Ekoloji 22, 87, 10-15 (2013)
doi: 10.5053/ekoloji.2013.872

# Assessment of the Heavy Metal Contents of *Sardina pilchardus* Sold in Izmir, Turkey

**Murat YABANLI**

Mugla Sitki Kocman University, Fisheries Faculty 48000, Mugla- TURKEY

*Corresponding author: myabanli@gmail.com

**Abstract**
The total heavy metal (chromium (Cr), copper (Cu), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb)) present in the muscle tissue of sardine was determined by inductively coupled plasma-mass spectroscopy (ICP-MS) after microwave wet digestion during the winter season of 2010. The mean concentrations of heavy metals in the sardine muscle tissues was 0.21 mg Cr/kg, 1.00 mg Cu/kg, 1.49 mg As/ kg, 0.46 mg Cd/kg, 0.03 mg Hg/kg, and 0.14 mg Pb/kg wet weight. It was found that the cadmium concentrations in the sardine fish samples provided from four fish markets with the exception of one fish market, exceeded the value of 0.05 mg Cd/kg (wet weight) limit which is permitted nationally and internationally. The level of arsenic for one of each group of sardines sampled was higher than the permitted limit of arsenic according to the Australia Food Standard (1.00 mg As/kg). As a result, the comprehensive and periodic controls of heavy metals in fish are important in terms of public and environmental health.
**Keywords**: Heavy metal, Izmir (Turkey), *Sardina pilchardus*.

**İzmir'de (Türkiye) Satışı Yapılan Sardina pilchardus Balıklarının Ağır Metal İçeriği Yönünden Değerlendirilmesi**

**Özet**
2010 yılı kış sezonunda sardalya balığı kas dokusunda toplam ağır metal varlığı [krom (Cr), bakır (Cu), arsenik (As), kadmiyum (Cd), civa (Hg) ve kurşun (Pb)], mikrodalga yaş yakma işleminden sonra indüktif olarak eşleştirilmiş kütle spektrometresi (ICP-MS) kullanılarak belirlenmiştir. Sardalya kas dokusu örneklerinde ortalama ağır metal konsantrasyonları 0,21 mg Cr/kg, 1,00 mg Cu/kg, 1,49 mg As/kg, 0,46 mg Cd/kg, 0,03 mg Hg/kg ve 0,14 mg Pb/kg yaş ağırlık olarak tespit edilmiştir. Kadmiyum miktarı (bir balık market hariç) ulusal ve uluslararası izin verilen limit olan 0,05 mg Cd/kg (yaş ağırlık) değerini aşmıştır. Her bir grup için arsenik seviyeleri Avustralya Gıda Standardına göre önerilen limit değerden (1,00 mg As/kg) yüksek bulunmuştur. Sonuç olarak, balıkların ağır metaller yönünden kapsamlı ve düzenli olarak kontrolleri çevre ve halk sağlığı açısından önemlidir.
**Anahtar Kelimeler**: Ağır metal, İzmir (Türkiye), *Sardina pilchardus*.

Yabanli M (2013) Assessment of the Heavy Metal Contents of Sardina pilchardus Sold in Izmir, Turkey. Ekoloji 22 (87): 10-15.

## INTRODUCTION

Nowadays increasing industralization and human activities intensify the emission of various pollutants into the environment (Demirayak et al. 2011).

There has been a growing interest in determining heavy metal concentrations in the aquatic environment, and attention has been drawn to the measurement of contamination levels in fish (Iwegbue et al. 2009, Ferreira-Cravo et al. 2010, Kayhan et al. 2010).

Heavy metals naturally occur in seawater in very low concentrations, but concentration levels have increased due to anthropogenic pollutants (Kargın et al. 2001).

Fish and other aquatic life forms are constantly exposed to chemicals in polluted and contaminated waters (Burger et al. 2002). At the top of the aquatic food chain, fish are known to be able to accumulate large amounts of toxic contaminants from their living environment (Suhaimi et al. 2005).

Humans may be exposed to harmful non-essential elements such as arsenic, silver, lead, mercury, cadmium, and nickel mainly through drinking water, consumption of fresh and processed foods and through occupational exposures (Ikem and Egiebor 2005). Some heavy metals, such as cadmium and lead, injure the kidney and cause symptoms of chronic toxicity, including impaired organ function, poor reproductive capacity, hypertension, tumors, and hepatic dysfunction (Abou-Arab et al. 1996). Sub-lethal effects of heavy metals are of concern as they accumulate and are transferred through the food-chain to humans

Received: 27.01.2012 / Accepted: 02.12.2012

(Yılmaz and Yılmaz 2007).

The marine ecosystem is sensitive to exposure from toxic contaminants (Allen and Moore 2004). Among animal species, fishe are the inhabitants that cannot escape the detrimental effects of the pollutants (Olaifa et al. 2004). The toxic effects of heavy metals on aquatic organisms have been reviewed. Most sublethal toxicity appears to be of a biochemical origin and causes morphological, physiological (growth, swimming performance, respiration, and reproduction), and behavioral changes (Lall 2002).

The Sardine is an important fish species in Turkey and it is often consumed by people because of it's delicious taste and lowprice.

The aim of this study was to determine the concentrations of some heavy metals (chromium, copper, arsenic, cadmium, mercury, and lead) in the flesh of the sardine (*Sardina pilchardus*) commercially sold in İzmir, since this fish is an important dietary component in this city. It is hoped that the obtained results will help in generating data needed for the assessment of heavy metal intake from this source.

## MATERIAL AND METHODS

The sardine samples (length: 11.7±1.64 cm and weight: 28.8±2.35 g) in this study were purchased at four local fish markets in Izmir (Turkey) during the winter season of 2010. Forty-five individuals were collected randomly from each of the four local fish markets. The fishwere then immediately taken to the laboratory on ice and stored at -20°C until the microwave digestion process.

An ELGA Model deionizer system was used to prepare deionized water (resistivity: 18.0 MΩ cm). A Berghof speedwave MWS-3 microwave digestion system with DAP 60+ vessels was used to digest fish samples prior to metal analysis. Inductively coupled plasma-mass spectroscopy (Agilent 7700x) with auto sampler (Agilent ASX-500) was used to analyze the digested samples for total metals. The operating conditions for ICP-MS are shown in Table 1.

All reagents used were of analytical grade (nitric acid, 65% suprapur Merck and hydrochloric acid, 30% Suprapur Merck). Multi-element calibration solutions of all investigated elements were prepared daily by dilution of 10 mg/L mix element standard stock solution (AccuTrace MES-21-1) and 10 mg/L mercury standard stock solution (AccuTrace MES-21-HG-1).

The procedure by Matek and Blanuša (1998) was used for the microwave digestion of the fish samples, with the addition of some minimal modifications. The muscle tissues of the sardine samples were homogenized throughly in a laboratory blender (Waring trade marker) with stainless steel cutters. Each homogenized sample group was composed of thirty individuals. Three replicate analyses were carried out for each group. For each homogenized samples, between 0.5 g homogenate (wet weight) was weighed and placed in polytetrafluorethylene (PTFE) vessel with 5 mL of 65% nitric acid. The material was then subjected to a microwave program (Table 2). The digest was finally made up with a 2% nitric acid and 0.5% hydrochloric acid solution to 50 mL in acid washed standard flasks and then placed in 50 mL polypropylene centrifuge tubes. The in-house method recovery repeatability tests are described below.

The fish samples were spiked with 1000 μg/kg concentration of mixed heavy metals for the recovery repeatability tests. Ten homogenized blank and spiked samples were subjected to the microwave digestion process. Then, the digested samples were analyzed using ICP-MS. The obtained values are given in Table 3.

## RESULTS

The homogenized muscle tissues of the sardines purchased from the four local fish markets were analyzed for chromium, copper, arsenic, cadmium, mercury, and lead. Good recoveries of the spiked samples demonstrate the accuracy of the methods. The levels of all heavy metals studied are given in Figure 1. The total concentrations of chromium, copper, arsenic, cadmium, mercury, and lead are expressed in milligram per kilogram wet basis (mg/kg wet weight). The maximum concentration and mean values of the standard deviations of studied heavy metals in the sardine muscle tissue are summarized in Table 4. The levels of analyzed heavy metals in sardines from other studies are compared in Table 5.

## DISCUSSION

The concentrations of chromium, copper, arsenic, cadmium, mercury, and lead in the sardine flesh from the four local markets were compared to national and international standards.

Chromium is considered as a heavy metal and pollutant as well as an essential micronutrient

**Table 1.** ICP-MS instrument operating conditions.

| | |
|---|---|
| Radio frequency power | 1550 W |
| RF matching | 2.1 V |
| Sample depth | 8 mm |
| Carrier gas | 1.05 L/min |
| S/C temperature | 2°C |
| Nebulizer type | MicroMist |

**Table 2.** The microwave digestion process.

| Step | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Temperature (°C) | 160 | 190 | 190 | 100 |
| Ramp (min) | 5 | 1 | 1 | 1 |
| Hold (min) | 5 | 5 | 10 | 10 |

**Table 3.** The recoveries of studied heavy metals.

| | Blank mean (n = 10)±SD* (μg/kg) | Spike mean (n = 10)±SD (μg/kg) | Recovery (%) |
|---|---|---|---|
| Chromium | 41.95±3.28 | 1004.88±64.97 | 96.44 |
| Copper | 131.92±13.37 | 1105.37±76.73 | 97.65 |
| Arsenic | 295.40±27.99 | 1273.40±98.76 | 98.30 |
| Cadmium | 1.08±0.16 | 1003.28±73.53 | 100.22 |
| Mercury | 7.60±0.89 | 1079.25±26.65 | 107.11 |
| Lead | 65.41±2.91 | 988.95±61.36 | 92.82 |

*SD: Standart deviation

(Yılmaz et al. 2010). The mean level of homogenized muscle tissues of the sardine (0.21 mg/kg) obtained in this study was lower than values obtained (2.22 mg/kg) for sardine in the northeast Mediterranean Sea (Canli and Atli 2003). There is no information about the maximum chromium levels for fish samples in the Turkish Standards (Anonymous 2008). But, the critical chromium limit for human health is 8.00 mg/kg (Tüzen 2009). The obtained chromium levels in this study were below the critical chromium limit.

Copper is an essential element. However, it can be potentially toxic to aquatic organisms when in excess in water (Martins et al. 2011). In the Turkish Food Codex (Anonymous 2002) the recommended limit for copper in fish is 20 mg/kg. In this study the maximum copper content for sardine was 2.04 mg/kg and the obtained copper levels in the sardine samples were found to be lower than the set limits. The mean concentration of copper reported in this study (1.00 mg/kg) was lower than the values reported by several investigators (Tarley et al. 2001, Canli and Atli 2003).

Arsenic, a well-known water pollutant, has been associated with various human health problems (Celino et al. 2008). The maximum arsenic level permitted is 1.00 mg/kg according to Australia Standards (Anonymous 1998). Arsenic was the most abundant of the heavy metals examined. The maximum concentration of arsenic in the muscle tissue obtained (1.96 mg/kg) was higher than the permitted arsenic level. Falcó et al. (2006) reported

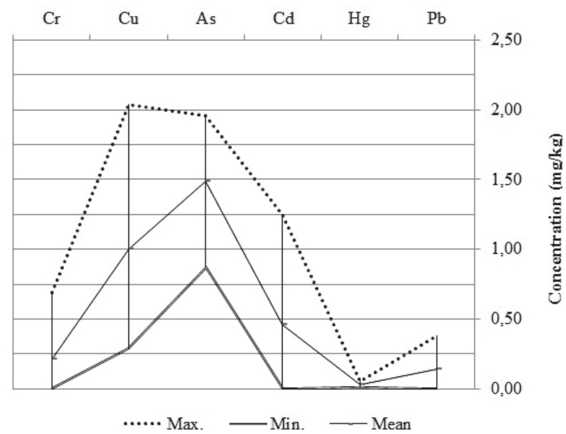


**Fig. 1.** The maximum (Max.), minimum (Min.) and mean concentrations of the heavy metals in the sardine muscle tissue from this study.

**Table 4.** The maximum concentration and mean values of the studied heavy metals in the sardine muscle tissue (mg/kg).

| Heavy metals | N | Mean±SD | Minimum | Maximum |
|---|---|---|---|---|
| Chromium | 4 | 0.21±0.29 | ND | 0.63 |
| Copper | | 1.00±0.79 | 0.29 | 2.04 |
| Arsenic | | 1.49±0.53 | 0.87 | 1.96 |
| Cadmium | | 0.46±0.56 | ND | 1.25 |
| Mercury | | 0.03±0.02 | 0.01 | 0.05 |
| Lead | | 0.14±0.17 | ND | 0.38 |

ND: Not detected

**Table 5.** The maximum levels of studied heavy metals for sardine in the literature and this study.

| Material | Maximum levels of heavy metals (mg/kg) | | | | | | Literature |
|---|---|---|---|---|---|---|---|
| | Cr | Cu | As | Cd | Hg | Pb | |
| Sardine muscle tissue | 2.22[a] | 4.17[a] | --- | 0.55[a] | --- | 5.57[a] | Canli and Atli 2003 |
| Sardine muscle tissue | | | | 0.003 | | 0.05 | Celik et al. 2004 |
| Sardine (edible parts) | --- | --- | 3.94 | 0.01 | 0.09 | 0.08 | Falcó et al. 2006 |
| Sardine | 0.63 | 2.04 | 1.96 | 1.25 | 0.05 | 0.38 | This study |
| Limits | 8.00[b] | 20.00[c] | 1.00[d] | 0.10 | 0.50 | 0.30 | Anonymous 2008 |

[a]Mean levels of heavy metals.

[b]Health critera (Tüzen 2009)cTurkish Food Codex (Anonymous 2002) dAustralia standard (Anonymous 1998).

the maximum arsenic value of the edible parts of sardine as 3.94 mg/kg. This value was higher than our observed level. The continuous exposure to arsenic from sardine consumption can cause health risks for example; skin, vascular, and nervous system disorders. The rate of fish consumption in Turkey 6.92 kg/year was low, for 2010 (Anonymous 2012). Nevertheless, the arsenic concentration in fish has to be controlled by the authorities.

Cadmium is a highly toxic element for all mammals and fish. Accumulation of cadmium in living organisms is a major ecological concern, especially because of its ability to accumulate very quickly (Beširović et al. 2010). Furthermore, seafood is the main source of cadmium for people. In this study, the obtained maximum value of cadmium content (1.25 mg/kg) in the muscle tissue of the sardine is higher than the recommended limit for cadmium in fish (0.05 mg/kg) according to the Commission of The European Union (Anonymous 2006) and Turkish Food Codex (Anonymous 2008). But, the maximum allowed cadmium doses for an adult are 0.5 mg/week (Anonymous 1976). The mean level of cadmium in our samples (0.46 mg/kg) was lower than values reported by Canli and Atli (2003), 0.55 mg/kg. However, the mean values of cadmium we obtained were higher than values reported by Soliman (2006), 0.03 mg/kg.

Mercury, a very toxic metal, is present at trace levels in living organisms. It is generally accepted that seafood represents one of the major sources of mercury in the human food chain (Plessi et al. 2001). The concentration levels of mercury found in sardine range from 0.01 to 0.05 mg/kg. The maximum concentrations of mercury permitted in marine organisms are similar in the majority of the Mediterranean countries, ranging between 0.50 and 0.70 mg/kg (Anonymous 1984). Mercury levels in the analyzed sardine samples was found to be lower than the legal limits, 0.50 mg/kg (Anonymous 2008). The maximum mercury contents of sardine in the literature was reported as 0.05 mg/kg (Bordajandi et al. 2004) and 0.09 mg/kg (Falcó et al. 2006, Shiber 2010), respectively.

Lead is a potentially toxic chemical that may be directly ingested by man or indirectly through aquatic animals like fish and shellfish (Olaifa et al. 2003) and is one of the most important heavy metal pollutant found in the environment, including the aquatic environment (Cigerci et al. 2010). In this study the maximum level of lead in sardine was 0.38 mg/kg. According to the Turkish Food Codex, the maximum lead level permitted for fish is 0.30 mg/kg (Anonymous 2008), However, the maximum lead level permitted is 0.50 mg/kg by the Food and Agriculture Organization, FAO (Anonymous 1983). Celik et al. (2004) has repeorted a mean lead concentration of 0.05 mg/kg in the sardine from İzmir Bay (Turkey) compared to the 0.12 mg/kg we observed in our study. At this point we can say that the concentration of lead in İzmir has increased in recent years.

## CONCLUSION

This pilot study improves the baselines data and information on some heavy metal concentrations in the muscle tissue of sardine which is commonly consumed in İzmir (Turkey). Furthermore, this data can be useful to investigators who study about public health in this area. According to the obtained data, copper had the highest concentration followed by arsenic, cadmium, chromium, lead, and mercury. However, the levels of arsenic, cadmium, and lead for the studied samples were higher than the recommended legal limits. The higher concentrations of arsenic, cadmium, and lead in the sardine from the markets samples could be related to industrialization, transportation, and agricultural activities in the area. The periodical control of heavy metals in the sardine is needed both for the assessment of toxic metal intake from these fish by humans and for generating data for further studies.

## REFERENCES

Abou-Arab AAK, Ayesh AM, Amra HA, Naguib K (1996) Characteristic levels of some pesticides and heavy metals in imported fish. Food Chemistry 57(4): 487-492.

Allen JI, Moore MN (2004) Environmental prognostics: Is the current use of biomarkers appropriate for environmental risk evaulation? Marine Environmental Research 58: 227-232.

Anonymous (1976) List of maximum levels recommended for contaminants by the joint FAO/WHO codex alimentarius commission. Second series 3, Rome.

Anonymous (1983) Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fishery Circular No: 464, Food and Agriculture Organization of the United Nations, Rome.

Anonymous (1984) Meeting on the Biogeochemical Cycle of Mercury in the Mediterranean. Report No. 325, Food and Agriculture Organization of the United Nations, Rome.

Anonymous (1998) Australia New Zealand Food Authority, Food Standards Code. Standard A12 Metals and Contaminant in Food, Issue 37, Canberra, Australia.

Anonymous (2002) Regulation of Setting Maximum Levels for Certain Contaminants in Foodstuffs. Turkish Official Gazette, 23 September 2002, Issue 24885, Ankara, Turkey.

Anonymous (2006) Setting maximum levels for certain contaminants in foodstuffs. Commission Regulation (EC) No: 1881/2006, Publication Office of European Union, Luxembourg.

Anonymous (2008) Regulation of Setting Maximum Levels for Certain Contaminants in Foodstuffs. Turkish Official Gazette, 17 May, 2008, Issue 26879, Ankara, Turkey.

Anonymous (2012) Fishery Statistics 2011. Turkish Statistical Institute, Printing Division, Ankara.

Beširović H, Alić A, Prašović S, Drommer W (2010) Histopathological effects of chronic exposure to cadmium and zinc on kidneys and gills of brown trout (Salmo trutta m. fario). Turkish Journal of Fisheries and Aquatic Sciences 10: 255-262.

Bordajandi LR, Gómez G, Abad E, Rivera J, Fernández-Bastón MD, Blasco J, González MJ (2004) Survey of persistent organochlorine contaminants (PCBs, PCDD/Fs, and PAHs), heavy metals (Cu, Cd, Zn, Pb, and Hg) and arsenic in food samples from Huelva (Spain): Levels and health implications. Journal of Agricultural and Food Chemistry 52: 992-1001.

Burger J, Gaines KF, Shane Boring C, Stephens WL, Snodgrass J, Dixon C, McMahon M, Shukla S, Shukla T, Gochfeld M (2002) Metal levels in fish from the Savannah river: potential hazards to fish and other receptors. Environmental Research 89: 85-87.

Canli M, Atli G (2003) The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution 121: 129-136.

Celik U, Cakli S, Oehlenschläger J (2004) Determination of the lead and cadmium burden in some northeastern Atlantic and Mediterranean fish species by DPSAV. European Food Research and Technology 218: 298-305.

Celino FT, Yamaguchi S, Miura C, Miura T (2008) Testicular toxicity of arsenic on spermatogenesis in Fish. In: Murakami Y, Nakayama K, Kitamura SI, Iwata H, Tanabe S (eds), Interdisciplinary Studies on Environmental Chemistry – Biological Responses to Chemical Pollutants, Terrapub, Tokyo, 55-60.

Cigerci IH, Konuk M, Kutlu HM (2010) Lead toxicity and biochemical characterization of ALAD on endemic prawn, Palaemonetes turcorum. Ekoloji 19(77): 16-22.

Demirayak A, Kutbay HG, Kılıc D, Bilgin A, Hüseyinova R (2011) Heavy metal accumulation in some natural and exotic plants in Samsun city. Ekoloji 20(79): 1-11.

Falcó G, Llobet JM, Bocio A, Domingo JL (2006) Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. Journal of Agricultural and Food Chemistry 54: 6106-6112.

Ferreira-Cravo M, Ventura-Lima J, Sandrini JZ, Amado LL, Geracitano LA, Rebelo M, Bianchini A, Monserrat JM (2009) Antioxidant responses in different body regions of the polychaeta Laeonereis acuta (Nereididae) exposed to copper. Ecotoxicology Environmental Safety 72(2): 388–393.

Ikem A, Egiebor NO (2005) Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). Journal of Food Composition and Analysis 18: 771-787.

Iwegbue CMA, Nwajei GE, Arimoro, FO, Eguavoen O (2009) Characteristic levels of heavy metals in canned sardines consumed in Nigeria. Environmentalist 29: 431-435.

Kargın F, Dönmez A, Çoğun HY (2001) Distribution of heavy metals in different tissues of the shrimp Penaeus semiculatus and Metapenaeus monocerus from the Iskenderun Gulf, Turkey: seasonal variations. Bulletin of Environmental Contamination and Toxicology 66: 102–109.

Kayhan FE, Muslu MN, Colak S, Koc ND, Colak A (2010) Lead (Pb) Levels in Liver and Muscle Tissues of Atlantic Bluefin Tuna (Thunnus thynnus Linnaeus, 1758). Ekoloji 19(76): 65-70.

Lall SP (2002) The minerals. In: Halver JE, Hardy RW (eds), Fish Nutrition, Academic Press, New York, 259-308.

Martins CMG, Barcarolli IF, Menezes EJ, Giacomin MM, Wood CM, Bianchini A (2011) Acute toxicity, accumulation and tissue distribution of copper in the blue crab Callinetes sapidus acclimated to diffirent salinities: in vivo and in vitro studies. Aquatic Toxicology 101: 88-99.

Matek M, Blanuša M (1998) Comparison of two methods for destruction of biological material for determination of selenium. Archives of Industrial Hygiene and Toxicology 49: 301-305.

Olaifa FE, Olaifa AK, Lewis OO (2003) Toxic stress of lead on Clarias gariepinus (African catfish) fingerlings. African Journal of Biomedical Research 6: 101-104.

Olaifa FE, Olaifa AK, Adelaja AA, Owolabi AG (2004) Heavy metal contamination of Clarias gariepinus from a Lake and Fish farm in Ibadan, Nijeria. African Journal of Biomedical Research 7: 145-148.

Plessi M, Bertelli D, Monzani A (2001) Mercury and selenium content in selected seafood. Journal of Food Composition and Analysis 14: 461-467.

Shiber JG (2010) Arsenic, cadmium, lead and mercury in canned sardines commercially available in eastern Kentucky, USA. Marine Pollution Bulletin 62(1): 66-72.

Soliman ZI (2006) A study of heavy metals pollution in some aquatic organisms in Suez Canal in Port-Said Harbour. Journal of Applied Sciences Research 2(10): 657-663.

Suhaimi F, Wong SP, Lee VLL, Low LK (2005) Heavy metals in fish and shellfish found in local wet markets. Singapore Journal of Primary Industries 32: 1-18.

Tarley CRT, Coltro WKT, Matsushita M, Souza NE (2001) Characteristic level of some heavy metals from brazilian canned sardines (Sardinella brasiliensis). Journal of Food Composition and Analysis 14: 611-617.

Tüzen M (2009) Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. Food and Chemical Toxicology 47: 1785-1790.

Yılmaz AB, Turan C, Toker T (2010) Uptake and distribution of hexavalent chromium in tissues (gill, skin, and muscle) of a freshwater fish, Tilapia, Oreochromis aureus. Journal of Environmental Chemistry and Ecotoxicology 2(3): 28-33.

Yılmaz AB, Yılmaz L (2007) Influences of sex and seasons on levels heavy metals in tissues of green tiger shrimp (Penaeus semisulcatus de Hann, 1844). Food Chemistry 101: 1664-1669.