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Use of Biochemical Markers in *Capoeta trutta* (Heckel, 1843) for the Assessment of Aquatic Pollution in Munzur River, Tunceli, Turkey

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The potential utility of biomarkers for monitoring both environmental quality and the health of organisms inhabiting polluted ecosystems has received increasing attention during the recent years. In this study, it was aimed to investigate the possible effect of pollution on biochemical parameters of *Capoeta trutta* captured in two environments with different levels of pollution in Munzur River, Tunceli, Turkey. One of them, hereby named reference site, there is no sewage discharge. Other side is denominated as polluted site is characterized by discharge of domestic sewage. Levels of amylase, alkaline phosphatase, lactate dehydrogenase, high-density lipoprotein cholesterol, cholesterol, albumin, total protein, direct bilirubin, total bilirubin, γ-glutamyl tranferase, aspartate aminotransferase, alanine aminotransferase, urea, creatinine, glucose, triglycerides and uric acid were checked in blood samples. Plasma biochemical changes showed significantly differences between reference and polluted sites in alkaline phosphatase, lactate dehydrogenase, high-density lipoprotein cholesterol, cholesterol, alanine aminotransferase and glucose values. These results indicate that both domestic waste water and agricultural activities can alter the serum biochemical contents of *Copeata trutta* fish in Munzur River. Continuous production of pollutants resulting in impaired ability to respond to different environment stress factors and diseases. Present groups of researchers suggest that pollution levels should be monitored regularly.

Key Words: Biochemical parameters, Water pollution, *Capoeta trutta*, Munzur River.

INTRODUCTION

Increasing environmental pollution and public awareness, have forced scientists to study the direct and indirect effects of the disposal of industrial and other wastes on the aquatic environment[1]. Biomarkers, suitable for the analysis of the chemical toxicity of water, would be particularly useful for studying the physiological disturbances caused in fish by naturally or accidentally modified environmental factors[2]. The exposure to xenobiotic contamination in aquatic ecosystems can have dramatic effects on the inhabiting organisms[3]. Biochemical variables are of fundamental importance in the physiopathological evaluation of animals. Biochemical parameters were often used when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances[4,5]. It is difficult to determine different kinds of organic and inorganic pollutants and their by-products in the environment. Some enzymes are commonly used as key biomarkers of environmental pollution in recent years[6]. Biochemical parameters such as lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase may also be useful biomarkers to determine pollutional effects on fish[7]. Cholesterol is a steroid lipid found in the cell membranes of all body tissues and transported in the blood plasma[8]. Triglycerides are used to evaluate nutritional status, lipid metabolism and its high concentrations may occur with nephritic syndrome or glycogen storage disease[9]. Changes in glucose concentrations are most often associated with renal injury. Plasma concentrations of glucose are regulated by complex interactions of hormones such as glucagons and cortisol[10]. Lactate dehydrogenase is a tetrameric enzyme recognized as a potential marker for assessing the toxicity of a chemical. Lactate dehydrogenase is an important glycolytic enzyme in biochemical systems and is inducible by oxygen stress[11]. Urea and creatinine could be used like good indicator of state of fish population in some water systems especially because they are also indicators of gill and kidney dysfunction, respectively[12]. The rise in creatinine and uric acid might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism[13].

Elevated levels of serum bilirubin suggest liver damage[14]. γ-Glutamyl tranferase has several uses as a diagnostic marker in medicine. Elevated serum γ-glutamyl tranferase activity can be found in diseases of the liver, biliary system and pancreas[15]. Blood serum amylase may be measured for purposes of medical diagnosis. A higher than normal concentration may reflect one of several medical conditions, including acute inflammation of the pancreas. Low albumin (hypoalbuminemia) may be caused by liver disease, nephrotic syndrome, burns, protein-losing enteropathy, malabsorption, malnutrition, late pregnancy, artifact, genetic variations and malignancy. High albumin (hyperalbuminemia) is almost always caused by dehydration[16]. uric acid may be a marker of oxidative stress[17].

Herbivorous fish of the genus *Capoeta* are widely distributed in southern China, northern India, Turkmenistan, Lake Aral, Middle East and Anatolia, inhabiting gravel and stony zones of fast flowing rivers. There are five species and six subspecies of this genus in the inland waters of Turkey. They are the object of commercial and sport fishery[18].

With Uzuncayir Dam Lake in Tunceli, it is aimed to observe especially domestic waste, directly discharged into Munzur River and pollution threat due to the absence of a treatment facility and some prospective and negative changes in the water quality of Munzur River are expected to be observed.

In this study, biochemical parameters of fishes, taken from dam lake area and points that will be located outside the dam area, were analyzed. It was aimed to determine effects of domestic waste water and agricultural activities on some biochemical parameters of *Capoeta trutta* at two stations in Munzur River.

EXPERIMENTAL

The experiment was organized on Aquaculture Department, Fisheries Faculty and Faculty of Engineering, Department of Environmental Engineering on Tunceli University (Tunceli, Turkey).

Locality: The localities were illustrated in Fig. 1. The fish was caught from reference (I) and polluted (II) sites of Munzur River with gill net (20 m length and 10 cm diameter) on 10 April 2010.

Fish: In this study, wild fishes of *Capoeta trutta* (Heckel, 1843) were used for biomonitoring purpose. The fish (for each site 10 ♀ and 10 ♂) was caught from their natural areas in Munzur river (Tunceli, Turkey). These fishes had been anaesthetized immediately 0.7 g/L benzocaine dissolved in ethyl alcohol[19] and observed anesthesia of fish being deep sedation, losing of swimming actions and partial losing of equilibrium[20]. The animal experiments were performed in accordance with the guidelines for animal research from the National Institute of Health and were approved by the Committee of Animal Research at Firat University, Elazig, Turkey.

Blood sampling: Blood samples were collected by piercing in the ventro-lateral side of the caudal peduncle with 5 mL disposable syringe and transferred to gelled and vacuumed tubes (5 mL) for biochemical analyses. The tubes were closed and rinsed to prevent haemolysis and stored in cold until analysis[21].

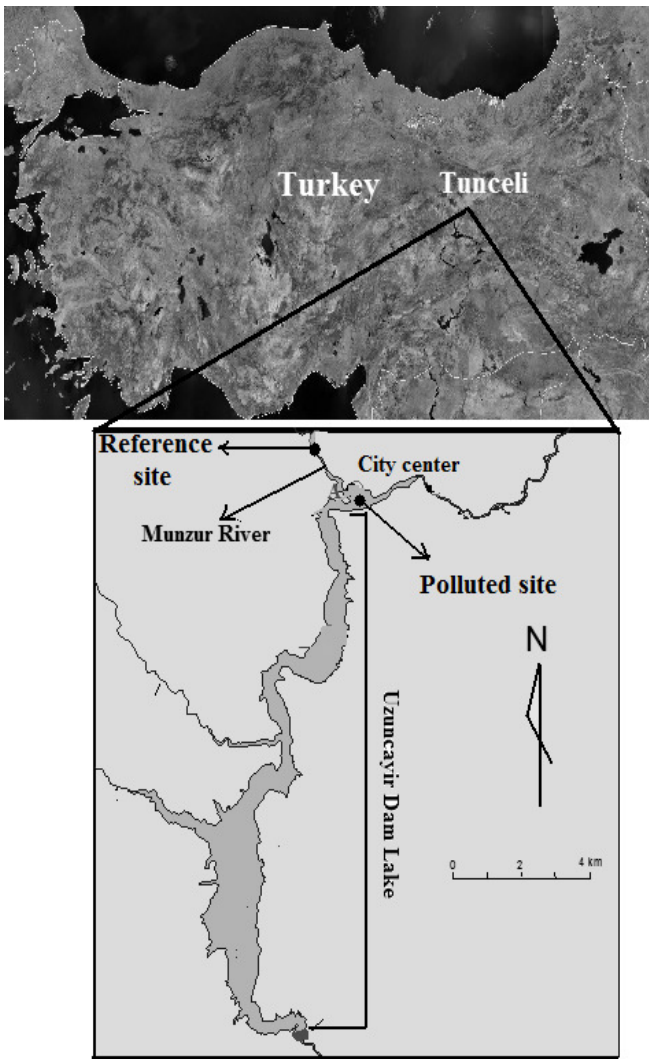

Fig. 1. Map of sampling sites on Munzur River, Tunceli, Turkey

Physico-chemical analyses of water on sampling sites: Temperature and pH of water was measured by pH meter. Water analyses were conducted at Tunceli Public Health Laboratories (Turkey).

Biochemical analyses: Biochemical analyses were conducted at Tunceli National Clinically Laboratories (Turkey). The blood sample was centrifuged at 4000 rpm for 20 min [centrifuge; Universal 320 R (Hettich Zentrifugen)] to separate the serum and analyzed. Amylase, alkaline phosphatase, lactate dehydrogenase, high-density lipoprotein cholesterol, cholesterol, total protein, direct bilirubin, total bilirubin, γ-glutamyl tranferase, aspartate aminotransferase, alanine aminotransferase, creatinine, urea, glucose, albumin, triglycerides and uric acid were measured by kits [lot numbers of kits; E504, E423, E518, HDL100101, E597, E467, F050, F145, F004, F097, F098, E338, E398, F027, E424, E418 and E628, respectively, (purchased Thermo Scientific)] using clinical chemistry analyzer (Konelab Prime 60I).

Statistical analysis: SPSS v13.0 statistical software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Data was statistically analyzed for standard error. Means were calculated and Duncan's new multiple range test was used to compare the sexuality and stations.

RESULTS AND DISCUSSION

Water quality criteria of references and polluted site were shown in Table-1.

TABLE-1
PHYSICO-CHEMICAL PROPERTIES OF WATER SAMPLES FROM SAMPLING SITES

Physico-chemical parameters	Reference site (I)	Polluted site (II)
Temperature (ºC)	12.3	13.0
pH	6.6	6.3
Turbidity	14.83	22.3
Conductivity (µS/cm)	211	220
Ammonium (mg/L)	0.4	0.5

When biochemical parameters were compared regarding sex and station differences; alkaline phosphatase levels were found to be higher for female and male in reference site ($p < 0.05$). Lactate dehydrogenase levels were increased for female in reference site ($p > 0.05$), but for male lactate dehydrogenase levels in polluted site were higher than references site ($p < 0.05$). In polluted site, high-density lipoprotein cholesterol levels were higher for female and male ($p < 0.05$). Cholesterol levels were found to be higher in polluted site for female ($p > 0.05$), but for male cholesterol levels were higher in polluted site ($p < 0.05$). Alanine aminotransferase levels were determined to be higher in polluted site for female and male ($p < 0.05$). Also, No significant variation was observed for amylase, triglycerides, total protein, direct bilirubin, total bilirubin, γ-glutamyl tranferase, aspartate aminotransferase, creatinine and urea (Table-2).

In general, the edible freshwater fishes constitute one of the major sources of nutritious food for humans. Analysis of biochemical parameters could help to identify target organs of toxicity as well as the general health status of animals. It may also to provide an early warning signal in stressed organism[22]. The source of these parameters is the indicators responding to the environmental effects and can also serve as markers for toxicant exposure and effect in fish. Oner *et al.*[23] investigated the effects of environmentally realistic metal exposures on serum biochemical parameters, demonstrated that fish serum could sensitively reflect environmental metal stress and they suggested that serum biochemical parameters could be used as important and sensitive biomarkers in eco-toxicological studies concerning the effects of metal contamination and fish health. Hadi *et al.*[24] investigated that the effects of aluminum chloride were investigated on the biochemical parameters in adult *Tilapia zilli* and indicate that sublethal levels of aluminum can alter the plasma biochemical contents of tilapia fish in acidified waters. Azmat *et al.*[25] determined the heavy metal pollution in marine and fresh water and their acute toxicity and its toxicological affects on survival, physiological and biochemical parameters of the widely consumed fresh water and marine water fishes of Sindh. They show that pollutants act by changing the structural or biological function of bio-indicator.

In this study, blood glucose levels were significantly higher in fish exposed to pollutant as compared to the control groups ($p < 0.05$). The depletion of liver glycogen (glycogenolysis) and the rise in blood glucose levels were reported in *T. zilli* as a consequence of water pollution[26]. Blood glucose levels have long been used as indicators of stress in fish. It is generally thought that, under conditions of stress, hyperglycemia may provide additional energy during times of high metabolic need such as a ' fight or flight ' response[27]. Consequently, we suggest that pollutants affect glucose dynamics in *Capoeta trutta* in order to obtain more energy to withstand and overcome the existing stress condition.

In present study, in polluted site high-density lipoprotein cholesterol levels were higher for female and male ($p < 0.005$). Cholesterol levels were found to be higher in polluted site for female ($p > 0.05$), but for male cholesterol levels were higher in polluted site ($p < 0.05$). No significant variation was observed for triglycerides. The rise of these energy reserves in response to pollution could be due to the fact that excess

TABLE-2
SOME BIOCHEMICAL BLOOD PARAMETERS OF *Capoeta trutta* CAUGHT FROM SAMPLING SITES ON MUNZUR RIVER

Parameter	Polluted site (n = 10)♀	Polluted site (n = 10)♂	Reference site (n = 10)♀	Reference site (n = 10)♂
Amylase (U/L)	396.50 ± 178.82^{a}	356.50 ± 58.36^{a}	383.00 ± 63.00^{a}	421.00 ± 101.25^{a}
Alkaline phosphatase (U/L)	21.50 ± 5.69^{b}	31.50 ± 1.32^{ab}	41.25 ± 1.25^{a}	40.15 ± 6.84^{a}
Lactate dehydrogenase (U/L)	1452.00 ± 464.15^{b}	2604.00 ± 75.12^{a}	1502.00 ± 30.61^{b}	2131.00 ± 408.22^{ab}
High-density lipoprotein cholesterol (mg/dL)	92.22 ± 11.71^{ab}	102.12 ± 6.43^{a}	87.20 ± 2.09^{ab}	72.20 ± 9.14^{b}
Cholesterol (mg/dL)	163.75 ± 27.77^{ab}	195.25 ± 8.01^{a}	146.25 ± 9.66^{ab}	124.25 ± 6.04^{b}
Albumin (g/dL)	1.30 ± 0.08^{a}	1.20 ± 0.04^{a}	1.05 ± 0.06^{a}	1.00 ± 0.14^{a}
Total protein (g/dL)	3.02 ± 0.27^{a}	3.30 ± 0.22^{a}	2.85 ± 0.15^{a}	2.75 ± 0.48^{a}
Direct bilirubin (mg/dL)	0.75 ± 0.36^{a}	0.83 ± 0.16^{a}	0.50 ± 0.23^{a}	1.17 ± 0.51^{a}
Total bilirubin (mg/dL)	1.53 ± 0.69^{a}	1.55 ± 0.35^{a}	1.38 ± 0.40^{a}	2.05 ± 1.15^{a}
γ-Glutamyle tranferase (U/L)	10.00 ± 2.44^{a}	12.25 ± 8.64^{a}	13.00 ± 3.43^{a}	8.25 ± 4.76^{a}
Aspartate aminotransferase (U/L)	40.75 ± 9.81^{a}	43.75 ± 2.95^{a}	44.50 ± 6.66^{a}	48.50 ± 8.31^{a}
Alanine aminotransferase (U/L)	124.25 ± 25.19^{a}	129.00 ± 3.5^{a}	44.50 ± 3.47^{b}	65.50 ± 16.19^{b}
Creatine (mg/dL)	0.32 ± 0.05^{a}	0.35 ± 0.06^{a}	0.27 ± 0.05^{a}	0.25 ± 0.05^{a}
Urea (mg/dL)	6.75 ± 0.25^{a}	6.00 ± 0.40^{a}	6.75 ± 0.85^{a}	7.00 ± 0.81^{a}
Glucose (mg/dL)	123.50 ± 19.34^{a}	112.75 ± 38.17^{ab}	95.75 ± 25.77^{ab}	50.25 ± 12.84^{b}
Albumin (g/dL)	1.30 ± 0.08^{a}	1.40 ± 0.07^{a}	0.97 ± 0.05^{a}	1.27 ± 0.41^{a}
Triglycerides (mg/dL)	156.00 ± 15.94^{a}	152.25 ± 11.79^{a}	176.00 ± 56.25^{a}	222.25 ± 56.62^{a}
Uric acid (mg/dL)	1.30 ± 0.44^{a}	1.70 ± 0.24^{a}	1.67 ± 0.70^{a}	1.65 ± 0.71^{a}

Mean values with different letters in the same line are significantly different ($p < 0.05$), SE = Standard error.

energy reserves (as glucose, triglycerides and cholesterol) are required by organisms to mediate the effects of stress[28]. Since homeostasis of lipids is one of the principal liver functions, any change in serum triglycerides concentration is used as an indicator of liver dysfunction[29].

Several reports have revealed decreased lactate dehydrogenase activity in tissues under various pesticide toxicity conditions[11]. This might be due to the higher glycolysis rate, which is the only energy-producing pathway for the animal when it is under stress conditions. In recent work, lactate dehydrogenase levels were increased for female in reference site ($p > 0.05$), but for male lactate dehydrogenase levels in polluted site were higher than references site ($p < 0.05$).

The quantity of protein is dependent on the rate of protein synthesis or on the rate of its degradation. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains[20,30]. In present study, there was no significant variation was observed for total protein.

Aspartate aminotransferase is normally found in a diversity of tissues including liver, heart, muscle, kidney and brain. It is released into serum when any one of these tissues is damaged. Alanine aminotransferase is, by contrast, normally found largely concentrated in liver and is released into the bloodstream as the result of liver injury. The increase in serum aspartate aminotransferase and alanine aminotransferase accompanied by decrease of alkaline phosphatase enzyme activity is related to the intensity of cellular damage due to chemical-induced cellular alteration varying from simple increase of metabolism to death of cell[31]. When biochemical parameters were compared regarding sex and station differences; alkaline phosphatase levels were found to be higher for female and male in reference site ($p < 0.05$). Alanine aminotransferase levels were determined to be higher in polluted site for female and male ($p < 0.05$). No significant variation was observed for aspartate aminotransferase. Abou El-naga *et al.*[32] investigated toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish *Mugil seheli*. It was showed that plasma enzymes (aspartate aminotransferase and alanine aminotransferase) were greatly affected of exposure to copper than cadmium. Glucose recorded high values of exposure to cadmium rather than copper. Plasma lipids and protein were affected by copper more than cadmium. Muscle glycogen, lipids and protein were affected by copper more than cadmium. Tilak *et al.*[33] investigated the effects of alachlor on biochemical parameters of the freshwater fish, *Channa punctatus* (Bloch). They show that the glycogen, total proteins, were all decreased but the activity of the enzymes aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase were all increased which is due to the toxic stress.

In present study, when amylase, creatinine, urea, uric acid, albumin, total bilirubin, direct bilirubin and γ-glutamyl tranferase parameters were compared regarding sex and station differences, there was no significant variation found ($p > 0.05$). Pollution affects physiological status of *Capoeta trutta* leading to increased susceptibility to infection. It can conclude that biochemical parameters could be ranked as possible biomarkers of pollution.

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