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RESEARCH ARTICLE

Determination of Essential and Toxic Metals from Vegetable, Fruits and their Daily Intake by the Population of Hyderabad City, Pakistan

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Abstract

The aim of present study was to determine the concentrations of essential metals such as iron (Fe), cupper (Cu), manganese (Mn), zinc (Zn), magnesium (Mg), calcium (Ca), sodium (Na) and potassium (K) and toxic metals (TMs) were detected from vegetables and fruit samples and prior to analyzed by electro thermal/flame Atomic/Cold Vapor Absorption spectrometry (ETAAS/FAAS/CVAAS). The samples of vegetables (14) and fruit (13) samples were randomly collected from different Markets of Hyderabad City, Pakistan. The mean concentration of vegetables were found to be higher in spinach (50.1±1.52, 39.7±0.55) for Ca and K respectively), (15.6±0.35 and 10.8±0.11) mg/Kg on dry weight basis for Na and Mg respectively). However concentrations of TMs (Cd and Pb) were found slightly higher in vegetables than fruits. The proposed method was validated by the analysis of certified reference material of Bush Branches and leaves (NIM-GBW07602) and Peach Leaves (NIST-1547). The determined values of CRM were found in good agreement with the certified values at p-value of 0.05. The CAD method was successfully applied to real samples.

Keywords: Vegetable; Fruit; Trace and Essential Elements; Pakistan

Introduction

Fruits and vegetables continue to be the major sources of nutrients, including proteins, vitamins, macro and essential trace elements, and minerals in human diet for proper growth, body development and maintenance of overall health and well-being [1,2]. Essential elements are generally found in different environments such as aquatic medium, vegetables and atmosphere [3]. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals [4,5]. Toxic elements like (Cd, Cr, Mn and Pb) are considered as hazardous element to the environment and human health. [6,7]. The main sources of metal contamination are industrial development and human activities [8,9]. Contamination with TMs is important, particularly in agricultural production systems and human health [10]. Several Factors influencing on the level of TMs in plants and fruits such as climate change, cultivated land adjacent to polluted area, irrigated water, environmental pollution, nature of the soil on which the plant is grown, anthropogenic activities, and harvesting time of the mature plant [11]. However, these toxic elements are entered into human body by ingestion of contaminated food, air and water, to create the vulnerability of vital organs of human body such as liver, kidney tissues and brain [12]. These elements can be very harmful to humans and animals even at low concentrations when ingested over a long period of time [13]. There is a relationship between long-term effects on health and the presence of these metals in foods [14]. Thus, it becomes essential that they are kept at acceptable and safe levels because they can create clinical problems for human health [15,16]. Analysis of metals in food samples has conventionally been performed by atomic absorption spectrometry (AAS) [17-19]. The determination of metal ions at trace levels by flame atomic absorption spectrometry (FAAS) has several advantages such as high selectivity, speed, and fairly low operational cost. However, the direct determination of trace elements by Graphite Furnace atomic absorption spectrometry (GFAAS) at extremely low concentrations is often very difficult due to insufficient sensitivity of the methods and matrix interferences [20-22]. The main objective of the present study was to determine the concentrations of essential and TMs metals from different fruits and vegetables, and further study was aimed to examine whether the fruits and vegetables were safe for the population of Hyderabad City.

Materials and Methods

Instrumentation

Instrumentation A Hitachi Model 5000 Z Flame Atomic Absorption Spectrometry was used for determination of Fe, Cu, Mn, Zn, Mg, Ca, Na and K in all understudy samples, ETAAS was used for Cd and Pb determination. However cold vapor technique was used for Hg determination. The instrument conditions and temperature programs of graphite furnace as are given in Table 1. The calibration curves for Fe, Cu, Mn and Zn (0.4-2.0 mg L^{-1}), Ca, K, Na and Mg (0.5-1.5 mg L^{-1}) Cd (0.5-2.0 ug/L), Pb (10-30 ug/L) and Hg (1-3 ug/L) were established by using of working standard solutions. Hollow cathode lamps were used and operated at recommended current.

S. No.	Metals	Wave length (nm)	Regression Equation	Regression $(R)^2$	Limit Of Detection (LOD)	Limit Of Quantification (LOQ)
1	*Fe	238.204	0.03x+0.0001	0.9999	0.026	0.087
2	*Cu	327.395	0.0116x-0.00002	0.9999	0.06	0.2
3	*Mn	257.61	0.0554x-0.0002	0.9999	0.009	0.032
4	*Zn	213.857	0.0529x+0.0002	0.9996	0.041	0.139
5	*Ca	396.847	0.002x+3E-05	0.9992	0.189	0.632
6	*Mg	280.491	0.0559x-0.0001	0.9996	0.0101	0.034
7	*Na	588.995	0.0115x+0.0001	0.9995	0.042	0.138
8	*K	766.491	0.0305x+0.0004	0.996	0.017	0.056
9	**Pb	253.7	0.0005x+0.0001	0.9999	1.41	4.69
10	**Cd	228.8	0.0052x+0.00003	0.9995	0.128	0.427
11	**Hg	283.3	0.0129x-0.0003	0.9996	0.043	0.144

*=mg/Kg; **=µg/Kg

Table 1: Regression

Sampling

In the present study, fruit and vegetable were collected from the local market of Hyderabad city, Pakistan. The samples were collected on random basis in the year of 2016-2017. Before analysis, all collected samples were first washed with tap water, and then twice washed with distilled water.

Analysis of Fresh Fruit and vegetable Samples: (0.5-2.0) g of vegetable and fruits samples was weighed in 100 ml of the conical flask and then added 10 ml of HNO_3 to this mixture and then left the sample solution for overnight. After acid addition, samples were heated on an electric hot plate at 250 °C for 2 hr until a complete colorless solution occurred. After cooling, the resulting solutions were filtered through a Whatman 42 filter paper into a 25 ml volumetric flask and were diluted with distilled water up to the mark. Afterwards, the subsequent determination of under studied metals was performed by ETAAS/FAAS and CVAAS spectrometry. Blank samples were also treated in same way as samples were treated. The proposed procedure was also applied to CRMs and real samples.

Statistical Evaluations

The statistical analysis was performed by computer program of Excel. Student's t test was used to assess the significance of the differences between concentrations of elements in fruits and vegetables.

Results and Discussion

Metal concentrations in fruits and vegetables

Fruits and vegetables play significant role and widely used for dietary purposes and they contain important vitamins, minerals, plant chemicals and some metals that need in the daily food as a dietary supplementation. When these minerals and trace metals were found in small/high concentrations become toxic with bad effects on human health. The present study aims to detect Essential and toxic metals and find these elements their concentrations in some fruits that is most consumed in Hyderabad city. Concentrations of Fe, Cu, Mn, Zn, Ca, Mg, Na, K, Cd, and Hg in different vegetables collected from different areas of Hyderabad City as given in (Table 2a,2b,3a and 3b). Concentrations in these samples are varied quietly such as Fe (0.032–0.63), Cu (0.01-0.25), Mn (0.005-0.09), Zn (0.028-0.35), Mg (3.2-10.8), Ca (4.13-50.1), Na (3.22-15.6) K (4.11-39.7), Pb (0.54-6.98), and Cd (0.01-0.91) mg/Kg. The obtained results of the present study illustrated that the amount of Fe (0.634±0.05) in the potato vegetable is considerably high than other vegetables at p-value of ($p < 0.05$). Spinach vegetable contains high levels of Ca (50.00±1.55) and Na (15.56±0.55), which is useful for maintaining bone health. However, K level was found higher in ginger (39.75±0.55) mg/Kg (Table 2a and 2b). The concentration of Cu (0.256±0.0004 mg/Kg) found nearly twice in vegetable similarly, the mean concentration of Mn (0.005-0.09) and Zn (0.028-0.35) mg/g found to be in vegetables.

S. No.	Samples	Iron (Fe)	Copper (Cu)	Manganese (Mn)	Zinc (Zn)	Magnesium (Mg)	Calcium (Ca)	Sodium (Na)	Potassium (k)
1	Potato	0.63±0.05	0.095±0.001	0.021 ±0.0005	0.334±0.01	9.02±0.16	22.9±0.23	12.1±0.17	4.11±0.15
2	Tomato	0.12±0.005	0.011±0.0002	0.09±0.002	0.124±0.002	4.04±0.08	10.25±0.15	8.81±0.41	35.4±0.45
3	Cabbage	0.11±0.004	0.015±0.0001	0.02±0.0003	0.088±0.002	4.64±0.08	18.6±0.55	10.9±0.35	35.3±0.45
4	Cucumber	0.33±0.005	0.25±0.004	0.08±0.007	0.312±0.312	5.91±0.15	9.25±0.14	8.81±0.41	35.2±1.11
5	Turaii	0.13±0.004	0.0114±0.0002	ND	0.088±0.002	4.96±0.05	8.51±0.16	5.72±0.15	33.9±1.23
6	Tendey	0.14±0.005	0.0142±0.0002	0.011±0.0002	0.35±0.01	3.37+_0.05	20.88±0.75	9.65±0.42	32.9±0.74
7	Ladyfinger	0.156±0.005	0.031±0.001	0.010±0.0002	0.076±0.076	10.8±0.11	50.1±1.55	9.71±0.25	23.5±1.15
8	Mint	0.36±0.01	0.0656±0.001	ND	0.158±0.003	9.13±0.13	34.9±0.45	10.1±0.26	34.4±0.44
9	Garlic	0.06±0.002	0.0642±0.001	0.012±0.0002	0.134±0.003	3.21±0.09	4.13±0.08	3.22±0.12	26.1±0.45
10	Spinach	0.43±0.02	0.054±0.001	ND	0.194±0.003	8.33±0.17	50.1±1.52	15.5±0.55	37.4±1.22
11	Ginger	0.031±0.001	0.0574±0.001	0.005±0.0001	0.028±0.001	5.79±0.07	8.02±0.15	7.45±0.22	39.7±0.55
12	Lemon	0.088±0.002	ND	0.018±0.0003	0.25±0.01	4.34±0.15	29.2±0.52	4.91±0.15	23.1±0.51
13	Onion	0.093±0.004	ND	0.012±0.0002	0.07±0.002	6.04±0.09	22.7±0.25	5.01±0.21	25.4±0.57

Data are not significantly different as determined by sample t- test. at P < 0.05 level

Table 2a: Determination of Fe, Cu, Mn, Zn, Mg, Ca, Na, and K in different in Vegetables (mg/Kg)

S. N0.	Vegetables Samples	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)
		Mean± STD		
1	Potato	1.68±0.05	1.13±0.06	0.026±0.002
2	Tomato	1.78±0.06	0.88±0.03	0.012±0.001
3	Cabbage	2.05±0.08	1.18±0.04	0.021±0.002
4	Cucumber	2.25±0.07	0.76±0.06	0.011±0.001
5	Turaii	3.04±0.14	1.21±0.07	0.031±0.002
6	Tendey	3.12±0.15	0.233±0.01	0.011±0.001
7	Ladyfinger	1.49±0.09	0.35±0.01	0.016±0.001
8	Mint	2.89±0.11	0.76±0.02	0.036±0.003
9	Garlic	1.46±0.07	0. 45±0.02	0.029±0.001
10	Spinach	1.07±0.05	0.51±0.03	ND
11	Ginger	1.25±0.06	0.61±0.03	0.023±0.001
12	Lemon	1.12±0.04	1.32 ±0.09	ND
13	Onion	1.93±0.004	0.62±0.04	0.023±0.001

Each value is a mean of three replicates (n = 3) ± Std. Data are not significantly different as determined by sample t- test at P < 0.05 level; ND: not detected

Table 2b: Determination of Cd, Pb and Hg in Vegetables (μg/Kg), (n = 3)

In the fruit samples, the concentration of Mg (8.21±0.15), K (42.3±0.7), Ca (37.1±1.2), and Na (7.72±0.18) mg/kg was found to be the higher in banana, pomegranate, and watermelon (Table 3a and 3b). The lower concentrations of Mg (1.87±0.04), Ca (3.75±0.05), Na (3.09±0.12) and K (10.8±0.1) were observed in Chico, apple and strawberry as shown in (Table 3a and 3b). Iron contents in fresh fruits especially in pear (0.12±0.002) and plum (0.12±0.005) record the minimum values (Tables 1,3a and 3b) as compared to other fruits, whereas, the amount of Fe was found to be significantly higher in pineapple (0.48±0.005) and melon (0.19±0.005) mg/Kg respectively. Although content of Zn was observed highest in grapefruit (0.43±0.007) and lowest concentration was found to be in chikoo (0.003±0.0001) mg/kg. Zn is an essential element for plants; its presence in excessive amounts can be detrimental for plant growth [23].

The concentration of Fe, Cu, Mn, Zn, Mg, Ca, Na, and K in fruit samples were found in the range of 0.01-0.48 mg/Kg (95% confidence interval [0.0348, 0.1904] 0.006–0.051 [95% CI 0.02411, 0.03898], 0.003-0.018 [0.00496, 0.01013], 0.003-0.43 [-0.0042, 0.1356], 1.87-8.21 [4.033, 6.401], 3.75-37.1 [12.95, 27.66], 3.09-7.72 [4.361, 5.920], 10.8-42.3 [19.19, 30.59] mg/Kg. The contents of Fe, Cu, Mn, Zn, Mg, Ca, Na, and K in vegetable samples were found to be in the range 0.031-0.63, [95% CI 0.0987, 0.3138] , 0.01-0.25 mg/Kg [95% CI 0.0106, 0.0930], 0.005-0.09 [0.00587, 0.04109], 0.028-0.35 [0.1040, 0.2349], 3.2-10.8 [4.319, 7.589], 4.13-50.1 [13.03, 31.52], 3.22-15.5 [6.581, 10.633] 4.1-39.7 [24.01, 35.42], respectively.

In order to inter elements distribution, data of fruits and vegetable are not significantly different as determined by sample t- test at P < 0.05 level as can be seen in Figure 1.

S. no	Fruits samples	Iron (Fe)	Copper (Cu)	Manganese (Mn)	Zinc (Zn)	Magnesium (Mg)	Calcium (Ca)	Sodium (Na)	Potassium (K)
1	Chikoo	0.162 ±0.005	0.006 ±0.0002	0.006 ±0.0001	0.003 ±0.0001	3.85±0.04	3.7±0.05	6.30±0.21	23.1±0.26
2	Strawberry	0.112±0.005	0.023±0.001	0.012±0.0002	0.014±0.0003	1.87±0.04	14.2±0.16	5.14±0.16	10.8±0.15
3	Melon	0.19 ±0.005	0.031±0.001	0.004±0.0001	0.054 ±0.001	6.34±0.22	17.7±0.24	7.12±0.24	31.2±0.34
4	Mango	0.052±0.005	0.029±0.001	0.005±0.0001	0.056 ±0.001	5.91±0.11	11.9±0.45	5.74±0.16	24.3±0.55
5	Grapefruit	0.022±0.001	0.022±0.001	0.006±0.0001	0.431±0.007	3.99±0.06	18.6±0.35	5.21±0.15	23.1±0.53
6	Peach	0.11±0.005	0.041±0.001	0.010±0.0001	0.016±0.0003	4.10±0.06	7.88±0.21	4.96±0.15	29.1±0.35
7	Pear	0.01±0.0001	0.042±0.001	0.006±0.0001	0.006±0.0001	7.66±0.22	34.6±1.11	4.28±0.15	14.6±0.22
8	Watermelon	0.078±0.005	0.038±0.001	0.003±0.0001	0.06± 0.002	7.66±0.22	34.5±1.11	7.72±0.18	38.5±1.15
9	Plum	0.01±0.0001	0.032±0.001	0.004±0.0001	0.018±0.0003	4.85±0.08	14.7±0.42	4.51±0.15	27.65±0.33
10	Apple	0.038±0.0004	0.034±0.001	0.011±0.0001	0.011±0.0002	2.58±0.05	5.13±0.11	3.09±0.12	14.3±0.25
11	Pineapple	0.48±0.005	0.051±0.001	0.018±0.0001	0.028±0.0005	4.94±0.07	29.4±0.65	4.44±0.14	16.2±0.44
12	Banana	0.18±0.005	0.044±0.0005	0.012±0.0002	0.142 ±0.005	8.21±0.15	34.6±0.85	4.13±0.15	42.3±0.75
13	Pomegranate	0.019±0.0003	0.016±0.0002	0.007±0.0003	0.018±0.0005	5.86±0.11	37.1±1.21	4.26±0.17	28.7±0.35

Data are not significantly different as determined by sample t- test at P < 0.05 level

Table 3a: Determination of Fe, Cu, Mn, Zn, Mg, Ca, Na, and K in different Fruits (mg/Kg), (n=3)

S. NO	Fruits samples	Lead (Fe)	Cadmium(Cu)	Mercury (Hg)
		µg/Kg		
1	Chikoo	1.56±0.11	0.085±0.005	ND
2	Strawberry	1.87±0.12	0.035±0.002	ND
3	Melon	1.05±0.03	0.032±0.001	ND
4	Mango	1.13±0.04	0.25±0.01	ND
5	Grapefruit	1.89±0.05	0.011±0.001	0.025±0.001
6	Peach	1.03±0.06	0.014±0.001	0.019±0.001
7	Pear	0.15±0.01	0.031±0.001	ND
8	Watermelon	0.36±0.015	0.066±0.003	ND
9	Plum	0.64±0.013	0.064±0.003	ND
10	Apple	0.43±0.01	0.054±0.002	ND
11	Pineapple	0.32±0.01	0.057±0.002	0.022±0.001
12	Banana	0.58±0.03	ND	ND
13	Pomegranate	0.67±0.03	0.005±0.0002	0.017±0.001

Data are not significantly different as determined by sample t- test at P < 0.05 level; ND: not detected

Table 3b: Determination of Cd, Pb and Hg in Fruits (µg/Kg), (n=3)

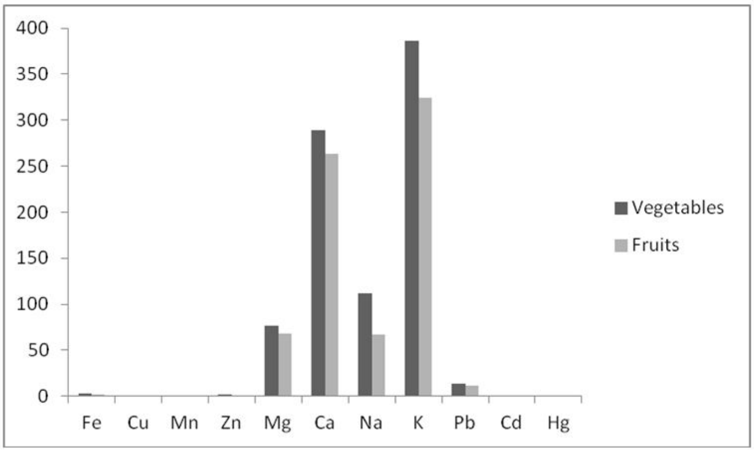

Figure 1: Mean level of essential and toxic metals in vegetables and fruits

The accumulation of essential metals was obtained slightly below in fruits and vegetable samples as compared to permitted limits. The contents of TMs in most of the samples were found within safe. However, some researchers have found high contents of the elements in plants grown on contaminated soils [24-27]. The results of present study indicate that the Pb level was found to be slightly higher in vegetable therefore Pb is the major component contributing the environmental pollution [28]. The low uptake of heavy metals by fruit and vegetables shows that the health risks for human are insignificant. The need to effectively monitor the contents of TMs in fruit and vegetables is not only of environmental concern, but also a public health safety interest [29]. Toxic metals are released into water, plants, soil, and food by natural and human activities [30]. Due to that reason, it is important to determine the concentrations of TMs in fruit and vegetables. An excess ingestion causes neurological anomalies, hepatic and renal disturbances [31].

TMs concentrations including (Cd, Pb and Hg) understudy samples were significantly lower/not detected. Although in this study, the essential metals were found under safe limits. Although the soil in agricultural lands near Hyderabad city has a pH value above 7, this is an advantage for preventing the toxic effects of TMs (Table 4). The present study demonstrated that the intake of essential and TMs is fruit and vegetables of Hyderabad city, Pakistan and provides specific information on the average dietary intake of studied metals as given in Table 5. Moreover, the present study showed that toxic TMs were detected in fruit and vegetable but these metals were founded at a low level as compared to TDIs permitted levels (Table 5). These TMs are most responsible for major and minor contribution to cause serious health issues, as these metals are considered as a toxic even in ultra-small doses.

Elements	Certified Value	Obtained Value	% Recovery	% Relative Standard Deviation (RSD)
	x ± ts/√n	x ± ts/√n		
Bush Branches and leaves (NIM-GBW07602)				
*Ca	2.2	2.15±0.11	97.7	5.12
*Mg	0.29	0.286±0.02	98.6	6.99
*Na	1.1	1.08±0.03	98.2	2.78
*K	0.85	0.86±0.03	101	3.49
**Cu	5.2	5.11±0.21	98.3	4.11
**Fe	1020	1015±59	99.5	5.81
**Zn	20.6	19.8±1.3	96.1	6.57
**Mn	58	57.1±2.8	98.4	4.9
Peach Leaves (NIST-1547)				
**Cd	0.026	0.025±0.001	96.2	4
**Pb	0.87	0.85±0.04	97.7	4.71
**Hg	0.031	0.029±0.002	93.5	6.9

*=% values; **=mg/Kg

Table 4: The results of certified reference material (CRM), (n=6)

Daily dietary intake estimate of fruits and vegetable samples

$$\text{Estimated daily dairy intake} = \frac{\text{level of element } (\mu g/g) \times \text{mass of food consumption (g)}}{\text{per person per day}}$$

These foods will provide the recommended daily amounts of essential elements as shown in Table 5. The average consumption of these studied samples was estimated by the population of different areas of Hyderabad city, Pakistan. Essential and TMs in fruit and vegetables are usually expressed in terms of the provisional tolerable daily intake (PTDI). The FAO/WHO have set a limit for the essential and TMs intake based on average per person. The daily intakes of studied metals were calculated on the consumption of a minimum 80 gm/person/day of fruit and vegetables and compared with permitted levels which recommended by WHO/FDA [32].

Elements	Vegetables	Fruits
Fe*	2.67	1.46
Cu*	0.67	0.41
Mn*	0.28	0.098
Zn*	2.14	0.85
Mg	76.2	67.8
Ca	289.2	264
Na	111.9	66.9
K	386	324
Pb*	14.1	11.7
Cd*	0.67	0.704
Hg*	0.01912	0.00664

*µg (Including intake limits)

Table 5: Estimated daily dietary intake of metals (mg/person/day) from food samples

Conclusion

The CAD method was used for determination of essential and toxic metals in fruit and vegetables which were collected from the different area of Hyderabad City, Pakistan. In vegetable samples essential metals were detected within safe limits, while concentrations of TMs such as Pb, Cd and Hg were found below the maximum permitted limits of WHO/FDA. However, in most of the fruit samples the content of Hg was not detected (ND). Regular consumption of the investigated fruits and vegetables will provide the adequate amounts of the essential metals needed for humans, but found within permitted limits and to ensure the global public safety. In addition to, an excess ingestion of these vegetables may pose serious effect on human health. Regarding the polluted environment, humans are exposed the TMs by ingestion of food which may lead to serious health risks. The study concludes that the levels of TMs in vegetables and fruits having no potential health hazards to consumers of locally produced foodstuffs.

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