International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2014 IJFAS Journal-2014-3-1/60-65/ 31 January, 2014
ISSN 2322-4134 ©2014 IJFAS

Nitrate and Heavy Metal Contents In Eggplant (Solanum melongena) cultivated in the farmlands in the south of Tehran-Iran

Fereshteh Saeedifar[1], Parisa Ziarati[2], Yousef Ramezan[1]

1. Food Science & Technology Department, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
2. Medicinal Chemistry Department, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran

Corresponding author: Parisa Ziarati

ABSTRACT: Egg plant, *Solanum melongena* is a common and popular vegetable crop grown in the different parts of Iran and is a common vegetable on Iranian diet. Due to the importance role of Nitrate and heavy metals play in health status of the human body, the present study was initiated to investigate the levels of these contaminants and then determine and emphasis on their toxicological implications in eggplant *(Solanum melongena*) cultivated in the farmlands in the south of Tehran-Iran. To assess the levels of Cadmium and Lead in peeled and unpeeled eggplants, a systematic survey of heavy metal and nitrate concentrations in 880 samples was carried out. Results were compared with the permissible limits (PL), acceptable daily intake (ADI) and provisional maximum tolerable daily intake (PMTDI) as set by World Health Organization (WHO), Food and Drug Administration (FDA) and Joint FAO/WHO expert committee on food additives (JECFA). Analysis of variance (ANOVA) was employed to detect significances among samples from different states and seasons. In autumn studied samples the lead , Cadmium and nitrate contents were much higher. Results showed a significant increase in the Lead concentration in the late of October and beginning of November (P _ value = 0.023) compared to that of August and September. There was also a significant increase in the amount of Cadmium in October (P_ value = 0.028).

***Keywords*:** Nitrate, Heavy metals, Eggplant, *Solanum melongena*, Risk assessment, Tehran

INTRODUCTION

Egg plant, *Solanum melongena* is a common and popular vegetable crop grown in the different parts of Iran and is a common vegetable on Iranian diet. The shape of fruit varies from ovoid, oblong, obovoid, or long cylindrical. Its composition has been recorded as per 100 g of edible portion is Calories 24.0 Kcal, Moisture content 92.7%, Carbohydrates 4.0%, Protein 1.4 g, Fat 0.3 g and vitamins 130g. Bitterness in egg plant is due to the presence of glycol alkaloids which are of wide occurrence in plants of Solanaceae family (Khemnani et al., 2012). It may contain certain medicinal properties because medicinal uses of egg plant have been reported. For example, white egg plant is good for diabetic patients. It can cure toothache if fried egg plant fruit oil is taken. It has also been recommended as an excellent remedy for those suffering from liver complaints and asthma (Khemnani et al., 2012). Vegetables as rich sources of vitamins, minerals, and fibers, and also have beneficial antioxidative effects, their consumption is increasing gradually, particularly among the urban community. However, intake of heavy metal-contaminated vegetables and crops may pose a risk to the human health. Analysis of pollution sources of nitrate shows that the heavily polluted regions are usually associated with larger uses of nitrogen fertilizer and household livestock or poultry (Zhao et al., 2008).This situation is existing for southern parts of

Tehran where provides the major part of the leafy vegetables of Tehran province. According to the recent, studies southern parts of Tehran has the highest levels of pollution of environmental contaminants especially heavy metals that are related to the use of urban and industrial wastewater and agricultural practices (Hani and Pazira, 2010) but to our knowledge there is no study on the nitrate levels of water, food and soil in this area. The sources of nitrites include vegetables, fruit, and processed meats therefore additional studies on the level of nitrate in food and water in populations with well-characterized exposures are urgently needed to further our understanding of cancer risk associated with nitrate ingestion (Ward, 2009). Prolonged accumulation of heavy metals through food source may lead to chronic effect in different organs such as kidney, liver, heart and nervous system of humans (WHO,1992; WHO, 1995; Jarup, 2003). Contamination of vegetables with heavy metal may be due to irrigation with contaminated water, soil pollution, and the addition of fertilizers and metal based pesticides, industrial emissions, transportation, the harvesting process, storage and/or at the point of sale (Khan et al., 2004; Zhuang et al., 2009; Behbahaninia and Mirbagheri ,2008 **).** Research findings show that at least 20 million hectare of land in North and South Africa, South America, Middle East, Southern Europe, South west America, Mexico and a significant part of Central and East Asia is irrigated with untreated sewage chiefly for cultivating vegetables (Carr 2005; Dayle 1998). Investigation of soil pollution levels in fields and farmlands in the country has reveal that the quantity of Cd and Pb in samples collected from contaminated areas in the provinces of Tehran, Guilan, Mazandaran, Zanjan, Esfehan (Kalantari et al., 2006; Charkhabi et al., 2008; Abdolhossein et al., 2010; Bigdeli and Seilsepour 2008). It has been reported that vegetables and water contamination to Pb and Cd around Gorgan city, Iran (Shahryari and Shehamat 2012). Accumulate metals at concentrations that are probably toxic to human health has been reported in Vegetables grown at environmentally contaminated sites in Tehran and Shahre Rey (Mousavi and Ziarati 2013; Bigdeli and Seilsepour 2008).

Due to the importance role of Nitrate and heavy metals play in health status of the human body, the present study was initiated to investigate the levels of contamination with them and then determine and emphasis on their toxicological implications in eggplant *(*Solanum melongena) cultivated in the farmlands in the south of Tehran-Iran in summer and autumn 2013 .

MATERIALS AND METHODS

Sampling method

At harvest time (August to the beginning of November, 2013) Eggplant *(Solanum melongena*) samples were randomly collected from ten vegetable farmland located in agricultural areas in Tehran province in the south parts of Tehran city(each farm was more than 10 hectares) and each sample consists of 10 subsamples . All sample sites were recorded using a hand-held Global Position System (GPS). 880 samples were collected in three consequent months: last days of August, September ,October and beginning of November 2013. All peeled and unpeeled samples have rinsed 4 times then followed by the procedure.

Study Area

The location and description of all 10 vegetable farmland sites in the south of Tehran in this study are provided in figure 1.

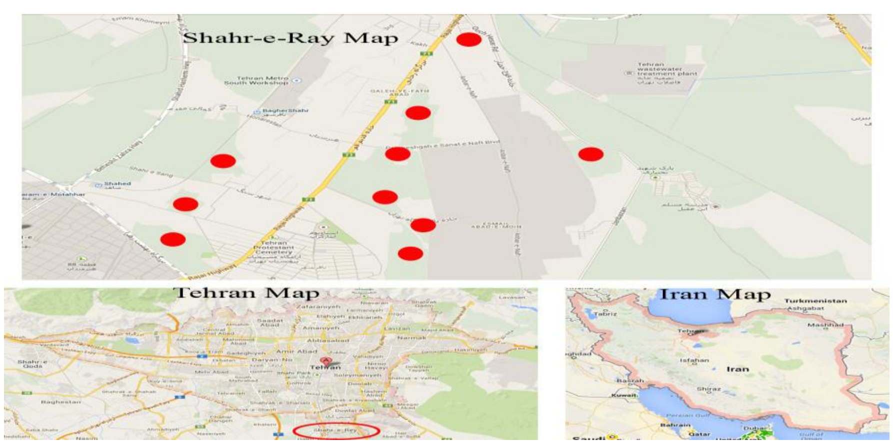

Figure 1. Description of Eggplant sampling

Quantitative determination of Heavy Metals

For heavy metal analyses approximately 50.0 g of each sample (peeled and unpeeled samples) accurately weighed and digested in accordance with Analar grade nitric acid (Merck 65%), hydrogen peroxide (Merck about 30%) and concentrated Hydrochloric acid were used for the digestion. Application of concentrated HNO_3 along with thirty percent hydrogen peroxide H_2O_2 for mineralization of samples to the complete digestion of samples following Environmental Protection Agency (EPA) Method 3052 was done. All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid at least overnight, and rinsed abundantly in deionized water and dried in such a manner to ensure that any contamination does not occur. Five-point calibration curves (five standards and one blank) were constructed for each analyte. The calibration curve correlation coefficient was examined to ensure an $r^2 \geq 0.998$ before the start of the sample analysis. The digested samples were diluted with 10% HNO_3 and brought up to 50 mL and analyzed by a graphite furnace atomic absorption spectrophotometry, (GFAAS). The measurements were performed using a PerkinElmer PinAAcle 900T atomic absorption (AA) spectrophotometer and using at least five standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines (AOAC ,1998). A certified standard reference material (Alpha – Line, Chem Tech Analytical, England) was used to ensure accuracy, and the analytical values were within the range of certified values. All recoveries of the metals studied were greater than 95%.

Quantitative determination of nitrate:

For nitrate analysis, sub-samples were chapped and mixed with a food processor. 50 to 100 grams of sub-sample were weighed and placed into a mixer. Deionized water was added to the samples (nine times than exact the sample weight) and the water and sub-sample were homogenized for 10 minutes. A 30 gram sample of homogenate was placed in a centrifuge tube, and 0.5 ml of H_2O_2 was added and the tube was capped and shaked well by the hand after adding H_2O_2. All samples were centrifuged at 3500 rpm for 3 min. The supernatant was then separated and filtered with filter paper wattman ≠ 1 and nitrate concentration in the filtrate was determined calorimetrically by a flow injection analysis system (Leif, 1979). Nitrate content was expressed as mg nitrate per kg on a fresh weight basis (mg NO_3/kg FW) unless otherwise stated. Nitrate concentration in eggplant samples was calculated from nitrate content in peeled and unpeeled samples separately.

Statistical Method

State differences on the basis of the states (peeled and unpeeled) of eggplant samples and the season of study (summer and autumn) were determined by student t-test. The changes were calculated by one way Anova and for analysis of the role of multiple factors univariate analysis was used by SPSS 17.Probability values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Results

Both Cd and Pb are considered as the most significant heavy metals affecting vegetable crops. In Australia and New Zealand, the maximum level (ML) of Cd and Pb allowable in vegetable crops has been set by the Australian and New Zealand Food Authority (ANZFA) (Kachenko and Singh 2006; ANSTAT ,2001). For Cd the ML is 0.1 mg /kg fresh weight (FW) for all vegetable types. The ML of Pb is also 0.1 mg/ kg FW for all vegetable types excluding Brassicas (0.3 mg /kg FW). The Commission of the (European Communities ,2001) and the Codex Alimentarius Commission (2001, 2004) sets similar levels of Cd and Pb for vegetable crops. Both organizations set the ML for Cd as 0.2 mg /kg FW for leafy vegetables and fresh herbs, 0.1 mg kg − 1for stem and root vegetables and 0.05 mg/ kg for the remaining ungrouped vegetables. For Pb, both organizations set the ML of 0.3 mg/ kg FW for brassicas, leafy vegetables and herbs, and 0.1 mg/ kg FW for all remaining vegetables. The level of Cd in vegetables of commercial growing regions located within the Sydney Basin has been extensively studied (Jinadasa et al., 1997). WHO prescribed limit for Cd contents in medicinal plant at 0.3 mg/kg [WHO 1989; Ziarati and Asgarpanah ,2013) and the maximum acceptable concentration for food stuff is around 1 ppm (Neil 1993; Ziarati and Asgarpanah ,2013) . Cadmium intoxication can lead to kidney, bone and pulmonary damages (Godt et al., 2006 ;Ziarati and Asgarpanah ,2013) .

The mean concentration of lead found in peeled and unpeeled eggplant samples is shown in figure 2. Concentrations were expressed in terms of mg/Kg on a dry weight basis. The results indicated that the lead content in unpeeled samples was much higher than peeled samples and lead contents in both states of peeled and unpeeled samples in autumn season were much higher.

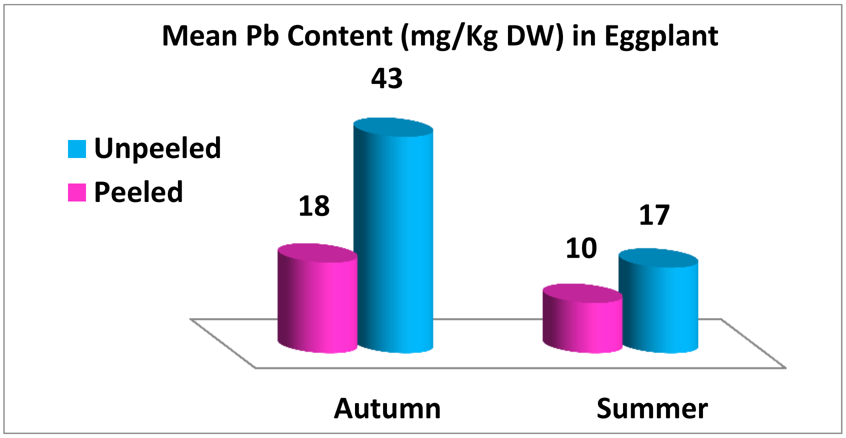

Figure 2. The mean of Lead content (mg/Kg DW) in peeled and unpeeled Eggplant samples

The results also revealed that the Cadmium content in peeled samples was much higher than unpeeled samples and in autumn all samples had more cadmium contents in comparison to summer samples (figure 3).

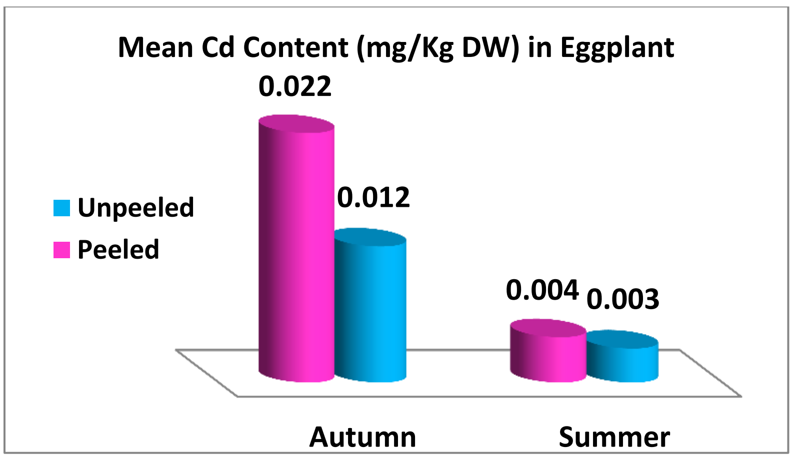

Figure 3. The mean of Cadmium content (mg/Kg DW) in peeled and unpeeled Eggplant samples

According to the current maximum levels are laid down in Annex, section 1 of Commission Regulation (EC) No 1881/2006 of 19 December 2006 the nitrate contents compared . In figure 4 , the results of nitrate content in 2 different seasons and states are indicated.

The intakes of nitrate and nitrite from food were calculated as a global level on the basis of mean food consumption in the GEMS/Food regional diet (WHO, 1998). Intake from drinking water was added, assuming a water consumption of 2 L/day. The mean concentration in water that was used in the intake calculation was 4 mg/L for nitrate which was representative of the usual concentrations found in water (WHO ,1998). An average body weight of 60kg was used for the global intake assessment (Speijers and van den Brandt ,1993). As we have no documented data for dietary intake in Iran, nitrite intakes from peeled eggplant according to consumption of 2.8 (g/day) in Middle Eastern (WHO Food Additives Series: 50) estimated in table 1.

Table1. Nitrite intakes from sources other than food additives in the Middle Eastern

State of Eggplant	Nitrate (mg/kg FW)	Summer Consumption (g/day)	Intake (mg/day)	Nitrate (mg/kg FW)	Autumn Consumption (g/day)	Intake (mg/day)
peeled	248.44	2.8	0.696	311.09	2.8	0.871
unpeeled	267.56	2.8	0.749	289.23	2.8	0.810
ADI *			50			50

* ADI: acceptable Daily Intake

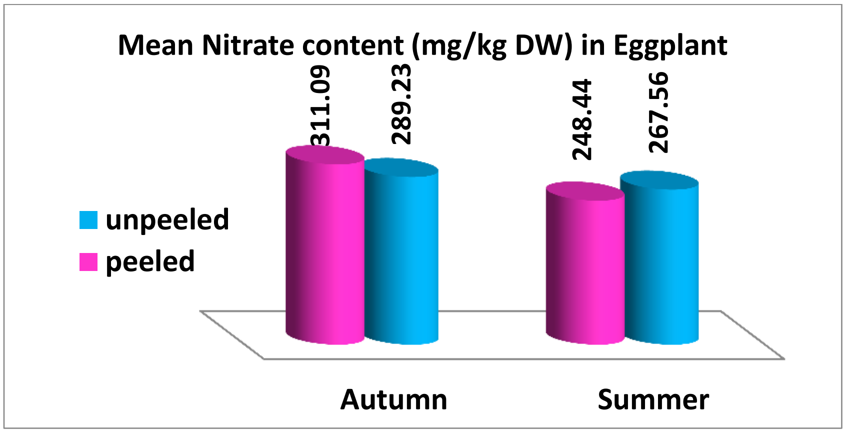

Figure 4. The mean of Nitrate content (mg/Kg FW) in peeled and unpeeled Eggplant samples

DISCUSSION

All the samples examined had lead concentration above the permissible limit by FAO/WHO in the food (FAO/WHO 2001; Commission regulation (EC) No 466/2001 ; Codex Alimentarius Commission 2001 and 2004; CODEX ALIMENTARIUS COMMISSION 1994; Godt et al., 2006 ;Ziarati and Asgarpanah ,2013) .In autumn studied samples the lead , Cadmium and nitrate contamination was much higher. Results showed a significant increase in the Lead in the late of November and beginning of October (P _ value = 0.023) compared to that of August and September. There was also a significant increase in the amount of Cadmium in October (P_ value = 0.028). The increasing amount of nitrate in the Eggplant was not significant in the two seasons of summer and autumn. The different agricultural areas in the south of Tehran in this research show no significant effect on the nitrate content in eggplant samples tested. Growing conditions like temperature, stress (drought), hot or dry winds effect the nitrate accumulation in plants (WHO,1995), it has been found that vegetables grown in winter, the time of year with low temperature and less sunlight has a higher Nitrate content (European commission,1998). In this study we found higher nitrate content in small size of most crops. Probably during initial growth, the nitrate taken up by the plant for root and shoots development process. In this stage, the roots are able to take up more nitrate than required and nitrate accumulates in the leaves and stems of the crop. As the plants growing up, the leaves would be able to convert more nitrates into plant protein and therefore fewer nitrates can be found in the plant by maturing progress.

It can be suggested that the consumption of average amounts of these contaminated eggplant does not pose a health risk for the consumers as the values obtained are below the FAO/WHO limits for heavy metals and nitrate intake. Heavy metals have a toxic impact, but detrimental impacts become apparent only when long-term consumption of contaminated vegetables occurs. Because of the vegetables is not alone source of dietary heavy metals, certain groups of consumer such as elderly with cardiovascular problems and kidney deficiency who may intake these food items for long term should be extra cautions as they are more susceptible to toxicities. It is therefore suggested that regular monitoring of nitrate and heavy metals in vegetables and other food items should be performed in order to prevent excessive buildup of these heavy metals in the human food chain. Appropriate precautions should also be taken at the time of production, transportation and marketing of vegetables.

ACKNOWLEDGEMENTS

This research work was supported by Pharmaceutical Sciences Branch, Islamic Azad University.

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