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Determination of Heavy Metal Levels, Oxidative Status, Biochemical and Hematological Parameters in *Cyprinus carpio* L., 1758 from Bafra (Samsun) Fish Lakes

[1]Sevket Kandemir, [2]M. Ilker Dogru, [2]Ibrahim Örün, [3]Arzu Dogru, [4]Levent Altas,
[2]Kenan Erdogan, [5]Gulnihal Orün and [6]Nazmi Polat
[1]Science Teaching Programme, Faculty of Education,
Amasya University, Amasya, Turkey
[2]Department of Biology, Faculty of Science and Letters,
[3]Science Teaching Programme, Faculty of Education,
[4]Department of Environmental Engineering, Faculty of Engineering,
Aksaray University, 68100 Aksaray, Turkey
[5]Vocational School, Aksaray University, 68100 Aksaray, Turkey
[6]Department of Biology, Faculty of Science and Letters,
Ondokuz Mayis University, Kurupelit, 55139, Samsun, Turkey

Abstract: Aquatic ecosystems can be considered as an indicator of health in both animals and humans. In this 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 *Cyprinus carpio* L., 1758 living in Bafra fish lakes. In addition, antioxidant enzyme activities (CAT, GSH-Px and SOD), MDA levels, blood biochemical and hematological parameters were studied. All heavy metal results were lower than the limits prescribed in European Communities and the Turkish Food Codex in muscle tissue, except arsenic. Moreover, some heavy metal levels (Cd, Zn) were higher in other tissues (liver, kidney, gill and gonad) as well. The changes in the other parameters of study were also discussed in terms of heavy metal levels. In conclusion, it is possible that heavy metals levels may increase in fishes living in Bafra fish lakes because of both domestic waste water and agricultural activities. Therefore, we suggest that heavy metal levels should be monitored regularly.

Key words: Heavy metals, oxidative stress, biochemistry, hematology, *Cyprinus carpio*, Bafra fish lakes

INTRODUCTION

Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystems (Oymak *et al*., 2009). Due to their toxicity, long persistence, bioaccumulative and non-biodegradable properties in the food chain, heavy metals constitute a core group of aquatic pollutants (Uysal *et al*., 2008). Different kinds of organisms may be used to determine the mechanisms of action of pollutants on specific physiological functions (Gul *et al*., 2004).

Fishes are often at the top of the aquatic chain and may concentrate large amounts of some metals from the water. 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. Hence, it is important to determine the concentrations of heavy metals in commercial fishes in order to evaluate the possible risk of fish consumption (Yilmaz *et al*., 2007). The accumulation of heavy metals in the tissues of organisms can result in chronic illnesses and cause potential damage to the population. Additionally, exogenous pesticides, heavy metals, chemical mutagens, radiation and various stress factors cause to increase of free radicals and oxidative stress. As a result of increasing this stress, lipid peroxidation, protein denaturation and DNA damages occur in the cells of living organisms. These changes could be a great risk for the organisms' life and productivity (Fidan *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 he, rainy seasons is about 9250 he. 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). In the present study, it was aimed to evaluate the pollution level of Bafra fish lake via determining the accumulation of heavy metals

Corresponding Author: M. Ilker Dogru, Department of Biology, Faculty of Science and Letters, Aksaray University, 68100 Aksaray, Turkey

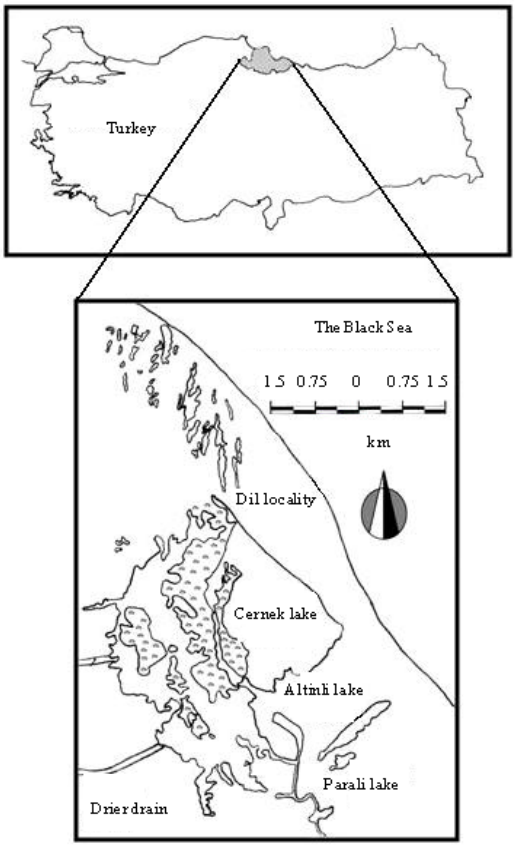

Fig. 1: Map of Bafra fish lakes

and oxidative status in *Cyprinus carpio* samples. The indicative parameters of oxidative stress Catalase (CAT), Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD) enzyme activities and Malondialdehyde (MDA) levels were determined. Also, hematological and biochemical parameters of blood samples were studied.

MATERIALS AND METHODS

The fish samples were collected by fish nets from Bafra fish lakes. 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.

Biochemical assay: Blood samples (approximately 2 mL) were drawn from the caudal vein of each fish. Before collecting blood samples no anesthetic was applied to fish as it may affect blood parameters and cause hemolised tissues. Blood samples were stored in glass tubes containing anticoagulant (EDTA) stored in cooled bags and blood analysis were carried out immediately after sampling. The blood was centrifuged at 3000 g, at 4°C for 5 min. ALP (Alkaline Phosphatase), AST (Aspartate Aminotransferase = SGOT; Serum Glutamic-Oxaloacetic Transaminase), ALT (Alanine Transaminase = SGPT; Serum Glutamate Pyruvate Transaminase) activities and BUN (Blood Urea Nitrogen) total protein, albumin, globulin, sodium, potassium, calcium and chloride levels in blood plasma were assayed by Olympus AU 600 autoanalyser (Olympus Optical Corp., Shizuoka-ken, Japan) using commercially available kits (Roche).

Hematological assay: Blood samples were stored in glass tubes containing anticoagulant (EDTA) and analysis were carried out immediately after sampling. The determination of erythrocytes and thrombocytes (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. Leucocyte 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 (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 six cycles (20-s sonications and 40-s pause on ice). The homogenate was centrifuged (15 000 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/cm) (Lampreht and Trautschold, 1963). One unit of catalase

activity is defined as the amount of enzyme catalysing the degradation of 1 μmol of H_2O_2 min^{-1} at 37°C and the specific activity corresponding to transformation of substrate (in imol) (H_2O_2/min/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 homogenates were subjected to lipid peroxidation assay immediately. The analysis of the lipid peroxidation was carried out as described (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^{-1} protein in the homogenate. The protein content of the samples was determined using the colorimetric method of Bradford using BSA as the standard (Bradford, 1976). All analysis 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 and 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 AND DISCUSSION

The heavy metal concentrations in tissues of *Cyprinus carpio* are presented in Table 1. Maximum cadmium levels are 0.05 and 0.1 mg kg^{-1} according to EU Commission Regulation (2001) and TFC, (2002), respectively. In this study cadmium levels in liver and kidney were found higher than EC and TFC. On the other hand according to FAO (1983) the maximum permissible cadmium levels for fish are 0.05-5.5 mg kg^{-1}. In this study cobalt levels were obtained between 0.155 and 0.249 μg g^{-1}. There is no information about maximum permissible cobalt limits in fish tissues in TFC.

In the study of Turkmen *et al.* (2008) the literature cobalt levels in different fish species were reported between 0.003-0.67 mg kg^{-1}. The cobalt levels are in agreement with the literature. The minimum and maximum nickel levels were 0.045 and 0.081 μg g^{-1} in this study. There is no information about maximum nickel levels in fish samples in TFC.

But it is reported that maximum nickel level in some food samples is 0.2 μg g^{-1}. Reported nickel levels in literature are in the range of 0.03-0.69 mg kg^{-1} for muscles of fish (Sivaperumal *et al.*, 2007), 0.66-1.59 mg kg^{-1} for muscles of fish (Turkmen *et al.*, 2006) 0.009-0.011 mg kg^{-1} for muscles and 0.07-0.10 mg kg^{-1} for livers of fish (Turkmen and Ciminli, 2007). The lowest and highest copper contents were found to be 0.297 and 1.449 μg g^{-1}. The maximum copper level permitted for fish is 20 mg kg^{-1} according to TFC (2002).

Iron levels were ranged from 2.847-22.530 μg g^{-1}. There is no information about maximum permission iron concentrations in fish tissues in TFC.

But the iron results were in agreement with the literature reported as 8.87-18.8 mg kg^{-1} for muscles of fish (Turkmen *et al.*, 2006) and 1.49-3.69 mg kg^{-1} for muscles and 19.5-21.6 mg kg^{-1} for livers of fish (Turkmen and Ciminli, 2007). Selenium concentrations ranged from

Table 1: The heavy metal concentrations (μg g^{-1} wet weight) in tissues of *Cyprinus carpio* from Bafra fish lakes

Metals	Muscle	Liver	Kidney	Gill	Gonad
Cd	0.089±0.003^{c}	0.146±0.025^{b}	0.190±0.012^{a}	0.096±0.002^{c}	0.094±0.004^{c}
	(0.083-0.09)	(0.116-0.177)	(0.173-0.203)	(0.092-0.098)	(0.09-0.098)
Co	0.165±0.005^{c}	0.244±0.014^{a}	0.249±0.017^{a}	0.155±0.006^{c}	0.197±0.035^{b}
	(0.158-0.173)	(0.224-0.263)	(0.225-0.27)	(0.15-0.165)	(0.158-0.248)
Ni	0.081±0.028	0.045±0.018	0.061±0.010	0.053±0.017	0.062±0.060
	(0.052-0.127)	(0.025-0.065)	(0.045-0.07)	(0.03-0.075)	(0.023-0.165)
Cu	0.297±0.075^{b}	1.449±0.450^{a}	1.487±0.141^{a}	0.363±0.026^{b}	0.620±0.195^{b}
	(0.24-0.428)	(0.848-2.055)	(1.282-1.63)	(0.338-0.398)	(0.39-0.863)
Fe	2.847±0.834^{d}	22.530±2.917^{a}	18.564±3.408^{b}	4.721±1.254^{d}	3.894±1.526^{d}
	(2.248-4.248)	(18.92-26.138)	(12.94-22.08)	(3.5-6.6)	(1.59-5.67)
Se	1.689±0.237^{a}	1.061±0.138^{c}	1.094±0.127^{c}	1.421±0.190^{b}	1.669±0.056^{a}
	(1.283-1.883)	(0.892-1.23)	(0.95-1.25)	(1.133-1.62)	(1.59-1.747)
Zn	11.465±2.555^{e}	614.28±52.789^{a}	350.58±26.83^{b}	242.84±9.343^{c}	101.82±64.67^{d}
	(8.31-15.375)	(545.93-682.65)	(305.25-375.0)	(230.0-256.0)	(36.45-173.475)
Cr		0.014±0.030^{ab}		0.010±0.023^{ab}	0.054±0.074^{a}
	ND	(0.0-0.068)	ND	(0.0-0.053)	(0.0-0.15)
Mn		0.060±0.134		0.090±0.128	
	ND	(0.0-0.3)	ND	(0.0-0.278)	ND
As	2.462±0.451^{a}	0.375±0.838^{b}			0.256±0.332^{b}
	(2.0-3.202)	(0.0-1.875)	ND	ND	(0.0-0.81)

Values in parentheses indicate the minumum and maximum levels. Values with different letters in each line are significantly different at $p<0.05$ level

Table 2: MDA levels and antioxidant enzyme activities in tissues of *Cyprinus carpio* from Bafra fish lakes

Tissues	MDA nmol mg^{-1} protein	CAT U mg^{-1} protein	GSH-Px U mg^{-1} protein	SOD U mg^{-1} protein
Muscle	1.47±0.39^{b}	1.20±0.09^{d}	4.17±0.07^{c}	2.68±0.71^{b}
Liver	0.58±0.08^{c}	54.11±0.18^{a}	38.02±9.66^{a}	5.68±0.05^{a}
Gonad	0.79±0.22^{c}	24.97±1.12^{b}	5.90±0.42^{b}	1.51±0.03^{c}
Gill	2.49±0.02^{a}	4.79±0.03^{c}	4.98±0.05^{bc}	5.51±0.14^{a}

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

1.061-1.689 μg g^{-1}. Tuzen and Soylak (2007) reported the minimum and maximum selenium levels as 0.96 and 3.64 μg g^{-1}, respectively. There is no information about maximum selenium levels in fish samples in TFC.

The highest and the lowest zinc contents were found to be 614.28 and 11.465 μg g^{-1}. The maximum zinc level permitted for fish is 50 mg kg^{+} according to TFC (2002). Zinc levels were found to be higher than the both TFC and the levels (reported 30-100 mg kg^{-1} for fish) reported by FAO (1983) in all tissues, except muscle. Moreover, Uysal *et al*. (2008) reported zinc levels ranged from 4.27-339.76 mg kg^{-1} in muscle, skin and gills of different fish species. We couldn't determine chromium levels in muscles and kidneys of fish samples. In the other tissues the lowest and highest chromium concentrations in analyzed fish were 0.010 and 0.054 μg g^{-1}. There is no information about maximum permissible chromium limits in fish tissues in TFC. On the other hand, the maximum permissible chromium levels for fish are 1.0 mg kg^{-1} reported by FAO (1983).

Manganese levels were only detected in liver and gill tissues which were 0.060 and 0.090 μg g^{-1}, respectively. Sivaperumal *et al*. (2007) and Turkmen and Ciminli (2007), reported manganese levels in the range of 0.14-3.36 mg kg^{-1} for muscles of fish and 0.89-3.32 mg kg^{-1} for livers respectively. There is no record on maximum permissible manganese concentrations in fish tissues in TFC. Arsenic levels were determined in muscle, liver and gonad tissues as 2.462, 0.375 and 0.256 μg g^{-1}, respectively. The maximum arsenic level permitted for fish is 1 mg kg^{-1} according to TFC. The muscle results were higher than TFC. MDA levels and antioxidant enzyme activities are shown in Table 2. Mean MDA levels were in tissues were as gill>muscle>gonad> liver. Both CAT and GSH-Px enzyme activities were similar and as follows: liver>gonad>gill>muscle while SOD enzyme activities followed the order liver>gill>muscle> gonad, respectively. Hermatological and biochemical parameters of blood samples of *Cyprinus carpio* are shown in Table 3 and 4, respectively.

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 kidney (Turkmen *et al*., 2009). Metals, such as Fe, Cu, Zn and Mn are essential metals since they play important roles in biological systems. But the essential metals can also produce toxic effects ah high concentrations (Turkmen *et al*., 2009). We have found elevated Cd levels in liver and kidney and Zn levels in all tissues except muscle. The heavy metals accumulate mainly in metabolically active tissues such as the liver and kidney. In these organs, metals are bound to metallothioneins, low molecular weight proteins with high cysteine content

Table 3: Hematological parameters of *Cyprinus carpio* from Bafra fish lakes

Parameters	Mean±SD
Total leukocyte count (10^3 mm^{-3})	13.53±1.21
Granulocyte (%)	83.10±4,38
Agranulocyte (%)	16.90±4.38
Erythrocyte count (10^6 mm^{-3})	1.73±0.28
Hemoglobin (g dL^{-1})	8.34±0.64
Hematocrit (%)	37.93±3.25
Erythrocyte index	
MCV	219.24±1.78
MCH	48.20±0.46
MCHC	21.98±1.95

Table 4: Biochemical parameters of *Cyprinus carpio* from Bafra fish lakes

Parameters	Mean±SD
Glucose (mg dL^{-1})	169.6±7.82
BUN (mg dL^{-1})	2.84±0.45
Creatine (mg dL^{-1})	0.26±0.11
Uric acid (mg dL^{-1})	1.62±0.13
Total Protein (g dL^{-1})	3.28±0.32
Cholesterol (mg dL^{-1})	200.00±48.08
TGS (mg dL^{-1})	102.00±3.16
AST (IU L^{-1})	255.4±18.64
ALT (IU L^{-1})	34.20±1.64
ALP (IU L^{-1})	12.40±2.07
LDH (IU L^{-1})	788.00±78.37
P (mmol L^{-1})	20.88±1.40
Fe (mmol L^{-1})	34.20±2.77
Na (mmol L^{-1})	117.60±5.12
K (mmol L^{-1})	1.02±0.13
Cl (mmol L^{-1})	83.00±5.52
Ca (mmol L^{-1})	11.08±1.23

(Oymak *et al*., 2009). Moreover, gills are the first organs which come in contact with environmental pollutants. Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver (Pandey *et al*., 2008). The antioxidant enzyme activities and MDA levels of gill and liver are in agreement with this situation. While the antioxidant enzymes activities of liver are higher than gill, MDA levels of liver are lower than gill. It is well known that fish muscle is not an active tissue in accumulating heavy metals (Uysal *et al*., 2008). But we found higher arsenic levels in muscle than the liver and gonad. On the other hand, arsenic levels couldn't be detected in kidney and gill. The metal concentration in muscle tissue is important because it is the chief edible part of the fish. The source of As in the fish may result from pesticides and herbicides present in agricultural effluents. For this reason, it is important to evaluate the heavy metal concentrations in Bafra fish lakes continuously.

CONCLUSION

This study was carried out to provide information on heavy metal concentrations in *Cyprinus carpio* from Bafra fish lakes. Except as, all results were below the limits in muscle tissue for fish proposed by EU Commission Regulation (2001), TFC (2002) ant the literature. In this regard, the low metal concentrations found in studied fish samples are in sufficient to have any toxicological effects on human health when these fish are included in the diet. The results of this study supplied valuable information on the metal levels in *Cyprinus carpio* from Bafra fish lakes. This could be considered a bio-indicator of environmental contamination within this zone by estimating the biovailability of metal to the freshwater biota.

RECOMMENDATIONS

It is suggested that heavy metal pollution of the lake should be checked and oxidative stress of the fish controlled regularly for food safety and environmental pollutions.

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