

# Effects of Cooking Methods on the Concentrations of Lead, Chromium and Cadmium in Whitefish (*Rutilus frissi kutum*) from the Caspian Sea, Iran

**Mehdipour, Seyedeh Zahra**

*Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, I.R. IRAN*

**Shokrzadeh, Mohammad**

*Department of Toxicology, Faculty of Pharmacy, Mazandaran Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, I.R. IRAN*

**Khazadi, Saeid\*<sup>+</sup>; Shahsavani, Davar**

*Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, I.R. IRAN*

**ABSTRACT:** *Today seafood plays a significant role in feeding people worldwide. Identification nutritional value of these products over other protein intake is increasing day by day. In parallel with the increase in fish consumption, assessment of hygiene and health of these products is important. In this study, Heavy metals (Pb, Cr, and Cd) were investigated in white fish (*Rutilus frissi kutum*) from the Caspian Sea in Mazandaran province. Different food treatments were used (frying, salting, grilling, microwaving, boiling and steaming). The results obtained were statistically compared with those of raw fish. An ANOVA test was used to compare the mean metal concentrations between and within groups. The results indicated that the heavy metal content in white fish decreased on various cooking methods. Concentrations of grilled, microwaved and boiled fish were considerably decreased.*

**KEYWORDS:** *Whitefish; Heavy metals; Cooking methods.*

## INTRODUCTION

Environmental pollution and its hazards are the most significant problems of societies. Increased population with the development of technology and production can cause a lack of attention to environmental safety [1].

It is well known that fish has low cholesterol, high protein content and polyunsaturated fatty acids (PUFAs) such as an Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). It plays an important role in the human diet [2-5].

---

\* To whom correspondence should be addressed.

+ E-mail: khazadi@um.ac.ir

1021-9986/2018/1/141-147

7/\$/5.07

DOI:

Industrial waste, municipal and agricultural sewage, mining of metals, means of transportation emissions via shipping traffic, all caused the heavy metals as non-biodegradable toxic substances to enter aquatic environments and bioaccumulation by marine inhabitants, especially over the last few decades. Heavy metals present in the environment can be absorbed into living organisms from the surrounding water, sediment, and diet. Therefore population exposure to heavy metals via seafood consumption is unavoidable and may pose a threat to human health. Unfortunately, heavy metal pollution effect on marine ecosystems and humans are intense and very extensive [6-9].

Heavy metals are confused neurological disorders particularly in children and in the foetus, which can lead to weakend performance in IQ test and behavioural changes. Fish can be considered as considerable indicators in water for the estimation of metal contamination level [10].

Heavy metal, such as cadmium and lead are toxic when present in traces for human health [11, 12]. Many metals found in the marine environment pose a risk to human health and animals [13] through the consumption of fish, wherein pollutant and exposure are significant [14]. Pb toxicity can lead to neuronal defects, anemia in children and growth retardation. Also, neurotoxicity, nephrotoxicity and hepatotoxicity, can be occurred following Pb chronic toxicity. Cadmium is a nonessential heavy metal. It is considered as toxic water pollutants and could cause toxicity at each level in human [15]. Cadmium was shown to be associated with inhalation toxicity, osteomalacia, nephrotoxicity and prostatic cancer [16]. Chromium is one of the most widely used industrial metals in addition, it is one of the most important contaminants in various hazardous waste sites universal [17]. Additionally, Cr was reported to be associated with some disorders, such as nasal septa defect [16].

Since heavy metal, such as cadmium, lead, and chromium are harmful to human health, the consumption of seafood will impose wellbeing dangers around people. Large portions metals also metallic exacerbates found in the marine environment pose a risk to human health through the use of seafood, where pollutant concentration and exposure are significant [14]. The pollution of water by toxic heavy metals is an important problem in the world [18]. Because fish is the last chain of the aquatic food web, the level of heavy metals in the marine environment should regularly be monitored to evaluate the possible risks of fish

consumption for human health. Thus it is important to find out the concentrations of heavy metals in marketable fish [8, 19]. Fish is occasionally eaten raw, but it is usually consumed by various processes, such as boiling, frying and microwave oven for cooking. These processing has increased significantly during these days [20, 21]. Several researchers have investigated the presence and the daily intake of chemical pollutants through seafood consumption. In some studies, analyses were performed on raw seafood products. Although in many cases fish consumed after being cooked [22]. The heavy metal analysis of raw samples does not adequate for estimating the intake of heavy metals by a human through seafood consumption. Therefore numerous studies were carried out the effects of culinary practices on heavy metal concentrations in fish and seafood. For example in sea bass [23], sea bass and red seabream [24], sardine, hake, and tuna [25], rainbow trout [26] and African catfish [27, 28]. However, it should be noted that to our knowledge, no studies concerning the effects of cooking on heavy metal content of Caspian whitefish have been carried out.

Therefore, the aim of this study was to evaluate the effects of different culinary practices on the concentrations of Cd, Cr and Pb in white fish (*Rutilus frissi* kutum), which is among the most commonly marketed and consumed in the Caspian Sea coast. The metal concentrations of the cooked samples were compared to those determined in the raw ones.

## EXPERIMENTAL SECTION

### Sample preparation

The white fish, (45 to 50 cm length and 1 to 2 kg weight), used in this study were obtained from Sari coastal water of the Caspian Sea ( Mazandaran Province, Iran). They were kept in cold iced boxes and transported to the laboratory within 2 h. On arrival at the laboratory, the fresh fish were washed with tap water and then prepared using a handling process, i.e. beheading and eviscerating. Samples were filleted and cut into pieces, and then fish fillets were divided into seven groups. Then they were dried in the open air for ready to cook [23].

### Cooking methods

The first group was uncooked. The other six groups (two repetitions) were cooked in the microwave oven (2450 MHz, 10 min), grilled in the oven (180 °C, 20 min),

**Fig. 1: Mean concentration of metals (ppm) in fish muscle tissues in different standards and ecosystems.**

Location (species)	pb	cd	cr	references
Rutilus frisi Kutum	$2.7 \times 10^{-1}$	0.000	-	[31]
Acipenser persicus	$6 \times 10^{-3}$	$2 \times 10^{-3}$	-	[32]
Rutilus frisi Kutum	$8 \times 10^{-3}$	$10^{-3}$	$3.3 \times 10^{-3}$	[33]
WHO	$5 \times 10^{-1}$	$10^{-1}$	$2 \times 10^{-1}$	[34]

fried in sunflower oil (180 °C, 4 min) [23], boiled in a stainless steel container (5min) [26], steamed in a rice cooker (10 min) and salted in a 10% density of brine for 1h with a sample: brine ratio of 1:1 (w/v) [29]. Raw and cooked samples were homogenized in a stainless-steel meat grinder, and each group was analyzed in the same way.

#### Digestion procedures

Fish sample digestion was carried out according to the method described by Tuzen [30] with some modifications. 5 g of the dry homogenized sample was placed in a 50 mL digestion tube, and 4.5 ml of concentrated HNO<sub>3</sub> (65%) were added, where necessary. The samples were placed overnight in the laboratory. The next day 1.5 mL of 72% perchloric acid was added to the samples, then the samples were heated slowly by the hot plate for 6 hours at 150 °C to dissolve. The digest was quantitatively transferred to a 25 ml volumetric flask with deionized water and filtered with Whatman No. 42 filter paper. A blank digest was carried out in the same way. All metals were determined against aqueous standards.

#### Analytic procedure

Determinations of all metal concentrations were performed by atomic absorption spectrometry. Metal concentrations were expressed in milligram per kilogram (mg/kg) dry weight.

#### Statistical analysis

Analysis of variance was used to evaluate the study data, and significant differences among means were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test ( $p = 0.05$ ) (SPSS 13.0 for Windows) [23, 28].

## RESULTS AND DISCUSSION

Mean concentration of metals in fish muscle tissues in different ecosystems are shown in Table 1.

The metal concentrations in cooked white fish in all samples were less than those of uncooked cases. The Pb concentration of raw fish was found to be  $4.62 \times 10^{-4}$  mg/kg. As shown in Fig. 1, despite the decrease in cooked samples, there were no significant differences in Pb concentrations between the raw sample with fried, salted and steamed fish. Although, in comparison with raw samples the decrease in Pb concentration was significant ( $P < 0.05$ ) for grilled, boiled and microwave cooking methods.

The Cd concentration of raw samples was  $3.26 \times 10^{-3}$  mg/kg. According to Fig. 2, in the grilling and boiling methods, the concentration of Cd decreased significantly. So that, the amount of cadmium in these processes were  $2.56 \times 10^{-3}$  and  $2.52 \times 10^{-3}$  respectively.

In our study, the Cr concentration of raw fish was found to be  $2.8 \times 10^{-3}$  mg/kg. The highest value found in the raw samples; while the lowest value was in the boiling samples ( $2.00 \times 10^{-3}$  mg/kg). As shown in Fig. 3, the decrease in Cr concentration was significant ( $P < 0.05$ ) for grilled, boiled and microwave-cooked samples, while it was insignificant for other methods.

#### Discussion

In the present study, we have investigated the effects of different culinary practices on the concentrations of Cd, Cr and Pb in whitefish (*Rutilus frissi* kutum). The mean concentrations of heavy metals analyzed in our study were lower than the maximum permitted concentrations recommended by FAO/WHO [34], but some studies shown that the concentration of heavy metals in another region of Caspian sea and in another species was higher than the standards [35, 36]. So, in our study, we tried to find the best method of cooking to decrease the heavy metals. Recently, many studies have investigated the concentrations of metals in seafood from different countries. However, most of these surveys

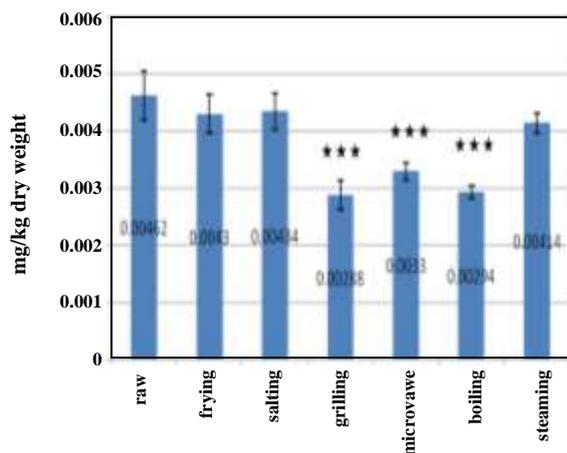


Fig. 1: Pb concentrations (mg/kg) in raw and processed whitefish samples, \*\*  $p < 0.01$ , \*\*\* $p < 0.001$ .

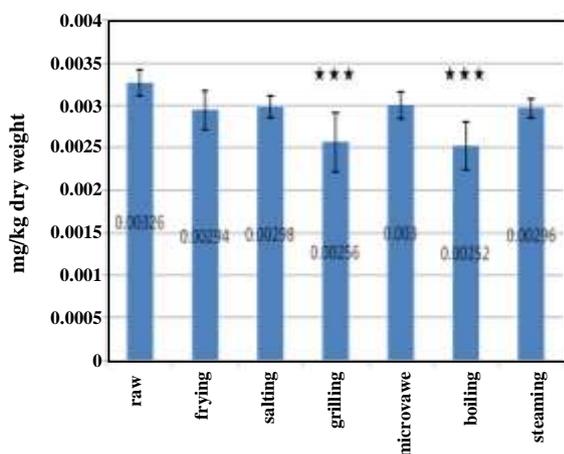


Fig. 2: Cd concentrations (mg/kg) in raw and processed whitefish samples, \*\*  $p < 0.01$ , \*\*\* $p < 0.001$ .

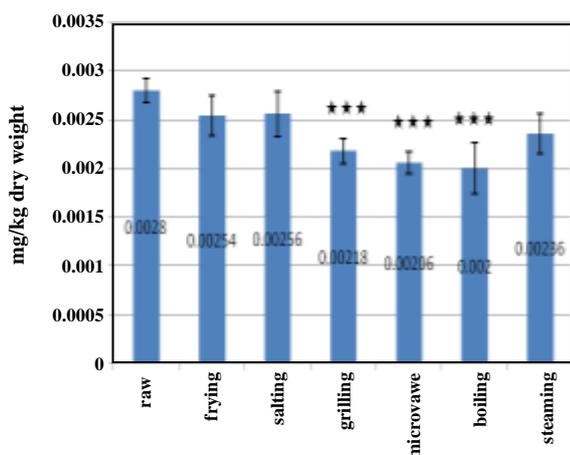


Fig. 3: Cr concentrations (mg/kg) in raw and processed whitefish samples, \*\*  $p < 0.01$ , \*\*\* $p < 0.001$ .

reported the levels of heavy metal concentration on an uncooked fish [2, 5, 14, 37]. There is limited information on the influence of the cooking processes on metal concentrations in seafood. It is well established that in certain conditions, cooking may change the amount of chemical pollutants in foods [20, 38, 39]. About the effects of cooking practices on metals in seafood, a distinguished number of the studies found in the scientific literature have been focused on fishes. Similar to our study, *Ersoy et al.*; have been reported that concentrations of Pb decrease significantly in the sea bass fillets after baking and microwaving when compared to the raw fish [23]. Also, *Ersoy*, showed that grill and microwave heating, reduced the Cr content in African catfish, but no significant difference was shown in Pb level between the raw and cooked fish [27]. *Alberti-Fidanza et al.*, reported that Cd content in raw and cooked foods showed slight differences [40]. *Amiard et al.*, revealed that bioaccessibility was significantly lowered for Cd after cooking a total of seven species of mollusks from, U.K., France and Hong Kong [41]. *Atta et al.*, observed that steaming and baking lead to a decrease in heavy metal concentrations including Cd and Pb after processing in different organs of fish [42]. Thermal processing leads to loss of weight and water, and therefore, chemical contaminants may also be affected by the heat processing [43, 44]. *Gokoglu et al.*, found that cooking had significant affect on the mineral contents [26]. According to *Giovanna Lomolin*, the concentration of Cr decreases during cooking [11]. There are antithetically results about the association between trace element content of cooked and uncooked seafood reported in the literature. For example *Perello et al*, shown that the concentration of Cd, Pb, Mercury (Hg) and Arsenic (As) in sardine, hake and tuna fish determined by inductively coupled plasma-mass spectrometry (ICP-MS) increased during cooking (frying, grilling, roasting, and boiling) [25]. In another study *Kalogeropoulos et al.*, reported that the metal concentrations in cooked fish following pan frying and grilling were higher than those of raw ones [45]. On the other hand, in the study of *Ersoy et al.*, as concentration increased after frying and Pb concentration decreased during baking of sea bass [23]. According to *Kalogeropoulos et al.*, Cr and Cd in some of the samples increase after grilling and pan-frying [46]. *Kalogeropoulos et al.*, stated that a possible explanation of the antithetical results could be the size of cooked fish. They described

that the size of fish was inversely related to the oil uptake and water loss during culinary practice. Therefore during cooking of small portions, higher water loss takes place which in turn leads to increase in metal concentrations [45].

### Conclusions

The concentrations of Pb, Cr and Cd decreased in all cooking methods. Therefore, these methods were found to be appropriate. The investigation indicates that the reductions were statistically significant for the heavy metal and iron of grilled, microwaved and boiled fish. It can be recommended that grilling, boiling and microwaving are the most suitable methods for fish cooking. The reduction depends on cooking conditions, such as temperature, time, cooking methods, etc. Therefore, the metal in fish and seafood can be reduced by choosing an appropriate method of cooking.

### Acknowledgment

This work was supported by the Ferdowsi University of Mashhad. The authors also wish to express their gratitude to the Mazandaran University of Medical Sciences, Sari, for its cooperation support.

Received: Mar 12, 2017; Accepted: Jul. 10, 2017

### REFERENCES

- [1] Tabari S., Saravi S.S.S., Bandany G.A., Dehghan A., Shokrzadeh M. [Heavy metals \(Zn, Pb, Cd and Cr\) in Fish, Water and Sediments Sampled from Southern Caspian Sea, Iran](#), *Toxicology and Industrial Health*, **26**(10):649-56 (2010).
- [2] Ersoy B., Çelik M., [Essential Elements and Contaminants in Tissues of Commercial Pelagic Fish from the Eastern Mediterranean Sea](#), *Journal of the Science of Food and Agriculture*, **89**(9):1615-21 (2009).
- [3] Ersoy B., Çelik M., [The Essential and Toxic Elements in Tissues of Six Commercial Demersal Fish from Eastern Mediterranean Sea](#), *Food and Chemical Toxicology*, **48**(5):1377-82 (2010).
- [4] Fallah A.A., Rahnama M., [Determination of Copper, Zinc and Iron Levels in Edible Muscle of Three Commercial Fish Species from Iranian Coastal Waters of the Caspian Sea](#), *Journal of Animal and Veterinary Advances*, **8**(7):1285-8 (2009).
- [5] Hajeb P., Jinap S., Ismail A., Fatimah A., Jamilah B., Rahim M.A., [Assessment of Mercury Level in Commonly Consumed Marine Fishes in Malaysia](#), *Food Control*, **20**(1):79-84 (2009).
- [6] Idris A.M., Eltayeb M., Potgieter-Vermaak S.S., Van Grieken R., Potgieter J., [Assessment of Heavy Metals Pollution in Sudanese Harbours Along the Red Sea Coast](#), *Microchemical Journal*, **87**(2): 104-12 (2007).
- [7] Mendil D., Demirci Z., Tuzen M., Soylak M., [Seasonal Investigation of Trace Element Contents in Commercially Valuable Fish Species from the Black Sea, Turkey](#), *Food and Chemical Toxicology*, **48**(3):865-70 (2010).
- [8] Uysal K., Emre Y., Köse E., [The Determination of Heavy Metal Accumulation Ratios in Muscle, Skin and Gills of Some Migratory Fish Species by Inductively Coupled Plasma-Optical Emission Spectrometry \(ICP-OES\) in Beymelek Lagoon \(Antalya/Turkey\)](#), *Microchemical Journal*, **90**(1):67-70 (2008).
- [9] Vieira C., Morais S., Ramos S., Delerue-Matos C., Oliveira M., [Mercury, Cadmium, Lead and Arsenic Levels in Three Pelagic Fish Species from the Atlantic Ocean: Intra-and Inter-Specific Variability and Human Health risks for Consumption](#), *Food and Chemical Toxicology*, **49**(4):923-32 (2011).
- [10] Tiimub B.M., Afua M.A.D., [Determination of Selected Heavy Metals and Iron Concentration in Two Common Fish Species in Densu River at Wejja District in Greater Accra region of Ghana](#), *Am. Int. J. Biol.*, **1**:29-34 (2013).
- [11] Lomolino G., Crapisi A., Cagnin M., [Study of Elements Concentrations of European Seabass \(Dicentrarchus Labrax\) Fillets After Cooking on Steel, Cast Iron, Teflon, Aluminum and Ceramic Pots](#), *International Journal of Gastronomy and Food Science*, **5**:1-9 (2016).
- [12] Djedjibegovic J., Larssen T., Skrbo A., Marjanović A., Sober M., [Contents of Cadmium, Copper, Mercury and Lead in Fish from the Neretva River \(Bosnia and Herzegovina\) Determined by Inductively Coupled Plasma mass Spectrometry \(ICP-MS\)](#), *Food Chemistry*, **131**(2):469-76 (2012).
- [13] Sanchooli M.M., Rahdar S., Taghavi M., [Cadmium Removal from Aqueous Solutions Using Saxaul Tree Ash](#), *Iranian Journal of Chemistry & Chemical Engineering (IJCCE)*, **35**(3): 45-52 (2016).

- [14] Al-Saleh I., Shinwari N., Preliminary Report on the Levels of Elements in Four Fish Species from the Arabian Gulf of Saudi Arabia, *Chemosphere*, **48**(7):749-55 (2002).
- [15] Rashed M., Cadmium and Lead Levels in Fish (*Tilapia nilotica*) Tissues as Biological Indicator for Lake Water Pollution, *Environmental Monitoring and Assessment*, **68**(1):75-89 (2001).
- [16] Shokrzadeh M., Saeedi Saravi S., The Study of Heavy Metals (Lead, Cadmium, and Chromium) in Three Species of Most Consumed Fish Sampled from Gorgan Coast (Iran), 2008. *Toxicological & Environmental Chemistry*, **92**(1):71-3 (2010).
- [17] Medeiros M., Rodrigues A., Batoreu M., Laires A., Rueff J., Zhitkovich A., Elevated Levels of DNA-Protein Crosslinks and Micronuclei in Peripheral Lymphocytes of Tannery Workers Exposed to Trivalent Chromium, *Mutagenesis*, **18**(1):19-24 (2003).
- [18] Haq Nawaz B., Rubina K., Muhammad Asif H., Biosorption of Pb (II) and Co (II) on Red Rose Waste Biomass, *Iranian Journal of Chemistry and Chemical Engineering (IJCCCE)*, **30**(4):81-7 (2011).
- [19] Cid B.P., Boia C., Pombo L., Rebelo E., Determination of Trace Metals in Fish Species of the Ria de Aveiro (Portugal) by Electrothermal Atomic Absorption Spectrometry, *Food Chemistry*, **75**(1):93-100 (2001).
- [20] García-Arias M., Alvarez-Pontes E., García-Fernández M.C., Sánchez-Muniz F.J., Freezing/Defrosting/Frying of Sardine Fillets. Influence of Slow and Quick Defrosting on Protein Quality, *Journal of the Science of Food and Agriculture*, **83**(6):602-8 (2003).
- [21] García-Sartal C., del Carmen Barciela-Alonso M., Moreda-Piñeiro A., Bermejo-Barrera P., Study of Cooking on the Bioavailability of As, Co, Cr, Cu, Fe, Ni, Se and Zn from Edible Seaweed, *Microchemical Journal*, **108**:92-9 (2013).
- [22] Domingo J.L., Influence of Cooking Processes on the Concentrations of Toxic Metals and Various Organic Environmental Pollutants in Food: A Review of the Published Literature, *Critical Reviews in Food Science and Nutrition*, **51**(1):29-37 (2010).
- [23] Ersoy B., Yanar Y., Küçükgülmez A., Çelik M., Effects of Four Cooking Methods on the Heavy Metal Concentrations of Sea Bass Fillets (*Dicentrarchus Labrax* Linne, 1785), *Food Chemistry*, **99**(4):748-51 (2006).
- [24] He M., Ke C.-H., Wang W.-X., Effects of Cooking and Subcellular Distribution on the Bioaccessibility of Trace Elements in Two Marine Fish Species, *Journal of Agricultural and Food Chemistry*, **58**(6):3517-23 (2010).
- [25] Perelló G., Martí-Cid R., Llobet J.M., Domingo J.L., Effects of Various Cooking Processes on the Concentrations of Arsenic, Cadmium, Mercury, and Lead in Foods, *Journal of Agricultural and Food Chemistry*, **56**(23):11262-9 (2008).
- [26] Gokoglu N., Yerlikaya P., Cengiz E., Effects of Cooking Methods on the Proximate Composition and Mineral Contents of Rainbow trout (*Oncorhynchus Mykiss*), *Food Chemistry*, **84**(1):19-22 (2004).
- [27] Ersoy B., Effects of Cooking Methods on the Heavy Metal Concentrations of the African Catfish (*Clarias Gariepinus*), *Journal of Food Biochemistry*, **35**(2): 351-6 (2011).
- [28] Ersoy B., Özeren A., The Effect of Cooking Methods on Mineral and Vitamin Contents of African Catfish, *Food Chemistry*, **115**(2):419-22 (2009).
- [29] Goulas A.E., Kontominas M.G., Combined Effect of Light Salting, Modified Atmosphere Packaging and Oregano Essential Oil on the Shelf-Life of Sea Bream (*Sparus Aurata*): Biochemical and Sensory Attributes, *Food Chemistry*, **100**(1):287-96 (2007).
- [30] Tüzen M., Determination of Heavy Metals in Fish Samples of the Middle Black Sea (Turkey) by Graphite Furnace Atomic Absorption Spectrometry, *Food Chemistry*, **80**(1):119-23 (2003).
- [31] Monsefrad F., Namin I., Heidary S., Concentration of Heavy and Toxic Metals Cu, Zn, Cd, Pb and Hg in Liver and Muscles of Rutilus Frisii Kutum During Spawning Season with Respect to Growth Parameters, *Iranian Journal of Fisheries Sciences*, **11**(4):825-39 (2012).
- [32] Agusa T., Kunito T., Tanabe S., Pourkazemi M., Aubrey D.G., Concentrations of Trace Elements in Muscle of Sturgeons in the Caspian Sea, *Marine Pollution Bulletin*, **49**(9):789-800 (2004).
- [33] Anan Y., Kunito T., Tanabe S., Mitrofanov I., Aubrey D.G., Trace Element Accumulation in Fishes Collected from Coastal Waters of the Caspian Sea, *Marine Pollution Bulletin*, **51**(8): 882-8 (2005).

- [34] Koning H.W.de., "Setting Environmental Standards: Guidelines for Decision-Making", Division of Environmental Health World Health Organization Geneva, Switzerland (1987).
- [35] Elsagh A., Determination of Some Heavy Metals in *Rutilus Frisii* Kutum and *Cyprinus Carpio* Fillet from South Caspian Sea, *Pajouhesh And Sazandegi*, **24**(4): 33-44 (2011).
- [36] Elsagh A., Bioaccumulation of Heavy Metals Levels in Muscles of *Rutilus Frisii* Kutum and *Cyprinus Carpio* Fishes of Coastal Waters of the Mazandaran Province, *Caspian Sea, Veterinary Journal*, **2**(95): 41-48 (2012).
- [37] Anand M., Kumaraswamy P., Analysis of Heavy Metals in Fish Samples Along the East Coastal Region of Valinokkam, Ramanathapuram, District Tamilnadu, *Adv Appl Sci Res.*, **4**(6):178-183 (2013).
- [38] Bayen S., Barlow P., Lee H.K., Obbard J.P., Effect of Cooking on the Loss of Persistent Organic Pollutants from Salmon, *Journal of Toxicology and Environmental Health, Part A*, **68**(4):253-65 (2005).
- [39] Zabik M., Zabik M., Booren A., Daubenmire S., Pascall M., Welch R., et al. Pesticides and Total Polychlorinated Biphenyls Residues in Raw and Cooked Walleye and White Bass Harvested from the Great Lakes, *Bulletin of Environmental Contamination and Toxicology*, **54**(3): 396-402 (1995).
- [40] Alberti-Fidanza A., Burini G., Perriello G., Trace Elements in Foods and Meals Consumed by Students Attending the Faculty Cafeteria, *Science of the Total Environment*, **287**(1):133-40 (2002).
- [41] Amiard J.-C., Amiard-Triquet C., Charbonnier L., Mesnil A., Rainbow P.S., Wang W.-X., Bioaccessibility of Essential and Non-Essential Metals in Commercial Shellfish from Western Europe and Asia, *Food and Chemical Toxicology*, **46**(6):2010-22 (2008).
- [42] Atta M., El-Sebaie L., Noaman M., Kassab H., The Effect of Cooking on the Content of Heavy Metals in Fish (*Tilapia Nilotica*), *Food Chemistry*, **58**(1): 1-4 (1997).
- [43] Cabañero A.I., Madrid Y., Cámara C., Selenium and Mercury Bioaccessibility in Fish Samples: an in Vitro Digestion Method, *Analytica Chimica Acta*, **526**(1):51-61 (2004).
- [44] Lind Y., Glynn A.W., Engman J., Jorhem L., Bioavailability of Cadmium from Crab Hepatopancreas and Mushroom in Relation to Inorganic Cadmium: a 9-Week Feeding Study in Mice, *Food and Chemical Toxicology*, **33**(8):667-73 (1995).
- [45] Kalogeropoulos N., Andrikopoulos N.K., Hassapidou M., Dietary Evaluation of Mediterranean Fish and Molluscs Pan-Fried in Virgin Olive Oil, *Journal of the Science of Food and Agriculture*, **84**(13):1750-8 (2004).
- [46] Kalogeropoulos N., Karavoltos S., Sakellari A., Avramidou S., Dassenakis M., Scoullou M., Heavy Metals in Raw, Fried and Grilled Mediterranean Finfish and Shellfish, *Food and Chemical Toxicology*, **50**(10):3702-8 2012.