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The Effect of Pollution on the Arginase Activity in Tissues of Freshwater Crayfish (*Astacus leptodactylus* Esch. 1823) in the Keban Dam Lake

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ABSTRACT: In the present study, the effects of pollution on the arginase activity in the tissues of *Astacus leptodactylus* in the Keban Dam Lake were investigated. The arginase activity was found to be higher in the hepatopancreas ($p<0.05$) and gill ($p<0.001$) tissues at the contaminated station as compared to that of the uncontaminated one. No significant difference in the levels of arginase in muscle was observed between the stations. However, the arginase activities at the contaminated station were found to be statistically higher in the hepatopancreas ($p<0.001$), gill ($p<0.01$) and muscle ($p<0.05$) of female crayfish, as well as in the gill ($p<0.001$) of male crayfish.

Key Words: *Astacus leptodactylus*, Arginase, Keban Dam Lake, Pollution.

Keban Baraj Gölü'ndeki Tatlısu İstakozlarının (*Astacus leptodactylus* Esch. 1823) Dokularındaki Arginaz Aktivitesi Üzerine Kirliliğin Etkisi

ÖZET: Bu çalışmada, Keban Baraj Gölü'nde bulunan *Astacus leptodactylus*'un dokularındaki arginaz aktivitesi üzerine kirliliğin etkisi araştırıldı. Arginaz aktivitesi, temiz bölge ile karşılaştırıldığında kirli bölgedeki kerevitlerin hepatopankreas ($p<0.05$) ve solungaç ($p<0.001$) dokularında daha yüksek bulundu. Kastaki arginaz seviyesinde istasyonlar arasında hiçbir fark gözlenmedi. Buna ilave olarak, kirli bölgedeki dişi kerevitlerin hepatopankreas ($p<0.001$), solungaç ($p<0.01$) ve kasında ($p<0.05$), erkek kerevitlerin ise solungacındaki ($p<0.001$) arginaz aktivitesinin istastistiksel açıdan daha yüksek olduğu tespit edildi.

Anahtar Kelimeler: *Astacus leptodactylus*, Arginaz, Keban Baraj Gölü, Kirlilik.

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INTRODUCTION

Crustaceans are generally used as bioindicators in various aquatic systems (Borković et al., 2007). Since increasing varieties of industrial, agricultural and other chemicals are entering into the aquatic environment, they are taken up into the tissues of invertebrates and vertebrates (Livingstone, 2001). Therefore, biological investigations are necessary to analyze the health status of populations in ecosystems. Reddy et al. (1988), Ozan et al. (1993) and Lee and Chen (2004) reported that one of the biochemical parameters affected with pollution was the arginase enzyme. Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) catalyses the hydrolysis of L-arginine to form L-ornithine and urea in the final reaction of the urea cycle (Powers and Meister, 1982). Arginase enzyme is also found in organs and organisms not synthesizing the urea (Aminlari and Vaseghi, 1992). Although crayfish are ammonotelic organisms and do not contain an active urea cycle, they have an active arginase enzyme (Erişir et al., 2006).

Keban Dame Lake is one of the largest dam lakes of Turkey, and it has been established for irrigation purposes and production of electrical energy. Its area and volume are about 687.31 km^2 and 30.6 milliard m^3, respectively (Anonymous, 1994). *A. leptodactylus* is one of the commercial species in the reservoir. But, the production of *A. leptodactylus* dramatically decreased (from 5000 tons to 200 tons annually) in the most Turkish Lakes after the year 1985 (Harlıoğlu, 2004). As the human population is ever increasing, it means that less crayfish per capita will be available every year.

This study was designed to investigate whether the pollution affect the arginase activity of freshwater crayfish (*Astacus leptodactylus*, Esch. 1823) or not. Our present research represents the first comprehensive report of arginase activity in tissues of *A. leptodactylus* collected from the Keban Dam Lake.

MATERIALS and METHODS

The arginase activities were measured in the samples of hepatopancreas, gill and muscle (in abdomen) of crayfish from the Keban Dam Lake (Elazıg, Turkey). The samples used were provided from the two stations (control station (station I): 38° 49^1 44 77^{11}N-39° 16^1 19 11^{11}E, contaminated station (station II): 39° 0^1 11 12^{11}N-38° 53 48^1 99^{11}E) at the lake in August 2007. Presumably, the pollution was originated from the high amount of heavy metal in wastewaters discharged from the Leather Factory (Agın). The amount of some heavy metals (Cu, Fe, Cr, Cd, Ar, Ni) in polluted site (Ağın, Elazığ, Turkey) has been found a hundred times higher than that of the control site (Canpolat, 2007). The control site was Aydıncık, Elazığ, Turkey. Crayfish captured with net were placed in plastic bags and transported to the laboratory in freezer bags with ice. The carapace length (50.08±0.41 mm) and weight (30.88±0.93 g) were recorded for each crayfish. The muscle, gills and hepatopancreas tissues of the crayfish were surgically removed and stored at –80 ^oC for biochemical assays.

Arginase activity was measured spectrophotometrically in optimised conditions for crayfish (Hartenstein, 1971) by the thiosemicarbazide diacetylmonoxime urea (TDMU) method of Geyer and Dabich (1971). One unit of arginase activity was expressed as the amount of enzyme catalysing the formation of one μmol of urea h^{-1} at 37 ^oC. The results are given as units/mg of protein. Protein was measured by the method of Lowry et al. (1951), using bovine serum albumin as standard.

The results were expressed as mean ± SEM. All the statistical analyses were performed with Analysis of Variance (ANOVA) followed by Duncan's test and Independent-Sample T Test by using SPSS 15 computer program. Differences were considered statistically significant when $p<0.05$ (Özdamar, 2001).

RESULTS and DISCUSSION

In the present study, the highest arginase activity was found in the tissues of hepatopancreas and gill as compared to that of the muscle tissue ($p<0.01$) (Figure 1). The hepatopancreas, gills and muscle were chosen for the study because the hepatopancreas are the main digestive gland and responsible for regulating the overall metabolism of the body (Muriana et al., 1993). Gills are

the major organs of biotransformation and respiration (Borković et al., 2007). However, the tissues of hepatopancreas and gills in the crustacean are more often considered as an environmental indicator of water pollution than any other organs (Borković et. al., 2007). Regarding the arginase activities measured in the hepatopancreas of 15 species of marine invertebrate, the highest activities have been found in the crustacea (Hanlon, 1975; Erişir et al., 2006). In some crustaceans, the arginase activities have been determined in the midgut, gill, muscle and hemolymph as well as in the hepatopancreas (Reddy, et.al., 1988; Lee and Chen, 2004; Erişir et al., 2006).

The results of the present study illustrate that the arginase activity in the tissues of hepatopancreas and gills was significantly higher (45.57% ($p<0.01$), 87.18% ($p<0.001$), respectively) at the station II as compared to that of station I. The arginase activity in the muscle of crayfish in the contaminated area (Agın) was found

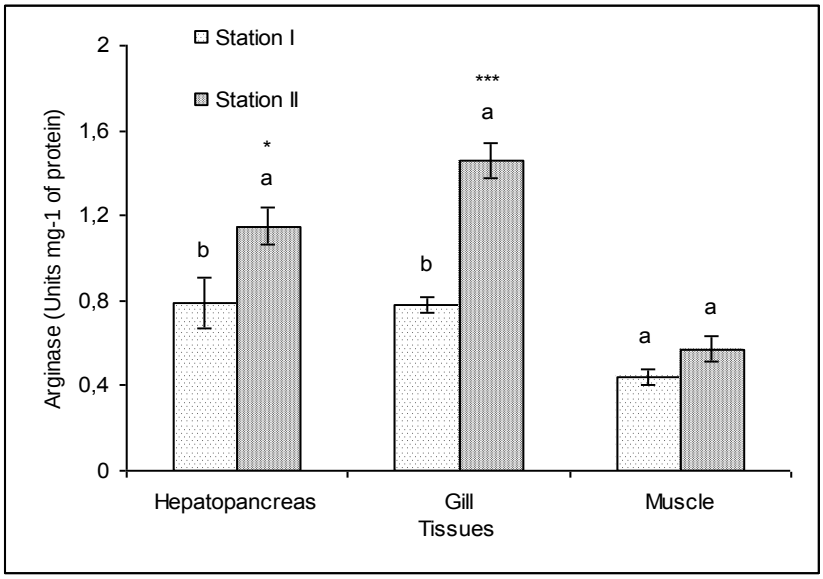

Figure I. Comparison of the mean levels of arginase in the tissues of hepatopancreas, gills and muscle of samples from different stations (Note: *= $p<0.05$, ***= $p<0.001$).

Şekil I. Farklı mevkilerden alınan örneklerin hepatopankreas, kas ve solungaç dokularındaki ortalama arginaz seviyelerinin karşılaştırılması.

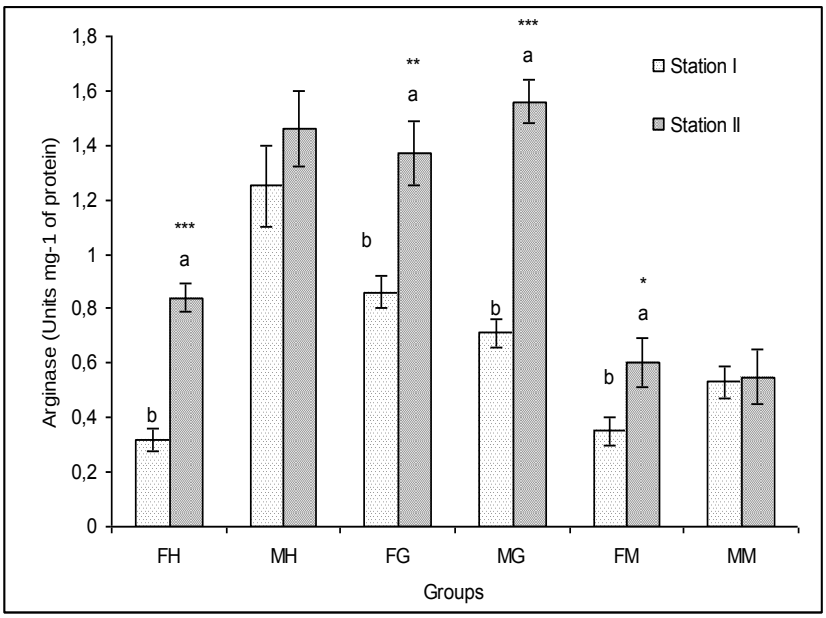

Figure II. The comparison of the mean levels of arginase according to tissues of the same gender groups of different stations (MH: The hepatopancreas of male crayfish, FH: The hepatopancreas of female crayfish, MG: The gill of male crayfish, FG: The gill of female crayfish, MM: The muscle of male crayfish, FM: The muscle of female crayfish) (Note: *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$).

Şekil II. Farklı mevkilerden alınan aynı cinsiyetin dokularının ortalama arginaz seviyelerinin karşılaştırılması. (MH: erkek kerevit hepatopankreası, FH: Dişi kerevit hepatopankreası, MG: Erkek kerevit solungacı, FG: Dişi kerevit solungacı, MM: Erkek kerevit kası, FM: Dişi kerevit kası), (Not: *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$).

relatively higher (29.55% ($p>0.05$) than that of the uncontaminated area (Aydıncık). However, the differences between the parameters were not statistically significant (Figure 1). Lee and Chen (2004) determined that the arginase specific activities increased directly in hepatopancreas and hemolymph of the kuruma shrimp (*Marsupenaeus japonicus*) exposed to pollution. Similarly, it has been determined that the arginase activities increased in the midgut gland, muscle and gill of penaeid prawn (*Penaeus indicus*) exposed to pollution (Reddy et al., 1988). In addition, Ozan et al. (1993) found that the difference in the arginase activity in several tissues of *Capoeta trutta* was statistically significant when the values from the uncontaminated and contaminated stations, in Keban Dam Lake- Turkey, were compared. Ozan et al. (1993) determined high arginase activity in gill, muscle, heart and kidney tissues of fish in contaminated station. Reddy et al. (1988) and Lee and Chen (2004) reported that the exposure of crustaceans to environmental pollution triggered the ammoniagenesis and activated alternative metabolic pathways such as an elevation in arginase activity to avoid from toxicity of ammonia.

The levels of arginase of *A. leptodactylu*s collected from both the station I and the station II was also compared in the same gender groups (male at the station I -male at the station II, female at the station I - female at the station II). The arginase level was found to be higher in the gill tissues (119.72%, $p<0.001$) of male and in the hepatopancreas (162.5%, $p<0.001$), gills (59.30%, $p<0.01$) and muscle tissues (71.41%, $p<0.01$) of female crayfish at the station II compared to station I (Figure 2).

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