in gill and liver tissues of four different fish species in the range of 2.06-26.55 and 0.73-7.08 μg g^{-1}, respectively. In another study, Mn levels were found as 0.89-3.32 mg kg^{-1} (Turkmen and Ciminli, 2007) for livers of fish. The levels of Co, Ni, Fe, Se and Mn in the present study were in agreement with these ranges afore mentioned.

CONCLUSION

This research focused on to provide information on heavy metal concentrations, oxidative status and hematological parameters in *Scardinius erythrophthalmus* (L. 1758) from Bafra fish lakes. Fish are one of the important nutrition sources for mankind and the markers of pollution where they live (sea, lake or stream) (Fidan *et al*., 2008). As researchers have found high levels of As, Cd and Zn in some tissues (muscle, gill, liver, kidney and gonad), studied fish samples may have toxicological effects on human health when these fish are included in the diet. The result of the study supplied information on the metal levels in *S. erythrophthalmus* from Bafra fish lakes. This can be considered a bio-indicator of environmental contamination within this zone. For this reason heavy metal pollution of the lake should be monitored and oxidative stress of the fish controlled regularly for food safety and environmental pollutions.

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