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EVALUATION OF ENVIRONMENTAL POLLUTION AT MUNZUR RIVER OF TUNCELI APPLYING OXIDATIVE STRESS BIOMARKERS IN *CAPOETA TRUTTA* (HECKEL, 1843)

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ABSTRACT

Aim of this study was to determine the extent of pollution in the Munzur River in Turkey was determined. The antioxidant (reduced glutathione (GSH), glutathione peroxidase (GSH-Px) catalase (CAT)) and the oxidant (MDA) status of hepatopancreas, gills, muscle, heart and kidney tissues of *C. trutta* were chosen as bioindicators. The fish samples were caught from contaminated (Station I) and uncontaminated (Station II) stations in Munzur River during April 2010. GSH levels were lower in gill, muscle and heart of the *C. trutta* collected at the contaminated station ($p<0.05$). The MDA levels were significantly higher in gill, muscle, kidney and heart of the *C. trutta* from the contaminated station ($p<0.05$). The CAT activity was significantly increased in the gill tissue at the contaminated station ($p<0.05$). GSH-Px activity was significantly decreased ($p<0.05$) in gill, muscle, kidney and heart tissues samples from *C. trutta* collected at the contaminated station compared to those collected from the uncontaminated station. Overall, the results demonstrate that alteration in the antioxidant enzymes, glutathione system and induction of lipid peroxidation reflects the presence of pollution, which may cause oxidative stress in the *C. trutta* from Munzur River. The results provide evidence those enzymatic and non-enzymatic biomarkers of oxidative stress.

Key words: *Capoeta trutta*; Munzur River; Pollution; Oxidant and antioxidant status.

INTRODUCTION

Aquatic pollution is a major contributor to oxidative stress in fish resulting from the redox cycling of pollution. In addition, it is known that xenobiotic metabolism causes continuous production of reactive oxygen species (ROS) even without pollution (Ahmad *et al.,* 2000). To cope with the continuous generation of ROS from normal aerobic metabolism, cells and tissues contain a series of cellular antioxidants with both enzymatic and non-enzymatic activities (Nordberg and Arner, 2001). To neutralize toxic effects of ROS on fish, like mammals, possess well developed antioxidant defence systems (Almeida *et al.,* 2002; Pandey *et al.,* 2003). This system includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), and glutathione reductase (GR). Also, numerous low-molecular weight antioxidants such as glutathione, b-carotene (vitamin A), ascorbate (vitamin C), and a-tocopherol (vitamin E) can participate in the process of eliminating oxyradicals (Van Der Oost *et al.,* 2003; Yildirim and Asma, 2010). Cellular antioxidant defense systems in biological systems are impaired when exposed to environmental pollutants, but the levels of antioxidants in living organisms can increase in order to restore the imbalance caused by oxidative damage. Levels of antioxidant enzymes can be used as an indicator of the antioxidant status of the organism and can serve as biomarkers of oxidative stress (Livingstone, 2001). When antioxidant defences are impaired or overcome, oxidative stress may produce DNA damage, enzymatic inactivation and peroxidation of cell constituents, especially lipid peroxidation (Halliwell and Gutteridge, 1989). Toxicity biomarkers, such as malondialdehyde (MDA), have been also proposed to reflect the oxidative status of exposed species (Sole *et al.,* 1996). MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation. An increase in MDA levels in organisms can be related to degradation of an environmental site by decreasing the water quality (Charissou *et al.,* 2004).

The level of antioxidant enzymes have been extensively used as an early warning indicator of lake pollution (Lin *et al.,* 2001). Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissue, directly from water through respiration and also through their diet. Fish are frequently subjected to prooxidant effects of different pollutants often present in the aquatic environment (Velkova-Jordanoska *et al.,* 2008). It was determined that the activities of SOD, CAT, GST, glutathione (GSH) concentration and MDA formation as indicators of environmental pollution in african cat fish (*Clarias gariepinus)* from Nigeria Ogun River. They demostrated that alteration in the antioxidant enzymes, glutathione system and induction of lipid peroxidation

reflects the presence of heavy metals which may cause oxidative stress in the *Clarias gariepinus* from Ogun River. (Farombi *et al.* 2007). Box *et al.* (2007) investigated the antioxidant enzyme response of the mussel *Mytilus galloprovincialis* to different degree of pollution. Antioxidant enzyme activities – CAT, GSH-Px, GR, SOD – and MDA concentration were measured in gills and digestive glands of mussels. Antioxidant enzyme activities showed an adaptive response to increase the activities in the more polluted areas. CAT, GR and SOD in gills and CAT and GR in digestive gland presented significant differences between polluted and non-polluted stations. No significant differences were observed in MDA concentration indicating that the antioxidant response is capable to avoid the lipid peroxidation. Yilmaz *et al.* (2006) determined the antioxidant enzymes activities in liver of *Cyprinus carpio* taken from different stations in the Karakaya Dam Lake. CAT and GSH-Px activities were found to be lower in *Cyprinus carpio* in relatively uncontaminated Imikusagi station than in *Cyprinus carpio* in Boran and Hasircilar.

Munzur River (Tunceli) in Turkey has been contaminated by domestic effluents and agricultural products. To obtain relevant results from the field studies, it is absolutely necessary to choose a common fish species that enables the measurement of both biochemical and physiological responses to pollutants. *C. trutta* is one of the most popular fish in the Munzur River and has an economical importance. *C. trutta* is of great commercial importance because it is the most common fresh water fish widely consumed in Tunceli. The aim of this work was to use the response of the antioxidant enzyme activities and the changes in lipid peroxidation of the *C. trutta* as biomarkers of pollution. In the present study, we have investigated the activity of CAT, GSH-Px and GSH concentration and MDA formation in hepatopancreas, gills, muscle, heart and kidney tissues of *C. trutta* in the relatively uncontaminated and contaminated waters in the Munzur River.

MATERIALS AND METHODS

Levels of GSH, MDA, GSH-P_X and CAT were measured in samples of the hepatopancreas, gill, muscle, heart and kidney tissues of *C. trutta* from Munzur River. *C. trutta* were sampled from two stations (Polluted site: 39º 06’ 2.45’’N, 39º 33’ 18.79’’E, Reference site: 39º 07’ 17.55’’N, 39º 30’ 56.18’’E) at Munzur River in April 2010. Captured fishes were placed in plastic bags, and anaesthetized immediately 0.7 g L-1 benzocaine dissolved in ethyl alcohol (Sardella et al., 2004) and observed anesthesia of fish being deep sedation, losing of swimming actions and partial losing of equilibrium (Altun and Danabas, 2006). Then, they transported to laboratory in freezer bags with ice. For biochemical assays, hepatopancreas, gill, muscle, heart, and kidney were removed and stored -80ºC until the assay. The homogenization of tissues was carried out in Glass-glass homogenizer with a buffer contain 1.15 % KCl to obtain 1/10 (w/v) whole homogenate. Homogenates were centrifuged 10 min at 700 g at +4 ºC to determine of GSH, MDA levels and GSH-P_X and CAT activities, 15 min at 10.500 g at +4 ºC for GSH-P_X activities. The supernatants were used for assays. Lipid peroxidation levels (as MDA) in the tissues were measured with the thiobarbituric-acid reaction using methods described by Placer *et al.* (1966). The quantification of thiobarbituric acid reactive substances was determined by comparing the absoption to the standart curve of malondialdehyde equivalents generated by acid catalysed hydrolysis of 1,1,3,3 tetraethoxypropane. The values of MDA were expressed as nmol g^{-1} tissue.

GSH-Px activity in supernatant was measured according to Lawrence and Burk (1976). GSH levels were measured according to Sedlak and Lindsay (1968). CAT activity in supernatant was determined according to the method of Aebi (1984) by monitoring the initial rate of disappearance of hydrogen peroxide (initial concentration 10 mmol) at 240 nm in a spectrophotometer. Results were reported as rate constant per second per milligram protein (k g^{-1} protein). Protein was measured by method of Lowry *et al.* (1951).

Data were expressed as mean ± standard deviation (SE) and analyzed with the SPSS 10.0 software. Independent-samples t test was used for the evaluation of measurement data between Station I and Station II. P values (<0.05) were regarded as statistically significant. Analysis of variance (ANOVA) and Duncan’s multiple Range test was used to compare oxidative stress biomarkers data at all tissues in same stations (Duncan, 1955).

RESULTS AND DISCUSSION

Physico-chemical parameters of water samples from two stations are shown in Table I. The oxidant and antioxidant status of tissues of *C. trutta* were investigated in gill, hepatopancreas, muscle, kidney and heart. As shown in Table II, GSH levels decreased in Station I in gill, muscle and heart tissues ($p<0.05$) when compared to Station II but this decrease was insignificant in hepatopancreas and kidney ($p>0.05$.) MDA levels in Station I were increased in gill, muscle, kidney and heart ($p<0.05$) but in hepatopancreas MDA levels were decreased when compared to Station II ($p>0.05$) (Table II). In gill, heart, kidney and muscle, GSH-Px activty was significantly decreased in Satation I when compared Station II ($p<0.05$) but in hepatopancreas GSH-Px activity increased ($p>0.05$) (Table III). Lenartova *et al.* (1997) investigated antioxidant and detoxifying fish enzymes as biomarkers of river pollution. They indicated that total GSH-Px activity was 1.8 fold higher in the

polluted fish than in reference animals. CAT activity was increased in gill and hepatopancreas but this increase was significant only in gill ($p<0.05$) (Table III). Padmini *et al.* (2008) determined the liver oxidative stress status of grey mullets living in heavy-metal-rich polluted Ennore Estuary compared with unpolluted Kovalam Estuary. CAT was detected in the liver of fish from the polluted estuary (Ennore) compared to fish from the unpolluted estuary (Kovalam) during the summer. Fish living in the polluted estuary had significantly higher lipid oxidation products, conjugated dienes, lipid hydroperoxides, and lipid peroxides than those of the unpolluted estuary during the summer.

Stressful conditions leads to the formation of excessive free radicals which are major internal threat to cellular homeostasis of aerobic organisms. Envionmental stress has been demonstrated to cause an increase in the oxidative stress, an imbalance in the antioxidant status (Yildirim *et al.,* 2010). Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution (Dautremepuits *et al.,* 2004). The present study was designed to determine oxidant and antioxidant status of tissues of *C. trutta*, with a view to using them as potential future biomarkers of reservoir health. The oxidant and antioxidant status of tissues of *C. trutta* were investigated in gill, hepatopancreas, muscle, kidney and heart. Levels of GSH, MDA, GSH-Px and CAT were measured in samples of the hepatopancreas, gill, muscle, heart and kidney tissues of *C. trutta* from Munzur River.

The biological membrane that contains polyunsaturated fatty acid as its major constituent is considered to be the target for the reactive oxygen species-driven oxidation process (Abele and Puntarulo, 2004). The significant increase in lipid oxidation markers may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules. The clear increase in lipid oxidation and its markers may also be due to the decrease in antioxidant enzyme activities. In our study, in general, MDA concentration in Station I (polluted site) were found to be higher than Station II (Reference site) and the highest MDA levels were found in gill, compared to other tissues (Table II). Borkovic *et al.* (2008) suggest that gills exhibit a low-threshold response to oxidative stress, as they are the first tissues to come into contact with water-borne contaminants. The gills are more exposed to contaminated water and as such metal can penetrate through their thin epithelial cells (Nwaedozie, 1998; Gul *et al.,* 2004). Under acute oxidative stress, the toxic effects of the pollutants may overwhelm the antioxidant defenses (Bebianno *et al.,* 2004). Furthermore, the apparent decrease in glutathione detoxification system in the gill, the first point of contact with environmental xenobiotics indicates that this system is a sensitive biochemical indicator of environmental pollution (Kono and Fridovich, 1982). As a result of our

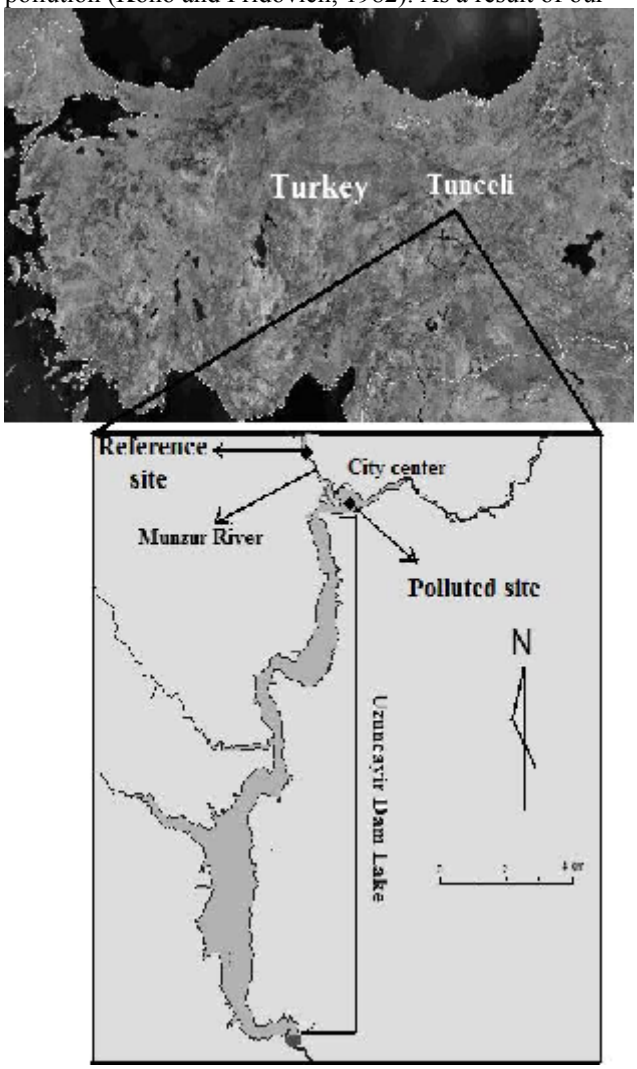

Fig. 1: Map of sampling sites on Munzur River, Tunceli, Turkey

study, GSH levels were decreased in Station I when compared to Station II (Table II). GSH-Px activity was decreased in Station I were found to be higher than Station II. (Table III). Due to altered glutathione redox ratio and hence a decrease in reduced GSH levels, the glutathione-mediated detoxification process may also be affected. This might be a factor responsible for the lack of elimination of toxic compounds that enter the fish and thus result in their accumulation, aggravating oxidative stress (Nammalwar, 1992). Barim *et al.* (2009) suggested that low activity of GSH-Px in gill may demonstrate the inefficiency of this organ in neutralizing the impact of peroxides, resulting in increased LPO. However, decreased GSH-Px activity in the gill may also be related to the de-creased availability of GSH needed to reduce the ROS impact. Long-term exposure of fish to pollutants

may also be a possible reason for the decrease in antioxidant enzyme levels. In the present study, a decline in tissue GSH content during exposure to pollution may be due to an increased utilization of GSH, which can be converted into oxidized glutathione (GSSG), and in-efficient GSH regeneration. However, severe oxidative stress may suppress GSH levels due to the impairment of adaptive mechanisms (Zhang *et al.,* 2004).

Table 1: Physio-chemical parameters of water samples from stations

Physio-chemical Parameters	Station I Polluted site	Station II Reference site
Temperature (^{o}C)	13.00	12.30
pH	6.30	6.60
Turbidity	22.30	14.83
Conductivity (μS cm^{-1})	220	211
Ammonium (mg L^{-1})	0.50	0.40

At the polluted site CAT activity was found to be higher than the references site (Table III). Antioxidant systems may be induced after exposure to pollutants, possibly as an adaptive response to environmental change (Van Der Oost *et al.,* 2003). Increase in the activity of CAT and SOD is usually observed in the face of environmental pollutants since SOD-CAT system represents the first line of defense against oxidative stress (McCord, 1996). The decreased CAT activity may be due to the flux of superoxide radicals, which have been shown to inhibit CAT activity (Stanic *et al.,* 2005) Maintenance of high constitutive levels of antioxidant enzymes like superoxide dismutase and catalase is essential to prevent oxyradical-mediated lipid peroxidation (Lushchak *et al.,* 2001).

This is the first comprehensive report of levels of enzymatic antioxidants (GSH-Px, CAT) non-enzymatic antioxidants (GSH) and MDA in five different tissues (hepatopancreas, gill, muscle, heart and kidney) of *C. trutta* in Munzur River from Turkey. In conclusion, estimation of oxidative stress biomarkers in fish, as in the present study, could provide a useful indicator of pollution of water bodies but oxidative stress is a broad topic. Further studies are needed to be better understanding modulating oxidative stress in fish exposed to pollutants.

Table 2: The mean levels of GSH and MDA in gill, hepatopancreas, muscle, kidney and heart tissues of *Capoeta trutta* from reference and polluted sites

Tissues	GSH (nmol g^{-1} tissue)			MDA (nmol g^{-1} tissue)		
	Polluted site (Station I) n=10	Reference site (Station II) n=10	P values	Polluted site (Station I) n=10	Reference site (Station II) n=10	P values
Gill	1.40±0.06^{d}	1.78±0.02^{c}	0.004*	60.53±8.70^{a}	28.90±7.32^{b}	0.02*
Hepatopancreas	2.35±0.17^{a}	2.38±0.12^{a}	0.86	27.61±4.63^{b}	28.83±3.94^{b}	0.84
Muscle	1.61±0.04^{c}	1.89±0.02^{bc}	0.002*	24.13±4.23^{b}	11.28±2.44^{c}	0.04*
Kidney	1.93±0.08^{b}	2.07±0.01^{b}	0.16	67.44±7.98^{a}	44.73±2.40^{a}	0.04*
Heart	1.72±0.02^{bc}	1.86±0.04^{c}	0.03*	66.30±10.36^{a}	36.78±4.46^{ab}	0.03*

Independent-samples t test was used for the evaluation of measurement data between station I and station II, *P values <0.05 were regarded as statistically significant. Means in the same column followed by different letters are significantly different according to Duncan's test (p<0.05).

Table 3: The mean activity of GSH-Px and CAT in gill, hepatopancreas, muscle, kidney and heart tissues of *Capoeta trutta* from reference and polluted sites

Tissues	GSH-Px (IU g^{-1} protein)			CAT (k g^{-1} protein)		
	Polluted site (Station I) n=10	Reference site (Station II) n=10	P values	Polluted site (Station I) n=10	Reference site (Station II) n=10	P values
Gill	17.42±2.33^{b}	37.10±4.57^{b}	0.009*	17.89±6.43^{b}	0.17±0.01^{b}	0.04*
Hepatopancreas	18.79±2.17^{b}	16.74±2.73^{c}	0.57	397.65±26.91^{a}	327.23±83.27^{a}	0.45
Muscle	37.32±7.97^{a}	72.21±7.74^{a}	0.001**	9.39±4.44^{b}	22.82±4.54^{b}	0.06
Kidney	2.51±0.16^{c}	9.93±2.49^{c}	0.04*	18.52±1.34^{b}	27.06±4.28^{b}	0.11
Heart	21.67±3.30^{b}	61.77±5.88^{a}	0.01*	18.63±2.94^{b}	8.23±3.77^{b}	0.06

Independent-samples t test was used for the evaluation of measurement data between station I and station II, *P values <0.05 were regarded as statistically significant. Means in the same column followed by different letters are significantly different according to Duncan's test (p<0.05).

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