

Essential and Toxic Heavy Metals Status in Some Fruits from Turaba District (Saudi Arabia), Health Risk Assessment

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Abstract: This study was carried out to determine the essential (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) and the toxic heavy metals (Al, As, Cd and Pb) in fruit samples of commonly consumed in Turaba District, (Saudi Arabia). Samples were digested by microwave assisted reaction system using (3:1) HNO₃:H₂O₂ mixture. The metals were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES). Studied samples includes bananas (*Musa acuminata*), tomatoes (*Solanum lycopersicum*), guava (*Psidium guajava*), grapes (*Vitis spp*), date palm (*Phoenix dactylifera*), mangos (*Mangifera indica*), cantaloupe melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), orange (*Citrus maxima*), mandarin (*Citrus reticulata*), lemon (*Citrus limon*) and pomegranate (*Punica Granatum*). The method was validated in terms of linearity, accuracy and precision, limit of detection (LOD) and limit of quantification (LOQ). The recovery (%) was found to be between 91.6–103.4%. It was found that Ca (~14.79 mg/kg), Mg (~10.46 mg/kg), Na (~6.327 mg/kg), K (~166.33 mg/kg) and Zn (2.85 mg/kg) were predominant among the major elements, while, Cr (~0.001 mg/kg), Cu (~0.147 mg/kg), Fe (~0.104 mg/kg) and Mn (~0.010 mg/kg) were comparable. The concentration of toxic heavy metals (Cd, Al, As and Pb) were mostly below LOD and they may not develop any health problems, while Co and Ni were not detected in all studied fruit samples. Moreover, the estimated concentrations of all metals in the present study were lower than the limits that permitted by Saudi Food and Drug Authority (SFDA) and World Health Organization (WHO). This is results is also confirmed by the estimated daily dietary elements intake (EDDEI) values. This indicate that, fruit types of commonly consumed in Turaba District and other parts in Saudi Arabia may not rises any health risk to consumers. Student's t-test, ANOVA test at 95% confidence interval and Microsoft excel were employed to estimate the significance of values obtained.

Keywords: Toxic Heavy Metals, Fruits, Estimated Daily Intake, Atomic Emission Spectrophotometer, Saudi Arabia

1. Introduction

Mineral nutrient are fundamentally metal and other inorganic compounds. The life cycle of these nutrients begins in soil that provide minerals to plants (fruits) and through them minerals go to humans. Based upon their requirements these minerals were classified into three different categories. Major elements such as potassium (K), magnesium (Mg), sodium (Na) and calcium (Ca) are required in amounts of up to 10 g/day. While the requirement of secondary and micro minerals ranges from 400 to 1500 mg/day and 45 ug/day to 11 mg/day, respectively [1]. It is well known that nutritional elements are essential regulators of several physiological and metabolic reactions that are important for maintaining good health. Approximately one-third of all the human proteins require the presence of metal ions/nutritional elements to function appropriately [2]. The human body cannot synthesize nutritional elements, and hence, the diet must contain nutrient elements supply their regular amounts.

Fruits such as tomatoes, date palm, orange and lemon are important source in the daily diet and they continue to be the major sources of nutrients. They contribute essential mineral nutrients for maintaining good health [3, 4]. In addition, they contains 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 [5, 6]. Current development of human health related studies requires a growing number of elements to be monitored in food matrices. Most of the literature conducted on bioaccumulation and toxicity of heavy metals as the heavy metal pollution is global concern [7-9]. This increasing pattern of heavy metals have adverse health effects for humans [10-13]. Different elements are present in our diet, they are actually necessary for good health, but others may cause acute or chronic toxicity. For instance, Ca and Mg are necessary for proper development of bone and structural tissue formation and play important roles in glucose and protein absorption and metabolism [14]. While Zn, Cu and Mn play important roles in maintaining proper human health. Moreover, the toxicity of some metals like Pb and Cd can reduce mental and central nervous function, and damage to blood composition, lungs, kidneys, liver, and other vital organs [15].

The measurement of essential and toxic heavy metals is increasingly attracting interest from physicians because deviations in elements uptake and/or metabolism may related to certain dysfunctions. Moreover, a great effort has been expended on developing analytical procedures for elemental measurements in food samples and improving their sensitivity and specificity [16]. Furthermore, the determination of essential and toxic heavy metals like Pb, Cd, As, Cr and Mn in various food items has been widely reported elsewhere [17-25]. The need to effectively monitor the concentrations of heavy metals in foods and natural products is not only of environmental concern, but also of a considerable global public health safety interest. Because various health related issues including cancer diseases, cardiovascular problems, children low intelligent

quotients, depression, hematic, gastrointestinal and renal failure, osteoporosis, tubular and glomerular dysfunctions and other health issues have been directly linked to high levels of heavy metals in humans [26-30].

Foods can potentially be contaminated through environmental pollution, industrial activity or the absorption of heavy metals from contaminated soils, industrial effluent or contaminated irrigation water [18, 21-23]. For example, varying concentrations of toxic heavy metals have been detected in several food items in both developed and under developed countries [18-22, 24, 31-33]. In addition, foods has a central role in health and its supplementation prevent various types of diseases due to the presence of secondary metabolites, vitamins and nutritional elements [34-37]. Many health-related issues including cancers, cardiovascular problems, depression, hematic, gastrointestinal and renal failure, osteoporosis, tubular and glomerular dysfunctions have been directly linked to high levels of heavy metals in humans [26-30, 38]. Therefore, imperative to focus on proper food quality assurance and quality control protocols that ensure the intake of adequate amounts of essential elements and prevent the consumption of toxic heavy metals from food products. However, the residual contents of elements and their inorganic salts in such common fruits like tomatoes and lemon, which are daily consumed, have not been reported in many areas. In addition, no information about the mineral composition of fruits from Turaba District has been reported up to date. Thus, simple analytical procedures for sample preparation and measurement of elemental species are highly needed.

Sample digestion is of great importance for obtaining desirable results for the desired analytes. Wet digestion and dry ashing procedures are quite slow difficult to follow consistently [39]. In recent years, the microwave assisted leaching technique in closed vessel has becoming more popular in the digestion of various food matrices. Since this method provides simple and rapid dissolution of the sample matrix with powerful for extraction of elements from samples. In addition, it requires low oxidizing reagent volume and causes minimal contamination of the sample before the elemental analysis step. Furthermore, this technique helps in preventing losses due to volatilization of elements [16].

In literature there are many techniques has been reported that used for elemental determination, such as flame atomic absorption spectroscopy (FAAS) and/or electrothermal atomic absorption spectroscopy (ETAAS). These techniques are the most commonly used for conventional method of elements analysis [40]. However, FAAS and ETAAS suffers from poor detection limit this hindering there uses for detection of elements at ultra-low concentrations. Keeping in mind the health implications of acute and chronic exposure to toxic elements in humans, the use of a more efficient, accurate, and sensitive analytical protocol that is capable of detecting these elements at trace and ultra-trace levels in food is required. Consequently, an inductively coupled plasma optical emission spectroscopy (ICP-OES), which has better detection limits for many elements compared to FAAS and ETAAS was used for

elements analysis [5, 15, 16]. In addition to that, it combines fast analysis time, relative simplicity with low sample volume requirements and good analytical performance [40-43]. Moreover, the use of ICP-OES will allowed the accurate analysis and determination of elements at low levels [15, 16].

The chemical composition of the fruits is a good indicator of their quality, acceptability and the health status of consumers. There are many factors that influence the chemical composition and the nutritional value of fruits, such as fruit variety, soil quality and production area, farming practices, the quality of irrigation water, local climate conditions and storage and commercialization conditions. In order to avoid the influence of these factors, studied fruit samples were collected from only one market (vegetables and fruits market in Turaba District), during one harvest season.

In literature there are many works concerning the determination of essential and toxic heavy metals status in some fruit but their a lack of the consistency between the reported results. This may be attributed either to the differences in the geographical origin or variety or the use of unappropriated analytical methodologies. According to the above mentioned reasons the current work was proposed to determine and to compare (for the first time) the content of the essential (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) and the toxic heavy metals (Al, As, Cd and Pb) of eighteen fruit samples, under the climate conditions of Turaba District (Saudi Arabia) using ICP-OES. In addition, it is imperative to focus on proper food quality assurance and quality control protocols that ensure the intake of adequate amounts of essential trace elements and prevent the consumption of toxic heavy metals from food products. Therefore, daily dietary elements intake (DDEI) values were estimated and the potential human health risks for the consumption of studied fruit were evaluated according to Saudi Food and Drug Authority (SFDA 2018) [44] and World Health Organization (WHO 2010) [27].

2. Materials and Methods

2.1. Instrumentation

A multi-vessels microwave-assisted reaction system (model

MARS 5, CEM corporation, Matthews, USA) programmable for time and power between 800 and 1600 W, that equipped with 12 high pressure Teflon vessels (model Easy Prep xp-1500 plus, CEM corporation, Matthews, USA) was used for samples digestion. A quadruple Elan DRC II (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) and Inductively coupled plasma optical emission spectrometer (Perkin Elmer Model Optima 2100 DV, USA) with CCD detector was used to analyze the blank, standard and sample solutions. The instrument was used with a high-efficiency sample introduction system equipped with a quartz cyclonic spray chamber and an additional mixing peristaltic pump (Apex-IR, Omaha, NE, USA). The operating conditions of microwave assisted reaction system (MARS-5) (Table 1) and of ICP-OES (Table 2) were carefully selected and well optimized in order to maximize the sensitivity for the desired elements and to obtain the best precision and accuracy. The selected spectral wavelengths (nm) for the target elements were shown in Table 3. Selection was performed in terms of sensitivity and absence of interferences. Some instrumental operating conditions of MARS-5 and ICP-OES were set according to manufacturer guidelines.

Table 1. ICP-OES operating conditions

Parameter	Value
RF incident power	1.6 (KW)
Frequency	40.68 (MHz)
Nebulizer argon flow rate	0.60 (L/min)
Plasma argon flow rate	15.0 (L/min)
Auxiliary argon flow rate	0.2 (L/min)
Pump flow rate	2.0 (mL/min)
Number of replicates	3

Table 2. Heating program of MARS-5 for digestion of samples

Parameter	Condition
Temperature	220 (°C)
Pressure	800 (pis)
Ramp time	25 (min)
Holding time	10 (min)
Ventilation	10 (min)
Acid/oxidant mixture	6 mL HNO ₃ (65%) + 2 mL H ₂ O ₂ (30%)

Table 3. Figures of merit of ICP-OES method showing the wavelength, R², the average recovery values (%), LODs and LOQs of each element

Element	Wavelength (nm)	R ²	Added volume (ml) from multi-element standard solution (1 mg/L)		Added volume (ml) from multi-element standard solution (1 mg/L)		Average recovery (%) before and after digestion	LODs (mg/L)	LOQs (mg/L)
			Before digestion	After digestion	Before digestion	After digestion			
Al	308.212	0.9993	10.0	10.0	25.0	25.0	106±4	0.0556	0.174
Pb	220.353	0.9997	10.0	10.0	25.0	25.0	98±5	0.0062	0.086
Cd	226.502	0.9990	10.0	10.0	25.0	25.0	104±3	0.0007	0.019
As	188.979	0.9998	10.0	10.0	25.0	25.0	102±5	0.0054	0.016
Cr	267.716	0.9996	10.0	10.0	25.0	25.0	100±4	0.0012	0.042
Mn	257.610	0.9993	10.0	10.0	25.0	25.0	108±6	0.0005	0.006
Ni	231.604	0.9991	10.0	10.0	25.0	25.0	106±5	0.0011	0.004
Co	238.892	0.9997	10.0	10.0	25.0	25.0	95±3	0.0008	0.003
Ca	317.361	0.9973	10.0	10.0	25.0	25.0	101±4	0.162	0.499
Cu	324.700	0.9995	10.0	10.0	25.0	25.0	107±6	0.003	0.009
Fe	259.939	0.9997	10.0	10.0	25.0	25.0	103±2	0.015	0.051
Mg	279.145	0.9984	10.0	10.0	25.0	25.0	99±5	0.026	0.078

Element	Wavelength (nm)	R ²	Added volume (ml) from multi-element standard solution (1 mg/L)		Added volume (ml) from multi-element standard solution (1 mg/L)		Average recovery (%) before and after digestion	LODs (mg/L)	LOQs (mg/L)
			Before digestion	After digestion	Before digestion	After digestion			
Na	588.995	0.9988	10.0	10.0	25.0	25.0	104±5	0.045	0.137
K	766.491	0.9956	10.0	10.0	25.0	25.0	97±6	0.068	0.212
Zn	213.857	0.9992	10.0	10.0	25.0	25.0	100±4	0.009	0.029

Results are expressed as mean $\pm ts/\sqrt{n}$, whereas: t is the Student's t (95 % confidence level), s is the standard deviation, n is the number of replicates (3), LODs is the Limit of Detections, LOQs is the Limit of Quantifications, R² is the correlation coefficient.

2.2. Reagents and Solutions

All solvents and reagents were of the highest commercially available purity grade. Ultrapure deionized distilled water (DDW), (i.e., with conductivity lower than 18 MΩ/cm) was obtained from a Milli-Q Plus water purification system (Millipore Inc., Paris, France) and used throughout the analyses to prepare blank, standard and sample solutions. Suprapur grade 65% (m/m) HNO₃ (d=1.40 kg/L) and 30% (m/m) H₂O₂ (d=1.11 kg/L), (Merck, Germany) were used for sample digestions. Multielement, high-purity grade V (Atomic Spectroscopy Standard) consist of 2 mg/L stock solutions of Pb, 5 mg/L of Cd, 10 mg/L of As and Cr, 15 mg/L of Mn, 20 mg/L of Zn, 25 mg/L of Cu, 40 mg/L of Ni, 50 mg/L of Co, 100 mg/L of Fe, 200 mg/L of Al and 500 mg/L of Ca, Mg, K and Na were purchased from PerkinElmer, Shelton, CT, USA) was used for preparing standards for calibration curves and spiking of some samples for recovery test. The calibration standards were prepared by diluting the stock multi-element standard solution with DDW in 2% HNO₃. The calibration ranges were modified according to the expected concentration values of investigated elements.

All laboratory plastic/glassware were decontaminated by soaking in 10% solutions of purified HNO₃ for ~24 hrs and were rinsed thrice with DDW. The purity of the plasma torch argon that has been used as auxiliary, plasma and samples introduction was greater than 99.99% (v/v).

2.3. Samples Collection and Preservation

Samples of commonly consumed were collected randomly from vegetables and fruits market in Turaba District (Saudi Arabia). Some of samples (e.g. tomatoes, watermelon, and mandarins) were produced locally and they were obtained directly from local producers. Different import companies that guarantee the origin traceability were purchased the rest of samples like bananas, mangos and strawberries. Samples were collected in clean polyethylene containers according to their types and preserved in the refrigerator prior to processing for drying.

Immature and broken samples were discarded, the rest were washed (cleaned) thrice with DDW to remove the dirt and dust particles from the fruit surfaces. Then, samples were cuts (chopped or peeled) with clean stainless steel knife into small pieces (~2-3 mm size), kernels and seeds were removed, well mixed and dried in an oven at ~100°C for ~24 hrs to remove moisture in order to prevent food decay and microbial activity [5]. Three dried samples of each type were

subsequently grounded into a fine powder and homogenized using a clean commercial kitchen grinder (Philips, Indonesia). The grounded samples were properly labeled and stored in pre-nitric acid washed and dried polyethylene bags at ~ -20°C prior to any further laboratory analysis or until used for acid digestion. For determination of moisture content, a apportion from each sample was dried at ~100°C in oven until constant weight was obtained [45]. Dry matter of the samples were calculated as dry weight from the moisture content. The results were reported based on dry weight basis.

2.4. Microwave Digestion of Fruit Samples

For digestion, 0.5 gm of each dried and ground sample in triplicate were weighed accurately using 0.01 mg sensitive weighing analytical balance and inserted directly into a dry and clean Teflon separate microwave assisted digestion vessel. Six-mL HNO₃ (65%) and 2.0 mL H₂O₂ (30%) (in the ratio 3:1) were added drop wise to each sample. The contents were shaken carefully before closing the vessels. Then the vessels were kept for ~30 min (pre-digestion time) at room temperature before digestion. After this, the closed vessels were placed inside the rotor of microwave oven unit. Samples were digested following a one stage digestion program (Ramp time [min]/Power level [W]/Temperature [°C]/Pressure [pis]/Hold time [min]: 25/1600/220/800/10, 10 min ventilation) (Table 2). After digestion, clear solutions were cooled to room temperature; reactors were opened to eliminate nitrous vapors. After that, the interior walls of the vessels were washed down with a little DDW and vessels were swirled through the digestion to keep the wall clean and to prevent the loss of the samples. Then, the contents of the vessels (a transparent clear solution) were quantitatively transferred to 50 ml volumetric flask and diluted to the mark with DDW. Several analytical blanks consisting of DDW, HNO₃ and H₂O₂ were also prepared in the same way as the samples and analyzed to characterize instrumental drift. Three replicate measurements were made for each sample. This procedure was partly modified from that of Sayim and Cagran [41] and that recommended by Bressy group [46], which applied for determination of trace elements in tomato samples, with minor modifications. In all cases, the digestion was complete and no solid residues were observed. To avoid cross-contamination, Teflon vessels were carefully cleaned with 10% HNO₃ solution before to proceed with the sample treatment. In addition, for safety purposes, sample and blank solutions were prepared in a Class-100 laminar flow hood. The elemental contents were determined by ICP-OES

(Perkin-Elmer Model Optima 2100 DV, USA).

2.5. Standard Preparation (Calibration)

Quantitative analyses of the samples were carried out by external calibration. Standard solutions were prepared in HNO₃ (65%) by diluting a multi-elemental standard containing the analytes. Reagent blank was prepared in the same manner as standards. Under the optimized conditions, seven concentrations (mg/L) of working standards within the linear dynamic range were measured, and calibration curves for each analyte were plotted from the limits of detection (LODs). To avoid error, a slight instrumental drift monitored by analyzing calibration standards at regular intervals during analysis alongside samples were taken into account. All measurements were carried out using the full quantitative analysis mode.

2.6. Analysis of Samples

The multielement standard stock solution was used to prepare calibration standards. Calibration standards were diluted with DDW contain HNO₃ (65%). All digests were analyzed on a simultaneous Varian 710 ES axial ICP-OES with CCD detector. A Cetac auto sampler with 15-mL sample tubes was connected to the peristaltic pump. A Burgener Teflon Mira Mist-nebulizer (SCP Science) and glass cyclonic spray chamber were used for sample introduction. The operating conditions of ICP-OES were indicated in Table 2. The instrument detection limits were determined by measuring the emission intensities of seven blanks. Each sample was analyzed three times (n=3) for each element.

2.7. Statistical Analysis

The results were statistically evaluated by One Way Analysis of Variance (ANOVA) and Student t-test, (P=0.05), in addition, Microsoft Excel and Origin software's were also used. The concentration values obtained were expressed as average value ± confidence interval (at 95 % confidence). All statistical analysis was based upon triplicate measurements of all blank, standard and sample solutions (n=3).

2.8. Validation Studies

To evaluate the analytical method proposed for the elemental analysis of fruits by ICP-OES based techniques, some analytical figures of merit were estimated such as linearity, accuracy, precision, LOD and LOQ.

The linearity were determined by preparing the calibration curves of all analytes of the standards using non-weighted least-squares linear regression. The square correlation coefficient (R²) of the ICP-OES calibration equation (curve) of each analyte (concentrations versus emissions) was calculated (Table 3). Moreover, to estimate method accuracy a recovery test was performed by spiking some fruit samples at different concentration levels with a multi-element standard solution (before and after digestion steps) and passed through the same dissolution procedure. The recoveries (%) of the

different elements in selected fruit samples (Table 3) were calculated using Eq. (1) [47], [48].

$$\text{Recovery (\%)} = \frac{\text{ECSS} - \text{ECNSS}}{\text{SCE}} \times 100 \quad (1)$$

Where as: ECSS is the mean value of element concentration in spiked solution (mg/L). ECNSS is the mean value of element concentration in non-spiked solution (mg/L), SCE is the spiked concentration value of element (mg/L).

The precision (the closeness of agreement between mutually independent test results) of the method was estimated by means of the relative standard deviation (RSD). The RSDs were calculated from the elemental concentrations obtained after the analysis of the five independent replicates of each sample. Therefore, RSDs takes into account not only the precision of the analytical method but also the homogeneity of samples. The instrument limits of detection (LODs) and quantification (LOQs) of each analyte was determined by measuring the emission intensities of the analyte that corresponded to three and ten times, the standard deviation (σ) of ten independent measurements (ten replicates) of the independent reagent blank solutions, divided by the slope (m) of the calibration curve of each element, respectively [49, 50]. Each sample was analyzed three times (n= 3) for each element.

2.9. Health Risk Assessment

Health risks for the essential and toxic elements by consumption of bananas, tomatoes, guava, grapes, date palm, mangos, cantaloupe melon, watermelon, mandarin, lemon, and pomegranate were assessed by the determination of estimated dietary daily element intake (EDDEI) values. The EDDEI values were calculated and their values were compared with the recommended dietary and provisional tolerable intake values of the elements that provided by Gupta and Gupta [1] and Alzahrani and co-authors [5] in order to evaluate the element dietary intake and fruit consumption pattern of the indigenes of Saudi Arabia according to Eq. (2) [5, 51].

$$\text{EDDEI} = \frac{C \text{ (mg/Kg)} \times \text{FIR (Kg/day)}}{\text{BW (per person per day)}} \quad (2)$$

Where as: EDDEI is the estimated dietary daily element intake of food ingestion rate (mg/person/day), C is the level or concentration (mg/kg) of the essential and toxic elements present in the fruit, FIR is the food ingestion or consumption rate (~1.5 Kg/day), C is the concentration (mg/kg) of the essential and toxic elements present in the fruit, and BW represents the body weight (~60 kg) of an adult consumer (per person per day).

Moreover, EDDEI values were also compared with the safety limits that recommended by the guidelines of SFDA 2018 [44] and WHO 2010 [27] were also considered.

3. Results

3.1. Calibration Curves, LODs and LOQs

Taking into account, the different analytical methods and techniques for the elemental analysis of fruit samples have been reported in the literature. It is found that most of the methodologies employed have not been validated. Therefore, the evaluation of the analytical method proposed in the present work is mandatory in order to ensure the quality of the results afforded. The ICP-OES method has been evaluated in terms of linearity, accuracy, precision, and LOD/LOQ. Table 3 shows the wavelengths, R^2 , LODs and LOQs obtained for each element. Obviously the figures of merit of the calibration curves are excellent, under the optimal ICP-OES operating conditions (Table 1) for multi-elements measurement with their analytical lines used (Table 3), differences in the suitable calibration ranges of these elements were established with good linearity ($R^2 > 0.9956$ or better), (Table 3). Moreover, Table 3 also, shows the method LODs and LOQs estimated for the tested elements. They were determined by analyzing seven portions of standard solutions simultaneously following the general procedure. The LODs and LOQs in mg/Kg were calculated as $3\sigma/m$ and $10\sigma/m$, respectively, where σ is the standard deviation of the intensity of seven blanks and m is the slope of the calibration curve for each element. The LODs of the elements ranged between 0.0007 mg/L for Cd and 0.162 mg/L for Ca while LOQ ranged between 0.003 mg/L and 0.499 mg/L for Co and Ca, respectively. The low LODs clearly demonstrate the high sensitivity and linear range of ICP-OES method for elemental analysis in fruit samples. Unfortunately, no comparison with other analytical methodologies can be performed since LODs for multi-element analysis of fruits by ICP-OES based technique have been previously reported in the literature.

3.2. Method Validation, Accuracy and Precision

All necessary precautions were taken during sample collection, preparation, digestion, and analysis in order to preserve the sample integrity and to ensure accurate results. First, the fruit samples were collected in pre-nitric acid washed and dried polyethylene bags. The samples were immediately oven dried to eliminate sample decomposition or microbial activity. The samples were prepared in a clean, dust free laboratory to avoid contamination as much as possible. All glassware were pre-soaked in 6 M HNO_3 for at least 24 hrs and thoroughly rinsed with DDW before use. Each fruit sample was analysed in triplicate ($n=3$). As it can be observed in Table 3, the average recoveries (95 % confidence level, $n=3$) obtained for all analytes in the samples tested were almost complete, ranging between 95 ± 3 % (Co in Strawberry) and 108 ± 6 % (Mn in Pomegranate). Therefore, it can be concluded that, the method is free of interferences and no analyte losses are produced during the acid digestion step. No comparison with other date analysis methodologies can be performed due to the lack of data reported. Nevertheless, analyte losses during the sample treatment were expected to

be reduced when using a closed vessel microwave assisted digestion method when compared with those widely used in the literature, i.e., dry ashing procedure at temperatures above 500°C [52] and open-vessel acid digestion [39] were employed a closed-vessel microwave assisted digestion procedure, but unfortunately, no accuracy data was provided by the authors. As regards the precision of the method, RSD values for most of the tested elements do not exceed 8 % (Table 3).

3.3. Elemental Contents of Fruit Samples

Fruits are considered as a good source of dietary minerals. For this reasons eighteen fruit samples were analyzed for the determination of essential and toxic heavy metals in fruit samples. The average concentration (mean \pm SD, mg/kg d.w.) of essential and toxic heavy metals in eighteen fruit samples were shown in Table 4. Each sample was analyzed three times ($n= 3$) for each element. The concentrations of the essential elements varied widely in the fruit samples. As expected, Ca, Mg, Na, and K had the highest concentrations in each of the tested fruit samples, while the reverse were said for As, Al, Cd, Fe, Ni and Co. In general, the highest concentrations of Ca was found in orange (47.00 ± 2.91 mg/kg), while the lowest one was found in pomegranate (2.995 ± 0.993 mg/kg). The concentration of Mg and K were high in tomatoes (22.85 ± 2.02 mg/kg and 504.8 ± 6.5 mg/kg respectively) and low in apple (b) (3.718 ± 0.885 mg/kg and 59.54 ± 3.17 mg/kg respectively). Moreover, Na concentration was high in cantaloupe (22.89 ± 2.90 mg/kg) and low in dates (1.781 ± 0.759 mg/kg), while Zn and Cu were abundant in most samples, with elevated concentration of Zn in apple (b) (37.77 ± 2.35 mg/kg) and a moderate concentration of Cu (0.390 ± 0.095 mg/kg) in grape (a). In contrast, Cu was not detected in mangoes, watermelon (a) and (b), while Zn was not hound in grape (b), guava and lemon. Furthermore, Mn was detected only on strawberry (0.052 ± 0.011 mg/kg), grape (b) (0.025 ± 0.007 mg/kg), guava (0.006 ± 0.001 mg/kg) and tomatoes (0.016 ± 0.004 mg/kg). Also, Fe was slightly high in pomegranate (0.465 ± 0.047 mg/kg) and low in apple (b) (0.022 ± 0.003 mg/kg), but unfortunately not found in apple (a), banana, mandarin, strawberry, cantaloupe, guava, watermelon (a) and orange grape (a) and (b). In contrast, Al was moderately high in apple (a) (1.015 ± 0.915 mg/kg) and slightly low in dates (0.119 ± 0.055 mg/kg), but not detected in apple (b), grape (b), guava, apricot, lemon, watermelon (b), tomatoes, orange and mangoes. In addition, Co and Ni were not detected in all studied fruit samples or they may present in very low concentrations, while Cr was found only in grape (a) (0.005 ± 0.001 mg/kg). As unexpected Cd, Pb and As were found in most studied fruit samples but with very low concentrations (average concentration of 0.046 ± 0.001 mg/kg, NC and 0.093 ± 0.006 mg/kg respectively). The average concentration of Cd ($\sim 0.046\pm 0.001$ mg/kg) obtained were generally similar in almost all studied fruit samples. Besides that, tomatoes contained the highest K concentration (504.8 ± 6.5 mg/kg) and Mg (22.85 ± 2.02 mg/kg), which also

contained a relatively high Pb (and 0.161 ± 0.080 mg/kg). It was found that the average concentrations of most studied elements in bananas and dates was generally similar.

The results (Table 4) reveal that Co, Ni, Cr, Pb, As, Mn, Fe and Al concentrations were, in most cases were below ICP-OES method LOQ (i.e., they may present in concentrations below LOQ). For this reason, the average concentration of some elements Cr and Pb were not calculated (NC). In addition, from the results (Table 4) of the elemental analysis of all the fruit samples tested, a considerable variation were observed with regard to element concentrations in different studied fruit samples. The differences were significant for different samples at 95 % confidence level (i.e., $p < 0.05$), $n=3$. In addition, from the results three groups of elements can be distinguished attending to its concentration level. Major elements, with concentrations higher than 100 mg/kg (i.e., K); minor elements, with concentrations between 1 and 100 mg/kg (i.e., Ca, Mg and Na) and trace elements with concentrations less than 1mg/kg (i.e., Cu, Fe, Mn and Zn, Al, As, Cd, Co, Ni, Pb and Zn).

3.4. EDDEI of Fruits Samples

The daily intake of the elements investigated in this study were estimated in mg/person/day (Table 5) from the average concentration of each food along with the daily consumption of each food. The estimated EDDEI values were calculated and the results were compared with the recommended dietary and provisional tolerable intake values of the elements that provided by Gupta and Gupta [1]. The intake of essential elements must be closely monitored in the human diet, because both a deficiency and an excess can have negative health effects. In addition, elements such as Al, Pb, Cd, and as have no nutritional value or may have toxic effects.

From the estimated values (Table 5) it can be seen that the studied fruit samples provides the recommended daily amounts of essential elements such as Ca and K, but they will not provide high concentrations of potentially toxic elements like As and Cd. Furthermore, the EDDEI for some elements like Co and Ni were not calculated (NC) due to below detection limits (BDL). So that the lowest concentration for these metals was documented as BDL.

Table 4. Average concentrations of essential and toxic heavy metals in eighteen varieties of fruit samples

Sample	Concentration (mg/Kg dry wt.), mean \pm SD, n=3						
	Fe	Ca	Mg	Na	K	Cr	Mn
Apple (a)	BDL	13.30 \pm 1.51	5.04 \pm 0.927	4.25 \pm 0.95	80.57 \pm 3.01	BDL	BDL
Apple (b)	0.022 \pm 0.003	5.054 \pm 0.96	3.718 \pm 0.885	2.043 \pm 0.98	59.54 \pm 3.17	BDL	BDL
Banana	BDL	6.21 \pm 0.87	15.91 \pm 2.04	2.45 \pm 0.85	165.7 \pm 4.5	BDL	BDL
Mandarin	BDL	39.88 \pm 2.05	10.33 \pm 1.13	3.68 \pm 0.95	118.9 \pm 3.7	BDL	BDL
Strawberry	BDL	12.16 \pm 1.57	14.38 \pm 2.00	6.66 \pm 1.01	199.1 \pm 4.6	BDL	0.052 \pm 0.011
Cantaloupe	BDL	15.28 \pm 1.69	21.23 \pm 2.85	22.89 \pm 2.90	223.9 \pm 5.5	BDL	BDL
Grape (a)	BDL	6.59 \pm 0.91	3.88 \pm 0.837	3.28 \pm 0.88	89.39 \pm 3.02	0.005 \pm 0.001	BDL
Grape (b)	BDL	11.42 \pm 1.78	10.27 \pm 1.25	2.056 \pm 0.91	170.5 \pm 4.3	BDL	0.025 \pm 0.007
Guava	BDL	7.866 \pm 1.00	7.147 \pm 1.115	22.24 \pm 2.00	133.6 \pm 3.86	BDL	0.006 \pm 0.001
Apricot	0.037 \pm 0.005	13.50	10.92	3.799 \pm 0.991	220.2 \pm 4.6	BDL	0.089 \pm 0.009
Pomegranate	0.465 \pm 0.047	2.995 \pm 0.993	5.525 \pm 1.005	2.483 \pm 0.761	141.1	BDL	BDL
Lemon	0.271 \pm 0.087	38.62 \pm 2.076	9.75 \pm 1.63	5.069 \pm 1.076	132.5 \pm 3.5	BDL	BDL
Watermelon (a)	BDL	4.429 \pm 1.09	7.00 \pm 1.02	8.93 \pm 1.03	112.1 \pm 2.8	BDL	BDL
Watermelon (b)	0.197 \pm 0.065	20.97 \pm 2.08	19.75 \pm 2.784	3.758 \pm 0.889	323.8 \pm 5.2	BDL	BDL
Tomatoes	0.33 \pm 0.081	20.73 \pm 1.57	22.85 \pm 2.02	13.89 \pm 1.45	504.8 \pm 6.5	BDL	0.016 \pm 0.004
Orange	BDL	47.00 \pm 2.91	9.449 \pm 1.15	2.44 \pm 0.87	132.4 \pm 3.0	BDL	BDL
Dates	0.446 \pm 0.092	5.434 \pm 1.002	6.696 \pm 0.918	1.781 \pm 0.759	90.76 \pm 2.00	BDL	BDL
Mangos	0.112 \pm 0.058	6.698 \pm 1.705	4.464 \pm 0.970	2.189 \pm 0.905	95.35 \pm 2.45	BDL	BDL
Average	0.619 \pm 0.039	7.547 \pm 1.105	9.30 \pm 1.05	2.474 \pm 0.813	126.1 \pm 3.84	NC	NC

Table 4. Continue

Sample	Concentration (mg/Kg dry wt.), mean \pm SD, n=3							
	Zn	Cu	Ni	Co	Al	Pb	Cd	As
Apple (a)	37.77 \pm 2.35	0.38 \pm 0.088	BDL	BDL	1.015 \pm 0.915	0.006 \pm 0.002	0.030 \pm 0.001	BDL
Apple (b)	0.019 \pm 0.007	0.006 \pm 0.002	BDL	BDL	BDL	0.031 \pm 0.005	0.033 \pm 0.001	0.093 \pm 0.005
Banana	0.80 \pm 0.076	0.291 \pm 0.045	BDL	BDL	0.868 \pm 0.083	BDL	0.032 \pm 0.001	0.052 \pm 0.0015
Mandarin	0.12 \pm 0.088	0.237 \pm 0.076	BDL	BDL	0.936 \pm 0.075	0.099 \pm 0.006	0.033 \pm 0.001	0.061 \pm 0.003
Strawberry	0.453 \pm 0.075	0.082 \pm 0.009	BDL	BDL	0.907 \pm 0.088	BDL	0.032 \pm 0.001	BDL
Cantaloupe	1.014 \pm 0.915	0.115 \pm 0.069	BDL	BDL	0.918 \pm 0.071	BDL	0.033 \pm 0.001	0.085 \pm 0.004
Grape (a)	0.06 \pm 0.008	0.390 \pm 0.095	BDL	BDL	0.945 \pm 0.089	0.042 \pm 0.003	0.032 \pm 0.001	BDL
Grape (b)	BDL	0.088 \pm 0.007	BDL	BDL	BDL	BDL	0.032 \pm 0.001	BDL
Guava	BDL	0.230 \pm 0.087	BDL	BDL	BDL	0.071 \pm 0.004	0.032 \pm 0.001	BDL
Apricot	1.15 \pm 0.59	0.290 \pm 0.045	BDL	BDL	BDL	0.067	0.033 \pm 0.002	0.181 \pm 0.055
Pomegranate	0.417 \pm 0.078	0.144 \pm 0.065	BDL	BDL	0.257 \pm 0.078	BDL	0.033 \pm 0.001	0.091 \pm 0.005
Lemon	BDL	0.131 \pm 0.081	BDL	BDL	BDL	0.030 \pm 0.001	0.032 \pm 0.001	BDL
Watermelon (a)	0.214 \pm 0.055	BDL	BDL	BDL	0.877 \pm 0.090	0.076 \pm 0.006	0.033 \pm 0.001	0.082 \pm 0.004
Watermelon (b)	0.572 \pm 0.045	BDL	BDL	BDL	BDL	0.082 \pm 0.006	0.032 \pm 0.002	BDL
Tomatoes	1.652 \pm 0.451	0.141 \pm 0.095	BDL	BDL	BDL	0.161 \pm 0.080	0.033 \pm 0.001	BDL

Sample	Concentration (mg/Kg dry wt.), mean \pm SD, n=3							
	Zn	Cu	Ni	Co	Al	Pb	Cd	As
Orange	0.093 \pm 0.007	0.048 \pm 0.004	BDL	BDL	BDL	BDL	0.033 \pm 0.002	0.051 \pm 0.003
Dates	0.131 \pm 0.085	0.075 \pm 0.005	BDL	BDL	0.119 \pm 0.055	BDL	0.033 \pm 0.001	0.067 \pm 0.005
Mangos	6.837 \pm 1.409	BDL	BDL	BDL	BDL	0.071 \pm 0.005	0.034 \pm 0.002	BDL
Average	0.182 \pm 0.078	0.104 \pm 0.085	NC	NC	0.165 \pm 0.085	NC	0.046 \pm 0.001	0.093 \pm 0.006

Results are expressed as mean \pm ts/\sqrt{n} , whereas: t is the Student's t (95 % confidence level), s is the standard deviation, n is the number of replicates (3). BDL is below detection limit, Apple (a) is red type apple, Apple (b) is green type apple, Watermelon (a) is white type melon, Watermelon (b) is green type melon, Grape (a) is white type grape, Grape (b) is red type grape, NC is not calculated due to BDL.

Table 5. Estimated daily dietary elements intake (EDDEI) values (mg/person/day) from food samples

Sample	Estimated values (mg/person/day)														
	Fe	Ca	Mg	Na	K	Cr	Mn	Zn	Cu	Ni	Co	Al	Pb	Cd	As
Apple (a)	NC	33.25	126.0	106.3	2014.3	NC	NC	942.5	9.50	NC	NC	25.38	0.15	0.75	NC
Apple (b)	0.55	0.15	92.95	51.08	1488.5	NC	NC	0.475	0.15	NC	NC	NC	0.775	0.825	2.325
Banana	NC	155.3	397.8	61.25	4142.5	NC	NC	20.0	7.275	NC	NC	21.70	NC	0.80	1.30
Mandarin	NC	997.0	258.3	92.0	2972.5	NC	NC	3.00	5.925	NC	NC	23.4	2.475	0.825	1.525
Strawberry	NC	304.0	359.5	166.5	4977.5	NC	1.30	11.33	2.05	NC	NC	22.68	NC	0.80	NC
Cantaloupe	NC	382.0	530.8	572.3	5597.5	NC	NC	25.35	2.875	NC	NC	22.95	NC	0.825	2.125
Grape (a)	NC	164.8	97.0	82.0	2234.8	0.13	NC	1.50	9.75	NC	NC	23.63	1.05	0.80	NC
Grape (b)	NC	285.5	256.8	51.4	4262.5	NC	0.63	NC	2.20	NC	NC	NC	NC	0.80	NC
Guava	NC	196.7	178.7	556.0	3340.0	NC	0.63	NC	2.2	NC	NC	NC	1.775	0.80	NC
Apricot	0.925	337.5	273.0	94.98	5505	NC	2.23	28.75	7.25	NC	NC	NC	1.675	0.825	4.525
Pomegranate	11.63	74.88	138.1	62.08	3527.5	NC	NC	10.43	3.60	NC	NC	6.425	NC	0.825	2.275
Lemon	6.775	965.5	243.8	126.7	3312.5	NC	NC	NC	3.275	NC	NC	NC	0.75	0.80	NC
Watermelon (a)	NC	110.7	175.0	223.3	2802.5	NC	NC	5.35	NC	NC	NC	21.93	1.90	0.825	2.05
Watermelon (b)	4.925	524.3	493.8	93.95	8095.0	NC	NC	14.30	NC	NC	NC	NC	2.05	0.80	NC
Tomatoes	8.25	518.3	571.3	347.3	12620	NC	0.40	41.3	3.525	NC	NC	NC	4.025	0.825	NC
Orange	NC	1175.0	236.2	61.0	3310.0	NC	NC	2.325	1.20	NC	NC	NC	NC	0.825	1.275
Dates	11.15	135.9	167.4	44.53	2269.0	NC	NC	3.275	1.875	NC	NC	2.975	NC	0.825	1.675
Mangos	2.80	167.5	111.6	54.73	2383.6	NC	NC	171.0	NC	NC	NC	NC	1.775	0.850	NC
Total daily Estimate	0.619	7.547	9.30	2.474	126.1	NC	NC	0.182	0.104	NC	NC	0.165	NC	0.046	0.093

Where as: NC is not calculated due to BDL.

4. Discussion

The concentration of some heavy metals (Cd, Pb, As, Cr, Mn and Co) in studied fruit samples collected from vegetables and fruits market in Turaba District (Saudi Arabia) were found to be below the SFDA 2018 [44] and WHO 2010 [27] permissible concentrations given for fruits. Generally, the highest concentration of heavy metals was recorded in apricot, while the lowest one in lemon.

It is well known that if Cd accumulated in the human body it may causes prostate and breast cancer, kidney dysfunction, skeletal damage and reproductive deficiencies [53]. In the present study the amount of Cd found in all fruit samples were approximately equal (i.e., \sim 0.030 mg/kg) but not exceeded the limit (0.030 mg/kg) set by SFDA 2018 [44] and WHO 2010 [27]. Moreover, it is widely accepted that Pb is neurotoxic and found in paints, dyes, plastics in bibs and colouring sets. Its intoxication can result in disruption of certain cellular signaling processing, the generation of action potentials in certain nerve cells and the function of various enzymes and proteins [54]. The concentration of Pb ranged between (BDL-0.161 mg/kg), which is lower than the limit that set by the SFAD 2018 and WHO 2010 (0.01 mg/kg). In addition, As is highly toxic (carcinogenic) and can lead to a wide range of health problems, while Al is the most abundant

metal, in the earth crust and therefore is likely to be present at some level in most water. The correlation of Al consumption to nervous system disorders is being researched [18, 20]. Both As and Al were found in different ranges but in most studied fruit samples their concentrations were below detection limit (i.e., BDL-0.181 mg/kg and BDL-1.015 mg/kg respectively) and not exceed the limit that set by the SFAD 2018 and WHO 2010 (i.e., 0.01 mg/kg and 0.2 mg/kg respectively).

In the other side Cr is an essential trace element that plays an important role in glucose and cholesterol metabolism. It may improve insulin sensitivity, which can modify the risk of diabetes and cardiovascular disease. However, the deficiency of Cr can affect the glucose, lipid and protein metabolism and impaired growth [55]. In the studied fruit samples, the concentration of Cr is below the limit (i.e., 12–13 mg/kg) that set by SFAD. Moreover, it is well known that Co and Ni are beneficial to health, but in case of crossing the limits may causes lung cancer, nasal cavity and heart effects [56]. Nevertheless, both elements (i.e., Co and Ni) were below the detection limits of an ICP-OES (i.e., BDL) in all studied fruit samples.

Moreover, it is well known that small amount of Mn is needed for growth and prevention of cardiac arrest, heart attack, and stroke [55], but acute toxicity by Mn causes psychologic and neurologic disorder [53]. In present, study

the concentration of Mn in the studied fruit samples were ranged between BDL to 0.089 mg/kg. In addition to that, it is well known that Zn is an important element in human diet, but lower concentration may cause serious threat to human health [56]. While concentrations higher than 40 mg/kg of Zn may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite and nausea [57].

Furthermore, it is well known that Na at normal intake levels is beneficial to health, however, human with heart disease and/or hypertension should reduce sodium intake to lower the blood pressure [58]. Epidemiological and clinical studies show that a high K diet lowers blood pressure in individuals with both raised blood pressure and average population blood pressure. Prospective cohort studies and outcome trials show that increasing K intake reduces cardiovascular disease mortality [59]. In addition, both Ca and Mg play an essential role in muscle function, nerve transmission, regulation of heartbeat and dilation of blood vessels, bone and teeth formation and hormone secretion. Never the less, deficiency of both Ca and Mg is associated with weak bones and structural connective tissue formations [55, 60]. In addition, it is well known that Cu is an essential trace element; it forms an important constituent of many

metallo-proteins and metallo-enzymes of various organs and tissues [15]. Moreover, Fe is essential element and necessary for the production of hemoglobin, myoglobin and certain enzymes and it is deficiency causes anemia in human, weakness, inability to concentrate and susceptibility to infection [55]. It is found that the concentration of Fe (BDL-0.46 mg/kg) in the studied fruit samples was not sufficient to provide the recommended limit per day.

From the results, it was found that the essential elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) has the highest concentration, while the toxic heavy metals (Al, As, Cd and Pb) had the lowest one in the analyzed fruit samples. The lowest concentration of essential elements was recorded in apple (b), the moderate in dates and the highest in tomatoes. Furthermore, it was found that the average consumption of these fruit samples will provide the recommended daily allowances of almost all essential elements but will not expose consumers to toxic heavy metals. In addition, figure 1 shows the distribution of the average concentrations of the thirteen essential and toxic heavy metals in fruit samples under study. It shows that lower concentrations were observed for Fe, Cr, Mn, Ni, Co, Cd, Pb, As and Zn. The opposite was true for K, Mg, Na and Ca.

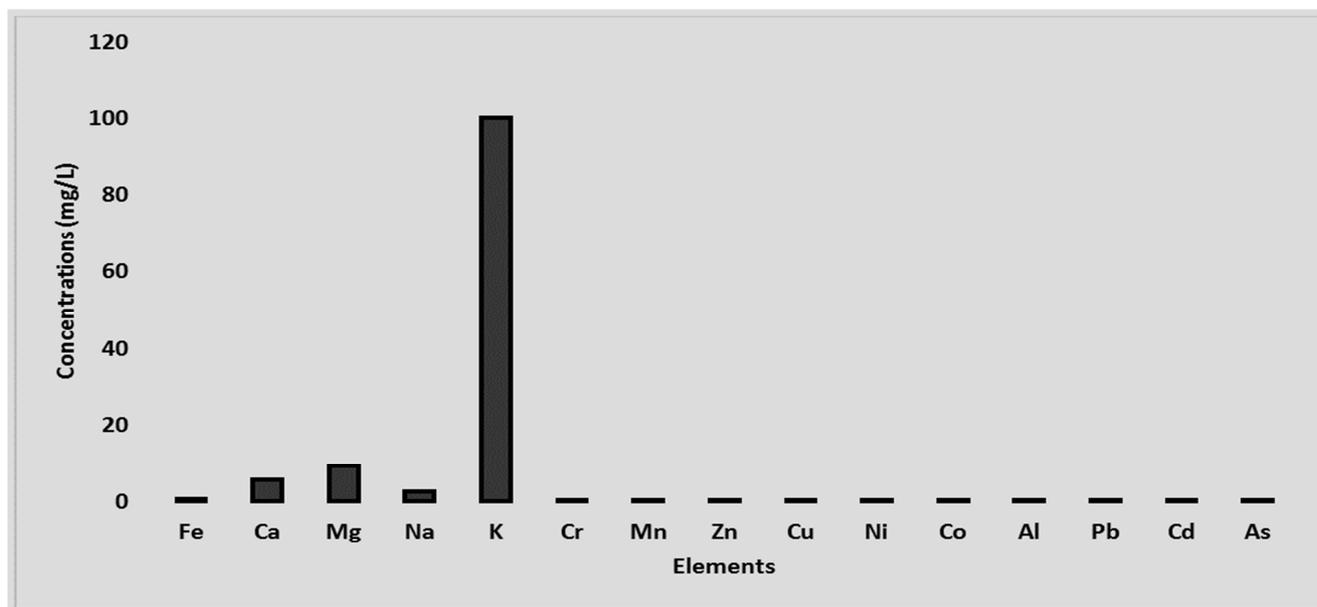


Figure 1. Illustrating graph showing bar plot of the essential and toxic heavy elements average concentrations in eighteen varieties of fruit samples.

5. Conclusion and Recommendations

The findings of the present study indicate the fruit samples that are consumed regularly by the local people in Turaba District and other parts in Saudi Arabia may not rise any health risk to consumers. The elevated level of heavy metals found in most edible fruit samples ultimately will not harm the human health. However, the present research recommends that the point sources of heavy metals in the Turaba Valley should be strictly monitored for protecting the health of riverine ecosystem along with fruit samples.

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