



# Health risk assessment of citrus contaminated with heavy metals in Hamedan city, potential risk of Al and Cu

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## Abstract

**Background:** Fruits especially citrus species are an integral part of human diet. Contamination of foodstuffs by heavy and toxic metals via environmental pollution has become an inevitable challenge these days. Therefore, the effect of pollutants on food safety for human consumption is a global public concern. In this regards, this study was conducted for Al and Cu health risk assessment through the consumption of citrus species (orange, grapefruit, sweet lime and tangerine) in Hamedan city in 2015.

**Methods:** After collecting and preparing 4 samples from each citrus species with acid digestion method, the concentrations of Al and Cu were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) with three replications. In addition, SPSS was employed to compare the mean concentrations of metals with maximum permissible limits (MPL) of the World Health Organization (WHO).

**Results:** The results showed that the mean concentrations of Al in citrus samples were  $3.25 \pm 0.35$  mg/kg and higher than WHO maximum permissible limits. The mean concentrations of Cu in citrus samples with  $0.16 \pm 0.05$  mg/kg are lower than WHO maximum permissible limits. Also, the computed health risk assessment revealed that there was no potential risk for children and adult by consuming the studied citrus.

**Conclusion:** Based on the results, consumption of citrus species has no adverse effect on the consumers' health, but as a result of the increased utilization of agricultural inputs (metal based fertilizers and pesticides, sewage sludge and wastewater) by farmers and orchardists, regular periodic monitoring of chemical pollutants content in foodstuffs are recommended for food safety.

**Keywords:** Citrus, Heavy metal toxicity, Health risk, Food safety, Hamedan

**Citation:** Rezaei Raja O, Sobhanardakani S, Cheraghi M. Health risk assessment of citrus contaminated with heavy metals in Hamedan city, potential risk of Al and Cu. Environmental Health Engineering and Management Journal 2016; 3(3): 131–135. doi: 10.15171/EHEM.2016.11.

## Article History:

Received: 3 June 2016

Accepted: 5 July 2016

ePublished: 2 August 2016

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## Introduction

Vegetables and fruits, as they supply the important and essential components for human health such as minerals, vitamins, tartaric acid, citric acid, malic acid, mineral salts, cellulose and woody fibers, tannins, gums, pectin, volatile oils and coloring matters are an integral part of human diet. Consumption of these products on regular basis is vital in providing essential nutrients to the human body for promoting health (1-3). Nowadays, contamination of heavy metals in foods has become an unavoidable challenge (4). Heavy metals are important environmental pollutants, especially in regions with high anthropogenic pressure. Their presence in the environment particularly atmosphere and water, even in trace amounts, can lead to serious problems to all organisms. Heavy metal accumulation in soils and resulting in foodstuffs such as vegetables and fruits through irrigation with polluted water, metal based fertilizers and pesticides, emissions from industrial,

transportation, harvesting process and storage of products can lead to adverse effects on food quality. So, heavy metals are among the major pollutants of food supply and may be considered as the most important problem to our environment (5-8).

Because heavy metals have long biological half-lives, non-biodegradable and persistent, they can be bioaccumulated via the soil-plant-food chains and the presence of high amount of heavy metals in the environment indicates a potential risk for human health and for the environment. Uptake of high amounts of heavy metals can lead to a number of serious adverse health effects such as depleting of some essential nutrients in the body, impaired psychosocial behaviors, decrease in immunological defenses, retardation in intrauterine growth, disabilities associated with undernourishment and a high spread of pharynx, larynx, esophagus, stomach and small intestine cancer (4,9). Although a number of heavy metals such as Cu, Fe,



Mn, Ni and Zn at lower quantities act as micronutrients, they become toxic at higher contents (10,11).

Potential health risk and food safety issues make this as one of the most serious health and environmental concerns (12). Therefore, over the past few decades, the increasing demand of food safety has motivated researches regarding the risk associated with the consumption of food products, contaminated by different types of environmental pollutants such as heavy and toxic metals and pesticides (13).

Aluminum is not considered to be an essential element in humans, however, it is prevalent in water, air, plants and consequently in all the food chain (7). Literally, Al is easily found at quantifiable concentrations in various tissues and biological fluids (14,15). It should be noted that high levels of Al can lead to a number of adverse health effects such as osteomalacia, colic, fatigue, dementia dialectica, anaemia and other blood disorders, neurodegenerative disorders, reduced renal function, dental caries, Parkinson and Alzheimer diseases along with kidney and liver dysfunctions, Al is considerably less toxic than other toxic metals (16-18).

Copper is one of most abundant trace elements with vitamin-like impact in human body and living systems and it is found in a wide range of foods we eat such as nuts, several fruits and vegetables, red meat, shellfish, as well as in many vitamin supplements (19). Although small amount of Cu (50-120 mg) is found in the human body, it plays a critical role in different kinds of biochemical processes (7). Copper, an essential micronutrient for growing plants can be applied via organic and artificial fertilizers and it is important for healthy hormone secretion, nerve conduction, along with the growth of bones and connective tissue. A constant diet of Cu, even at entirely allowable limits, can break down the barrier that keeps undesirable toxins from entering the brain, and an increase in the production of beta-amyloid. On the contrary, critical doses of Cu can result to inflammation in the brain tissues, anorexia, fatigue, hair loss, acne, allergies, depression, premenstrual syndrome, migraines, anxiety, childhood hyperactivity, panic attacks, kidney and liver dysfunction, strokes, elevated cholesterol, adrenal hyperactivity and insufficiency, learning disorders, as well as autism and cancer (18-20). Mausi et al reported that mango and orange fruits sold in Kenya posed no health risks to consumers based on their daily intake of metal levels, as the values were within PDTI standards of World Health Organization (WHO) (21). Chandorkar and Deota studied the health risk assessment of Ar, Cd, and Pb content of foods grown around the city of Vadodara (India) and noted that the study population was at risk of heavy metal toxicity through the consumption of food samples (22). Guerra et al studied the potential health risk assessment of Cd, Pb, Ni, Co and Cr in most regularly consumed vegetables in Brazil, and reported that the consumption of analyzed food samples can be considered safe and without adverse health effects to human (23).

Fruits as integral parts of human diet are widely con-

sumed, they contribute a large fraction to the heavy metals intake, and therefore, strict control of these elements is recommended. Therefore, this study aims to investigate the health risk assessment of Al and Cu in four citrus species (grapefruit, sweet lime, orange, and tangerine) marketed in Hamedan city in 2015.

## Methods

### Sampling and sample preparation

In this study, 16 samples of popular citrus species (grapefruit, lemon, orange, and tangerine) were bought from different markets in Hamedan city so as to analyze their Al and Cu content. All specimens were washed with deionized water to eliminate airborne pollutants. In order to reduce the water content of fruit samples, the edible parts of the each sample were weighed and air-dried for a day. Then, in order to remove the sample moisture, all specimens were oven-dried at 70–80°C for 24 hours. Finally, dried samples were powdered using a grinder and then sieved through a Muslin cloth and packed into polyethylene bags (4).

### Sample digestion

After weighing 0.5 g of each powdered citrus samples in three replicates for each sample and placed them in crucibles, the samples were digested with HClO<sub>4</sub> and HNO<sub>3</sub> (1:4) solution. The samples were left to cool and made up to a final volume of 25 mL with deionised water. Subsequently, the samples were well shaken and centrifuged at 3000 rpm to remove solid particles. The resulting homogenized samples were completely mixed before subsamples were taken for analysis in order to confirm the homogeneity of the mixture (4). Finally, the content of Al and Cu were analyzed in samples using the ICP-OES (Varian, 710-ES) with three replications.

### Statistical analyses

Data were statistically analyzed utilizing one sample *t* test to compare the mean concentrations of Al and Cu with WHO maximum permissible limits using the SPSS statistical package version 20.

### Human health risk assessment

Estimated average daily intakes (EADIs) of a metal in food and assumption of food consumption were used to investigate long term health risks to consumers. In this regard, for each type of exposure, the EADI was calculated by using Equation 1:

$$EADI = \frac{C \times F}{W \times D} \quad (1)$$

where *C* represents the concentration of metal in each commodity (mg/kg); *F* indicates the mean annual intake of food per person (the average daily intake of citrus species for adult and children is considered to be 0.01 kg per person per day); *D* indicates the number of days in a year (365 days); and *W* represents the mean body weight (70 kg for adult and 15 kg for children) (4,24).

The WHO has set values for toxicity, called acceptable daily intakes (ADIs) for a large number of chemicals, including some essential trace elements (25). The health risk indices were computed by dividing the EADI by the ADIs (mg/kg/day) established by FAO/WHO Codex Committee (24,26). The HI was computed by using Equation 2:

$$HI = \frac{EADI}{ADI} \quad (2)$$

When the HI >1; the food involved is considered a risk to the concerned consumers. When the HI <1, the food involved is considered as acceptable (no concern) to the concerned consumers (24).

## Results

The contents of Al and Cu in the analyzed citrus samples are shown in Table 1. Data in Table 1 showed that the percentage of metals contamination of citrus samples were approximately 100%. The contents of Al in samples were higher than WHO maximum permissible limits (MPL) (0.20 mg/kg) (14). The highest content of Al was found in grapefruit ( $3.65 \pm 0.82$  mg/kg) while the lowest was found in tangerine ( $2.94 \pm 0.37$  mg/kg). In addition, the Cu contents of citrus samples were lower than WHO MPL (4.0 mg/kg) (27). The highest content of Cu was found in sweet lime ( $0.20 \pm 0.18$  mg/kg) and the lowest in tangerine ( $0.12 \pm 0.03$  mg/kg).

The systemic health risk assessment due to heavy metals encountered in citrus species is summarized in Table 2. The results showed that the calculated EADIs ranged between  $1.55 \times 10^{-5}$  to  $2.20 \times 10^{-3}$  mg/kg/day, while the hazard indices (HI) ranged from  $3.87 \times 10^{-4}$  to  $2.36 \times 10^{-2}$  for the analyzed metals indicating no direct hazard to human health, in spite of their presence in the food.

## Discussion

Human exposure to heavy metals such as Al and Cu through environment or consumption of contaminated foods is known to be responsible for many human health problems (30,31).

The results of this study indicate that the mean concentrations of Al in citrus samples were  $3.25 \pm 0.35$  mg/kg and were higher than WHO maximum permissible limits. In this regard, studies have shown that in plant origin foodstuffs, the total Al content in plant tissues is under the influence of Al in soil (32). Moreover, It was verified that Al accumulation in plants is a problem when they are grown in acid soils (33). Saracoglu et al determined Al content in apricot samples were purchased from Turkey ranged from 0.08 to 0.22 mg/kg (7).

The findings of this study show that the mean concentrations of Cu in citrus samples were  $0.16 \pm 0.05$  mg/kg and lower than WHO maximum permissible limits. Researchers have determined Cu concentrations in various types of fruits from different countries in the world. Saracoglu et al determined Cu content in apricot samples purchased from Turkey, ranging from 0.92 to 6.49 mg/kg (7). Radwan and Salama surveyed the levels of Cu in various fruits sold in Egyptian markets, and reported that the Cu concentrations ranged from 0.90 to 25.90 mg/kg for Cu (5). Onianwa et al determined a mean Cu concentration of 2.13 mg/kg in some orange samples obtained from Nigerian markets (34). Parveen et al analyzed Cu content in some fruits marketed in Pakistan and reported that the mean concentrations of Cu in specimens were  $1.61 \pm 1.94$  mg/kg (35). Comparison of the results of this study with other studies is shown in Table 3.

As shown in Table 2, health index values of studied metals (Al and Cu) for children and adults are less than 1. Ac-

**Table 1.** Concentrations of heavy metals in the citrus samples (mg/kg)

Metal	Citrus species sample				Mean concentration $\pm$ SD
	Grapefruit	Sweet lime	Orange	Tangerine	
Al	$3.65 \pm 0.82$	$2.98 \pm 1.12$	$3.43 \pm 1.36$	$2.94 \pm 0.37$	$3.25 \pm 0.35$
Cu	$0.20 \pm 0.08$	$0.20 \pm 0.18$	$0.12 \pm 0.09$	$0.12 \pm 0.03$	$0.16 \pm 0.05$

**Table 2.** Acceptable and estimated daily intakes and health index for metals found in citrus samples

Metal	ADI (mg/kg/day) (14,28,29)	Mean Concentrations (mg kg <sup>-1</sup> )	EADI (mg/kg/day) (Children)	HI (Children)	EADI (mg/kg/day) (Adult)	HI (Adult)
<b>Grapefruit</b>						
Al	0.02	3.65	$2.20 \times 10^{-3}$	$1.10 \times 10^{-1}$	$4.71 \times 10^{-4}$	$2.36 \times 10^{-2}$
Cu	0.04	0.20	$1.21 \times 10^{-4}$	$3.01 \times 10^{-3}$	$2.58 \times 10^{-5}$	$6.46 \times 10^{-4}$
<b>Sweet Lime</b>						
Al	0.02	2.98	$1.80 \times 10^{-3}$	$8.99 \times 10^{-2}$	$3.85 \times 10^{-4}$	$1.92 \times 10^{-2}$
Cu	0.04	0.20	$1.21 \times 10^{-4}$	$3.01 \times 10^{-3}$	$2.58 \times 10^{-5}$	$6.46 \times 10^{-4}$
<b>Orange</b>						
Al	0.02	3.43	$2.07 \times 10^{-3}$	$1.03 \times 10^{-1}$	$4.43 \times 10^{-4}$	$2.21 \times 10^{-2}$
Cu	0.04	0.12	$7.23 \times 10^{-5}$	$1.81 \times 10^{-3}$	$1.55 \times 10^{-5}$	$3.87 \times 10^{-4}$
<b>Tangerine</b>						
Al	0.02	2.94	$1.77 \times 10^{-3}$	$8.86 \times 10^{-2}$	$3.80 \times 10^{-4}$	$1.90 \times 10^{-2}$
Cu	0.04	0.12	$7.23 \times 10^{-5}$	$1.81 \times 10^{-3}$	$1.55 \times 10^{-5}$	$3.87 \times 10^{-4}$

**Table 3.** Comparison of present mean values in fruit species with other studies result

Fruit Sample	Area	Metals (mg/kg)	
		Al	Cu
Grapefruit	Present study	3.65	0.20
Sweet Lime		2.98	0.20
Orange		3.43	0.12
Tangerine		2.94	0.12
Grapefruit	Turkey <sup>a</sup>	-	5.12
Lemon		-	4.80
Finike orange		-	5.01
Valansiya orange		-	3.86
Yafa orange		-	4.28
Papaya		-	1.44
Water melon	Ghana <sup>b</sup>	-	1.90
Banana		-	3.23
Mango		-	1.24
Pear		-	1.48
Pineapple		-	4.23
Lima orange		-	46.80
Pera orange		Brazil <sup>c</sup>	-
Tahiti lime	-		69.00
Sweet lime	-		25.30
Orange	Nigeria <sup>d</sup>	-	29.70
Avocado		-	4.65
Pawpaw		-	6.50
Banana		-	10.20
Orange	Nigeria <sup>e</sup>	-	0.23
Avocado pear		-	3.10
Pawpaw		-	5.29
Pineapple		-	0.64

<sup>a</sup> Özcan et al (6); <sup>b</sup> Bempah et al (11); <sup>c</sup> de Moraes Barros et al (36); <sup>d</sup> Ihesinachi et al (37); <sup>e</sup> Ihesinachi and Eresiya (38).

According to USEPA, the HI <1, implies that there will be no obvious health risks for consumers (39). The results of this study reveal that the average HI value was 0.01 for adults and 0.05 for children. So, it can be concluded that the Al and Cu in fruit samples do not present carcinogenic health risks to consumers. Nevertheless, the non-carcinogenic risks resulting from the utilization of citrus were greater for children than for adults.

### Conclusion

This study investigates the levels and health risk assessment of Al and Cu in four commonly consumed citrus species (orange, grapefruit, sweet lime and tangerine) in Hamedan city, and can help in creating awareness of the detrimental effects caused by heavy metals. In line with the results of this study, it can be concluded that the percentage of metals contamination of citrus samples were about 100%. Furthermore, according to the results, no direct hazard to adult and children health was observed, due to the consumption of analyzed citrus species, despite the presence of Al and Cu in the samples.

Although controlled consumption of studied citrus species has no adverse effect on the consumers' health, due to the increased use of agricultural inputs, especially metal based fertilizers and pesticides, sewage sludge and wastewater by farmers and orchardists, regular periodic moni-

toring of chemical pollutants content specially heavy metals in foodstuffs are recommended for food safety.

### Acknowledgments

The authors would like to appreciate the Hamedan Branch, Islamic Azad University for providing the necessary facilities to conduct and complete this study.

### Ethical issues

The authors certify that all data collected during the study are presented in this manuscript, and no data from the study has been or will be published separately.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

All authors participated in the study design, literature search, writing of the manuscript, data acquisition, analysis, and interpretation. All authors critically reviewed, refined, and approved the manuscript.

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