Bull. Chem. Soc. Ethiop. **2023**, 37(3), 533-551. ISSN 1011-3924

Printed in Ethiopia
DOI: https://dx.doi.org/10.4314/bcse.v37i3.1 Online ISSN 1726-801X

MAJOR AND HEAVY METALS CONTENTS AND HEALTH RISK ASSESSMENT OF PUMPKIN PEEL, FLESH AND SEED BY MICROWAVE PLASMA-ATOMIC EMISSION SPECTROSCOPY

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(Received August 15, 2022; Revised November 30, 2022; Accepted December 16, 2022)

ABSTRACT. The aim of this study was to determine the levels of major (Na, Mg, K, Ca) and heavy (Cr, Mn, Fe, Ni, Cu, Zn, Cd) metals and health risk assessment of the peel, flesh and seed of pumpkins cultivated in Ethiopia, using microwave plasma-atomic emission spectrometry. Dried powdered samples (0.5 g) were wet digested with 69% HNO_3 and 30% H_2O_2 in microwave digester. The percent recovery for the three parts of pumpkin samples were 90-110%. The mean concentration of metals in pumpkin peel, flesh, seed samples, respectively, were K (27806±9767, 29531±2452, 10667±1133), Ca (5267±2092, 3190±900, 2919±397), Mg (4716±469, 848±349, 4668±413), Fe (158±29, 111±46, 225±56), Na (167±116, 63.1±15, 171±123), Mn (28.9±1.4, 9.61±3.2, 33.4±4.9), Zn (24.2±4.4, 10.6±4.0, 67.5±12.3), Cu (8.14±1.1, 15.1±9.3, 10.8±0.9), Ni (3.79±2.4, 2.14±1.3, 3.44±2.8), Cr (0.28±0.1, 0.36±0.2, 0.69±0.1), Cd (0.24±0.1, 0.46±0.1, 1.39±0.2) mg/kg. The hazard index (HI) of pumpkin flesh and seed indicated ingestion of pumpkin is safe from non-carcinogenic risk. The total cancer risk (CR) due to consumption of pumpkin flesh and seed through Cr and Cd from the three sampling sites are in the range $1.1 \times 10^{-4} - 1.8 \times 10^{-4}$, indicating a non-risk of exposure to cancer due to the consumption of pumpkin grown in the sampling areas.

KEY WORDS: Pumpkin, Mineral content, Health risk, Microwave plasma-atomic emission spectrometry

INTRODUCTION

Pumpkin belongs to the family of *Cucurbitaceae*. It is a wide family with about 130 types and 800 species. *Cucurbita*, includes five major species: *C. maxima* (large orange pumpkin), *C. pepo* (summer squash), *C. moschata* (winter squash), *C. ficifolia* and *C. turbaniformis*. The most common species of pumpkins are *Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata*. These three species are cultivated worldwide and have high production yields compared with other vegetables. Pumpkin fruits exhibit large variation in size and shape and the average fruit weight fluctuates between 8 and 10 kg, sometimes even up to 20 kg have been noted. Botanically speaking, pumpkins are fruits, as they contain seeds and develop from the flower-producing part of the plant. However, people usually cultivate and consume pumpkin as a vegetable because it is not sweet as other fruits. In Ethiopia, it is known as *Dubba*.

Pumpkin is widely cultivated as garden and farm vegetable [1]. Pumpkins are widely used in the food arts, since many different parts of the plant are edible, like, flowers, leaves, shoots, roots, immature and mature fruits, peel and seeds. Pumpkin is eaten as raw and in the form of preserves, such as soups, smoothies and juices. Pumpkin flesh is also used as an additive to bread, cakes, cookies, chocolates and candies [2].

Ethiopia possesses highly-diversified agro-ecological conditions which are suitable for the production of various types of fruit and vegetables. Hence, almost all types of fruits and vegetables are grown in Ethiopia. In most regions of Ethiopia, pumpkins had been cultivated since several years ago and the matured fruit of the plant is used as edible parts [3].

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Fruits and vegetables provide antioxidants such as vitamin A, C and E that are important in neutralizing free radicals (oxidants) known to cause cancer, cataracts, heart disease, hypertension, stroke and diabetes. Pumpkins have a lot of biologically active compounds: vitamin C, vitamin A, vitamin E, minerals, pectin, antioxidant and carotenoids [4, 5]. They have huge concentration of β-carotene which protect against certain cancers and are powerful ally against degeneration aspect of aging [6]. Pumpkin has no cholesterol, low in fat and sodium and rich in vitamins. Pumpkin seeds are used for medicinal purpose for internal as well as external treatment and also used for production of soap, perfumes and lotions, food flavorings, food preservation, nutraceuticals, pharmaceuticals and cosmeceuticals. Pumpkins contain about 90% water and 2% seed. Pumpkin seeds contain 52% oil [7]. Pumpkin flesh is characterized by a low content of fat (2.3%), carbohydrates (66%), proteins (3%), and high-carotenoids content with values of 172 to 462 $\mu g.g^{-1}$ [8].

Many different parts of pumpkin are edible, e.g., flowers, leaves, fruits, peels and seeds. Pumpkin is eaten raw and in the form of preserves, such as soups, smoothies and juices. Pumpkin flesh is also used as an additive to bread, cakes, cookies, chocolates, candies [2]. Pumpkins can be processed into jerky and flour which has a longer shelf life. The flour can be used for its flavor, sweetness, deep yellow orange color and considerable amount of dietary fiber. It can be also used to supplement cereal flours in bakery products, soups, sauces, instant noodles and also as a natural coloring supplement for food. In Ethiopia matured fruit of the plant is used as edible parts as house holding spice stew (wat) consumed with Ethiopian traditional spongy thin-layer bread (injera) made from cereal grain called teff and roasted pumpkin seeds are used as snack [9].

Minerals are inorganic substance required as essential nutrients by all the body organs to perform functions necessary for the sustenance of life. Minerals are considered to be essential in human nutrition and they are important constituents of bones, teeth, tissues, muscles, blood and nerve cells. Generally, minerals help in the maintenance of acid-base balance, the response of nerves to physiological stimulation, physiological, catalytic, and regulatory functions of the body [10].

Nowadays scientists are exploring un-noticed vegetables which are rich sources of phytochemicals, which fight the deadly diseases like cancer and cardiovascular disease. Pumpkin is one of such vegetables gaining popularity as its medicinal and nutritional characteristics are equal or even better than those of widely cultivated vegetables and fruits [11]. Pumpkin possesses a significant amount of valuable minerals. Different parts are rich in K and relatively lower in Na, high in Ca, P, and Mg. Pumpkin is also good source of trace elements such as Zn and Fe. Heavy metals such as Zn, Cu, Mn, and Fe possess antioxidant properties. The low Na and high K content in pumpkin are used to a significant clinical implication for improving cardiovascular health. Zinc is essential in male reproduction and cellular protection [12, 13].

Recently several studies have been done on the major and heavy metal contents of vegetables and fruits cultivated and consumed in Ethiopia [14–23]. There is limited information about the major and heavy metal contents of different parts of pumpkin cultivated in Ethiopia [24].

Heavy metals are very important for the proper functioning of biological systems. But the high level of trace metals causes various threats to plants, animals and human life. Heavy metals intake in humans is associated with blood acidity, cancer, kidney problems, growth retardation and ultimately death [25]. Previous studies showed that the consumption of contaminated vegetables can lead to various diseases [26]. In particular, the Cd and Pb have carcinogenic effects and they can also cause neurological conditions or kidney, bone and cardiovascular diseases [27]. Other heavy metals such as Cu and Zn are considered non-carcinogenic, but excess amounts can be harmful to human health, causing liver failure, stomach pains and altering the immune system [28].

Among the health risk assessment indices applied to evaluate the non-cancer and cancer risk of heavy metals through the consumption of contaminated vegetables, the hazard quotient (HQ), the hazard index (HI), that analyzes the carcinogenic potential resulted from a lifetime exposure of an individual, have been widely used in the literature [28–31]. Thus, risk evaluation involves comparing the results of the risk analysis to specified risk criteria to decide if extra action is needed [32]. This study aims to investigate the levels of potentially toxic elements hazard quotients (HQ) and hazard index (HI) in pumpkin to quantify the noncancer risk. Also a daily intake of pumpkin by human were calculated and compared with the recommended dietary intakes.

The objectives of this study were to (i) determine the content of major and heavy metals in pumpkin peel, flesh and seed samples by microwave plasma-atomic emission spectroscopy, (ii) compare minerals found in different parts of pumpkin from three areas of Ethiopia, (iii) compare results of this study with reported results in the literature, (iv) compare the results of this study with other vegetables, and (v) estimate non-carcinogenic and carcinogenic risk due to life time ingestion of trace metals from pumpkin.

EXPERIMENTAL

Apparatus and equipment

A digital analytical balance (Mettler Tolledo) with ± 0.0001 g precision was used to weigh pumpkin samples. Milestone Ethos up microwave digester with Maxi 44 Rotor (Milestone Inc, USA) was used for the digestion of samples. A microwave plasma-atomic emission spectrometer (MP-AES) (Agilent Technologies, model 4200, USA) was used for the determination of metals.

Reagents and chemicals

All the chemicals and reagents used were high purity analytical grade reagents. Nitric acid (69%, w/w, HNO_3, MRS Scientific, UK) and hydrogen peroxide (30%, w/v, H_2O_2, England) were used for digestion of sample and blank. 1000 mg/L stock standard solution (Agilent Technologies, USA) of Na, Mg, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn and Cd metals were used for preparation of standard samples and in the spiking experiments. Deionized water was used for preparation of standard and sample preparation.

Description of the study area

Pumpkin samples were collected in November 2020 from Amhara region (North Shewa, Minjar Shenkora), Oromiya region (West Shewa, Woliso) and South region (Gamo Gofa, Arbaminch). The reason for the selection of Woliso and Arbaminch is the high production, commercial availability in urban markets, especially in Addis Ababa (capital city of Ethiopia) and high popularity in consumption of pumpkin compared to other parts of Ethiopia. Minjar Shenkora is selected to compare the composition of minerals with large, cultivated area, even though the local people do not use as an important vegetable. The map of sampling sites is shown in Figure 1.

Sample preparation

One piece (4 to 10 kg weight) of pumpkin fruit samples were collected from each site (Minjar Shenkora, Woliso and Arbaminch) in November 2020. The sample was washed with tap water followed by distilled water to remove adsorbed dust. The sample was cut into half and detached the seed from the flesh using bare hand and washed the seed sample with tap water to remove staked flesh and rinsed with distilled water. The sample was peeled to separate the flesh and cut into small pieces. The three separated parts (peel, flesh and seed) of pumpkin were air-dried for one month at room temperature. The dried sample were ground and homogenized into powder using mortar and pestle and sieved by using 300 mesh size sieve. The sieved sample was stored in polyethylene bottles and kept in a desiccator until the time of digestion. The samples were transported to the chemical analysis laboratory of Ethiopian Conformity Assessment Enterprise for digestion and analysis.

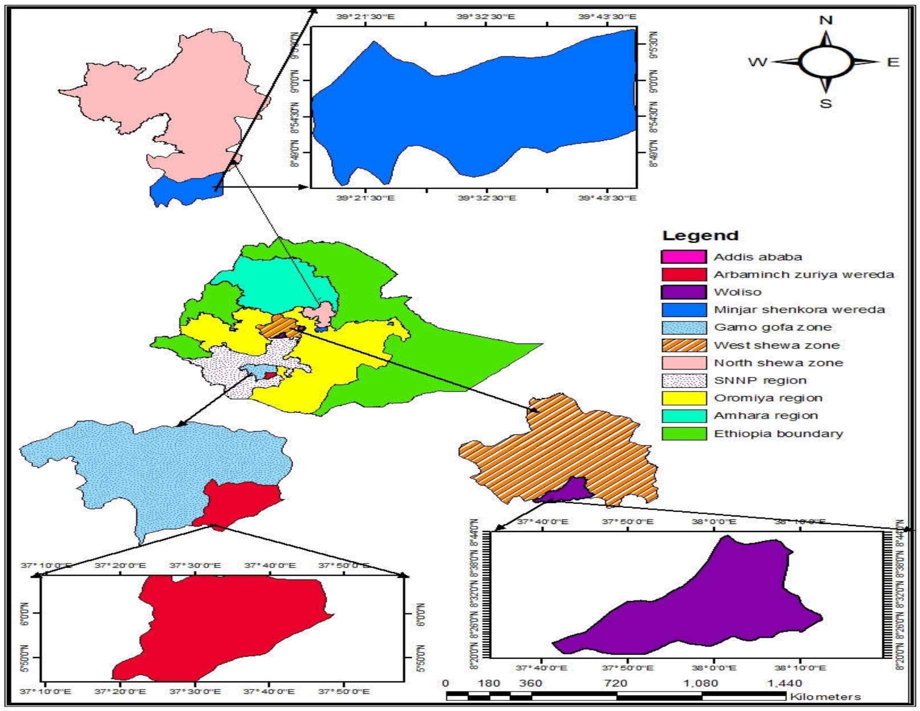


Figure 1. Map of pumpkin sampling sites.

Optimization of digestion procedure

For each part of pumpkin samples, 0.5 g dried and powdered sample was weighed and put in to clean nitric acid-soaked digestion vessel and different volumes of 69% (w/w) nitric acid and 30% (w/v) hydrogen peroxide at specified proportion were added and subjected to microwave digestion program at different temperature, time and power. Based on this, the optimized digestion condition for pumpkin peel was 7 mL HNO_3:1 mL H_2O_2 volume ratio of reagents, 1800 W power, 200 °C digestion temperature, 25 min at 1st stage; 1800 W, 200 °C, 20 min 2nd stage and 1800 W, 200 °C, 15 min for 3rd stage. For pumpkin flesh the optimized condition was 6 mL HNO_3: 2 mL 30% H_2O_2 reagent ratio, 1200 W, 200 °C, 25 min 1st stage; 1200 W, 200 °C, 20 min 2nd stage and 1200 W, 200 °C, 15 min for 3rd stage. For pumpkin seed the optimized sequence was 6 mL HNO_3: 1 mL H_2O_2 volume ratio, 1600 W, 200 °C, 25 min 1st stage; 1600 W, 200 °C, 20 min 2nd stage and 1600 W, 200 °C, 0 min for the 3rd stage.

Digestion of samples

A 0.5 g pumpkin peel was transferred to 100 mL microwave digestion vessel. The optimized volume of 7 mL 69% (w/w) HNO_3 and 1 mL of 30% (w/v) H_2O_2 was added to pumpkin peel in the vessel. The mixture was shaken, and the vessel was properly closed. The vessel was placed into a rotator and subjected to microwave digester. The microwave program was adjusted in the sequence of 1800 W, 200 ºC, 25 min; 1800 W, 200 ºC, 20 min and 1800 W, 200 ºC, 15 min in three stages. The vessel was allowed to stand for 15 min for cooling after the completion of digestion. The vessel was opened and rinsed by distilled water and the digested solution was filtered into 50 mL volumetric flask and made up to the mark of volumetric flasks. The flask was kept into refrigerator until analysis. Pumpkin flesh and pumpkin seed were also digested by the same procedure as pumpkin peel. The difference for each case was the optimized condition (volume ratio used, power and temperature adjusted) during digestion. The optimized condition for pumpkin flesh was 6 mL 69% nitric acid to 2 mL hydrogen peroxide reagent volume ratio, 1200 W, 200 ºC, 25 min in 1st stage; 1200 W, 200 ºC, 20 min in the 2nd stage and 1200 W, 200 ºC, 15 min in the 3rd stage. For pumpkin seed the volume ratio of reagent was 6 mL nitric acid to 1 mL hydrogen peroxide, 1600 W, 200 ºC, 20 min 1st stage; 1600 W, 200 ºC, 20 min 2nd stage and 1600 W, 200 ºC, 5 min for 3rd stage.

Preparation of standard solution

The working standards of each element were prepared from 1000 mg/L standard stock solution which was Agilent technologies wavelength calibration solution for ICP-OES and MP-AES in 5% HNO_3 certified reference material (CRM) by serial dilution with deionized water. 10 mg/L standard stock solution for Cr, Cd, Mn and Ni; 100 mg/L for Na, K, Ca, Mg, Cu, Fe and Zn was prepared by diluting stock solution with deionized water. 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2 mg/L for Cr, Cd, Mn, Ni; 2, 4, 6, 8, 10 mg/L for Mg; 1, 2, 3, 4, 5, 6, 7 mg/L for K, Cu, Zn, Fe; 2, 3, 4, 5, 6, 7 mg/L for Na and Ca were prepared in the same manner. Different concentrations of calibration standards were used for different metals because of differences in their linear ranges of calibration curves.

Determination of metals in pumpkin samples

After calibrating the instrument, the concentration of selected metals in the samples was determined by MP-AES. Triplicate analysis was carried out for each sample. Na, Mg, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn and Cd were determined by emission/concentration mode after properly calibrating the instrument using calibration blank and seven working calibration standard solutions of each metal. The determination of metals in the digested blank solution was also done in parallel with the pumpkin samples keeping all parameters the same by using the same procedure.

Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) of metals of interest in this study were determined from nine replicate blank solutions. LOD was calculated by multiplying the pooled standard deviation of the reagent blank (SD_{blank}) by three (LOD = 3 x SD_{blank}). Limit of quantification (LOQ) was calculated as ten times the standard deviation of blank solution (LOQ = 10 x SD_{blank}). The limit of detection of all eleven elements in this study (Table 1) was lower than the obtained sample concentration results.

Method validation

Analytical parameters for the determination of metals were evaluated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and linearity. The accuracy was evaluated by spiking the sample matrix of interest with a known concentration of analyte standard. From 100 mg/L stock solution 0.5 mL of Ca, Mg, Na, K, Cu, Zn, Fe and 2.5 mL of Mn, Ni, Cr, Cd were added to 0.5 g of each pumpkin peel, flesh and seed samples. The spiked and non-spiked samples were digested and analysed in the same condition using the method being validated. Accuracy was calculated as percent recovery. Table 2 displays the percent recoveries for different parts of pumpkin samples and were found in the range 90–108% which are within the acceptable range and indicates the efficiency of the optimized procedure.

Table 1. Wavelength, LOD, LOQ, correlation coefficient and calibration curve equation for determination of metals using MP-AES instrument.

Metals	Wavelength (nm)	LOD (mg/L)	LOQ (mg/L)	Correlation coefficient	Calibration curve equation*
Na	588.995	0.251	0.836	0.9988	I = 371193C - 3059
Mg	285.213	0.026	0.087	0.9974	I = 150761C + 47644
K	766.491	0.003	0.010	0.996	I = 14653C - 1652
Ca	393.366	0.026	0.087	0.9992	I = 405009C - 256
Cr	425.433	0.003	0.009	0.9998	I = 45651C - 253
Mn	403.076	0.004	0.012	0.9999	I = 50537C - 89.0
Fe	371.993	0.148	0.495	0.9999	I = 10584C - 245
Ni	352.454	0.026	0.087	0.9999	I = 14226C - 11.4
Cu	324.754	0.032	0.106	0.9998	I = 104245C + 3397
Zn	213.857	0.011	0.036	0.9971	I = 8197C + 1512
Cd	228.802	0.000	0.000	0.9996	I = 14735C + 34.1

*In the calibration equation, I = emission intensity and C = concentration (mg/kg).

Table 2. Recovery results for pumpkin peel, flesh and seed samples.

	Peel				Flesh				Seed			
Metal	Un spiked (mg/kg)	Added (mg/kg)	Spiked (mg/kg)	Reco-very (%)	Unspi-ked (mg/kg)	Added (mg/kg)	Spiked (mg/kg)	Recovery (%)	Un spiked (mg/kg)	Added (mg/kg)	Spiked (mg/kg)	Recovery (%)
Na	131	82.9	207	91±1.8	54.6	82.9	144	108±1.2	167	82.9	254	106±0.7
Mg	5243	4893	9637	90±1.8	447	4894	5473	103±0.3	4296	4895	9008	96±0.1
K	19123	4993	23692	92±5.3	32195	4994	37671	110±8.3	9824	9990	19953	101±2.1
Ca	6374	4910	10985	94±2.9	3845	4994	9109	105±0.0	2797	9990	13626	108±0.0
Cr	0.43	49.8	50.8	101±2.0	0.2	49.8	46.6	93±0.4	0.73	49.9	49.7	98±0.2
Mn	30.2	49.9	81	102±1.2	8.77	49.9	60.9	104±0.6	29.2	49.9	81.8	105±2.2
Fe	184	90.4	273	98±11	92.5	81.8	175	101±12	218	92.0	301	90±4.4
Ni	3.21	49.9	53.7	101±0.6	1.46	49.9	49.1	95±1.2	2.20	50.0	52.3	100±1.4
Cu	7.24	97.8	110	105±1.1	25.3	99.5	119	94±1.3	11.8	99.9	107	96±0.1
Zn	20.6	99.9	126	106±2.8	6.19	99.2	97.5	92±0.6	53.9	99.9	159	105±2.3
Cd	0.4	49.9	48.4	96±1.2	0.5	49.9	48.9	97±2.1	1.53	50.0	48.0	93±0.1

Health risk assessment

Health risk assessment is used to determine the magnitude and probability of an adverse health effect from exposure. It is very important to safeguard the public health [33]. Toxic metals enter to human body through ingestion (food, water), inhalation (air) and absorption through dermal contact [34]. The non-carcinogenic and carcinogenic health risk assessment of metals ingested from pumpkin edible parts were calculated based on the assumption that an adult of 61 kg (African adult average body weight) [35] ingested 0.2 kg of pumpkin and 0.1 kg fresh weight pumpkin seed per day for 96 days per a year (2 days per week) for 67 years (East African life expectancy). The fresh weight of pumpkin was converted into dry weight by multiplying with conversion factor 0.085 [36]. Non-carcinogenic adverse effects are characterized by hazard quotient (HQ), a ratio of average daily intake (ADI) and reference dose (RfD) due to exposure to toxicants:

$$HQ = \frac{ADI}{RFD} \quad (1)$$

where HQ – hazard quotient, ADI – average daily intake, RFD – reference dose. RFD is the estimated maximum permissible dose for human through daily exposure established by the Joint FAO (Food and Agriculture Organization)/WHO (Word Health Organization) Expert Committee on Food Additives [36]. Non-carcinogenic effects would occur when HQ ≥ 1 and HQ < 1 indicates no adverse effects. The average daily intake (ADI, mg/(kg day)) was estimated by using the equation:

$$ADI = \frac{C*IR*ED*EF}{BW*AT} \quad (2)$$

where C – metal concentration (mg/kg); IR – ingestion rate; ED – exposure duration; EF – exposure frequency; BW – body mass and AT – average time (ED*EF). The values of parameter are listed in Table 3.

The hazard index (HI) was calculated to evaluate the potential risk of adverse health effects from a mixture of chemical elements in edible part of pumpkin. The HI was calculated as the sum of HQ (assuming additive effects):

$$HI = \sum HQ \quad (3)$$

The cancer risk (CR) was calculated by multiplying the average daily intake (in mg/(kg day) over a lifetime) with a cancer slope factor (SF). It is incremental probability of an individual developing cancer over a lifetime.

$$CR = ADI * SF \quad (4)$$

If multiple carcinogenic elements are present, the summation of cancer risks is considered for total cancer risk (TCR).

$$TCR = \sum CR \quad (5)$$

TCR values from 1.0 x 10^{-6} to 1.0 x 10^{-4} is the acceptable range for cancer risk [37]. Table 3 shows the oral RFD and SF value for minor and trace metals in food.

Table 3. Values of parameters for ADI input and reference doses (RfD) and slope factors (SF) of selected metals.

Values of parameters for ADI input			Reference doses (RfD) and slope factors (SF) of selected metals		
Factor	Value	Unit	Metals	RfD (mg/kg/day)	SF $(mg/kg.d)^{-1}$
IR	0.2/0.1	kg/day	Cr	1.50	0.50
ED	67	year	Mn	0.01	-
EF	96	day/year	Ni	0.02	-
AT	6432	day	Cu	0.04	-
BW	61	kg	Zn	0.30	-
			Cd	0.001	0.64

RESULTS AND DISCUSSION

Concentration of metals in different parts of pumpkin sample collected from three areas

A total of 11 metals were determined by microwave plasma-atomic emission spectroscopy (MP-AES). In general, the most abundant elements were K, Ca, Mg, Na and Fe. From the overall mean concentrations, pumpkin flesh had relatively higher concentration of K (29531 ± 2452 mg/kg) and

Cu (15.1 ± 9.3 mg/kg). Pumpkin seed had higher concentration of Na (171 ± 123 mg/kg), Cr (0.69 ± 0.1 mg/kg), Mn (33.4 ± 4.9 mg/kg), Fe (225 ± 56 mg/kg), Zn (67.8 ± 12.3 mg/kg) and Cd (1.39 ± 0.2 mg/kg) and pumpkin peel had higher concentration only of Mg (4716 ± 469 mg/kg), Ca (5268 ± 2092 mg/kg) and Ni (3.79 ± 2.4 mg/kg).

Table 4 shows that there is a large variation in concentration of metals within pumpkin parts (peel, flesh, seed) and location of sampling sites (Woliso, Minjar Shenkora and Arbaminch). This variation can be mainly ascribed to the variation in the mineral composition of soil and water since it was collected from the compound of farmer's residence and environmental factors.

The concentration patterns of metals in pumpkin peel collected from Woliso, Minjar Shenkora and Arbaminch were in the order: K > Mg > Ca > Na > Fe > Mn > Zn > Cu > Ni > Cr > Cd; K > Ca > Mg > Fe > Na > Zn > Mn > Cu > Ni > Cr > Cd and K > Ca > Mg > Fe > Na > Mn > Zn > Cu > Ni > Cr > Cd, respectively. Concentration of K was the highest in pumpkin peel for all the sites followed by Ca, Mg, Fe and Na in Minjar Shenkora and Arbaminch site where as in Woliso pumpkin peel Mg was the second abundant metal followed by Ca, Na and Fe. The average concentration of metals in pumpkin peel sample from Woliso, Minjar Shenkora and Arbaminch, respectively, were K (27806), Ca (5268), Mg (4716), Fe (158), Na (167), Mn (28.9), Zn (24.2), Cu (8.14), Ni (3.79), Cr (0.28), Cd (0.24) mg/kg (Table 4).

The metal concentration patterns in pumpkin flesh samples from Woliso, Minjar Shenkora and Arbaminch were K > Ca > Mg > Fe > Na > Mn > Zn > Cu > Ni > Cd > Cr; K > Ca > Mg > Na > Fe > Zn > Cu > Mn > Ni > Cr > Cd and K > Ca > Mg > Fe > Na > Cu > Mn > Zn > Ni > Cd > Cr, respectively. The average concentration of metals in pumpkin flesh from Woliso, Minjar Shenkora and Arbaminch, were K (29531), Ca (3191), Mg (848), Fe (111), Na (63.1), Cu (15.1), Mn (9.63), Zn (10.6), Ni (2.14), Cd (0.46) and Cr (0.36) mg/kg.

Table 4. Mean concentration (mg/kg) with standard deviation of each sample.

Sample	Mean ± SD (mg/kg)										
	Na	Mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
WPP	297±3.05	4344±49.9	25914±1926	2854±14.4	0.17±0.12	27.5±0.33	163±3.79	6.44±0.87	9.34±0.67	22.8±0.55	0.13±0.12
MPP	73.6±0.58	4560±28.8	38380±610	6574±104	0.23±0.15	29.0±0.67	126±41.7	1.71±0.08	7.84±0.70	29.1±1.75	0.20±0.10
APP	131±1.53	5243±49.9	19123±528	6374±104	0.43±0.15	30.2±0.20	184±6.06	3.21±0.18	7.24±1.39	20.6±1.36	0.40±0.10
WPF	54.9±1.73	1010±4.72	27367±350	3562±57.7	0.37±0.06	13.1±0.05	163±1.71	3.70±0.10	7.09±0.10	11.8±1.08	0.47±0.06
MPF	79.9±1.73	1086±6.10	29032±650	2164±57.7	0.50±0.00	6.97±0.03	77.3±0.76	1.27±0.06	12.8±0.06	13.9±0.15	0.40±0.10
APF	54.6±0.58	447±4.72	32195±425	3845±0.00	0.20±0.00	8.77±0.08	92.5±6.89	1.46±0.06	25.3±1.19	6.19±0.44	0.50±0.10
WPS	296.0±1.15	5112±76.3	11955±153	2597±0.00	0.80±0.10	38.8±0.91	283±11.7	6.66±0.23	10.5±0.41	77.6±1.62	1.23±0.12
MPS	49.3±0.58	4595±0.00	10223±208	3363±57.7	0.53±0.40	32.1±1.74	173±6.95	1.47±0.38	10.1±0.33	71.8±2.94	1.40±0.10
APS	167±2.00	4296±0.00	9824±208	2797±0.00	0.73±0.06	29.2±1.06	218±3.74	2.20±0.10	11.8±0.08	53.9±1.26	1.53±0.06

WPP - Woliso pumpkin peel, MPP - Minjar Shenkora pumpkin peel, APP - Arbaminch pumpkin peel, WPF - Woliso pumpkin flesh, MPF - Minjar Shenkora pumpkin flesh, APF - Arbaminch pumpkin flesh, WPS - Woliso pumpkin seed, MPS - Minjar Shenkora pumpkin seed, APS - Arbaminch pumpkin seed, SD - standard deviation, RSD - relative standard deviation.

Pumpkin seed metal concentration patterns from Woliso, Minjar Shenkora and Arbaminch are K > Mg > Ca > Na > Fe > Zn > Mn > Cu > Ni > Cd > Cr; K > Mg > Ca > Fe > Zn > Na > Mn > Cu > Ni > Cd > Cr and K > Mg > Ca > Fe > Na > Zn > Mn > Cu > Ni > Cd > Cr, respectively. Pumpkin seed average metal concentration from the three sites Woliso, Minjar Shenkora and Arbaminch, respectively, were K (10667), Mg (4668), Ca (2919), Fe (225), Na (171), Zn (67.8), Mn (33.4), Cu (10.8), Ni (3.44), Cd (1.39) and Cr (0.69) mg/kg. As can be seen from the trend, all parts of pumpkin had highest concentration of K and also a good source of major, minor and trace metals.

Comparisons of metal concentration of different parts of pumpkin

Most metals (Cu, Zn, Fe, Mg, Na, Mn, Ni, Cr and Cd) are present in higher amounts in seed than in flesh and peel. Calcium and potassium are higher in Woliso pumpkin flesh. From Minjar Shenkora sample Mg, Fe, Zn, Cd, Mn, and Cr are higher in seed and K, Ca and Ni are higher in peel where as Na and Cu are higher concentration in flesh. Na, Fe, Zn, Cd and Cr had high concentration in Arbaminch pumpkin seed; Pumpkin peel of Arbaminch sample is high in Mg, Ca, Mn and Ni concentration whereas K and Cu are higher concentration in Arbaminch pumpkin flesh.

Comparisons of metal concentration of pumpkin from different areas

The concentration of Mg, Fe, Cr, Mn, and Cd are the highest in pumpkin peel from Arbaminch sample than Minjar Shenkora and Woliso pumpkin peel. Na, Ni and Cu are found in high concentration in Woliso pumpkin peel. While K, Ca, and Zn are higher concentration in Minjar Shenkora pumpkin peel. In general, most minor and trace metals are higher concentration in Arbaminch pumpkin peel sample. The concentration of Na, Mg, Zn and Cr are higher in Minjar Shenkora pumpkin flesh than the other two sites. Arbaminch pumpkin flesh accumulates high concentration of K, Ca, Cu and Cd when compared to Woliso and Minjar Shenkora whereas Woliso pumpkin flesh had higher concentration in Fe, Mn and Ni than Arbaminch and Minjar Shenkora. Except Ca, Cu and Cd, all determined metal concentrations in pumpkin seed from Woliso is higher than Minjar Shenkora and Arbaminch pumpkin seed. Ca is higher in Minjar Shenkora pumpkin seed while Cu and Cd are higher in Arbaminch pumpkin seed.

Comparisons of the metal concentration of different parts of pumpkin with other reported values

The levels of metal content in different parts of pumpkin have been reported in different countries. The comparison of results of this study with the literature data are given in Table 5. From the reported data K is the most abundant metal in all parts of pumpkin sample. The mean levels of Na in this study are comparable to those reported in Bangladesh [38] indigenous pumpkin peel and lower than those reported in Egypt [10] and Bangladesh [38] hybrid pumpkin peel. Fe has similar concentration to Bangladesh hybrid pumpkin peel and higher than Egypt pumpkin peel. Zn is higher than Egypt and lower than Bangladesh hybrid pumpkin. K, Mn and Cu have comparable concentration to Bangladesh and Egypt reported by Amin *et al.* [38] and Hashash *et al.* [10], respectively. Mean concentration of Na in this study (5.5 mg/100 g in Woliso and Arbaminch) is comparable with Indian pumpkin flesh (5.6 mg/100 g) reported by Dhiman *et al.* [39] and is lower than other report from Ethiopia [40], Egypt [10] and Bangladesh [38]. Higher concentration of Mg is reported by Belete *et al.* [40] from Ethiopia than this study but the level of Mg in this study is higher than those reports from Egypt, India, and Bangladesh. Ni is not included in those literature and K, Mn, Cu and Zn concentration are comparable with reported literature values. The concentration of Na in this study is lower than reported from Egypt [10], Nigeria [11] and Austria [41] and comparable with other Ethiopian [42] and Croatian [41] results. Mg, K, Cr, Mn, Cu and Zn concentration in this study are comparable with other report from Ethiopia [42]. Ca is higher in this study from all other reports from Ethiopia, Egypt, Nigeria, Zimbabwe, Croatia and Slovenia.

Comparisons for metal concentration of pumpkin flesh with other vegetables

In this study, the selected metals (Na, Mg, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn and Cd) concentration of pumpkin peel, flesh and seed were determined. But in most countries only pumpkin flesh is cooked and served as a vegetable. Accordingly the comparison of heavy metals concentration in pumpkin flesh with other vegetables which are more commonly consumed in Ethiopia has been done. The data in Table 6 shows the concentration of Cd is comparable with other vegetables listed in the table. Cr, Mn, Fe, Ni, Cu and Zn were less in pumpkin than other vegetables.

Table 5. Comparison of metal concentrations of different parts of pumpkin with those reported in the literature (mg/100 g).

Sample part	Concentration, (mg/100 g)						Method	Country	Reference
	Na	Mg	K	Ca	Cr				
Peel	68.9	32.6	153	5.32	-		ICP-AES, EFP	Egypt	[10]
	9.65	3.35	687	1.36	-		AAS, FP (Na)	Bangladesh indigenous	[38]
	60.6	3.66	1233	0.96	-		AAS, FP(Na)	Bangladesh hybrid	[38]
	16.7	**472**	**2781**	**527**	**0.03**		**MP-AES**	**Ethiopia**	**This study**
Flesh	13.3	221	3869	439	-		AAS	Ethiopia	[40]
	76.5	25.1	189	5.5	-		ICP-AES, EFP	Egypt	[10]
	20.8	5.64	1616	0.82	-		AAS, FP (Na)	Bangladesh indigenous	[38]
	24.8	4.77	1518	0.74	-		AAS, FP (Na)	Bangladesh hybrid	[38]
	5.6	38	139	10	-			India	[39]
	6.31	**84.8**	**2953**	**319**	**0.04**		**MP-AES**	**Ethiopia**	**This study**
Seed	7	592	809	46	-			Ethiopia	[42]
	155	60.5	224	6.65	-		ICP-AES, EFP	Egypt	[10]
	1.35	4.34	435	4	-		AAS, FP (Na)	Bangladesh indigenous	[38]
	0.98	3.69	558	3.76	-		AAS, FP (Na)	Bangladesh hybrid	[38]
	170	67.4	237	9.78	-		AAS, AES	Nigeria	[11]
	68	345	-	141	-		ICP-AES	Zimbabwe	[43]
	7.97	35.5	22.5	13.4	0.01		ICP-OES	Croatian	[41]
	45.8	32.1	42.4	9.48	0.01		ICP-OES	Slovenia	[41]
	149	115	85.5	33.6	-		ICP-OES	Austria	[41]
	17.1	**467**	**1067**	**292**	**0.07**		**MP-AES**	**Ethiopia**	**This study**
Peel	Concentration (mg/100 g)								
	Mn	Fe	Ni	Cu	Zn	Cd			
	1.25	4.95	-	0.49	0.05	-	ICP-AES, EFP	Egypt	[10]
	0.36	4	-	0.03	0.15	-	AAS, FP (Na)	Bangladesh indigenous	[38]
	0.38	15.7	-	0.02	18.8	-	AAS, FP(Na)	Bangladesh hybrid	[38]
	2.89	**15.7**	**0.37**	**0.81**	**2.41**	**0.01**	**MP-AES**	**Ethiopia**	**This study**
Flesh	1.27	8.99	-	0.94	1.42	-	AAS	Ethiopia	[40]
	1.35	6.48	-	0.55	0.65	-	ICP-AES, EFP	Egypt	[10]
	0.45	42.1	-	0.06	0.23	-	AAS, FP (Na)	Bangladesh indigenous	[38]
	0.43	4.79	-	0.06	0.21	-	AAS, FP (Na)	Bangladesh hybrid	[38]
	0.05	0.44	-	0.05	0.26	-		India	[39]
	0.96	**11.1**	**0.215**	**1.5**	**1.06**	**0.04**	**MP-AES**	**Ethiopia**	**This study**
Seed	4.54	8.82	-	1.34	7.81	-		Ethiopia	[42]
	4.55	12.4	-	0.85	0.85	-	ICP-AES, EFP	Egypt	[10]
	1.35	6.02	-	0.31	18.8	-	AAS, FP (Na)	Bangladesh indigenous	[38]
	0.98	5.51	-	0.26	16.4	-	AAS, FP (Na)	Bangladesh hybrid	[38]
	0.06	3.75	-	-	14.1	-	AAS, AES	Nigeria	[11]
	_	12	-	-	1.24	-	ICP-AES	Zimbabwe	[43]
	0.19	2.7	-	0.74	6.77	0.14	ICP-OES	Croatian	[41]
	0.18	2.66	-	0.01	0.01	-	ICP-OES	Slovenia	[41]
	0.56	2.65	-	-	-	-	ICP-OES	Austria	[41]
	3.34	**22.5**	**0.34**	**1.07**	**0.07**	**-**	**MP-AES**	**Ethiopia**	**This study**

Table 6. Comparison of pumpkin flesh metal concentrations with other vegetables those reported in the literature from Ethiopia.

Sample	Concentration (mg/100 g)							Reference
	Cr	Mn	Fe	Ni	Cu	Zn	Cd	
Cabbage	6.20	22.6	101	1.5	10.4	40.1	0.04	44]
Potato	6.66	0.9		20.4	11.3	56.7	6.01	[45]
Tomato	5.80	2.42	-	13.9	2.01	24.6	2.43	[45]
Ethiopian kale	13.3	30.6		11.1	2.41	88.3	6.25	[45]
Swiss chard	2.20	869	159	11.6	11.6	219	0.10	[44]
Carrot	0.82	53	403	2.44	7.68	44.9	0.06	[46]
WPF	0.04	1.31	16.3	0.37	0.71	1.18	0.05	This study
MPF	0.05	0.70	7.73	0.13	1.28	1.39	0.04	This study
APF	0.02	0.88	9.25	0.15	2.53	0.62	0.05	This study

WPF - Woliso pumpkin flesh; MPF - Minjar Shenkora pumpkin flesh; APF - Arbaminch pumpkin flesh.

Daily intake of metals from pumpkin edible parts

Table 7 shows the metal concentration recommended daily intake (mg/day) for different age group and the upper limit concentration established by FAO/WHO jointly. A 0.4 kg of vegetable intake per day is recommended by the Joint Food and Agriculture Organization/World Health Organization [47]. Assuming an average adult person takes 0.2 kg pumpkin flesh as part of vegetable and 0.1 kg pumpkin seed as snack per day, the amount of Na, Ca, Zn that a person can get is lower than the daily recommended value, indicates pumpkin alone cannot satisfy the daily requirement. K, Cr, Fe, Ni, Cu, Cd are higher than daily recommended values but still below the upper limit, indicates pumpkin in the selected area is good sources of these elements. Mg is above the upper limit and Mn is within the recommended range.

Table 7. The average metal concentration (mg/200 g) recommended daily intake (mg/day) and upper limit values which are recommended by IOM [48], FAO/WHO [42], Nordic Council of Ministers [49].

Metals	mg/200 g		Recommended daily intake (mg/day)					Upper limit (mg/day)	
	Flesh	Seed	1-9 year	10-18 year	Adults (>19 year)			Children	Adult
					Female	Male	Pregnancy		
Na	12.6	34.2	1500 - 1900	2300	1500	1500	1500	2300	2300
Mg	170	934	60 - 100	220 - 230	310 - 320	400 - 420	350 - 400	65 - 110	350
K	5906	2133	2000 - 2300	2500 - 3000	3500 - 4700	3500 - 4700	2900	ND	ND
Ca	638	584	500 - 800	1300	1000 - 1300	1000 - 1300	1200	2500	3000
Cr	0.07	0.14	0.05 - 0.07	0.06 - 0.08	0.10	0.03 - 0.1	0.03	0.25	0.25
Mn	1.92	6.67	1.80	1.80	2.10	3.00	2 - 2.6	11.0	11.0
Fe	22.2	44.9	3.9 - 17.8	8 - 18	8-18	8.00	10.0	40.0	45.0
Ni	0.43	0.69	0.08 - 0.10	0.1 - 0.14	0.106 - 0.109	0.136 - 0.139	0.12	0.30	1.00
Cu	3.01	2.16	0.34- 0.44	0.7 - 0.9	0.90	0.90	1.00	8.00	10.0
Zn	2.13	13.6	8 - 11	8 - 11	8.00	11.0	3.4 - 20	23-28	45.0
Cd	0.09	0.28	0.002-0.0031	0.004	0.01	0.06	0.01	0.03	0.20

Health risk assessment of metals from pumpkin edible parts

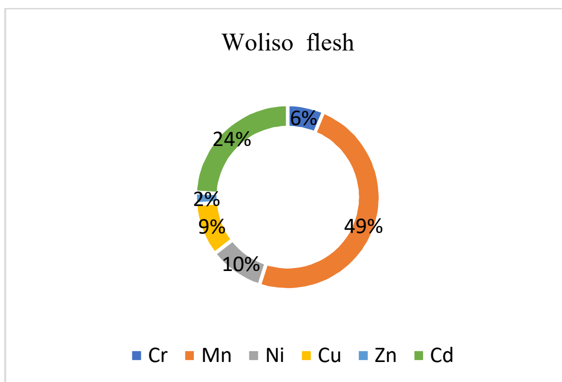
Hazard quotients (HQ) and cancer risk (CR) are used to assess the potential health risks for humans. The non-carcinogenic adverse health effects of minor and trace metals for hazard quotient (HQ) and hazard index (HI) in edible part of pumpkin (flesh and seed) are presented in Table 8. All calculated values of HQ from three sites were less than the permissible limit (1.0) indicating no adverse carcinogenic health risk due to consumption of the pumpkin from selected areas. The HI (the sum of individual metals HQ for pumpkin flesh) were found less than unity, with HI = 0.54, 0.42, 0.53 for Woliso, Minjar Shenkora and Arbaminch flesh, respectively. HI values from pumpkin seed were = 0.71, 0.62 and 0.62 due to the consumption of pumpkin seed from Woliso, Minjar Shenkora and Arbaminch, respectively. HI value > 1.0 indicates potential health influence consequences and when HI > 10 a serious chronic health impact has been suggested. HI from pumpkin edible part in all selected area has no adverse health impact which is less than one. This indicates ingestion of pumpkin from selected areas is safe from non-carcinogenic risk. Figure 2 shows the percent contribution of each metal to the total hazard index value in different parts of pumpkin from three sampling sites.

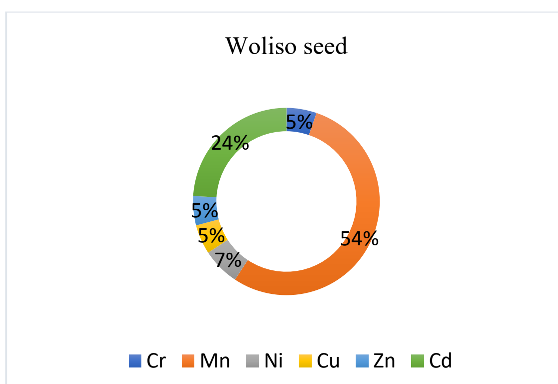
Table 8. Calculated HQ and HI values of edible parts of Woliso, Minjar Shenkora and Arbaminch pumpkin.

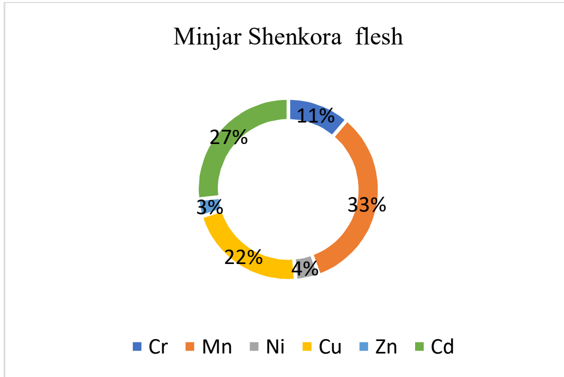
Metals	Woliso				
	Edible part	Conc. (mg/kg)	ADI (mg/kg/day)	RFD (mg/kg/day)	HQ
Cr	Flesh	0.37	0.0001	0.003	0.03
	Seed	0.80	0.0001		0.04
Mn	Flesh	13.1	0.0037	0.01	0.26
	Seed	38.8	0.0054		0.39
Ni	Flesh	3.70	0.0010	0.02	0.05
	Seed	6.66	0.0009		0.05
Cu	Flesh	7.09	0.0020	0.04	0.05
	Seed	10.5	0.0015		0.04
Zn	Flesh	11.8	0.0033	0.30	0.01
	Seed	77.6	0.0108		0.04
Cd	Flesh	0.47	0.0001	0.001	0.13
	Seed	1.23	0.0002		0.17
			HI	Flesh	0.54
				Seed	0.73
Metals	Minjar Shenkora				
	Edible part	Conc. (mg/kg)	ADI (mg/kg/day)	RFD (mg/kg/day)	HQ
Cr	Flesh	0.50	0.0001	0.003	0.05
	Seed	0.53	0.0001		0.02
Mn	Flesh	6.97	0.0019	0.01	0.14
	Seed	32.1	0.0045		0.32
Ni	Flesh	1.27	0.0004	0.02	0.02
	Seed	1.47	0.0002		0.01
Cu	Flesh	12.8	0.0036	0.04	0.09
	Seed	10.1	0.0014		0.04
Zn	Flesh	13.9	0.0039	0.30	0.01
	Seed	71.8	0.0100		0.03
Cd	Flesh	0.40	0.0001	0.001	0.11
	Seed	1.40	0.0002		0.19
			HI	Flesh	0.42
				Seed	0.61
Metals	Arbaminch				
	Edible part	Conc. (mg/kg)	ADI (mg/kg/day)	RFD (mg/kg/day)	HQ
Cr	Flesh	0.20	0.0001	0.003	0.02
	Seed	0.73	0.0001		0.03
Mn	Flesh	8.77	0.0024	0.014	0.17
	Seed	29.2	0.0041		0.29
Ni	Flesh	1.46	0.0004	0.02	0.02

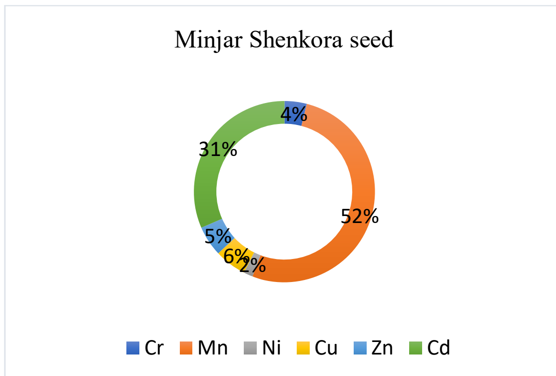
	Seed	2.20	0.0003		0.02
Cu	Flesh	25.3	0.0071	0.04	0.18
	Seed	11.8	0.0016		0.04
Zn	Flesh	6.19	0.0017	0.3	0.01
	Seed	53.9	0.0075		0.03
Cd	Flesh	0.50	0.0001	0.001	0.14
	Seed	1.53	0.0002		0.21
			HI	Flesh	0.54
				Seed	0.62

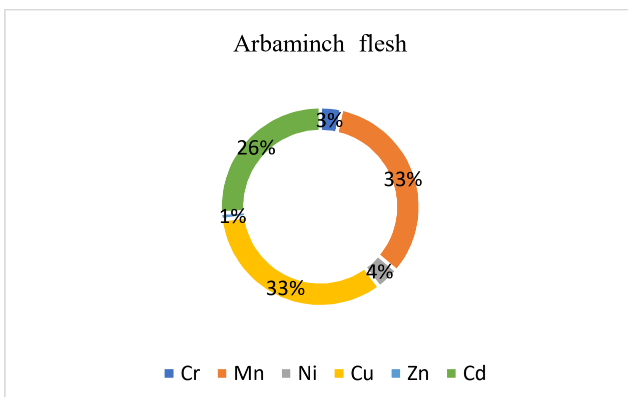
The cancer risk (CR) due to the exposure of toxic metals (Cd and Cr) through the consumption of pumpkin were estimated by using ADI and oral cancer slope factor (SF) $(mg/kg/day)^{-1}$ as indicated under experimental section and using Equation (5). The target cancer risk due to the exposure to Cr from pumpkin flesh were 5.1 x 10^{-5}, 6.9 x 10^{-5} and 2.7 x 10^{-5}, from pumpkin seed were 5.5 x 10^{-5}, 3.7 x 10^{-5}, 5.1 x 10^{-5} for Woliso, Minjar Shenkora and Arbaminch sample, respectively. The cancer risk due to Cd from pumpkin flesh were 8.3 x 10^{-5}, 7.1 x 10^{-5}, 8.9 x 10^{-5} and whereas from pumpkin seed were 1.1 x 10^{-4}, 1.2 x 10^{-4}, 1.3 x 10^{-4} for Woliso, Minjar Shenkora and Arbaminch, respectively.






Minjar Shenkora flesh
11%
33%
4%
22%
3%
27%
Cr Mn Ni Cu Zn Cd


Minjar Shenkora seed
4%
52%
2%
6%
5%
31%
Cr Mn Ni Cu Zn Cd


Arbaminch flesh
3%
33%
4%
33%
1%
26%
Cr Mn Ni Cu Zn Cd

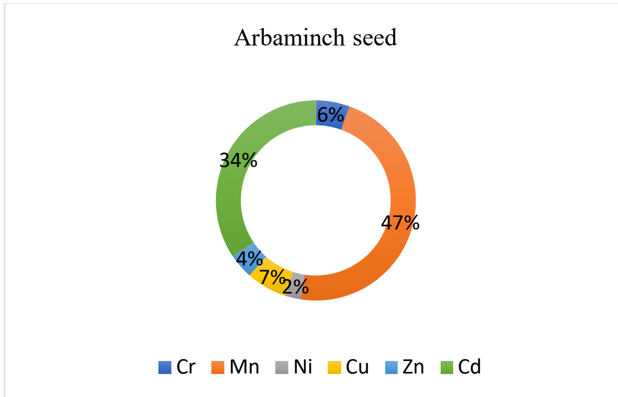


Figure 2. Percent contribution of each metal to the total HI.

Table 8 shows the TCR due to the consumption of pumpkin flesh and seed from all sampling area which is approximately equal to the maximum limit value (1 x 10^{-4}) indicates no risk of exposure to cancer due to the consumption of pumpkin from the selected area. According to the US EPA, human cancer over a 70-year lifetime (1.0 x 10^{-6} – 1.0 x 10^{-4}) is regarded as an acceptable or insignificant risk [34]. Gebeyehu and Bayissa [50] have reported a TCR value of 6.6 x 10^{-5} and 1.9 x 10^{-4} for Cd due to the consumption of tomato and cabbage which is more comparable data with reported in this study. The total Cr cancer risk value obtained from tomato and cabbage are 2.3 x 10^{-4} and 7.3 x 10^{-4}, respectively which is higher than this report.

Analysis of variance (ANOVA)

One way ANOVA was used to compare the mean value of the metals of each part of pumpkin between different sampling sites. The statistical analysis indicates that there is a significant difference between the mean concentration of Na, Mg, K, Ca, Mn, Ni and Zn found in the pumpkin peel collected from Woliso, Minjar Shenkora and Arbaminch ($p < 0.05$) whereas there is no significant difference in the mean concentration of Cr, Fe, Cu and Cd among the pumpkin peel samples from different areas. Except Cd all determined metals in this study (Na, Mg, K, Ca, Cr, Mn, Fe, Ni, Cu and Zn) have significant difference between mean of pumpkin flesh sample from three different areas.

The result showed there is a significant difference among means of all metals in pumpkin seed sample ($p < 0.05$) except Cr. Cr has insignificant difference between means of pumpkin seed samples from three areas. Significant difference in metal mean concentration of different parts of pumpkin may be the maturity of fruit, type of soil and the level of traditional fertilizer (dung), since the sample collected from the residence compound of farmers.

Pearson correlation coefficient of metals

In this study a Pearson correlation coefficient between metal concentrations of pumpkin peel sample showed a perfect positive relationship (+1 correlation value) between Na/Ni, Mg/Cr, Mg/Cd, Cr/Cd; a perfect negative relationship is observed between K/Fe; The strong negative correlation coefficient was observed in Na/Ca, Na/Mn, Mg/Cu, Ca/Ni, Ca/Cu, Cr/Cu, Mn/Ni, Mn/Cu, Fe/Zn, Cu/Cd and a strong positive relationship is between Na/Cu, Mg/Mn, K/Zn, Ca/Mn, Cr/Mn, Mn/Ca, Ni/Cu, with > 0.7 correlation values. A medium correlation is recorded in Na/Mg, Na/K, Na/Cr, Na/Zn, Na/Cd, Mg/k, Mg/Ni, Mg/Zn, K/Cr, K/Ni, K/Cd, Cr/Ni, Cr/Zn, Ni/Zn, Ni/Cd, Zn/Cd negatively and positive medium correlation was recorded between Na/Fe, Mg/Ca, Mg/Fe, Ca/Cr, Ca/Cd, Cr/Fe, Fe/Ni, Fe/Cd, with > 0.4 correlation values. Low correlation also observed between K/Ca, K/Cu, Ca/Zn, Mn/Fe, Fe/Ni, Cu/Zn positively and negative low correlation was among K/Mn, Ca/Fe, Mn/Zn/Fe/Cu with correlation values < 0.3.

The Pearson correlation coefficient between metal concentrations of pumpkin flesh samples showed that K/Cu and Fe/Ni have a perfect positive relationship (r = +1). Strong relationship is observed between Na/Mg, Na/Cr, Na/Zn, Mg/Cr, Mg/Zn, Ca/Mn, Ca/Fe, Ca/Cd, Cr/Zn, Mn/Fe, Mn/Ni, Cu/Cd positively and Na/Ca, Na/Mn, Na/Fe, Na/Ni, Na/Cd, Mg/K, Mg/Ca, Mg/Cu, Mg/Cd, K/Cr, K/Mn, K/Fe, K/Ni, K/Zn, Ca/Cr, Ca/Zn, Cr/Cu, Cr/Cd, Mn/Cu, Fe/Cu, Ni/Cu, Cu/Zn, Zn/Cd has a negative strong relation to each other with correlation value >0.4. Weak correlation also observed in between Na/K, Na/Cu, Cr/Mn, Cr/Fe, Cr/Ni, Mn/Zn negatively and a positive weak correlation is in between Mg/Mn, Mg/Fe, Mg/Ni, K/Ca, Ca/Cu, Fe/Zn, Fe/Cd, Ni/Zn, Ni/Cd with correlation value < 0.3.

The Pearson correlation coefficient between metal concentration of pumpkin seed samples exhibited a perfect positive correlation is detected in between Na/Fe, Mg/Mn and a negative perfect correlation is in between Mg/Cd and Ca/Cr. Most of the elements correlate strongly including Na/Mg, Na/K, Na/Cr, Na/Mn, Na/Ni, Mg/K, Mg/Fe, Mg/Ni, Mg/Zn, K/Cr, K/Mn, K/Fe, K/Ni, K/Zn, Cr/Fe, Cr/Ni, Cr/Cu, Mn/Fe, Mn/Ni, Mn/Zn, Fe/Ni, Ni/Zn, Cu/Cd, positively and Na/Ca, Na/Cd, Mg/Cu, K/Ca, K/Cd, Ca/Fe, Ca/Ni, Ca/Cu, Mn/Cu, Mn/Cd, Fe/Cd, Ni/Cd, Cu/Zn, Zn/Cd has strong negative relationship per correlation values > 0.4. It has also a weak positive correlation relation in between Na/Cu, Na/Zn, Mg/Cr, Ca/Zn, Ca/Cd, Fe/Cu, Fe/Zn and negative weak correlation in between Cr/Zn, Cr/Cd, and Ni/Cu with correlation values < 0.3.

CONCLUSION

This study evaluated the levels of macro, micro and toxic metals and the non-carcinogenic and carcinogenic health risk assessment of minor and toxic metals in different parts (peel, flesh, seed) of pumpkin collected from Woliso (Oromiya, West Shewa), Minjar Shenkora (Amhara, North Shewa) and Arbaminch (SNNP, Gamo Gofa) administrative region of Ethiopia. The optimized wet digestion method for different parts of pumpkin was evaluated through the recovery experiment by spiking known concentration of standards to solid samples and a good percent recovery (90–108%) was obtained. The overall mineral concentration was higher in peel compared to flesh and seed. From selected areas, Minjar Shenkora pumpkin contained the higher concentration of determined metals followed by Woliso pumpkin. Potassium was the most abundant and has higher concentration in all parts of pumpkin from selected area. Since the concentration of Cr, Mn, Fe, Ni, Cu, Zn and Cd obtained are higher than daily recommended values of guidelines of FAO/WHO [33] that may expose to health risk for people living around the area. The calculated non-carcinogenic risks factor (HI) for flesh and seed from three sampling areas was below one which is safe from a potential health influence for non-carcinogenic health risk. The total cancer risk (CR_t) due to the consumption of pumpkin flesh and seed due to Cr and Cd from all the sampling area are in the range $1.1 \times 10^{-4} – 1.8 \times 10^{-4}$ which is approximately equal to the maximum limit value of 1×10^{-4}, indicating the non-risk of exposure to cancer due to the consumption of pumpkin. According to ANOVA results, the level of some elements between and within the sampling sites were significantly different which could be ascribed to variation in different factors such as maturity of the pumpkin when collected, type of soil, the level of traditional fertilizer (dung), level of different wastes from household since the samples were collected from the residence compound of farmers. The Pearson correlation coefficient of most metals from three sites has strong relationship.

ACKNOWLEDGMENTS

The authors express their gratitude to the Department of Chemistry for providing financial support for sampling. Abeb Yetesha would like to thank the management and laboratory experts in Ethiopian Conformity Assessment Enterprise Testing Laboratory for permission to undertake the present study in their laboratories. Abeb Yetesha would like to express her deepest gratitude to National Metrology Institute of Ethiopia for sponsoring her study.

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