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Lead bioaccumulation in gill, muscle and hepatopancreas tissues of Mediterranean green crab, *Carcinus aestuarii* (Nardo, 1847).

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ABSTRACT

This study was conducted to determine lead bioaccumulation in gill, muscle and hepatopancreas tissues of the Mediterranean Green Crab, *Carcinus aestuarii* (Nardo, 1947) which exposed to sublethal concentrations of lead in vivo effect for 14 days. Lead accumulation in tissues of *C.aestuarii* was observed as gill>muscle>hepatopancreas in the end of 7th day and as gill>hepatopancreas>muscle in the end of 14th day. As a result of test, it was found that lead bioaccumulation in tissues of *C. aestuarii* that was exposed to different concentrations of lead (0.025; 0.05; 0.1 and 1.0 mg L^{-1}) was increased in line with the ambient lead concentration of and time of exposure. In addition, the most accumulation was determined in gills.

Introduction

Recently, metal contamination in aquatic systems are gradually increasing in consequences of natural and anthropogenic effects such as mining activities, agricultural applications, domestic and industrial waste discharges. Besides, coastal urban centers are important contaminant sources for aquatic environments (lp et al., 2005). Lead which is a non-essential metal for the metabolisms, can be obtained in different ways by the organisms. This toxic metal increases towards higher levels through the food chain. Thus, it accumulates tissues and organs, may cause structural and functional impairments in cellular or molecular levels, and even mortality (Rainbow and Wang, 2001).

Various types of aquatic macro invertebrates such as crustaceans have the potential for accumulation of metals within the body in different amounts according to their concentration in waters, whether they are essential or not (Rainbow, 2002). Environmental alterations that occur in waters may cause negative effects on benthic macro invertebrates which are in the third level of the food chain after phytoplankton and zooplankton. Therefore they constitute a significant part of fish feed as planktonic organisms (Cairns and Pratt, 1993).

Crustaceans can be used as monitors to give information on metal concentrations. *Carcinus aestuarii* (Nardo, 1847) that is widely spread around the Turkish coastal waters have been used as a model organism to determine metal accumulation and internal metal handling of decapod crustaceans due to living in estuarine systems and adaptability to environmental conditions (Bondgaard et al., 2000). A few studies were reported on the lead accumulation in benthic macro invertebrates, although there is no research about lead accumulation in different tissues of *C. aestuarii*. In this study, it has been aimed to determine the lead accumulation in different tissues (gill, muscle and hepatopancreas) of *C. aestuarii*, that was exposed to sublethal concentrations of lead.

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Material and methods

Individuals of *Carcinus aestuarii* (n=90 adult) were collected from Umurbey Stream estuary area (N 40°17'02" and E 26°35'22") near Umurbey, Çanakkale. They were adapted to environmental conditions for a month in the stock aquariums, each of them filled up with 20 lt filtered water (transferred from Umurbey Stream). They were separated to 15 aquariums (45x28x80 cm) to be six crabs in each and experimental design was established with three replications. During the test period crabs were exposed to different concentrations of lead as; control, 0.025, 0.05, 0.1 and 1 mg L^{-1} for 14 days. $Pb(NO3)_2$ (Merck) has been used for the preparing concentrations. Toxicity test has been designed as semi-static and water exchange was done for two times in a day to be ¾ of it in the morning and ¼ of it at night. Physico-chemical parameters of filtered water that is used intest were measured with YSI MPS 556 probe (temperature, salinity and dissolved oxygen) and HANNA C200 (HI 83200) photometer (pH) every day. Values were kept constant as 25°C±2 temperature, 19‰±1 salinity and 7.8±0.2 pH.

Sampling was carried out for 3 times to be in first day (T0), 7th day (T1) and 14th day (T2) in the test period. Then, in T1 and T2 days two crabs were taken from each aquarium to analyze lead in tissues. Tissues that were removed by dissection were dried at the incubator set to 100°C for 24 hours after measuring the wet weight. Subsequently, dry weights of samples were determined. Next, samples were burned over a hot-plate set to 70°C for 2 hours,

following the addition of 5 ml HNO_3. After the samples were burned homogenously and cooled, they were filtered in a 0.45 mm syringe and completed to 25 ml with distilled water (Anonymous, 1994). Finally for metal analysis in tissues has been determined with ICP-OES Varian Liberty Sequential.

Statistical Assessment

Values were expressed as mean±standard error (X±SE) for each parameter measured. Statistical assessment of results was carried out using Minitab 13. The data sets were analyzed by three-way Anova and Duncan multiple comparison test by using Mstat software. Between group variance was evaluated as $p<0.05$ (Logan, 2010).

Results

In this study, alterations depend on time and concentration were determined in *C. aestuarii* tissues which have been exposed to different concentrations of lead (0, 0.025, 0.05, 0.1 and 1 mg L^{-1}) in vivo effect for 14 days. Metal accumulation in crab's tissues (gill, hepatopancreas and muscle) which exposed to sublethal concentrations of lead has given in Table 1.

It has been indicated that, lead accumulation in gill tissues were increased depending on the concentration and time. Minimum (10.78 µg g^{-1}) and maximum (118.75 µg g^{-1}) accumulation in gill tissues were determined on T1 in C1 and on T2 in C4, respectively. Lead accumulation in gill tissue was very close to each other in C1 and C2, both on T1 and T2 days and there were no statistically significant differences ($p>0.05$). Lead accumulation in gill tissues has increased gradually from T0 up to T2. However, the difference between C4 and other doses were significant ($p<0.05$) (Table 1).

Lead accumulation in hepatopancreas has been increased depending on the concentration and time. Minimum and maximum accumulations were determined as 3.30 µg g^{-1} on T1 day at C1 and 35.53 µg g^{-1} on T2 at C4, respectively. There were non-significant differences between the accumulation of all of the doses, that crabs has been exposed on T1 ($p>0.05$). In this case, increase of lead accumulation in hepatopancreas tissue by increasing doses on T2, was significant ($p<0.05$) (Table 1). Minimum and maximum lead accumulations in muscle tissue were determined as 9.45 µg g^{-1} on T1 at C1 and 30.28 µg g^{-1} on T2 at C4, respectively. Lead accumulations of C1, C2 and C3 in muscle tissues were non-significant ($p>0.05$), although lead accumulations of C4 in muscle tissues, both on T1 and T2 were significant ($p<0.05$).

In 14 days toxicity test period, the differences between gill tissue and other tissues at C4 were significant ($p<0.05$). In addition the differences between muscle tissue and other tissues at C3 were significant ($p<0.05$). Consequently bioaccumulation of lead in tissues was determined on T1 day as gill>muscle>hepatopancreas and on T2 day as gill>hepatopancreas>muscle (Table 1).

Discussion

Metal accumulation in crustaceans can be realized in two routes; either via hepatopancreas during feeding or via gills (Silverstone et al., 2004). Metal accumulation in crab tissues were depends on the physiologic and endogeny factors (e.g. sex, age, tissue) and the external environmental factors (e.g. depth, salinity, temperature and concentration) (Rainbow, 2002). In this study an endogenous factor (tissue) and an external factor (concentration) have been investigated.

In aquatic organisms, gill plays an active role while uptaking metals to their bodies, due to the fact of the direct contact with water (Khan and Nugegoda, 2003). However, Torreblanca et al. (1989) have been reported high levels of lead accumulation in *Procambarus clarkii*, with the fact of resulting a structural defect on gill filaments. Also, high levels of lead accumulation in gill tissues were obtained in the present study. In other study, conducted with crabs collected from Manila Bay, high levels of lead and cadmium accumulation in gill tissues beside muscles were repertod (Su et al., 2009). Results of the present study indicate similarity with other studies (Canlı and Stagg, 1996; Khan and Nugegoda, 2003; Barrento et al., 2009).

Metals absorbed from gills have been transported to the tissues, especially liver via hemolymph as bounded to the carrier proteins. In crustaceans, function of liver tissue is undertaken by hepatopancreas. This organ has an important role in uptaking, detoxification and elimination of metals. In addition, it is the major organ that synthesizes metallothioneins (MT) (Bagatto and Alikhan, 1997). Many studies have shown that, metal elimination in hepatopancreas takes place more slowly beside muscle tissue (Tulasi et al., 1992; Cinier et al., 1999; Kalay and Canlı, 2000). In the present study, hepatopancreas was found to accumulate minimum lead along with muscle tissues. This can be explained by having not enough evidence about MT ability (Reichert et al., 1979; Roesijadi and Robinson, 1994). Essential metals in crustacean tissues such as Zn and Cu should be regulated actively,

Table 1. Pb accumulation (µg g^{-1} d.w.) in crab's tissues at sublethal concentrations (Three Way Anova/Duncan multiple comparison test).

		0 mg L^{-1}	0.025 mg L^{-1}	0.05 mg L^{-1}	0.1 mg L^{-1}	1 mg L^{-1}
Tissue	**Time**	**Control (X±SE)**	**C1 (X±SE)**	**C2 (X±SE)**	**C3 (X±SE)**	**C4 (X±SE)**
Gills	T0 (0. day)	2.38 ± 0.46^{AaI}	2.38 ± 0.46^{BaI}	2.38 ± 0.46^{BaI}	2.38 ± 0.46^{BaI}	2.38 ± 0.46^{CaI}
	T1 (7. day)	3.21 ± 0.23^{AbI}	10.78 ± 4.18^{BbI}	13.11 ± 4.93^{BbI}	14.52 ± 4.50^{BbI}	87.72 ± 6.30^{BaI}
	T2 (14. day)	2.43 ± 0.12^{AcI}	25.99 ± 4.47^{AbI}	29.25 ± 10.90^{AbI}	33.86 ± 11.29^{AbI}	118.75 ± 13.23^{AaI}
Hepatopancreas	T0 (0. day)	2.99 ± 0.55^{AaI}	2.99 ± 0.55^{AaI}	2.99 ± 0.55^{AaI}	2.99 ± 0.55^{BaI}	2.99 ± 0.55^{BaI}
	T1 (7. day)	2.10 ± 0.19^{AaI}	3.30 ± 0.25^{AaI}	4.11 ± 0.53^{AaI}	11.76 ± 1.55^{BaI}	14.68 ± 3.21^{BaII}
	T2 (14. day)	2.35 ± 0.32^{AbI}	5.72 ± 2.90^{AbII}	8.23 ± 0.91^{AbII}	33.07 ± 12.23^{AaI}	35.53 ± 8.38^{AaII}
Muscle	T0 (0. day)	5.97 ± 0.59^{AaI}	5.97 ± 0.59^{AaI}	5.97 ± 0.59^{AaI}	5.97 ± 0.59^{AaI}	5.97 ± 0.59^{AaI}
	T1 (7. day)	6.03 ± 0.31^{AbI}	9.45 ± 0.74^{AbI}	12.43 ± 1.05^{AabI}	13.16 ± 4.76^{AabI}	23.97 ± 2.16^{AaII}
	T2 (14. day)	4.64 ± 1.18^{AcI}	12.26 ± 2.85^{AbcII}	12.81 ± 4.13^{AbcII}	19.63 ± 6.09^{AabII}	30.28 ± 11.28^{BaII}

* Differences between times, that are shown with capital letters in the same tissues and concentrations are important ($p<0.05$).
** Differences between concentrations, that are shown with small letters in the same tissues and times are important ($p<0.05$).
*** Differences between tissues, that are shown with roman numbers in the same time and concentrations are important ($p<0.05$).

except non-essential metals such as Cd and Pb (Hopkin and Nott, 1979; Bu-Olayan and Subrahmanyam, 1997). Linde et al. (1999), who studying on copper and lead accumulation in aquatic organisms and effects of MT synthesis, has been reported that copper can bound to MT, but lead, do not cause synthesis of these proteins. Additionally, Talbot and Chegwidden (1982), studying over accumulation of Cd, Cu, Pb and Zn metals in *Portunus pelagicus* and it has been reported that, the accumulation of these metals are more in hepatopancreas.

Muscle tissue in aquatic organisms is less active in metal accumulation depends on low metabolic activity (Heath, 1987). However, in many studies made on decapods, it has been reported that, metal accumulation in muscles were less in comparison to other tissues (Wicklund et al., 1988; Khan and Negegoda 2003). In an other study, conducted on *Astacus astacus* which exposed to low doses of Pb (0.02 mg L^{-1}) for 10 weeks reported less accumulation in gut and muscle compared to carapace and gill (Meyer et al., 1991). Madigosky et al. (1991), also suggested that, muscle tissue of *Procambarus clarkii* accumulated less amounts of heavy metals (Pb, Cd and Al) as against other tissues. In the present study, minimum lead accumulation has been determined in muscle tissues of *C. aestuarii*. Accumulation of metals in aquatic organism tissues changes according to the exposure time and environmental concentrations. In this study the accumulation of lead in the crabs tissues were increased due to the time and concentrations.

Aquatic organisms has the ability of absorbing metals from water in active or inactive way and accumulating them in their bodies. Accumulation of metals in the tissues of aquatic organisms changes depending on the factors such as exposure time of metal, type of metal, water physico-chemical parameters (pH, temperature, salinity and hardness) and metabolic activity (absorption, detoxification and elimination) of tissues (Heath, 1987; Langston, 1990). Consequently, toxicity tests should be continued to consider the effects of environmental conditions within various external factor on both *C. aestuarii* and other macro invertebrates.

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