

## The Determination of Biochemical Indicators (Biomarkers) in the Common Carp (*Cyprinus carpio*) to the Physico-chemical Parameters of the Ceyhan River (Adana-Turkey)

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### Abstract

In this study, the levels of pollution in the Ceyhan River (Turkey), subjected to agricultural and industrial pollution, and the effects of these pollutants on the gill and liver tissues, used as biomarkers of *Cyprinus carpio* were analyzed. Water and fish samples were taken from the polluted region of the Ceyhan River (station II; polluted region) and from the area under the crest of the Aslantaş Dam (station I; control region) located on the same river. The research was carried out during the summer months and 40 fish from each region were studied. The physico-chemical parameters indicated that the water at station II had higher chemical oxygen demand (COD), nitrate, nitrite, pH, and soluble reactive phosphorus (SRP) than at station I. The biomarkers examined in the liver and gill tissues of the carp were superoxide dismutase (SOD), catalase (CAT), glucose-6-phosphate dehydrogenase (G6PD) as well as glutathione (GSH) and lipid peroxidation (LPO). The activities of CAT, G6PD, GST and GSH were observed by the high levels in the liver tissues of the fish in the polluted region. Also, SOD and LPO amounts ( $P < 0.05$  in gill and liver) were also detected as substantially high in the contaminated region.

**Keywords:** Biochemical indicators, Ceyhan River, Common carp (*Cyprinus carpio*), oxidative stress, physico-chemical parameters, pollution.

### Ceyhan Nehri (Adana-Türkiye)'nin Fiziko-Kimyasal Parametrelerine Karşı Doğa Sazan (*Cyprinus carpio*)'nda Biyokimyasal İndikatörler (Biyomarkerlar)'in Belirlenmesi

#### Ozet

Bu çalışmada, zirai ve endüstriyel kirliliğe maruz bırakılmış Ceyhan nehri'nin, kirlilik seviyeleri ve bu kirliliğin *Cyprinus carpio*'nun karaciğer ve solungaç dokularındaki çeşitli biyomarkerlar üzerindeki etkileri incelenmiştir. Su ve balık örnekleri, Ceyhan Nehri'nin kirli bölgesi (istasyon II; kirli bölge) ve aynı nehir üstünde bulunan Aslantaş Barajı (istasyon I; kontrol bölgesi)'nin kret altından alınmıştır. Araştırma yaz ayları boyunca devam etmiş ve her çalışma bölgesinden 40 balıkta çalışılmıştır. İstasyon-II deki fiziko-kimyasal parametrelerden kimyasal oksijen ihtiyacı, nitrit, nitrat, pH ve çözülmüş reaktif fosfor, istasyon I dekinden daha yüksek bulunmuştur. Sazanın karaciğer ve solungaç dokularındaki biyomarkerlarından Süperoksit Dismutaz (SOD), Katalaz (CAT), Glukoz-6-Fosfat Dehidrogenaz (G6PD), İndirgenmiş Glutasyon (GSH) ve Lipid Peroksidasyon (LPO) analiz edilmiştir. CAT, G6PD, GST ve GSH aktiviteleri, kirli bölgedeki balıkların karaciğer dokularında yüksek seviyelerde gözlenmiştir. Ayrıca SOD ve LPO miktarları (karaciğer ve solungaç için  $P < 0,05$ ) da kirli bölgede oldukça yüksek bulunmuştur.

**Anahtar Kelimeler:** Biyokimyasal indikatörler, Ceyhan Nehri, Doğa sazanı (*Cyprinus carpio*), fiziko-kimyasal parametreler, kirlilik, oksidatif stres.

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### INTRODUCTION

The generation of oxyradicals has a connection to the aetiology of several human diseases and is probably the same for fish (Gül et al. 2004). Apart from the normal metabolism in a living organism; carcinogens (pesticides, heavy metals), infections (bacterial, parasitical, viral, etc.), radiation damage, and environmental stress factors cause an increase in

free oxygen radicals and thus cause oxidative stress (Şahan et al. 2003, Karaytuğ et al. 2007). Biomarkers are defined as a change in a biological response, ranging from molecular to cellular and from physiological responses to behavioral changes, which can be related to the toxic exposure or to the toxic effects of environmental chemicals (Depledge et al. 1995, Cicik 2003). On the other hand,

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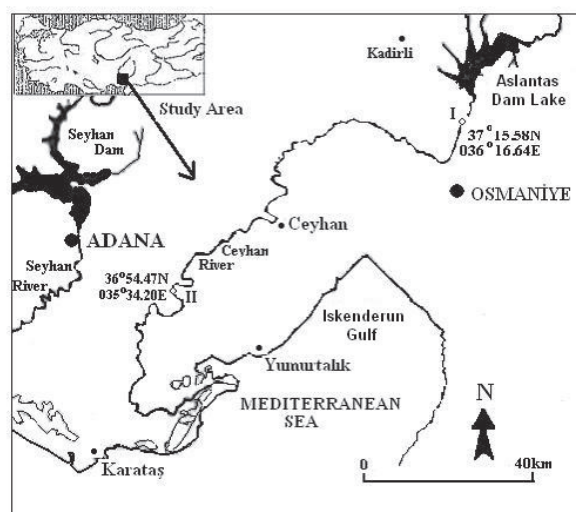
biomarkers are responses to environmental effects that occur at higher levels of the biological organization than sub-organism, and can be measured in the individual, population, community (primary production, disruption of the nutrient cycle), and ecosystem levels (Walker et al. 2001, Oertel and Salánki 2003). Recent findings show that the pollution toxicity in an aquatic organism may be connected to increased production of "reactive oxygen species" (ROS) that leads to oxidative stress (Kurutas et al. 2009). In this perspective, a number of studies confirmed the successful role of antioxidant enzymes and non-enzymatic antioxidant modulation in identifying environmental stress. In addition, LPO estimation in particular has also been found to have a high predictive importance as seen from a credible number of research papers describing its suitability as a biomarker (Ahmad et al. 2004, Santos et al. 2004, Ahmad et al. 2005). This study aims to reveal the oxidative stress response in the liver and gill tissues of carp collected from the polluted regions and also to identify that antioxidants are significant indicators of peroxidative damage.

Moreover, the suitability and sensitivity of fish oxidative stress biomarkers for the early detection of the health of the fresh water ecosystem was evaluated.

#### MATERIAL AND METHODS

**Area of Study:** The present study was carried out in the summer season (June, July and August) in the Ceyhan River because the pollution reaches its peak during this period (Yilmazer and Yaman 1999). Water and fish (*Cyprinus carpio* L. 1758) samples were obtained from two regions on the Ceyhan River. The regions were chosen because of the agricultural and industrial activities of the region. The first region was just under the crest of the Aslantas Dam on the Ceyhan River, where it was thought to be low in pollution (station I). The second region was receiving discharges from the agricultural and industrial areas and thought to be highly polluted (station II)(Fig. 1).

**Physico-chemical analysis:** Water samples were taken three times during the summer months with three repetitions. After that the samples were transported to the Water Quality and Chemistry Laboratory, Faculty of Fisheries, at Çukurova University in labeled and deep colored bottles (1 L) (in a  $\pm 4^{\circ}\text{C}$  carrying case). Then, the collected water



**Fig 1.** Sampling Regions on the Ceyhan River, Osmaniye-Adana, Turkey. Station I: Unpolluted (Control) Region, Station II: Polluted Region.

samples from the station I and station II were analyzed for some physico-chemical characteristics (Table 1) such as pH, nitrite ( $\text{NO}_2\text{-N}$ ), nitrate ( $\text{NO}_3\text{-N}$ ), ammonia ( $\text{NH}_3\text{-N}$ ), soluble reactive phosphorus (SRP), and chemical oxygen demand (COD), using procedures described in Anonymous (1998). The results of the water quality parameters were evaluated according to the criteria of the Anonymous (1996).

**Fish samples:** Forty fish (*Cyprinus carpio* L. 1758) samples, taken three times with three repetitions, were caught alive by using fishing line and an extension net from the crest of the Aslantas Dam Lake (station I) and the polluted region of the Ceyhan River (station II). Then, the fishes were transported in a conveying tank (270 L) reinforced with oxygen to the Fish Diseases Laboratory, Faculty of Fisheries at Çukurova University. The fish were anaesthetized using quinaldine (Sigma Chemical Company) at a dose of 20 mL/L, the weight of each fish was measured by using a balance with a sensitivity of 0.001g (Sado 1985). The mean body weight and total length of fish were respectively measured as  $158.82 \pm 41.64$  g and  $26.07 \pm 1.94$  cm for station I and  $121.82 \pm 18.78$  g and  $21.80 \pm 2.19$  cm for station II.

**Biological indices:** Once, the fish arrived at the laboratory, their body weight and length were determined and their liver and gills were excised. The condition factor (CF) was calculated by the formula of  $\text{CF} = \text{body weight (g)} \cdot [(\text{length (cm)})^3]^{-1}$

$\times 100$  and liver somatic index (LSI) (or liver-body weight rate) by  $LSI = [\text{liver weight (g)} \cdot \text{body weight (g)}^{-1}] \times 100$  (Bagenal and Tesch 1978, Sloof et al. 1983).

$CF = \text{body weight (g)} \cdot [(\text{length (cm)}^3)^{-1}] \times 100$

$LSI = \text{liver weight (g)} \cdot [\text{body weight (g)}^{-1}] \times 100$

Preparation of liver and gill homogenates: The liver and gill tissues of the fish were rapidly removed and frozen in a dry ice-refrigerated container. Tissue samples were immediately excised, weighed, mixed with 1.15% ice-cold KCl, and then homogenized in five volumes (w/v) of the same solution, using a Heidolph 50110 R2R0 homogenizer. The antioxidant systems and LPO assays were performed on the supernatant preparation in a Sorvall RC-2B and the centrifugation of the homogenate at 14,000 rpm for 30 min at +4°C (Gül et al. 2004).

Biomarker measurements: The G6PDH, CAT, and GSH levels were analyzed using the method of Beutler (1975) and the SOD activity was investigated according to Fridovich (1974). This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol-5-phenyl)tetrazolium chloride to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction. The GST activity was measured at 340 nm using 1 mM of 1-chloro-2,4-dinitrobenzene as the substrate (Mannervik and Guthenberg 1981). The LPO level was determined using the method of Ohkawa et al. (1979), which is based on the intensity of the colour after the treatment of the sample with TBA. The protein levels in the tissue samples were determined using Lowry's method (Lowry et al. 1951).

Statistical analysis: The statistical analysis of data was accomplished using an unpaired, one tailed Student's 't' test. The significance of the results were ascertained at  $P < 0.05$ .

## RESULTS

Water Quality: Table 1 shows the values of the physico-chemical parameters of both the Ceyhan River and the Aslantaş Dam water samples. The temperature, pH, SRP,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_3\text{-N}$ , and  $\text{NO}_2\text{-N}$  parameters of station II were found to have almost two or three-fold higher mean values when compared to station I values.

Enzyme profile: The mean SOD activities in the gill tissue of fish at station II was found to be significantly higher ( $P < 0.05$ ) when compared to

**Table 1.** Water quality parameters in the sampling regions on the Ceyhan River.

Physico-chemical parameters	Station I	Station II
Temperature (°C)	21.06±7.4*	26.8±7.5
pH	8.10±0.08	7.21±0.02
$\text{NO}_3\text{-N}$ (mgL <sup>-1</sup> )	0.55±0.01*	1.26±0.01
$\text{NO}_2\text{-N}$ (mgL <sup>-1</sup> )	0.007±0.002*	0.04±0.01
$\text{NH}_3\text{-N}$ (mgL <sup>-1</sup> )	0.25±0.20*	0.45±0.02
SRP (mgL <sup>-1</sup> )	0.001±0.001*	0.03±0.01
COD (mgL <sup>-1</sup> )	8.81±3.16*	37.75±6.41

Values are expressed as mean ±SD. \* $P < 0.05$  when compared with values at station II

station I. The activity of CAT in the liver tissues of the fish from station I was found to be higher than that in station II. However, the activity of CAT in the gill was found to be significantly low in station II ( $P < 0.05$ ) but, the activity of CAT was higher in the liver. The liver tissue collected from the fish in station II showed significantly higher GST values ( $P < 0.05$ ). While in the gill, the GST values were lower in the fish collected from station II ( $P < 0.05$ ) than that of station I. The activity of G6PD was found to be significantly higher in the liver of fish at station II but lower in the gills of fish from station II ( $P < 0.05$ ) (Table 2).

Reduced glutathione level (GSH): The values of GSH in the liver of fish from station II were significantly high ( $P < 0.05$ ) when compared to the values of the liver samples of fish from station I. On the other hand, the GSH values in the gill were lower in the fish collected from station II ( $P < 0.05$ ) when compared to the values in the fish collected from station I (Fig. 2).

Lipid peroxidation levels (LPO): The mean values of LPO in the liver and gills of fish *C. carpio* collected from station II were found to be higher when compared to the respective values from the same tissues of fish at station I. Particularly, in the gill tissue, the LPO level was found to be about eight-fold higher at station II than that at station I ( $P < 0.05$ ) (Fig. 3).

Physiological indexes: In this study, CFs were found in fish collected from both sites. The CF of the fish living in station II ( $1.04 \pm 0.15 \text{ g/cm}^3$ ) was lower than that of the fish in station I ( $1.30 \pm 0.15 \text{ g/cm}^3$ ). The LSI of fish living in station II ( $1.65 \pm 0.65$ ) was significantly higher than their counterparts in station I ( $0.65 \pm 0.14$ ).

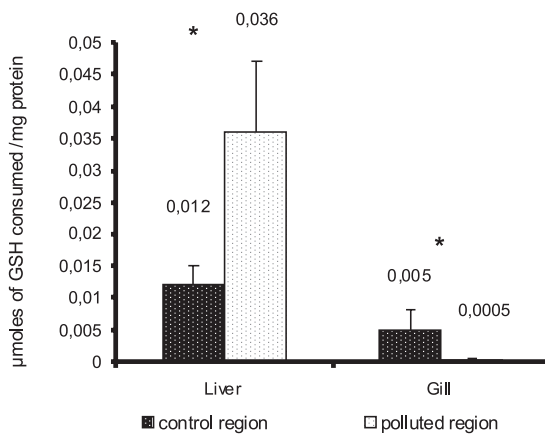
## DISCUSSION

The Ceyhan area has high agricultural potential

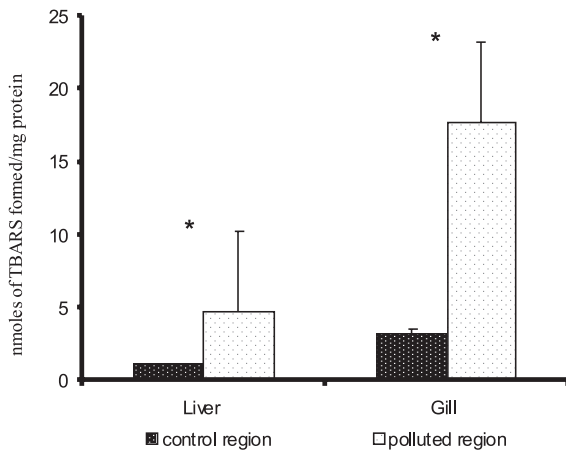
**Table 2.** Responses of oxidative stress biomarkers at the two stations in the liver and gill tissues of *C. carpio*

Biomarkers	Liver Tissue		Gill Tissue	
	Station I	Station II	Station I	Station II
SOD(U/mg protein)	2.69±0.70	3.62±0.41	1.13±0.16*	5.05±0.71
CAT(U/mg protein)	56.0±14.2*	176.2±27.2	3.40±0.66	0.99±0.22
GST(U/mg protein)	93.0±11.5*	359.7±88.9	8.95±1.06	1.95±0.87
G6PD(U/mgprotein)	0.0015±0.0005*	0.010±0.004	0.00016±0.00005	0.00002±0.00001
GSH(μmol/mgprotein)	0.012±0.003	0.036±0.011	0.005±0.003	0.0005±0.0002
LPO(nmol/mgprotein)	0.93±0.10	4.04±0.41	2.96±0.53	16.09±4.09

Values are expressed as mean ±SD. \*P<0.05 when compared with values at station II



**Fig 2.** GSH (μmol of GSH consumed /mg protein) in the liver and gills of *C. carpio*. Significant level: \*P<0.05.



**Fig 3.** LPO (nmol of TBARS formed/mg protein) in the liver and gills of *C. carpio*. Significant level: \*P<0.05.

and the river has been densely exposed to agricultural activities for almost four seasons. Because of its being under the effect of the Mediterranean climate, summer season in this area have high temperatures. In the study, the water quality parameters are, in general, at acceptable levels considering the criteria given in Anonymous (1998). The limits of nitrogen compounds were

found to be higher than the ones defined for 1st Class quality. Ammonia and nitrate nitrogen values in this study were harmonious with the results of Yilmazer and Yaman (1999). Ammonia and nitrate nitrogen values of our study as well as their results may be the indicators of the negative impacts of agricultural and industrial activities on the water quality of the Ceyhan River. In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg/L (Anonymous 1996). In this study, the SRP (soluble reactive phosphorus) average of station II exceeded (0.03±0.01 mg/L) that level. The water quality parameters of the Ceyhan River except for station I showed that the river is affected by the agricultural and industrial activities and discharge from domestic sources.

One of the features of antioxidant enzymes is their induction under conditions of oxidative stress, and such induction can be an important adaptation to pollutant-induced stress (Livingstone 2001).

SODs are a group of metalloenzymes that play a crucial antioxidant role and constitute a defense system against natural and chemical pollutants. An increase in SOD activity in the gill was found in the present work, and the higher SOD activity in the gill than in the liver could be an indicator of compensatory tissue response to pollution exposure. Oruc et al. (2004) also showed higher SOD activity in the gill compared to other tissues as a result of pollution.

The higher hepatic CAT activity in the gills of fish at station II, observed in this work, suggests that a metabolic increase was triggered to cope with polluted (metals or industrial waste)-induced oxidative stress. The high CAT activity may be a response to increased H<sub>2</sub>O<sub>2</sub> production to protect biological systems (Ritola et al. 2002).

The effects of pollutants on the GST activity have been somewhat inconclusive, showing induction, no change, or inhibition of this enzyme (Stephensen et al. 2000). Some studies showed that exposure to pollutants can lead to an increase of hepatic GST activity (Şen and Kırıkbakan 2004, Camargo and Martinez 2006). The values of hepatic GST activity of leaping mullet (*Liza saliens* Risso 1810) are similar to those found in the present work (Şen and Kırıkbakan 2004). In our study, the GST activity was found about three hundred-fold in the liver of the Common carp (*C. carpio*) at station II (polluted site) when compared to the gill. A positive

relationship between liver and gill CAT activities were found, which may reflect a reinforced response to oxidative stress in the liver.

The increased G6PD activity in the liver tissue demonstrates an increased production of NADPH for the detoxification process. This probably reflects an adaptation to oxidative conditions to which fish have been exposed (Lenartova et al. 1997). However, low gill G6PD observed in *C. carpio* at station II indicates that the low G6PD activity in pollution may aggravate oxidative stress. Therefore, oxidative damage in gill tissue may be present.

GSH, in addition to being a necessary cofactor for GST activity, is itself an effective protectant capable of quenching oxyradicals (Ross 1988). In our study, the higher hepatic glutathione concentration observed in *C. carpio* which is living in a pollution environment at station II indicates an adaptive and protective role for GSH, against oxidative stress induced by chemical contaminants. Similarly, Di Giulio et al. (1993) observed high levels of GSH in the catfish exposed to polluted sediment. The usefulness of fish glutathione has been reported by several researchers (Rodriguez-Ariza et al. 1993, Hasspielar et al. 1994). However, the decrease in GSH content observed in the gill tissues of fish at station II may be due to insufficient glutathione regeneration. The increase in LPO level, determined in this study, has been regarded as the result of the contamination. It is advisable to use a set of related biomarkers since an individual biomarker can provide a complete diagnosis of the impact of contaminants on fish.

Biological index parameters are often used in field toxicological studies to assess the general condition of fish. CF is a physiological index

parameter indicative of the health status of the whole body of the fish related to the environmental availability of food. In our study, the CF of fish living at station II was lower than those living in station I. That result indicates that *C. carpio* may be able to adjust themselves to low oxygen levels and poor food environments, although polluted water would not allow fish to access sufficient nutritional status. Van der et al. (2003) observed an increase in the LSI values in fish caught at contaminated sites; Our results readily agree with this trend. The increase in LSI of fish from station II may be interpreted as a consequence of the liver enlargement secondary to exposure to pollutants due to a compensatory proliferation processes (William and Iatropoulos 2002). An enlarged liver is not a disease but a sign of an underlying problem. The liver tissues in fish are more frequently propounded as an environmental indicator of water pollution than any other organs (Yilmaz et al. 2006). Yilmaz et al. (2006), have studied the metabolic organs of various fish species such as the liver, which stores heavy metals like Zn and Cu in order to prevent detoxification.

In conclusion, the present investigation suggests that oxidative stress biomarkers, especially estimation of antioxidant systems in fish could provide a useful indicator of the pollution of water bodies. The induction of antioxidant systems except for SOD (as observed in liver), as well as their inhibition (as observed in gill) should be considered as a clear indication of the presence of pollution and environmental health degradation. Measurement of LPO, which has been described as a biomarker of the effect of pollution in several studies, was also revealed as a useful indicator of the pollution load in the present study.

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