Determination of Heavy Metals in Some Fruits, Vegetables and Fish by ICP-MS

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Abstract

This study was designed to determine arsenic, lead, mercury and cadmium levels in Spinacia oleracea (spinach), Daucus carota (carrot), Malus pumila (apple), Solanum tuberosum (potato) and canned Thunnus thynnus (tuna fish), Engraulis encrasicolus (anchovy fish), Salmo trutta labrax (sea trout) and Salmo trutta (farm trout). Samples were mixed, blended raw, weighed and placed in labeled sample cups. All samples were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Arsenic, Pb and Hg concentrations were lowest in potato; As, Cd and Pb concentrations were highest in spinach. As and Cd loads were highest in anchovy fish and Pb load was highest in canned tuna. Lead concentrations of all fruits and vegetables exceeded the safe limits. Arsenic concentration in anchovy fish was above the acceptable limit. Some fruits, vegetables and fish species as analyzed in our study may threaten human health because of their heavy metal loads.

Keywords: Contamination, fish, fruit, heavy metal, vegetable.

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INTRODUCTION

The dissemination of heavy metals into the environment adversely affects the quality of foods. Trace metals such as copper (Cu), zinc (Zn), manganese (Mn), cobalt (Co) are necessary for healthy growth and development of humans and animals. In contrast; arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) are indicated as toxic metals for living things and for these toxic metals there is no known homeostasis mechanism in the body. (Morais et al., 2012).

Heavy metals can be spread to the environment in many different ways. Factors leading to the dissemination of heavy metals to the environment include natural degradation of the earth's crust, mining, soil erosion, industrial discharges, effect of industrial and domestic wastes blending in to the seas, urban flow, sewage wastes, harmful agents applied to plants, and air pollution (Morais et al., 2012). Heavy metal pollution in the soil and seas affects the nutritional quality of foods and can reach animals and humans through the food chain. The main sources that cause heavy metals to mix into nature are; mines, wastewater of various metal and paper industries, fertilizers, fossil fuels, pesticides, various chemicals and household waste (Afoakwa, 2008). Lead, As, Cd and Hg are the most common heavy metals that cause soil pollution (Wuana and Okieimen, 2011). Long-term accumulation of heavy metals in soil can lead to a reduction in buffering capacity and pollution of groundwater (Sun et al., 2010). Heavy metals present as particulates or vapor in the atmosphere can be taken into plants, although the uptake is typically less than that of from soil and water (Nagajyoti et al., 2010). For example, vegetables and fruits which have beneficial effects on health with rich vitamin mineral content can affect human health negatively as a result of heavy metal pollution. Same way while some heavy metals such as Cu, Zn, Fe are used by human metabolism in many events, heavy metals such as Hg, Cd and Pb which have no beneficial effect and function, can be transmitted to the water in various ways. At the same time, the presence of these metals in high amounts of water leads to the death of many living creatures, as well as disrupting the natural balance of life (Karayakar et al., 2017).

Heavy metals in food are not found in safe concentrations and the consumption by humans for a long time causes heavy metals to accumulate chronically in the human body. The accumulation of heavy metals in the body can cause many biochemical processes to deteriorate and cause kidney, liver, heart, nerve and bone diseases in humans. Therefore, the international and national regulations on food quality have reduced the permissible maximum levels of toxic metals in foodstuffs as these metals increase the risk of food chain pollution (Sharma et al., 2009).

The aim of this study was to determine the concentrations of As, Pb, Hg and Cd in one edible portion of some fruits, vegetables and some fish species frequently consumed in Turkey.

MATERIAL and METHOD

Sample Selection and Collection

The selection of vegetables and fruits was made in March 2017 and the selection of fish species was made in January 2018. One kg of each fruit and vegetable samples of the products were purchased from local markets in Kartal, Maltepe and Sarıyer districts of Istanbul. Potatoes, spinach and apples were grown at garden conditions, only carrots were grown in greenhouse conditions. For the fish samples; 324 g anchovy, 772 g farm trout, 1980 g sea trout and 80 g canned tuna were purchased from local markets and bazaars in Bakirkoy, Pendik and Uskudar districts of Istanbul. All fish species (except canned tuna fish) were obtained from Marmara Sea.

Sample Preparation

The fruit and vegetable samples were washed under flowing warm water for 3 minutes. Non-renewable parts were removed. The roots of spinach and pale leaves were discarded. Potatoes were peeled and damaged potatoes and the peelings were discarded. Two centimeters of the beginning and end of the carrot, and the skins were discarded. Samples taken from different district markets and bazaars were mixed and blended raw.

For the fish samples; the head, internal organs, bone and tail parts were discarded.Fish taken from different districts were blended homogenously. The samples were weighed with precision kitchen scale and placed in plastic sample cups and labeled. Sixty-three g of spinach, 113 g of potatoes, 130 g of apples, 107 g of carrots, 153 g of anchovy, 164 g of farm trout, 176 g of sea trout and 80g x 3 canned tuna were sent for analysis.

Chemical Analysis

Analysis of the samples was carried out in Yıldız Technical University Central Laboratory. Firstly, samples were digested by microwave digester. A wet ground weight (0.5 g) was digested with 6 mL of nitric acid (HNO_3, 65%) and 2 mL of hydrogen peroxide (H_2O_2, 30%). The digested samples were evaporated to dryness and diluated to 20 mL with deionized water. Concentrations of heavy metals (As, Hg, Pb and Cd) in the digested samples were analyzed with Agilent 7700 ICP-MS. Standard 1: Agilent 8500-6940 2A (10 ppm in 5% HNO_3): As, Cd and Pb, standard 2: Agilent 8500-6940 Hg (10 ppm in 5% HNO_3): Hg, internal satandard: Agilent 5188-6525(100 ppm in 5% HNO_3): Li, Sc, Ge, Rh, In, Tb, Lu, Bi and tune solution: Agilent 5185-5959: Li, Y, Tl, Ce, Co, Mg (1ppb in 2% wt HNO_3) solutions used during analysis.

Calculation

The results were reported as ng/g of fresh food. For this reason, the residual percentages found in the "Food Composition Table" were removed and after the net weight was found, the heavy metal load of one portion was calculated by multiplying the value per gram in the analysis report. The residual ratio of non-edible parts were; 8% for apple, 22% for carrot, 17% for spinach, 19% for potato, 23% for anchovy, 51% for farm trout and 62% for sea trout. There was no residue of non-edible parts for canned tuna.

RESULTS and DISCUSSION

Arsenic, lead and mercury concentrations were lowest in potato. In contrast, spinach had the greatest concentrations of As, Cd and Pb. Mercury levels were found low in all samples (shown in Table 1). Anchovy was found to have the highest levels of As and Cd. Canned tuna and farm trout had the highest concentrations of Pb (shown in Table 2).

According to the comparison of heavy metal levels of all samples with reference codex values; all fruit and vegetable samples had higher Pb concentrations than the values set by both Joint Expert Committee on Food Additives (JECFA) and Turkish Food Codex (TFC) limits (shown in Table 3). In all analyzed fish species, Zn, Cd and Hg values were found below the reference. Lead levels of canned tuna and farm trout were determined as close to the upper limit, and the anchovy's As level exceeded the reference limit (shown in Table 4).

Heavy metals found in food may cause heavy metal accumulation in human body. For this reason, food quality should be monitored within the framework of national and international regulations (Morais et al., 2012). The comparison of the results of heavy metal analysis of fruits and vegetables with the reference codex values is given in Table 3. According to the table, Cadmium level of apple was found to be higher than the reference value of Turkish Food Codex (Turkish Food Codex Regulation on Contaminants, 2011). Moreover, Lead levels of all fruits and vegetables were found to be over the limits of both Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2002) and Turkish Food Codex (Turkish Food Codex Regulation on Contaminants, 2011).

Investigation of heavy metal levels in aquatic organisms that are consumed by humans has great importance for human health. Heavy metals accumulate in the tissues and organs of the fish, and may cause dysfunctions at the cellular and molecular levels when consumed (Kayhan et al, 2010). One of the main causes of water pollution is petroleum. While the upper limit of the amount of petroleum in sea waters should be 10 μg /g, in a study conducted in 2017; it was found to be 540 μg/g in the Black Sea, 148 μg/g in the Marmara Sea, 1100 μg/g in the Bosphorus (İstanbul) and 592 μg/g in the Dardanelles. These results show that the seas are polluted with high amount of petroleum (TUDAV, 2018). In a study, it was stated that meat yields and fat ratios were negatively affected in fish exposed to heavy metal contamination, and the changes occurred differently according to the type and age of fish, and duration of heavy metal exposure (Kayhan et al., 2010).

Arsenic

In a study held in Bangladesh, As values for vegetables was found as 0.25 µg/g and 0.19 µg/g for fruits (Islam et al., 2014). In Osaili and collegues' study, As values found as; 0.31 µg/g in spinach, 0.6 µg/g in carrot and 0.13 µg/g in potato (Osaili et al., 2016). Another study held in 2016, As values were found as; <0.0007 µg/g in potato, <0.00073 µg/g in carrot, 0.01234 µg/g in spinach and <0.00074 µg/g in apple (Shaheen et al., 2016). The values found in this study were lower than the results of previous studies. Shaheen et al. conducted the study in Bangladesh and there may be differences in soil properties and soil characteristics in this region. Osaili and collegues (2016) pointed out that the use of atmospheric pollution and phosphate fertilizers may contribute to the residue of As in agricultural soils. Moreover, Islam et al. (2014) indicate that the As content in the soil was high due to the excessive use of As contaminated irrigation water. In this study, heavy metal loads of one portion of the foods examined were calculated based on the edible amount of nutrients. Arsenic load of 1 portion was found <0.057 µg in carrot, 1.5 µg in spinach, <0.051 µg in potato, <0.068 µg in apple. There is no limit value specified in the JECFA and Turkish Food Codex for arsenic.

In a study conducted in Iran in, the amount of As in tuna fish was analyzed with the atomic absorption spectrometry (AAS) method and the average value was found to be 0.128 µg/g (Khansari et al., 2005). It has been determined that the As level of anchovy was above the maximum consumable level. Moreover, this limit for canned tuna and farm trout were close to the consumable limit (Turkish Food Codex Regulation on Contaminants, 2011; 2002).

In a study conducted in 2013, anchovy were collected from the Black Sea shores of Turkey and Georgia during the hunting season (January, October and November) and analyzed. The As levels of anchovy's edible parts were found between 0.41-0.69 mg/kg. It is stated that the values in edible tissues are not above the values specified in the regulations and do not pose a health risk for consumers (Bat et al., 2014). In our study, in the anchovy sample collected in January, the As value was found as 1.48 µg/g, which is above the Food Codex limits.

Mercury

Hu et al., (2017) determine the Hg value in leafy vegetables as 0.002±0.001 µg/g, in rootstalk vegetables as 0.0003±0.0001 µg/g and in fruits as 0.0003±0.0002 µg/g. In this study, Hg values were found as <0.00032 µg/g in carrot, <0.00031 µg/g in spinach, <0.00031 µg/g in potato and <0.00033 µg/g in apple. Hg values determined in our study for carrot, potato and apple were higher, and Hg value for spinach was lower than the study of Hu and colleagues.. There is no reference limit specified in the Turkish Food Codex for mercury.

According to JECFA, the limits of one portion of the foods examined were 78 µg for carrots, 124.5 µg for spinach, 72.9 µg for potatoes and 92 µg for apples. In our study, these values are as follows: <0.025 µg/per portion in carrot, <0.038 µg/per portion in spinach, <0.023 µg/per portion in potato and <0.031 µg/per portion in apple. The Hg levels found in this study are below the values of JECFA.

Mercury levels were analyzed with the ICP-MS method in the anchovy fish that were caught every two months between October 2012 and April 2013 from the Marmara Sea and the average value was found within acceptable legal limits (0.04±0.00 mg/kg) (Türksönmez & Diler, 2017). In another study, the highest accumulation of heavy metals (lead, cadmium, mercury, copper and chromium) was observed in copper in tilapia fish obtained from Wadi Hanifah, Saudi Arabia in 2010, while the lowest was Hg bioaccumulation. The results were below safe limits for consumption (Abdel-Baki et al., 2011). In Khansari and colleagues' study, Hg level of canned tuna fish was found as 0.117 µg/g which is below the limits set by FAO and WHO (Khansari et al., 2005). In another study, metal levels in edible tissues of fish sold in Konya were analyzed by using inductively coupled plasma optical emission spectrometry (ICP-OES). As a result of the analysis, it has been determined that the Hg value of fish was at a level that will not cause any harm in terms of public health (Günhan and Yalçın, 2015).

Lead

In a study of Islam and colleagues, it was found that the Pb value for vegetables was 0.25 µg/g and 0.19 µg/g for fruits (Islam et al., 2014). Hu et al. (2017) determined the Pb value as 0.022±0,017 µg/g in leafy vegetables. Kim et al. (2012) determined the Pb value as 0.026 µg/g in tuberous vegetables. Osaili and collegues found the Pb value as 0.503 µg/g in spinach, as 1.26 µg/g in carrot and as 1.061 µg/g in potato (Osaili et al., 2016). In Shaheen and colleagues' study, it was found that the Pb value for carrots was 0.029±0.025 µg/g and 0.007±0.006 µg/g for potato (Shaheen et al., 2016). A study held in Serbia, Pb concentration in spinach leaves was found as 3.56 µg/g (Pajević et al., 2018). Adimula et al. (2019) found the Pb value as 0.459±0.03 mg/kg in carrot and 0.74±0.05 mg/kg in apple. In a study in Turkey by using inductively coupled plasma atomic emission spectroscopy (ICP-AES), Pb value was found as 2.21±0.75 µg/g in apple (Hamurcu et al., 2010). In our study, we found Pb values as 0.432 µg/g in carrot, as 0.430 µg/g in spinach, as 0.408 µg/g in potato and as 0.436 µg/g in apple. The Pb values found in our study are lower than what was found in the studies of Osaili et al. (2016), Pajević et al., (2018) and Adimula et al. (2019). However, our results for Pb is higher than the results of the study of Hu and colleagues (Hu et al., 2017). The reason for this result may be only the vegetables grown in the greenhouse were selected in the study of Hu et al. The maximum acceptable limits in Turkish Food Codex for lead and the general limits in JECFA for one portion are as 7.8 µg for carrots, 37.35 µg for spinach, 7.29 µg for potato and 9.2 µg for apple. In our study, the values of Pb were found as 33.6 µg/per portion in carrot, 53.5 µg/per portion in spinach, 29.7 µg/per portion in potato and 40 µg/per portion in apple. The Pb levels detected in our study were higher than permissible level and these results may indicate that some of the fruits and vegetables might cause a potential risk for human health in terms of Pb content. The soil properties and characteristics of the region where fruits and vegetables were grown can be shown among the reasons why the amount of lead is so high. Moreover, it is thought that the soil may have been exposed to lead especially by air.

In Türksönmez and Dilers' study (2017), Pb level of anchovy fish was found as 0.29 ± 0,03 mg/kg which is within the legal limits. For this reason, it has been concluded that the consumption of anchovy fish is not a problem that would threaten human health. Similarly, Khansari et al. (2005) found the Pb level of canned tuna fish within the acceptable limits (0.726 µg/g). In 2005, the Pb content of canned fish of different species from supermarkets in Turkey was analyzed by using AAS method.

The results were found in the range of 0.09-0.40 µg/g and compared with the values in the literature. The results were above the legal limits. As a result of the study, it was stated that metal levels can be reduced by processing raw materials and more frequent analyzes should be made in canned fish in Turkish supermarkets (Tüzen and Soylak, 2007). In our study, Pb level in canned tuna fish was determined as 0.18 µg/g, which is very close to the legal limits set by the Turkish Food Codex, 0.2 mg/kg.

Trout collected in Rize between April and September 2011 were analyzed with ICP-OES and the heavy metal levels of the fish in the muscles, liver, gills and skins were examined. According to the results of the study, it was concluded that the metal levels in trout were lower than the acceptable limit recommended by FAO and WHO and did not threaten human health (Dizman et al., 2017). In our study, heavy metal levels of sea trout were found below Turkish Food Codex limits. However, in farm trout, the Pb load was found to be 0.28 µg/g, which is close to the upper limit of 0.3 mg/kg.

Cadmium

Hu et al., (2017) determine the Cd value of leafy vegetables as 0.013±0.010 µg/g. Osaili and collegues (2016) found the Cd values as 0.129 µg/g in spinach, 0.07 µg/g in carrot and 0.08 µg/g in potato. These values found as 0.023±0.003 µg/g in carrot and 0.013±0.007 µg/g in potato in the study of Shaheen and colleagues (Shaheen et al., 2016). In the study of Pajević et al. the Cd concentrations were found as 0.48 µg/g in spinach leaves and 0.29 µg/g in carrot (Pajević et al., 2018). Adimula et al. (2019) found the Cd values as 0.108±0.02 mg/kg in carrot and 0.139±0.03 mg/kg in apple. In our study, Cd values were found as 0.055 µg/g in carrot, 0.088 µg/g in spinach, 0.069 µg/g in potato and 0.053 µg/g in apple. These values we found in our study are lower than the findings in the studies of Pajević et al. (2018) and Adimula et al. (2019), however, higher than the findings in the studies of Shaheen et al. (2016) and Osaili et al. (2016). This difference can be explained due to the differences in soil properties and soil characteristics in different regions. According to JECFA, the general limits for Cd in one portion were determined as 7.8 µg for carrot, 24.9 µg for spinach and 7.29 µg for potato, and there is no limit set for apple. In our study, Cd values were found as 4.3 µg/per portion in carrot, 10.9 µg/per portion in spinach, 5 µg/per portion in potato and 4.8 µg/per portion in apple. According to our results, the determined Cd levels were lower than the values of JECFA.

In Türksönmez and colleagues' study (2017), Cd level of anchovy fish was determined as 0.07 mg/kg which can be accepted as a safe limit. Similar to this study, Khansari et al. (2005) found the Cd level of canned tuna fish as 0.0223 µg/g and this value is below the acceptable limits of FAO and WHO. In the study of Tuzen and Soylak with different types of canned fish, Cd load was found as 0.25 µg/g and compared with previous studies. The results were found to be above the legal limits (Tüzen and Soylak, 2007). In contrast of this study, in another study held in Turkey, Cd values of edible parts of fish were analyzed by using ICP-OES and the results were found within the safe limits (Günhan and Yalçın, 2015). Similar to the previous study, Cd loads of the fishes in our study were found below the upper reference limits set by Turkish Food Codex.

One of the limitations of this study may be the foods (fruits, vegetables and fish) that were analyzed had a limited variety. On the other hand, one of the strength of this study may include these specific foods were selected as the most common ones that Turkish people consume.

Table 1. Heavy metal levels of fruits and vegetables

Heavy Metals (µg/g)	Fruits and Vegetables			
	Carrot *(Daucus carota)*	Potato *(Solanum tuberosum)*	Spinach *(Spinacia oleracea)*	Apple *(Malus pumila)*
Arsenic	<0.00073	<0.0007	0.01234	<0.00074
Cadmium	0.055	0.069	0.088	0.053
Lead	0.432	0.408	0.430	0.436
Mercury	<0.00032	<0.00031	<0.00031	<0.00033

Table 2. Heavy metal levels of fish species

Heavy Metals (µg/g)	Fish Species			
	Canned Tuna *(Thunnus thynnus)*	Anchovy *(Engraulis encrasicolus)*	Sea Trout *(Salmo trutta labrax)*	Farm Trout *(Salmo trutta)*
Arsenic	0.55	1.48	0.30	0.24
Cadmium	0.008	0.013	0.004	0.010
Lead	0.18	0.22	0.15	0.28
Mercury	0.126	0.064	0.065	0.065

Table 3. Comparison of the results of heavy metal analysis (µg/per portion) with reference codex values for fruits and vegetables

Fruits & Vegetables	**Arsenic**			**Cadmium**			**Lead**			**Mercury**		
	Analysis	*JECFA**	*TFC***	*Analysis*	*JECFA*	*TFC*	*Analysis*	*JECFA*	*TFC*	*Analysis*	*JECFA*	*TFC*
Carrot *(Daucus carota)*	<0.057	ND***	ND	4.3	7.8	7.8	***33.6***	7.8	7.8	<0.025	78	ND
Potato *(Solanum tuberosum)*	<0.051	ND	ND	5	7.29	7.29	***29.7***	7.29	7.29	<0.023	72,9	ND
Spinach *(Spinacia oleracea)*	1.5	ND	ND	10.9	24.9	12.45	***53.5***	37.35	37.35	<0.038	124.5	ND
Apple *(Malus pumila)*	<0.068	ND	ND	***4.8***	ND	4.6	***40***	9.2	9.2	0.031	92	ND

* *Joint FAO/WHO Expert Committee on Food Additives;* ***Turkish Food Codex;* ****Not determined.*

Table 4. Comparison of the results of heavy metal analysis (µg/per portion) with reference codex values for fish species

Fish Species	Arsenic		Cadmium		Lead		Mercury	
	Analysis	*TFC*,1,2*	*Analysis*	*TFC*,1,2*	*Analysis*	*TFC*,1,2*	*Analysis*	*TFC*,1,2*
Canned Tuna *(Thunnus thynnus)*	0.55	1	0.008	0.05	***0.18***	0.2	0.126	0.5
Anchovy *(Engraulis encrasicolus)*	***1.48***	1	0.013	0.30	0.22	0.3	0.064	0.5
Sea Trout *(Salmo trutta labrax)*	0.30	1	0.004	0.05	0.15	0.3	0.065	0.5
Farm Trout *(Salmo trutta)*	0.24	1	0.010	0.05	***0.28***	0.3	0.065	0.5

[*]Turkish Food Codex; [1]Turkish Food Codex Regulation on Contaminants, 2002; [2]Turkish Food Codex Regulation on Contaminants, 2011.

CONCLUSION

Lead concentrations in all fruits and vegetables that were analyzed were found to be higher than the reference codex values of both JECFA and TFC. Moreover, cadmium concentration of apple was determined as higher than the acceptable limit of TFC. Arsenic load of anchovy fish was found to be way higher than the safe limit of TFC. Furthermore, lead loads of canned tuna and farm trout were found to be close to the upper limits of TFC. This study conducted in Turkey has not enough data to comment on human health. There is very limited number of studies about the effects of foods with high heavy metal concentrations on human health. More comprehensive studies are needed to achieve the final results.

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