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TAM MAKALE

DETERMINATION OF SEASONAL CHANGES ON SOME HEAVY METAL (Cd, Pb, Cr) LEVELS OF SHRIMP AND PRAWN SPECIES FROM NORTH-EASTERN MEDITERRANEAN SEA, GULF OF MERSIN, TURKEY

Deniz AYAS^{1, 3}, Ali Rıza KÖŞKER², Mustafa DURMUŞ², Mısra BAKAN¹

¹Department of Seafood Processing Technology, Faculty of Fisheries, Mersin University, Mersin, Turkey

² Department of Seafood Processing Technology, Faculty of Fisheries, Çukurova University, Adana, Turkey

³ Ekosfer Environmental Consulting Limited, Mersin Technology Development Zone (Technoscope), Mersin, Turkey

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	E-mail: ayasdeniz@gmail.com	

Abstract:

The concentration levels of some potentially toxic trace elements such as Cd, Cr, and Pb in the muscle tissue of some shrimp and prawn species from Northeastern Mediterranean Sea, Gulf of Mersin, Turkey were seasonally determined in this study. The effects of seasons on the potentially toxic trace elements levels of regional commercially valuable five different shrimp and prawn species (green tiger prawn-Penaeus prawn-Marsupenaeus semisulcatus, kuruma japonicus, caramote prawn-Melicertus kerathurus, deepwater pink shrimp-Parapenaeus longirostris, speckled shrimp-Metapenaeus monoceros) were determined. 30 individuals of each species were caught by bottom trawling from the Mediterranean Sea (Gulf of Mersin) in four seasons in 2013. Shrimp and prawn samples were extracted using digestion method with concentrated nitric acid and per chloric acid and metal levels were measured using ICP/MS. The differences in Cd, Cr and Pb levels of muscle

tissue in all species were determined as $0.44-1.40 \,\mu g/g$ dw, 1.38-5.80 µg/g dw, and 1.99-13.42 µg/g dw, respectively. The quantitative relationships generally found in the metal levels of muscle in all the species were: Pb> Cr> Cd, but the concentration of each potentially toxic heavy metal varied seasonally. If the data obtained by this study were evaluated with respect to the data in U.S. Food and Drug Administration (2013), it would be obviously seen that all the shrimp and prawn species living in Gulf of Mersin were not contaminated with Cd and Cr but contaminated with Pb. However, if it is analyzed in accordance with European Commission (2011) and Turkish Food Codex (2011), all species except winter and spring samples of Parapenaeus longirostris are contaminated with Cd and Pb.

Keywords: Shrimp, Prawn, Season, Heavy metals, Cd, Cr, Pb

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Introduction

The concept of sustainable environment is currently being more significant depending on the negative effects of anthropogenic metal pollution. Numerous anthropogenic activities, such as growing use of pesticides and artificial fertilizers to improve the production yield in agricultural activities from past to present, using several methods to increase the efficiency in mining and excessive industrial activities during the industrial age lead dramatically to metal pollution of the global ecosystem. Metal contaminants with their negative effects have important role on human health and ecosystems. Metals as well as many other natural elements are kept in balance with biological and geological cycles. Many metals are essential to living organisms but some of them are highly toxic or become toxic at high concentrations. For example, Fe (hemoglobin), Cu (respiratory pigments), Zn (enzymes), Co (Vitamin B 12), Mo and Mn (enzyme), light metals Sodium (Na), Potassium (K) and Calcium (Ca) which play important biological roles. Transition metals Fe, Cu, Co and Mn which are essential but may be toxic at high concentrations. Metals such as Hg, Pb, Sn, Ni, Se, Cr and As are generally not required for metabolic activity and are toxic to living organisms at quite low concentrations. (Forster et al. 1983, Meria U. 1991). People's impacts on natural habitats by their industrial and mining activities can lead to disruptions in the natural biogeochemical cycles and also can damage human health.

The heavy metal contaminations not only constitute a significant risk especially for human health and nutrition but also can show some negative effects on the natural balances of the ecosystems. The negative effects of heavy metals on ecosystems when compared with the factors of other pollutants may not be observed in a short time, but effects can be observed more in a long term (Kucuksezgin et al., 2010). If the maximum limit value of heavy metals is exceeded, the heavy metals become hazardous elements for both human health and ecosystems. Heavy metal contaminations in human nutrition are serious problems that have and feel fairly large negative effects on human health for many years. Keeping under a fairly tight control studies due to intensive industrial activities in developed countries are carried out to reduce adverse effects of metal contamination on human and

ecosystems. Nevertheless, as a general belief seas are seen as a pollution compensatory system. This erroneous assumption has caused to increasing to the dramatic levels of the metal contaminations especially in the marine ecosystems. Although consumed marine organisms can serve as food source for people, they may be effective in the further spread of the pollution effects. Therefore, determining the levels of heavy metals in living organisms collected from their natural habitats is an important indicator for understanding the heavy metal contamination in the living area and the potential risks to human health. Determination of heavy metal levels using the indicator species not only in terms of the human health but also in terms of the sustainability of ecosystems is a necessary condition.

Heavy metals are potentially harmful factors to both marine organisms and people. In aquatic ecosystems various toxic elements such as heavy metals can accumulate via the food chain and they can create a health hazard when they are consumed by humans (Fernandes et al., 2007). Although people can be exposed to the metal through media as water, air and soil, they are exposed to them mostly through food (Alexander et al., 2010). Due to the fact that the epidemiological studies with heavy metals such as nickel, chromium, arsenic, cadmium and beryllium have been approved the carcinogenic effects on experimental animals, it is also believed to have carcinogenic effects even on humans. (Nordberg et al. 2011)

Heavy metal accumulations also occur at skeletal muscle as well as in internal organs such as liver, kidney and spleen. Muscle tissues of marine species are consumed denser by people with respect to the other parts of the organs. Knowledge of heavy metal levels of muscle is important for human health. Heavy metal levels in muscle tissues of marine species has been determined by many researchers (Ayas and Ozoğul 2011, Yılmaz *et al.* 2010, Ayas *et al.* 2009, Kalay *et al.* 2008, Çoğun *et al.* 2006, Türkmen *et al.* 2005, Turan *et al.*, 2009, Ersoy and Celik, 2010).

Even in the Gulf of Mersin as with other marine ecosystems there are several factors that affect pollution. Kalay *et al.* (2004) have showed artificial fertilizers and pesticides that are used

extensively in agricultural activities, domestic waste, waste of chrome, plastics, fertilizers, glass, industrial facilities in the region and intense maritime traffic of Mersin Port as the main sources of pollution in the Gulf of Mersin. The most of the related studies in Gulf of Mersin claimed that metal levels in some marine species showed seasonal variations (Kargin et al. 2001, Kalay et al. 2004, Yılmaz and Yılmaz, 2007). That is why, it is highly important to examine the metal levels of demersal invertebrates seasonally which may be the indicators particularly in terms of toxicity. In this study, it is aimed to detect and determine the seasonal changes of heavy metal levels such as Cd, Cr and Pb levels on the shrimp species caught in the Gulf of Mersin. Thus, it is also aimed to determine whether the values of Cd, Cr and Pb of shrimp and prawn species such as Penaeus semisulcatus, Marsupenaeus japonicus, Melicertus kerathurus, Parapenaeus longirostris, Metapenaeus monoceros are above the limit levels or not.

Materials and Methods

Five different shrimp and prawn species were trawling caught by bottom from the Mediterranean Sea (Gulf of Mersin) in four seasons in 2013 (Figure 1). Shrimp and prawn species were; green tiger prawn (Penaeus semisulcatus De Haan 1844), kuruma prawn (Marsupenaeus japonicus Bate 1888), caramote prawn (Melicertus kerathurus Forskal 1775), deepwater pink shrimp (Parapenaeus longirostris Lucas 1846), and speckled shrimp (Metapenaeus monoceros Fabricius 1798). In every season, 30

individuals of each species were caught and kept in polystyrene boxes with ice and transferred within ice to the laboratory. They were marketable size such as *Penaeus semisulcatus* 17.43 ±3.4cm, Marsupenaeus japonicus 15.4 ±4.1cm, Melicertus kerathurus 12.8 ±2.2cm, Parapenaeus longirostris 13.4 ±3.7cm, Metapenaeus Monoceros 12.6 ±2.6cm. Meat of abdominal muscle which is the main edible portion of shrimp and prawn was homogenized and metal analyses were done on this part of fresh samples. The analyses were performed in triplicate.

The muscle samples (0.1 g dry weight each) used for metal analysis were dried at 150°C to reach constant weight and then concentrated nitric acid (4 mL, Merck, Darmstadt, Germany) and per chloric acid (2 mL, Merck) were added to the samples, and they were put on a hot plate set to until all tissues were dissolved. 150°C Inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7500ce Model) was used to determine metals. The metal concentrations (Cd, Cr, Pb) in samples were detected as μg metal g^{-1} dry weight. The standard addition method was used to correct for matrix effects. High purity multi standard (Charleston, SC, USA) was used for determination of the metals.

Statistical analysis

Statistical analysis of data was carried out with the SPSS 16.0 Duncan's test was used to evaluate the species and season effects on metal levels.



Figure 1. The Map of Mersin Gulf (Google maps)

Results and Discussion

In this study, Cd, Cr and Pb levels on five different species of shrimp and prawn - green tiger prawn (Penaeus semisulcatus De Haan 1844), kuruma prawn (Marsupenaeus japonicus Bate 1888), caramote prawn (Melicertus kerathurus Forskal 1775), deepwater pink shrimp (Parapenaeus longirostris Lucas 1846), and speckled shrimp (Metapenaeus monoceros Fabricius 1798) - caught by bottom trawling from the Mediterranean Sea in the four seasons during the year 2013 in the Gulf of Mersin were determined and metal concentrations were calculated in microgram per gram dry basis ($\mu g/g$). The differences in Cd, Cr and Pb levels of muscle tissue in all species were determined as 0.44-1.40 µg/g, 1.38-5.80 µg/g, and 1.99-13.42 µg/g, respectively. The quantitative relationships generally found in the metal levels of muscle in all the species were: Pb> Cr> Cd (Table 1).

Results showed that the annual ranges of metal levels of green tiger prawn were: 1.05-1.40 μ g Cd g⁻¹, 2.81-5.80 μ g Cr g⁻¹, 5.01-10.80 μ g Pb g⁻¹. The annual ranges of metal levels of kuruma prawn were: 0.71-1.10 μ g Cd g⁻¹, 2.40-5.37 μ g Cr g⁻¹, 5.55-8.30 μ g Pb g⁻¹. The annual ranges of metal levels of caramote prawn were: 0.76-1.01 μ g Cd g⁻¹, 2.32-4.53 μ g Cr g⁻¹, 5.47-11.11 μ g Pb g⁻¹. The annual ranges of metal levels of deepwater pink shrimp were: 0.44-0.67 μ g Cd g⁻¹, 1.38-3.44 μ g Cr g⁻¹, 1.99-4.40 μ g Pb g⁻¹. The annual ranges of metal levels of speckled shrimp were: 0.55-0.97 μ g Cd g⁻¹, 2.38-4.38 μ g Cr g⁻¹, 7.29-13.42 μ g Pb g⁻¹ (Table 1).

Barrento et al. (2008) reported that Cd, and Pb levels of European (Homarus gammarus) and American (H. americanus) lobsters were 0.02- $0.02 \ \mu g/g \ dw$, and $0.10-0.10 \ \mu g/g \ dw$, respectively, in Scotland. Cd and Pb levels presented by those researchers were lower than the data obtained in our study. In a similar study, Kargin et al. (2001) reported Cd and Pb levels in muscle tissue of two Mediterranean shrimp species as 0.5-1.9 µg/g dw, and 5.7-21.6 µg/g dw, respectively, in Gulf of Iskenderun, northeasthern Mediterranean Sea. It can be seen that the results presented by them were similar to those in our study. The Cd limit levels reported by European Commission (2011) and, Turkish Food Codex (2011) for crustacean were 0.5 μ g Cd g⁻¹. It was noted that except spring and winter samples of Parapenaeus longirostris, all shrimp and prawn species living in Gulf of Mersin were contaminated with Cd. According to European Commission (2011) and Turkish Food Codex (2011), their toxicity levels exceed the levels appropriate for human consumption. However, U.S. Food and Drug Administration (2013) reported the Cd limit level as 3 µg Cd g⁻¹ for crustacean. The Cr limit level is reported as 12 μg Cr g⁻¹ for crustacean by U.S. Food and Drug Administration (2013). European Commission (2011) and, Turkish Food Codex (2011) set a Pb limit level for crustacean as 0.5 µg Pb g⁻¹. Thus, it can be stated that all species living in Gulf of Mersin were not contaminated with Cr but contaminated with Pb. The results of this study are as the same as the results found by Yılmaz and Yılmaz, (2007), for Cr level of P. semisulcatus species during the summer in Gulf of Iskenderun. In the same study the Cr levels are found as 9.3-12,0 µg/g in Autumn and 6.7-13,1 $\mu g/g$ in Winter. These results are higher than ours. While Pb levels reported by researchers in the springtime are parallel to our study, the values that have been found in our study are lower than those in the other seasons. Similarly, in another study in Iskenderun Gulf, the values of Cr and Cd for P. semisulcatus species have been found as 60.38 μ g/g and 16.72 μ g/g, respectively (Firat et al. 2008). The reported results of researchers are quite high compared to results of our study. These differences can be explained by the exposure to metal contamination at different levels in different Gulfs of species.

Özden (2010) has studied seasonal levels of the metal in the Marmara Sea for P. longirostris species and has stated the levels of Cd, Pb and Cr as in the range of 0.007-0.0098 µg/g, 0.0197- $0.230 \ \mu g/g$ and 1.262 to $1.502 \ \mu g/g$, respectively. The Cr results of researchers are similar to ours but their results of Pb and Cd levels are lower compared to our study. In a similar study in Marmara Sea for *P. longirostris* species by Dökmeci et al. (2013), the levels of Cd, Cr and Pb were found as 0.106 μ g/g, 0.77 μ g/g and 2.12 $\mu g/g$, respectively. While Pb levels reported by researchers are similar to the levels determined in our study, Cd and Cr levels were found lower. It has been evaluated that the differences between the results of the two studies described above with the results of our study can be caused by different pollution levels in the different marine ecosystems.

Metal	Spring	Summer	Autumn	Winter	Species
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X}\pm S_{\overline{X}}$	$\overline{\mathbf{X}}\pm\mathbf{S}_{\overline{\mathbf{X}}}$	$\overline{\mathbf{X}}\pm\mathbf{S}_{\overline{\mathbf{X}}}$	_
Cd	$1.05 \pm 0.22^{a,y}$	$1.40 \pm 0.12^{a,y}$	$1.11 \pm 0.44^{a,x}$	$1.07 \pm 0.25^{a,x}$	PS
	$0.91 \ {\pm} 0.32^{a,y}$	$1.10\pm\!\!0.29^{a,y}$	$0.71 \pm 0.33^{a,x}$	$0.80\pm\!\!0.15^{a,x}$	MJ
	$0.83 \pm 0.37^{a,y}$	$1.01 \pm 0.28^{\text{a},\text{y}}$	$0.76 \pm \hspace{-0.5mm} 0.47^{a,x}$	$0.82 \pm 0.22^{a,x}$	MK
	$0.44 \pm 0.11^{a,x}$	$0.67 \pm 0.21^{a,x}$	$0.50\pm\!\!0.22^{a,x}$	$0.47 \pm \hspace{-0.5mm} 0.37^{a,x}$	PL
	$0.55 \pm 0.14^{\text{a,x}}$	$0.97 \pm 0.22^{b,y}$	$0.69 \pm 0.14^{a,x}$	$0.63 \pm 0.11^{a,x}$	MM
Cr	$2.81 \pm 0.35^{a,y}$	$5.80 \pm 0.32^{d,q}$	$4.71 \pm 0.12^{c,y}$	$3.87 \pm 0.31^{b,y}$	PS
	$2.40 \pm 0.21^{a,y}$	$5.37 \pm 0.23^{d,q}$	$4.43 \pm 0.12^{c,y}$	$3.45 \pm 0.24^{b,y}$	MJ
	$2.32 \pm 0.23^{a,y}$	$4.53 \pm 0.15^{d,z}$	$4.34 \pm 0.32^{c,y}$	$3.53 \pm 0.22^{b,y}$	MK
	$1.38 \pm 0.31^{a,x}$	$3.44 \pm 0.11^{c,x}$	$2.38 \pm 0.51^{b,x}$	$2.44 \pm 0.29^{b,x}$	PL
	$2.38 \pm 0.17^{a,y}$	$4.31 \pm 0.24^{c,y}$	$4.38 \pm 0.26^{c,y}$	$3.35 \pm 0.31^{\text{b},\text{y}}$	MM
Pb	$5.01 \pm 0.92^{a,y}$	$10.80 \pm 1.02^{d,z}$	$8.71 \pm 1.04^{c,z}$	$7.87 \pm 0.55^{b,z}$	PS
	$6.29 \pm 0.44^{a,z}$	$8.30 \pm 1.37^{b,y}$	$6.91 \pm 0.51^{a,y}$	$5.55 \pm 0.92^{a,y}$	MJ
	$5.47 \pm 0.15^{a,y}$	$11.11 \pm 1.61^{c,z}$	$8.27 \pm 0.49^{b,z}$	$7.12 \pm 0.71^{b,z}$	MK
	$2.30 \pm 0.34^{a,x}$	$4.40 \pm 0.33^{b,x}$	$3.78\pm\!\!0.88^{b,x}$	$1.99 \pm 0.77^{a,x}$	PL
	$8.46 \pm 1.88^{\text{a},\text{q}}$	$13.42 \pm 0.45^{b,q}$	$9.34 \pm \! 0.44^{\mathrm{a},\mathrm{z}}$	$7.29 \pm 0.45^{\mathrm{a,z}}$	MM

Table 1. The effects of season on the potentially toxic metal levels of the shrimp and prawn ($\mu g g^{-1}$ DW)

Different letters (x-q) in the same columns and in the same rows (a-d) for each metal indicate significant differences (p<0.05). PS: *Penaeus semisulcatus*, MJ: *Marsupenaeus japonicus*, MK: *Melicertus kerathurus*, PL: *Parapenaeus longirostris*, MM: *Metapenaeus monoceros* $\overline{X} \pm S_{\overline{x}}$: mean \pm standard error

Numbers in bold means maximum and minimum values of each element obtained in this study.

Rahimi et al. (2013) have determined Cd level for P. semisulcatus species around of the coast of Iran as 0.054 μ g/g. Cd level, which we achieved in our study, is higher than the level reported by researchers. Although, Cd levels in Parapenaeus longirostris were obtained within limit values, Cd levels of other species exceeded the upper limit in our study. Since the shrimp species live in deep water, having higher values of heavy metal accumulation can be considered normal, but the reason of this higher level of Cd accumulation has been thought to be caused by the difference in geographic density and concentration of industrial activities. Compounds, batteries, and electronic components containing Cd and Pb are used as corrosion inhibitors for steel production and in nuclear power plants (Nordberg et al. 2011). Performed extensively iron and steel industry activities in the Gulf of Iskenderun are likely to direct negative impact on North Eastern Mediterranean.

Although Pb naturally occurs in nature, its polluting effect is generally enhanced by industrial activities carried out by people in the fields such as mining, casting industry, and battery manufacturing industry (Alexander *et al.* 2010). Similarly, the amount of Cd may be

increased by industrial and agricultural activities. Cr, Cd and Pb levels of species we have examined in the Gulf of Mersin have shown that Cd level of *P. longirostris* is close to the limit, while Cd levels in other species were found above the limit value. In terms of Pb values, contamination is concerned for all types of species. The reason of having high levels of Cd and Pb in the Gulf of Mersin is thought to be high due to the activities carried out by people.

In many countries throughout the world, some alternatives and restrictions for using and releasing of heavy metals have entered into force, due to the negative impact of industrial activities on marine ecosystems. For example, since the less toxic elements can be used instead of Cd, its use has been banned in some countries (Nordberg et al. 2011). Consequently, for the determination of metal contamination in marine ecosystems across the globe, many studies are carried out in the Mediterranean (D'Adamo et al. 2008, Fırat et al. 2008, Hanan et al. 2009, Ayas and Ozogul 2011). On the one hand, our studies contribute to the results of other studies; on the other hand, it also shows that metal toxicity levels are above the limits particularly in the Gulf of Mersin.

Conclusion

Metal levels of the marine species in the Gulf of Mersin have a potential threat on both human health and the marine ecosystem. It was observed that Cd and Pb levels were above the legal limit. In this situation, we are of the opinion that results from industrial activities and intense marine traffic. When the levels of Cd and Pb are over the legal limits, they have adverse effects on human health. Overall, Cd, Cr and Pb levels in this species were observed at higher levels in the summer. We believe that this situation results from increased ship traffic and tourism in the summer season. As a conclusion, the species in the Gulf of Mersin should be examined periodically in terms of potential threats of Cd and Pb.

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