

RESEARCH ARTICLE

Heavy metals in edible tissues of the brown shrimp *Crangon crangon* (Linnaeus, 1758) from the Southern Black Sea (Turkey)

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Abstract

In the present study, heavy metal concentrations (Cd, Pb, Cu, Zn, Co, Mn, Ni and Fe) have been measured in edible tissues of the brown shrimp *Crangon crangon* (Linnaeus, 1758) collected from Samsun coasts in the Black Sea coast of Turkey in 2010. Variations of heavy metal concentrations with seasons are compared. Cd and Pb concentrations in *C. crangon* were the lowest heavy metal concentrations, while Fe concentrations were highest level observed in all seasons. The concentrations of Cd, Pb, Cu, Zn, Co, Mn, Ni and Fe were in the range of 0.228-0.481, 0.291-0.491, 5.85-14.77, 18-36, 0.24-0.61, 6-15, 2-6 and 30-58 $\mu\text{g g}^{-1}$ wet weight, respectively. In general the heavy metal findings in the brown shrimp were below the Turkish Food Codex, Commission Regulation (EC) and MAFF maximum permitted levels for human consumption. It is concluded that the brown shrimps are suitable biomonitors to assess changes in metal pollution in the coastal area of the Black Sea.

Key words: Heavy metal, *Crangon crangon*, Black Sea, food codex.

Introduction

Heavy metals are known as a major source of marine pollution and have seriously increased in the Black Sea (Bakan and Büyükgüngör 2000; Altaş and Büyükgüngör 2007; Bat *et al.* 2009). Kızılırmak and Yeşilirmak, the two most important rivers of Black Sea Region, and a lot of big and little industries such as food, cement, fertilizer, pesticide, resin, plastic and textile exist in the middle Black Sea Region of Turkey (Altaş and Büyükgüngör 2007; Bakan *et al.* 2010). Altaş *et al.* (2001) pointed out that these factories have no treatment plant and

they have potential to create local problems of heavy metal pollution. Samsun is located in the middle of the Black Sea region in Turkey and considerable agricultural and industrial activities are carried out there. Its area is 9579 km² and its population is over 1,150,000 people. Fertilizers and pesticides are also heavily used in the agricultural fields in this region (Kurt and Özkoç 2004; Bakan *et al.* 2010). Samsun Port is one of the largest ports in the Black Sea region. It is known that metal contamination sources are typically derived from mining, industrial waste discharges, sewage effluent and harbour activities (O'Sullivan 1971; Bellinger and Benham 1978; Young *et al.* 1979).

Non-essential metals such as cadmium (Cd) and lead (Pb) have drawn considerable attention for its great different toxic effects on aquatic biota (Phillips and Rainbow 1994). Essential metals can also potentially be harmful to marine organisms and have been reported as hazardous environmental pollutants able to accumulate along the aquatic food chain with severe risk for aquatic organisms (Bryan 1976) and human health (Underwood 1977). Decapod crustaceans may concentrate large amounts of some metals from water and food in their tissues (Phillips 1977; Rainbow 1988; Phillips and Rainbow 1994). Decapod crustaceans like *Crangon crangon* have proved especially useful and are commonly employed in the monitoring of metal pollution (Culshaw *et al.* 2002; Junk and Zauke 2008) because they are consumed by human and they have a broad geographical range (Fish and Fish 1996; Cattrijsse *et al.* 1997; Oh *et al.* 2001) including in the Black Sea (Bilgin and Gönülğür-Demirci 2005).

Although some information is available on the bioaccumulation of metals in some decapod crustaceans (Öztürk *et al.* 1996), no data are available on heavy metal levels of *C. crangon* in the Black Sea. *C. crangon* has very high productivity (Oh *et al.* 2001) and is an important food source for many birds, fish and crustaceans (Fish and Fish 1996; Hufnagl *et al.* 2010). It is commercially important species for human consumption in Turkey (Turkish Statistical Institute 2011). Because of a wide range of biological and ecological aspects and their high commercial value (Oh *et al.* 2001), the investigation of the heavy metal concentrations in *C. crangon* through the accumulation of metals in their tissues remains a necessary basic study.

The aims of the present study were (1) to determine if accumulation levels of heavy metals in edible tissues of *C. crangon*, (2) to evaluate seasonal differences of heavy metals, and (3) to compare the international and national food safety standards for human consumption.

Materials and Methods

Brown Shrimp Collection and Sampling Procedure

The brown shrimp *C. crangon* were sampled seasonally, four times from January to March, April to June, July to September, and October to December in

2010, by beam trawl in the littoral zone of Samsun coasts of the southern Black Sea (Figure 1). The samples were placed into sterile bags and transported to the Hydrobiology Laboratory of Fisheries Faculty at Sinop University within 3 hours in an ice cooler to maintain a temperature of approximately 4-5°C and then measured. Shrimp samples 7.34 ± 0.94 cm in length and 3.16 ± 1.11 g in weight were washed thoroughly with double distilled water then stored at -21°C until heavy metal analysis. The total number of samples examined for the present study was 600.

Preparation of Brown Shrimps for Heavy Metal Analysis

Only edible parts of all the samples were analyzed. All tissue weights have been expressed as fresh weight in the present study. Upon thawing, the whole soft parts were removed using stainless steel instruments and were homogenized. All samples were run in triplicate. The precision of analysis was estimated by variations from the mean value reported and in all experiments several blanks were performed with the reagents used in order to check for possible contamination. To avoid these problems all glassware used for heavy metals analyses were washed with detergent, rinsed in distilled water, pre-soaked in 10% nitric acid for more than 24 h, rinsed with deionized water and allowed to air-dry at room temperature before use.

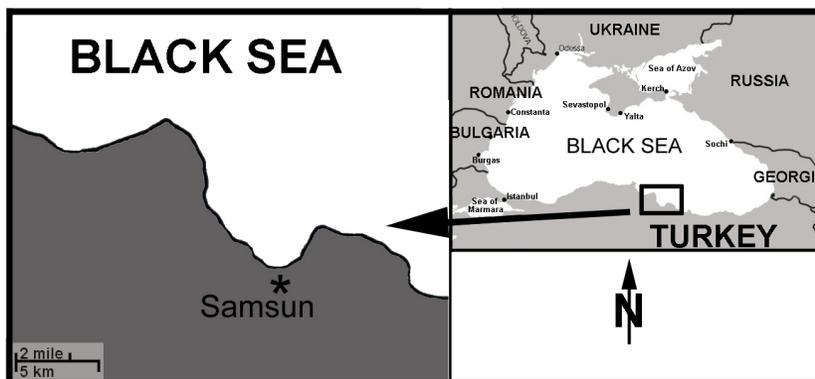


Figure 1. Study area for sample collection by beam trawl in the littoral zone of Samsun on the Black Sea coast of Turkey

Determination of Heavy Metals in Brown Shrimps

A modified version of the method used by Bernhard (1985) was employed in the present study. Ten gram samples of wet tissue were placed in silica flasks covered with glass plates and wet digested with 20 ml of $\text{HNO}_3:\text{HClO}_4$ (5:1) at 105°C for 24 h. The filtered extracts were brought to 25 ml with HNO_3 (70%)

and diluted with deionized water. Reagent blanks were processed simultaneously in triplicate.

The each residue was then filtered into volumetric flask, using Whatman filters. The solutions were analyzed by using an ATI Unicam 929 Atomic Absorption Flame Spectrophotometer, with background correction. The calibration curve was established by plotting the absorbance readings for a set of standards against the concentration. The blank solution was measured between the standards and samples, to ensure the stability of the base line. The theory and application of AAS have been described in detail by Cresser (1994) and Lajunen (1992).

Statistical Analyses

Statistical analyses of data were carried out using Statistica version 7.0 software. A one-way analysis of variance (ANOVA) was performed, followed by Duncan comparisons for the source of statistically significant differences of metal concentrations by seasons for each of the metal investigated in the present study. The significance was set at 0.05 and P-values less of 0.05 were considered statistically significant (Zar 1984). All values being expressed on a $\mu\text{g g}^{-1}$ are wet wt. basis.

Results and Discussion

Heavy Metal Concentrations in the Brown Shrimp Sampled in the Black Sea Coast in 2010

Figure 2 show the mean concentration of heavy metals in the brown shrimp *C. crangon* (Linnaeus 1758) collected off Samsun in the Black Sea coast of Turkey in 2010. Cd concentrations in *C. crangon* were the lowest heavy metal concentrations followed by Pb, while Fe concentrations were highest level observed in all seasons. The concentrations of Cd, Pb, Cu, Zn, Co, Mn, Ni and Fe were in the range of 0.228-0.481, 0.291-0.491, 5.85-14.77, 18-36, 0.24-0.61, 6-15, 2-6 and 30-58 $\mu\text{g.g}^{-1}$ wet weight, respectively. In general, the concentrations of the heavy metals were as follow: Fe>Zn>Mn>Cu>Ni>Co>Pb>Cd (Figure 2). *C. crangon*, like all crustaceans, require essential metals for biological functions.

C. crangon is generally a carnivorous nocturnal predator that buries in the sediment (Pihl and Rosenberg 1984) with only the eyes and antennae above the sediment surface (Pinn and Ansell 1993) and the diet of *C. crangon* consisted of 3 predominantly bottom-dwelling categories; demersal, epifaunal and infaunal organisms (Oh *et al.* 2001). *C. crangon* is also an omnivore feeder on detritus and plant material (Plagmann 1939). The stomach fullness of obligatory detritivores is known to be much higher than that of carnivores since substantial volumes of organic matter have to be consumed to meet their energy

requirements (Cattrijsse *et al.* 1997). It is well known that heavy metal concentrations generally increase with decreasing particle size and metals bound to organic matter in sediment (Buchanan 1984; Bryan and Langston 1992; Langston and Spence 1994). When *C. crangon* feeding on silt and clay sediment or alga or other organisms may have taken up the metals in their tissues. Rainbow (1988) noted that the gut contents of deposit-feeding crustaceans may present a significant component of the total body load due to they ingest potentially metal rich sediments.

Mainly due to industrialization, these metal concentrations in the upper layers of the sediment have risen over recent years (Bakan and Büyükgüngör 2000; Altaş and Büyükgüngör 2007). It was clearly seen that transportation of heavy metals from Kızılırmak and Yeşilirmak rivers to the Black Sea was rather of high quantity (Bakan and Büyükgüngör 2000; Altaş and Büyükgüngör 2007). Along with this enrichment, heavy metal accumulation in the shrimps of this habitat has taken place. However, the mean Pb and Cd levels of the brown shrimp samples were significantly lower ($p < 0.05$). A short period of food consumption in connection with a growth rate seems to be the main reason for the low Pb and Cd levels in this species. The low concentration of the non-essential metal Pb might be also due to the ability of decapods to actively release Pb into the environment (Marx and Brunner 1998). Even in a highly contaminated habitat (Bakan and Büyükgüngör 2000; Kurt and Özkoç 2004; Altaş and Büyükgüngör 2007; Bakan *et al.* 2010; Özkan and Büyükişik 2012), the other non-essential metal Cd intake via sediment, algae and organisms is not sufficient for the accumulation of relevant levels of Cd in the tissues in the present study.

Compared to data on the heavy metal levels in *C. crangon* from other habitats was shown in Table 1. The results of the present study, however, showed lower values of Zn, Cu and Cd than those in Severn Estuary and Bristol Channel, UK, higher values of Pb and Cd than those in the North Sea. Regional comparison for results must be made with caution because of variations in both quality of analytical data and in sampling procedure. More samples must be examined to assess geographical differences.

Seasonal Variation of Metals in the Brown Shrimp Sampled in the Black Sea Coast in 2010

The overall mean values for each of the eight metals (Cd, Pb, Cu, Zn, Co, Mn, Ni and Fe) in the brown shrimp *C. crangon* were compared by ANOVA to determine if there is a significant difference in the concentrations of these metals existed between seasons. Figure 2 shows that Ni concentrations in the shrimp sampled in Samsun coast of the Black Sea in 2010 did not vary significantly ($P > 0.05$) by season while other metals showed significant seasonal ($P < 0.05$) differences.

Pihl and Rosenberg (1984) and Szaniawska (1983) noted that diet composition was highly variable seasonally. There were important seasonal variations in feeding activity which were reflected in an increase in stomach fullness of *C. crangon* in autumn and winter (Oh *et al.* 2001). In the present study, the winter season showed an overall higher level of Cd, Cu, Mn and Zn in the shrimp samples relative to the summer season, whereas Co, Fe and Pb showed higher levels in the autumn season (Figure 2).

These results are similar to those reported by Culshaw *et al.* (2002). They observed that Cd levels in shrimp caught during autumn and winter were consistently high and suggested that the metal was highly bioavailable, which could lead to a rapid rate of Cd uptake and accumulation in the shrimp. It may also be concluded that the exceedingly high levels of Cd contamination of the shrimp is due to a combination of high Cd inputs and increased bioavailability of Cd in the winter months (Culshaw *et al.* 2002). In contrast to this, Marx and Brunner (1998) found that Pb values of shrimp caught in spring were even higher than those in autumn.

In winter, the salinity of the Samsun coastal waters is reduced (Bakan and Büyükgüngör 2000) which can lead to an increase in the proportion of toxic free metal ions (Rainbow 1997), that could be taken up by shrimp from water as well.

Maximum Permitted Limits of These Metals in Seafood

Several countries have developed maximum acceptable concentrations for commercialization and consumption of seafood such as the United States, Canada, European Countries and also Turkey. Significant differences were observed for all metals except Ni by seasons. However, there is no data in the literature to provide the levels of the heavy metals in shrimp for seasonal variations. All of the samples contained lower amounts of the investigated heavy metals than the levels of concern of international (Commission Regulation (EC) and MAFF) and local (Turkish Food Codex) standards as shown in Table 2.

Conclusion

In many environmental programmes of the Black Sea *Mytilus galloprovincialis* is used as biomonitor (Ünsal and Beşiktepe 1994; Bat *et al.* 1999; Bat *et al.* 2012). While very little information about such programmes for marine crustaceans can be found, at least they do not deal specifically with heavy metals. However, due to their great importance in food webs of the intertidal zone in the Black Sea, crustaceans such as the brown shrimp *C. crangon* merit further consideration. It is well known that *C. crangon* is a strong bioaccumulator of heavy metals (Marx and Brunner 1998; Culshaw *et al.* 2002; Jung and Zauke 2008). The result of the present study supply valuable

information on the heavy metal concentrations in the brown shrimp *C. crangon* from the southern of the Black Sea and indicated that *C. crangon* could be a suitable biomonitor for the heavy metals of its habitat sediment.

The differences in heavy metal concentrations in edible tissues of the brown shrimp are due to the length of the feeding period, the age of the shrimp (Marx and Brunner 1998), bioavailability of heavy metals in the habitat and time spent in contaminated water (Gökoğlu *et al.* 2008) and sediment.

The obtained data with guidelines showed that the metals concentration in *C. crangon* is below the permissible level defined by Commission Regulation (EC), MAFF and Turkish Food Codex. It may be suggested that continuous care must be taken to biomonitor the heavy metal levels in the brown shrimp *C. crangon* especially if they exceed international and local permissible limits for human consumption.

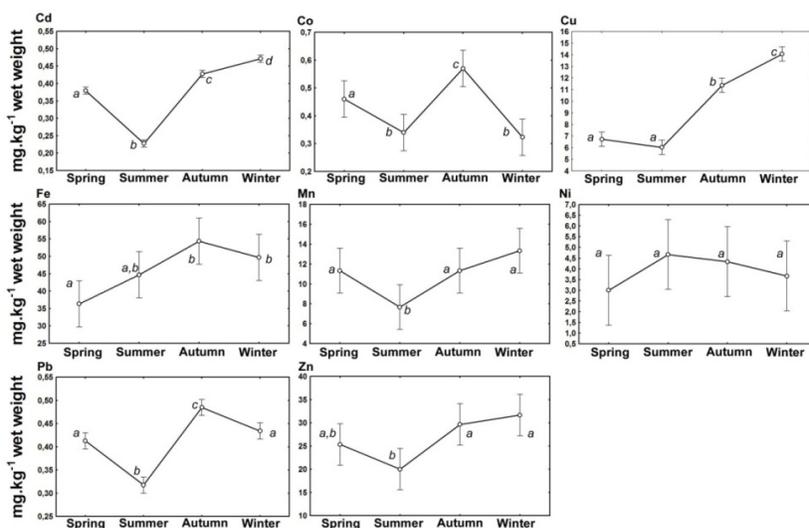


Figure 2. The means with standard deviations (vertical line) of Cd, Pb, Cu, Zn, Co, Mn, Ni and Fe concentrations ($\mu\text{g.g}^{-1}$ wet wt.) in the brown shrimp *C. crangon* from Samsun coastal waters of the Black Sea in 2010 (a, b, c, d, e, f = the same letters beside the vertical bars in each graph indicate the values are not significantly different ($p > 0.05$)).

Table 1. Heavy metal concentrations in *C. crangon* from different habitats

Area	Fe	Zn	Ni	Cu	Mn	Pb	Cd	Co	References	
Severn Estuary and Bristol Channel, UK (expressed in µg.g-1 dry wt)	--	29-136--	53.3-107.2--	--	--	--	1.40-15.36	--	Culshaw <i>et al.</i> 2002	
North Sea (expressed in µg.g-1 wet wt)	--	--	--	--	--	0.019-0.0110	0.043-0.026--	--	Marx and Brunner 1998	
Samsun coast of the Black Sea, Turkey (expressed in µg.g-1 wet wt)	30-5818-36	2-65	85-14	776-150	291-0	4910	228-0	4810	24-0	0.61 Present study

-- = not measured

Table 2. The tolerable values of heavy metals in Crustaceans (mg.kg⁻¹ wet weight)

Standards	Cd	Pb	Cu	Zn	Co	Mn	Ni	Fe	References
Turkish Food Codex	0.50	0.50	--	--	--	--	--	--	Anonymous 2008 ¹
Commission Regulation (EC)	0.50	0.50	--	--	--	--	--	--	Anonymous 2006
Turkish legislation	1	2	20	50	--	--	--	--	Anonymous 1995
The Food Safety	<0.2	10	20	50	--	--	--	--	MAFF 1995 ^{2,3}

1 = Notifications about maximum levels for certain contaminants in foodstuffs published in the Official Gazette of Turkey.
 Turkish Food Codex (Notification No: 2008/26), Issue: 26879 repeated (Anonymous 2012).

2 = Higher levels copper in food are permitted if copper is of natural occurrence

3 = Higher Zn levels are permitted in food which naturally contain more than 50 mg.kg⁻¹

Karadeniz'in güney kıyılarından toplanan teke karidesi (*Crangon crangon*) (Linnaeus, 1758) türünün yenilen dokularındaki ağır metaller

Özet

Mevcut bu çalışmada 2010 yılında Karadeniz'in Samsun kıyılarından örneklenen kahverengi teke karidesi *Crangon crangon* (Linnaeus, 1758)'un yenilebilir kas dokularındaki ağır metal (Cd, Pb, Cu, Zn, Co, Mn, Ni ve Fe) konsantrasyonları belirlenmiştir. Mevsimlere bağlı olarak ağır metal konsantrasyonları karşılaştırılmıştır. *C. crangon* türünün dokusunda Cd ve Pb konsantrasyonları en düşük seviyede Fe konsantrasyonları tüm mevsimlerde en yüksek düzeyde bulunmuştur. Cd, Pb, Cu, Zn, Co, Mn, Ni ve Fe konsantrasyonları sırasıyla 0.228-0.481, 0.291-0.491, 5.85-14.77, 18-36, 0.24-0.61, 6-15, 2-6 ve 30-58 $\mu\text{g}\cdot\text{g}^{-1}$ yaş ağırlık olarak belirlenmiştir. Genel olarak karideslerde tespit edilen ağır metal düzeyleri Türk Gıda Kodeksi Tebliği, Avrupa Birliği Komisyon Tüzüğü ve Tarım, Balıkçılık ve Gıda Bakanlığınca (MAFF) belirlenen gıda maddelerindeki bulaşanların maksimum limitlerinden daha düşük bulunmuştur. Karadeniz'in kıyısız alanlarında ağır metal kirliliği çalışmalarında teke karideslerinin biyomonitör olarak kullanılmasının uygun olduğu önerilmektedir.

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