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Determination of the Levels of Some Essential and Toxic Metals in Leaves, Seeds and Fruits of Pumpkin (Cucurbitapepo D.) Samples from Tepi South West Ethiopia

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Abstract: Vegetables have since ancient times, played a very important role in human life; they have been used for food, traditional medicines, and source of fiber and other purposes and also as fodder for domestic animals. Data about chemical composition of pumpkin itself is still insufficient, especially regarding the mineral content of pumpkin. As a result of such circumstances, there is a need for determination of essential and toxic metals in leaves, seeds and fruit of pumpkin, which was the main goal of this research. First the leaves, fruits and seeds were collected, dried, and digested by wet digestion method and levels of metals were determined by flame atomic absorption spectrophotometry. The accuracy of the procedure was evaluated by analyzing the digest of the spiked samples with standard solution and the percentage recoveries varied from 81.76% to 120.00%. The study found out that the levels of essential metals were in the order of Mg 454.280 mg/kg >Mn (202.222mg/kg > Fe (28.568mg/kg >Cu (23.600mg/kg) >Zn (7.673mg/kg) in the seed, Mg (255.233mg/kg) > Mn (187.360mg/kg) > Fe (31.467mg/kg) >Zn (11.93mg/kg) > Cu (8.200mg/kg) in the fruit Mg (524.60mg/kg) >Mn (190.222mg/kg) >Fe (34.62mg/kg) >Cu (20.01mg/kg) >Zn (8.62mg/kg) in the leaf samples respectively, whereas for non-essential metals the concentration of Cd was 0.147mg/kg >Pb (0.013mg/kg in the seed, Cd (0.088mg/kg) > Pb (0.014mg/kg) in the fruit and Pb (0.014mg/Kg) > Cd (0.012mg/kg) samples respectively. A pair wise t-test at 95% confidence level indicated that there is significant difference in the levels of metals among the two samples means. The amounts of all selected essential Minerals especially (Mg, Mn, Fe, Cu and Zn) and toxic non-essential metals (Cd and Pb) concentration analyzed in all pumpkin seed, leaf and fruit were in the range of the recommended values of WHO/FAO and detection limit of the instrument used. The result indicates that, all parts of the plant (seed, leaf and fruit) are rich in minerals and recommended to be taken for healthiness.

Keywords: Essential Metals, Minerals, Pumpkin, Concentration, Food

1. Introduction

Plants are the most important sources of food and nutrients for human beings. Among plant sources, Substitute this by vegetable and fruit sources are important sources of protein and minerals in the diets of millions of people in the world [1].

Vegetables are an important component in human's diet, especially in developing countries. It is needed to complement staples in diet, supplying essential minerals and vitamins that may not be obtained solely from staples. Vegetables are the fresh, edible and succulent parts of herbaceous plants. They are considered as special food sources owing to their valuable food ingredients that can be effectively utilized by the body. They contain appreciable number of vitamins and minerals that are highly beneficial for the maintenance of health and prevention of diseases [2].

They also contain high amount of dietary fiber and a minimal amount of protein. Knowledge of the nutritive values of local dishes, soup ingredients and local foodstuffs is necessary in order to encourage the increased cultivation and consumption of those that are highly nutritive [3]. Consuming the local foodstuffs like pumpkin plants, help to enhance the nutrients of the poor who cannot afford enough protein foods of animal origin. Vegetables are good sources of oil, carbohydrates, minerals and vitamins depending on the vegetable Consumed. Pumpkin is one of the vegetables belongs to the *Cucurbitaceous* family and grows widely around the world as a vegetable [4]. Pumpkin plant is an annual plant with leaf; which is locally known as "DUBA," is one of those plants that secretly carry some unbelievable health benefits. The delicate leaves are cooked like spinach or green verdant veggies and savored as a stable along with the main course meal. It has a climbing stem of up to 12 m long and fruit with a round fibrous flesh, is indicated in figure 1.

Figure 1. *Cultivated pumpkins.*

Pumpkin (Cucurbitapepo) is mostly used to refer to cultivars with round fruits, which are used in the mature state for baking or feeding livestock. It is famous for its edible seeds, fruit and greens. The most important part of pumpkin is its low-fat and protein-rich seeds [13]. The second most important part is its leaf and fruit. The immature fruit is cooked as a vegetable, while the mature fruit is sweet and used to make confectionery and beverages, sometimes alcoholic [3].

In some parts of the Ethiopia like sheka, pumpkins are vastly used for Halloween carvings and thanksgiving feasts. Most of its leaves are processed into cooked as common meal. However, the plentiful flat, oval seeds are generally discarded as agricultural residues [4]. Pumpkin seeds have been used in a number of countries either for oil or protein production. Outside Ethiopia, Yugoslavia, Austria, Hungary, and Nigeria are countries where pumpkin seed kernel has been used for extraction of edible oil [5]. Now a days grocery store are also selling these seeds as baked, sprouted, fermented, pumpkin protein concentrate and pumpkin protein isolate, as their richness in protein, minerals, polyunsaturated fatty acids, carotenoids and gamma-tocopherol is beginning to surface. It is being used in many epicurean delights, viz. chocolate, cereal bar, bread, cake, muffin, soup, pesto, stew and pasta garnish. Pumpkin seed and leaves are considered as a great alternative to peanut butter [4]. The different reviews strive to provide an updated account of the established and putative benefits, bottlenecks and scopes for broader uses [6]. The succulent, tasty leaves, stems, fruit, and nutritious seeds make pumpkin the most popular vegetable to millions of people, ranking as one of the most widely eaten vegetables at homes and in restaurants [7]. Pumpkin leaves used as food only sheka zone in Ethiopia.

Its seed is consumed directly as snack food in many cultures throughout the world but not in our country. Pumpkin fruits are variable in size, color, shape and weight; they have a moderately hard flesh with a thick edible flesh below and a central cavity containing the seeds [8].

Pumpkin seeds are also known for pharmacological activities like anti-fungal, anti-diabetic, anti-bacterial and anti-inflammation activities and anti-oxidant effects. Moreover, pumpkin seeds are loaded with amino acids like tryptophan, lysine, methionine, and tyrosine and also rich in iron, therefore these seeds are beneficial to adolescents to cure anemia caused due to iron deficiency [9].

Pumpkin seeds, leaves and fruits are richly endowed in essential elements (magnesium, phosphorus and calcium) and moderate amounts of essential trace elements (Iron, zinc and Copper) and thus the seed could be used as a valuable food supplement [10].

In most regions of Ethiopia, pumpkin (*Cucurbitapepo*) has been cultivated, since several years ago that matured fruits of the plant are used as edible parts. But people in different parts of south west Ethiopia, particularly in sheka Zone yeki woreda in Andnet kebele, don’t use the pumpkin seeds properly as food; rather they discard the seeds as waste product due to, the lack of awareness of seeds nutritional contents including essential metals [11].

2. Experimental Section

Matured pumpkin fruit and budes of yang pumpkin samples were collected from yeki district in sheka Zone of south west Ethiopia. We used a simple random sampling technique from farmers in the sampling area. Yeki woreda is one of the 3 Woreda in South Sheka Zone and 148 Woreda in Southern National Nationalities and people Regional State [from stat,]. The relative location of the Woreda is 611 km away from the capital city of the country, Addis Ababa and 683 km from Hawasa, the reginal capital city. This Woreda is found almost far from Hawasa compered to capital city Addis Ababa. The geographical location of the Woreda is 100 36’ 18 N and 370 55’ 02 E. The Woreda is bordered on the south by the Bench Maji Zone, on the west by the Gambela Region, on the north by Amderacha, and on the east by the Keffa Zone. The name 'Yeki' is named after the name of Sheko chief. The major town in Yeki is Tepi [40]. Tepi is also named after the name of Majang man who live in the center of the current Tepi town. It is specific places in indicated in figure 2.

The experiment was conducted at Mizan Tepi University department of Chemistry laboratory.

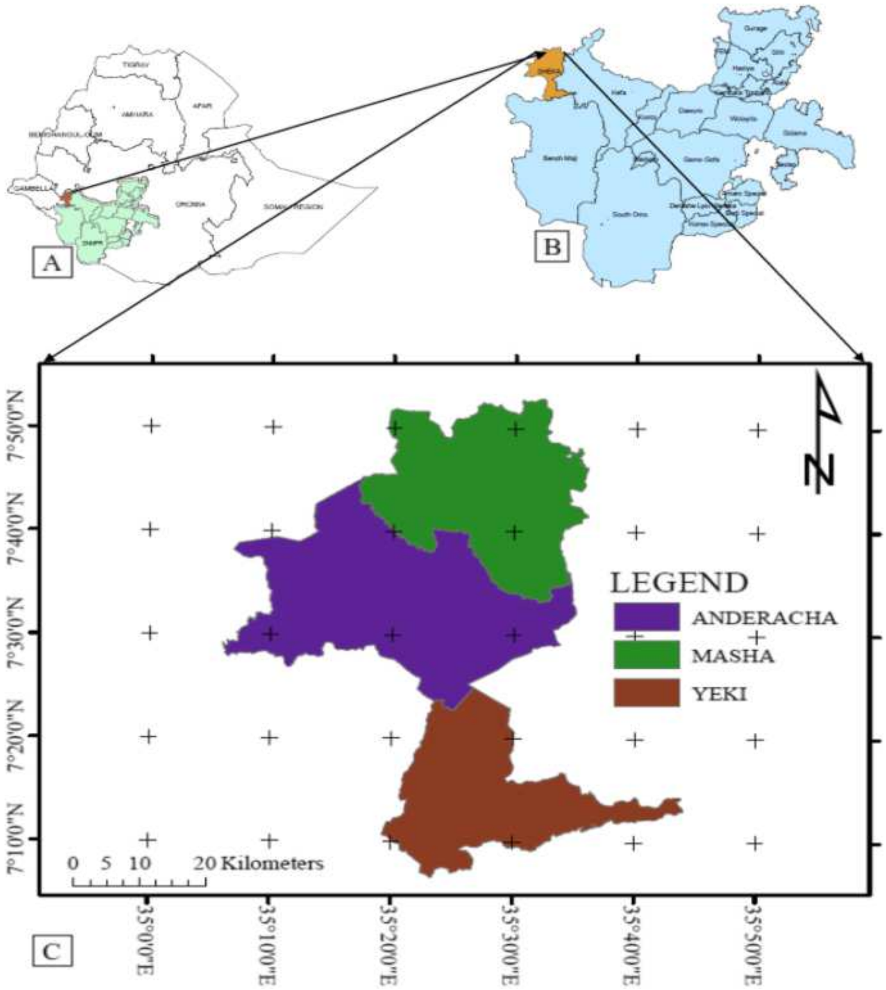

***Figure 2.** Map of study area.*

2.1. Sample Collection

Five fresh matured pumpkin fruits and small buds of young pumpkin leaves were collected by used simple random techniques from four kebeles such as hibrst, selam, adisalem and zinki in Sheka Zone. Because of large amount of pumpkins are growing in all kebeles with mixing in maize's in that of other kebeles of yeki woreda.

2.2. Sample Handling

The collected sample from the four kebele sample sites (hibrst, selam, adisalem and zinki) were mixed to obtain a representative bulk sample (care has be taken to prevent stinging). Then the leaf and fruit samples were washed with tap water followed by distilled water and dried with in an oven 80-110°C and 120-210°C for half an hour respectivly, until it completely dried. The dried sample was grinded to fine particles using a clean acid washed mortar and pestle.

2.3. Apparatus and Instruments

A drying oven was used to dry pumpkin seed, leaf and fruit samples. Mortar and pestle was used to grind and powder the dried pumpkin seed, leaf and fruit samples. Sieve mesh size (355μm) was used for separating small size samples from the large ones, and polyethylene plastic was used for sample holding. A digital analytical balance (ESJ 210-4) was used to weigh pumpkin seed, leaf and fruit samples. Volumetric flasks (50 mL, 100 mL, 250mL and 2000mL), pipette, measuring cylinder, micropipette (mode DRAGON MED 50-200 μL) conical flask (100 mL, 50mL), sample vials to measure volumes of sample and reagents were used during the lab work. Hot plate to digest the sample, stirrer to mix the sample, thermometer for measuring temperature, filter paper (what man 41 filter paper to filter the digest seed and fruit sample, and refrigerator (model-BXC-FW 300) were used in the practical work of this study. The instrument that was used during the analysis technique was Flame Atomic Absorption Spectrophotometer (model 210 VGP, Atomic Absorption Spectrophotometer) with air-acetylene flame.

2.4. Reagents and Chemicals

Reagents that were used in the analysis were all analytical grade. HNO_3 (65%), HCl (37%), H_2O_2 (30%) and H_2SO_4 (98%) were used for sample digestion. Lanthanium chloride heptahydrate ($LaCl_3 \cdot 7H_2O$) was to avoid refractory interferences (to release magnesium from their common phosphates (PO_4^{-3}) and sulphates (SO_4^{-2}) ions. Standard stock salt solutions containing 1000 mg/L of the metals such as for $MgCl_2 \cdot 6H_2O$ for Mg, $MnCl_2 \cdot 4H_2O$ for Mn, $CuSO_4 \cdot 5H_2O$ for Cu, $FeCl_3$ anhydrous for Fe, $Zn(NO_3)_2 \cdot 6H_2O$ for Zn, $CdCl_2$ for Cd and $Pb(NO_3)_2$ for Pb were respectively used for the preparation of serial calibration standards. Distilled and deionized water were used throughout the experiment for sample preparation, dilution, and rinsing apparatus prior to analysis.

2.5. Cleaning of the Apparatus

In this study, we gave special attention for cleaning of all apparatus to avoid contamination. This is because the contaminants from the apparatuses may contaminate the sample and so lead to false results and hence incorrect conclusions about the concentrations of that specific metal in the sample under analysis. Thus, cleanness of apparatuses was given higher emphasis in this study. All beakers, volumetric flasks, conical flasks and also other apparatuses used in this study were washed properly with tap water and distilled water followed by rinsing in 10% analytical grade nitric acid solution, to remove contaminants left on the surface of the apparatuses. Finally they were rinsed with de-ionized water and dried in the oven. They were then dried in hot air oven and stored in clean dry places free of contamination till use [25].

2.6. Sample Preparation

The collected sample from the sampling area was washed with tap water, so as to remove surface contaminants like soil, dust and spray residues. This washed pumpkin fruit and leaves were cut by sharp knife and then, the seeds and pieces of fruits of pumpkin were separate each other.

Blank solution was prepared following the same digestion procedure as for the samples. The powdered leaves, seeds and fruit sample of pumpkin also digested in the same procedure except the acids that were used (10mL of HNO_3 and HCl) in the ratio of 8:2. The digested samples were kept in the refrigerator until the level of all the metals in the samples solutions were determined by flam atomic spectroscope (AAS) [31].

$$3MgSO_4\downarrow + 2LaCl_3 \rightarrow 3MgCl_2 + La_2(SO_4)_3\downarrow$$

$$Mg_3(PO_4)_2\downarrow + 2LaCl_3 \rightarrow 3MgCl_2 + 2LaPO_4\downarrow$$

Blank solution was prepared following the same digestion procedure as for the samples. The powdered fruit sample of pumpkin also digested in the same procedure except the acids that were used (10mL of HNO_3 and HCl) in the ratio of 8:2.

2.7. Sample Digestion

0.5g of powdered pumpkin seed, leaf and fruit sample were taken separately and transferred to 100mL digestion conical flask. A freshly prepared 12mL HNO_3, H_2O_2, HCl and H_2SO_4 mixture in the ratio of 8:2:1:1 was added to the flask. The flask was shook slightly in order to mix the acidic sample mixture. The flask with the acid-sample mixture was then placed on hot plate for digestion. By setting the temperature first to 37°C and slightly increasing up to 95°C, the heating continued until the solution became clear and colorless. After 17 minutes of digestion, the mixture was allowed to cool to room temperature for about 41 minute. Then after, about 10 ml of deionized water was added in to 100ml volumetric flask. This was done by rinsing the neck of the flask which is in contact with the flask and then filtered with what man filter paper in to 100 mL volumetric flask [12]. After filtering the conical flask was rinsed with deionized water until the total volume reached to the 100 mL mark. 0.25 g of $LaCl_3 \cdot 7H_2O$ solution was filtered in 100 mL volumetric flask and then filled this with deionized water until the mark of the flask. The round bottom flasks were rinsed subsequently with deionized water until the total volume reached to the 100 mL mark. That means on the addition of $LaCl_3 \cdot 7H_2O$ to the sample solution the SO_4^{-2} and PO_4^{-3} were made precipitated so that the Mg^{+2} ions became free for atomization. The reaction chemical equation can be represented as follows [18]:

$$3MgSO_4\downarrow + 2LaCl_3 \rightarrow 3MgCl_2 + La_2(SO_4)_3\downarrow$$

$$Mg_3(PO_4)_2\downarrow + 2LaCl_3 \rightarrow 3MgCl_2 + 2LaPO_4\downarrow$$

Blank solution was prepared following the same digestion procedure as for the samples. The powdered fruit sample of pumpkin also digested in the same procedure except the acids that were used (10mL of HNO_3 and HCl) in the ratio of 8:2. The digested samples were kept in the refrigerator until the level of all the metals in the samples solutions were determined by flam atomic spectroscope [19, 25].

Table 1. *Instrumental operating conditions for determination of metals in cucurbitapepo seed and fruit samples using flame atomic absorption spectrophotometer.*

Element	λ (nm)	SW (nm)	Lc (mA)	Energy (ev)	Instrumental detection limit (mg/L)	Flame type
Lead	217	0.7	3	0.407	0.006928	Air- acetylene
Iron	248. 3	0.2	3.5	0.003	0.9232	Air- acetylene
Zinc	213. 9	0.7	0.2	0.1296	0.2503	Air- acetylene
Copper	324.7	0.7	1.5	0.556	0.006245	Air- acetylene
Cadmium	228.9	0.7	2	0.621	0.003	Air- acetyle
Magnesium	285.2	0.7	1	1.571	0.005	Air acetylene
Manganese	279	0.7	3	1.541	0.03	Air acetylene

λ =Wave length SW= slit width Lc=lamp current eV = electrovolt.

3. Results and Discussion

3.1. The Concentration of Essential and Toxic Non-Essential Metals in the Pumpkin Seed and Fruit Samples

The levels of essential (Mg, Mn, Fe, Cu and Zn) and toxic non-essential (Cd and Pb) metals in the seed and fruit samples of pumpkin were analyzed by flam atomic spectroscope. The average mineral concentrations, determined in the seed and fruit of pumpkin were reported below in (table 2).

Table 2. *Mean levels (mean± SD) of essential and non-essential metals (mg/km) in the two samples.*

Metal	seed	fruit	leaf
Mg	454.28±2.857	255.23±1.62	524.6±1.822
Mn	202.22±3.85	187.4±1.16	190.22±2.14
Fe	28.57±0.37	31.47±0.92	34.62±1.03
Cu	23.60±2.00	8.20±0.30	20.01±2.13
Zn	7.67±1.00	11.93±1.15	8.62±3.14
Cd	0.147±0.005	0.09±0.46	0.012±0.003
Pb	0.013±0.001	0.014±0.001	0.014±0.001

Pumpkin leaves contain higher amount of Mg, Mn, Fe and Cu than other analyzed ones. As seen from the (table 2 and figure 2), there were relatively higher concentrations of Mg and Mn in all (seed, leaf and fruit) samples in that of compared with another metals analyzed in the pumpkin samples. The mean concentration of Mg, Mn, Fe and Cu were 524.6 mg/kg, 190.22 mg/km, 34.62mg/and 20.01 mg/kg in the leaf sample respectively.

The concentration of magnesium was in different studies it was reached up to 592 mg/kg. Here in this study concentration of Mg in leaf sample of pumpkin was 524.6 mg/kg higher and 255.23mg/kg in fruit less [17].

This present study values lied in the range of other studies on the seeds and leaves of pumpkins. The relatively higher concentration of Mg in the pumpkin sample, dueto, in the cause of the soil which has been used for cultivating the plant might be highly fertilized with manure and organic residues as a result of the Woreda is highly covered with forest. Therefore, the high values of Mg in the present study were due to its accumulation highly in plants (pumpkin) or the nature of the plant.

This amount of magnesium is needed for energy production, oxidative phosphorylation, and glycolysis. It contributes to the structural development of bone and required for the synthesis of DNA, RNA, and the antioxidant glutathione [15, 28].

The level of Mn was also relatively high in the leaf and seed samples, which was compared with other minerals analyzed in it. The concentration of Mn in pumpkin leaf, seed and fruit was 190.22 mg/kg, 202.22 and 187.4 respectively. The highest amount of Mn, in the seed sample is used for Carbohydrate, Protein, and cholesterol metabolism, Cartilage and bone formation and Wound healing, production of sex hormones, fertility, reproductive health, and lactation, maintains healthy nerves, activates enzymes, maintains healthy nerviest [27].

The third highly concentrated mineral (metal) in the pumpkin sample was Fe; its mean concentration in the sample was 34.62mg/kg, 28.568mg/kg and 31.47 mg/kg in leaf, seed &fruit respectively. The pumpkin leaves have abundant iron that plays an essential role in strengthening the body's immune system. A strong immune system enables the body to fight against several disorders and infections. Moreover, iron helps heal the red blood cells, allowing better oxygen flow in cells, tissues, and organs [14].

Moderate amount of iron in the pumpkin seed, leaf and fruit samples are very important for; - blood building, essential component of hemoglobin, erythrocyte protein that transfers oxygen from the lungs to the tissues. As a component of myoglobin, a protein that provides oxygen to muscles, iron supports metabolism. Iron is also necessary for growth, development, normal cellular functioning, and synthesis of some hormones and connective tissue [26].

The fourth highly concentrated mineral (metal) in the seed sample was Cu; its mean concentration in the sample was 23.600 mg/kg.

Proper amount of Cu in the seed sample is essential for; - normal growth, pigmentation of hair and eyes, is a critical component in the formation of red blood cells. Copper also influences the functioning of the heart and arteries, helps prevent bone defects such as osteoporosis and osteoarthritis, and promotes healthy connective tissues (hair, skin, nails, tendons, ligaments and blood vessels), antioxidant, collagen and connective tissue formation, energy production, iron metabolism and nervous system function [29].

Excessive amount of Mg, Mn, Fe and Cu are toxic for humans, but the amount of these essential metals in the sample were, lied between in different reported data in different time in different counties and WHO or FAO recommended limits.

Relatively other analyzed metal, the level of other trace metal (Zn) in the seed sample was low but it is between other reported data and under WHO or FAO recommended values. Its concentration was 7.673 mg/kg.

Zinc is very essential for numerous aspects of cellular metabolism, the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division, supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system, cellular replication, the development of the immune response [16].

On the other hand the concentration of two toxic non-essential metals such as Cd and Pb were analyzed in the study. Therefore their mean concentrations were 0.147 mg/kg and 0.013 mg/kg of Cd and Pb respectively. The levels of minerals in the seed sample were listed in decreasing order as Mg > Mn >Fe > Cu and Zn for essential metals and Cd > Pb for non-essential metals.

As shown from the table above cucurbitapepo fruit contains higher amount of Mg followed by Mn and Fe respectively.

The amount or the levels of minerals in the fruit sample were 255.233 mg/kg, 187.360 mg/kg and 31.467 mg/kg of Mg, Mn and Fe respectively. The levels of the other trace minerals in fruit sample were 11.930 mg/kg, 8.600 mg/kg, of Zn and Cu respectively [14, 17].

On the other hand the concentration of toxic non-essential metals such as Cd and Pb were analyzed in the study. Therefore their mean concentrations were 0.088mg/kg and 0.014 mg/kg of Cd and Pb respectively. These concentrations of the two toxic non-essential metals, from the seed and fruit sample were below the recommended limit of WHO i.e 0.2 mg/kg for Cd and 0.3 mg/kg for Pb. The levels of the minerals in the fruit samples were listed in decreasing order as Mg > Mn > Fe > Zn > Cu for essential metals and Pb > Cd for non-essential metals.

Generally, as shown from above (table 2) the amounts or levels of the minerals (metals) in the seed and fruit sample of cucurbitapepo was strongly agreed with other studies in other countries and WHO or FAO recommended limits.

Existences or presences of Mg, Mn, Cu and Cd in the seed sample were higher than that of the fruit sample, but the existences or presences of Fe, Zn and Pb in the fruit sample were greater than that of the seed sample of the pumpkin. The following figure (bar graph) shows the comparison of levels of essential and toxic non-essential metals in the samples.

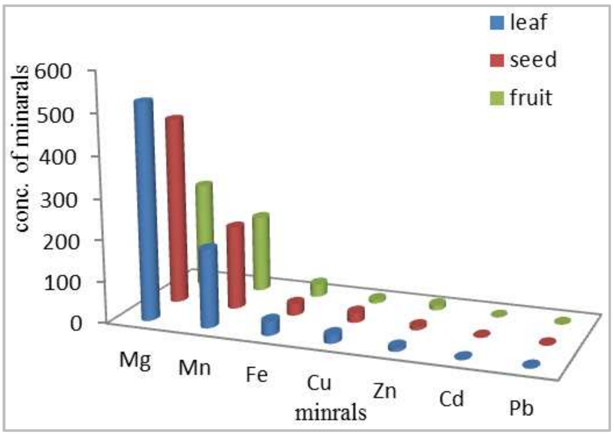

Figure 3. Comparison of minerals in the two samples.

As shown from the above (figure 3), essential metal element magnesium in the seed sample show higher concentrations, in that of magnesium in the fruit sample. Essential trace metallic elements iron and zinc in the fruit sample shows relatively higher concentration of iron and zinc in that of the seed sample. On the other hand, the tow essential trace metallic elements manganese and copper in the seed sample shows higher concentrations of manganese and copper in the fruit sample. This is due to the nature of higher accumulation of Fe and Zn in the fruit in that of the accumulation of Fe and Zn in the seed and the nature of accumulation of Mg, Mn and Cu in the seed sample are higher on that of the nature of accumulation of Mg, Mn and Cu in the fruit. The concentrations of the two toxic non-essential metals in the seed and fruit samples were below the recommended values of WHO/FAO [37, 43].

3.2. Evaluation of Analytical Results

3.2.1. Precision of the Measurements

The precision of a method is the degree of closeness of the results and is usually reported as percentage relative standard deviation [38]. It is often subdivided into repeatability and reproducibility. Repeatability expresses the closeness or agreement between a series of measurements obtained from multiple sampling and sample analysis. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. For this study the precision of the results were evaluated by the standard deviation, and relative standard deviation of the results of three replicate measurements (n = 3). These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with the corresponding standard deviation of six measurements for a blank sample and triplicate reading per sample and relative standard deviation. The% relative standard deviation of each metal is shown in the above (table 2).

As shown from above (table 2) percentage relative standard deviations (%RSD) of metals Mg, Mn, Fe, Cu, Zn, Cd and Pb of measurements were calculated. The%RSD value of Mg, Mn, Fe, Cu, Zn, Cd and Pb for the analysis of the two (seed and fruit) samples were found to be 0.629%, 1.903%, 1.293%, 1.192%, 7.049%, 3.149% and 8.627% for seed sample 0.633%, 0.616%, 0.029%, 5.773%, 0.010%, 5.239 and 7.278% for fruit sample respectively. This mean value% RSD indicated that the measurement for Mg and Cu were more precise than that of other measurements in the seed sample and the measurement for Zn and Fe were more precise than that of other measurements in the fruit sample. The overall result indicated that the precision was good i.e. % RSD ≤ 10. All the values were found under the recommended control limit ≤ 15% RSD. These results showed that the analytical method possesses the required precision and accuracy [33, 35, 41].

3.2.2. Recovery Test

In this study due to the absence of certified reference materials for pumpkin samples in the laboratory, the validity of the digestion procedure, precision and accuracy of flam atomic absorption were assured by spiking samples with standard of known concentration. The spiked and unspiked samples were digested following the same procedure employed in the digestion of the respective samples and analyzed in similar condition.

3.3. Method of Detection Limit

The minimum concentration of analyte that can be measured and reported with 99% confidence is greater than zero [22]. In other words, it is the lowest analyte concentration that can be distinguished from statistical fluctuations in a blank. In this study, two blank samples were digested following the same procedure as that was used in the digestion method and each of the blank samples were analyzed for metal concentrations of Mg, Mn, Fe, Cu, Zn, Cd and Pb by FAAS. The standard deviations for each metal were calculated from the two blank measurements to determine method of detection limit. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. As we can see in table 3 below the method of detection limit of each element is above the instrument detection limit.

3.4. Limit of Quantification (LOQ)

Limit of quantification is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test. It is the same as the concentration which gives a signal ten times the standard deviation of the blank. The quantification limit of each metal was calculated as ten times the standard deviation of the blank (i.e, LOQ=10xsdblank, n=3) [20, 21].

$$\text{LOD} = 3 \text{ x sd of the blank}, \text{LOQ} = 10 \text{ x sd of the blan}$$

Table 3. Instrument detection limit, Limit of detection and Limit of quantification.

Metals	IDL (mg/l)	DLM (mg/100g)	MQL (mg/100g
Zn	0.2652	12.09302	18.60465
Cd	0.004	0.746873	1.122054
Pb	0.028595	1.175706	1.192841
Fe	0.004	12.17368	12.67368
Cu	0.533988	0.837713	1.799557
Mg	0.5693	0.9542	1.0785
Mn	0.5692	0.9641	0.9892

DLM =detection limit, MQL= method of quantization, IDL=Instrumental detection limit.

As it can be seen from the table above, the LOQ values for each metal are higher than the LOD values indicating that the precision of the selected analytical method was good.

3.5. Comparison of Metals Within Seed and Fruit Samples

When the concentration of metals in the pumpkin were compared, the leaf has relatively higher concentration of Zn, Mg, and Fe. But, Mn which is slightly lower in leaf pumpkin samples. Whereas, all leaves, seeds and fruits have very low and relatively same Cd and Pb contents [table 6]. The pattern of concentration of elements in the leaf, seeds and fruits of pumpkin studied decreases in the following order for pumpkin leaf and seed: Mg > Mn > Fe > Cu > Zn > Cd &Pb. For pumpkin fruit: Mg > Mn > Fe > Zn > Cu > Cd & Pb.

The small amount of Cu found in fruit pumpkin does not contradict with the requirement of the metal for proper functioning of the body, because this metal is required in small amount (Cu = 0.3 mg/day) as a constituent of vitamin B12 and Cu = 3.5 mg/day [24]. As can be seen from table 6 magnesium is the most concentrated (454.28mg/kg) in both seeds and fruits. Iron is the most concentrated heavy metals in both seeds and fruits with values 28.568mg/kg for seeds and 31.467mg/kg while Pb the lowest. From this, generally we can say that the iron content of pumpkin is superior and it substitutes other grains like red tef, maize and others.

3.6. Instrument Calibration

The qualities of results obtained for essential and non-essential metal analysis using FAAS are seriously affected by calibration and standard solution preparation procedures [36, 39]. The instrument was calibrated using four series of working standards. The working standard solutions of each metal were prepared fresh by diluting the intermediate standard solutions. Calibration curves for Mg, Mn, Fe, Zn, Cu, Cd and Pb were obtained by using suitable standard solutions prepared from stock solutions. In addition, the calibration standards were prepared by taking into consideration the optimum working ranges of the metals. The correlation coefficient (R^2) values that are closer to the absolute value of 1 indicate that there is a strong relationship between the variables being correlated, whereas values closer to 0 indicate that there is no linear relationship. The correlation coefficients of the metals were determined using prepared standards versus their corresponding absorbance. In this study, the instrument was calibrated by using four series of working standard solutions prepared from intermediate solutions for each metal under study. The table below shows the working range of standard solutions and the value of correlation coefficients of calibration curves for each metal.

Table 4 Working standard concentration, correlation coefficient and equation of the calibration curves for determination of essential and non-essential metals by using flam atomic spectroscope.

Table 4. Series of working standards and correlation coefficients of the calibration curves for determination of metals in the nettle leaves varieties using FAAS.

Metal	Concentration of standards solutions (mg/L)	Correlation coefficient Values (R^2)	R (correlation coefficient) square root value	Regression equation Y=mX+b or A=mC+ b
Fe	0.4, 0.8, 1.2, 1.6	0.9956	0.99979	A=0.00288x+1.1443
Cu	0.4, 0.8, 1.2, 1.6	0.9812	0.9905	A=0.0066x+0.00777
Zn	0.4, 0.8, 1.2, 1.6	0.9968	0.9984	A=0.0043x+0.001
Cd	0.4, 0.8, 1.2, 1.6	0.9979	0.9989	A=0.0057x+0.0029
Pb	0.4, 0.8, 1.2, 1.6	0.99664	0.9983	A=0.0009x+0.0.00007
Mg	0.4, 0.8, 1.2, 1.6	0.9918	0.99589	A=0.0068x+0.459
Mn	0.4, 0.8, 1.2, 1.6	0.9941	0.99704	A=0.0065x+0.7695

A-absorbance, C- concentration (mg/ml).

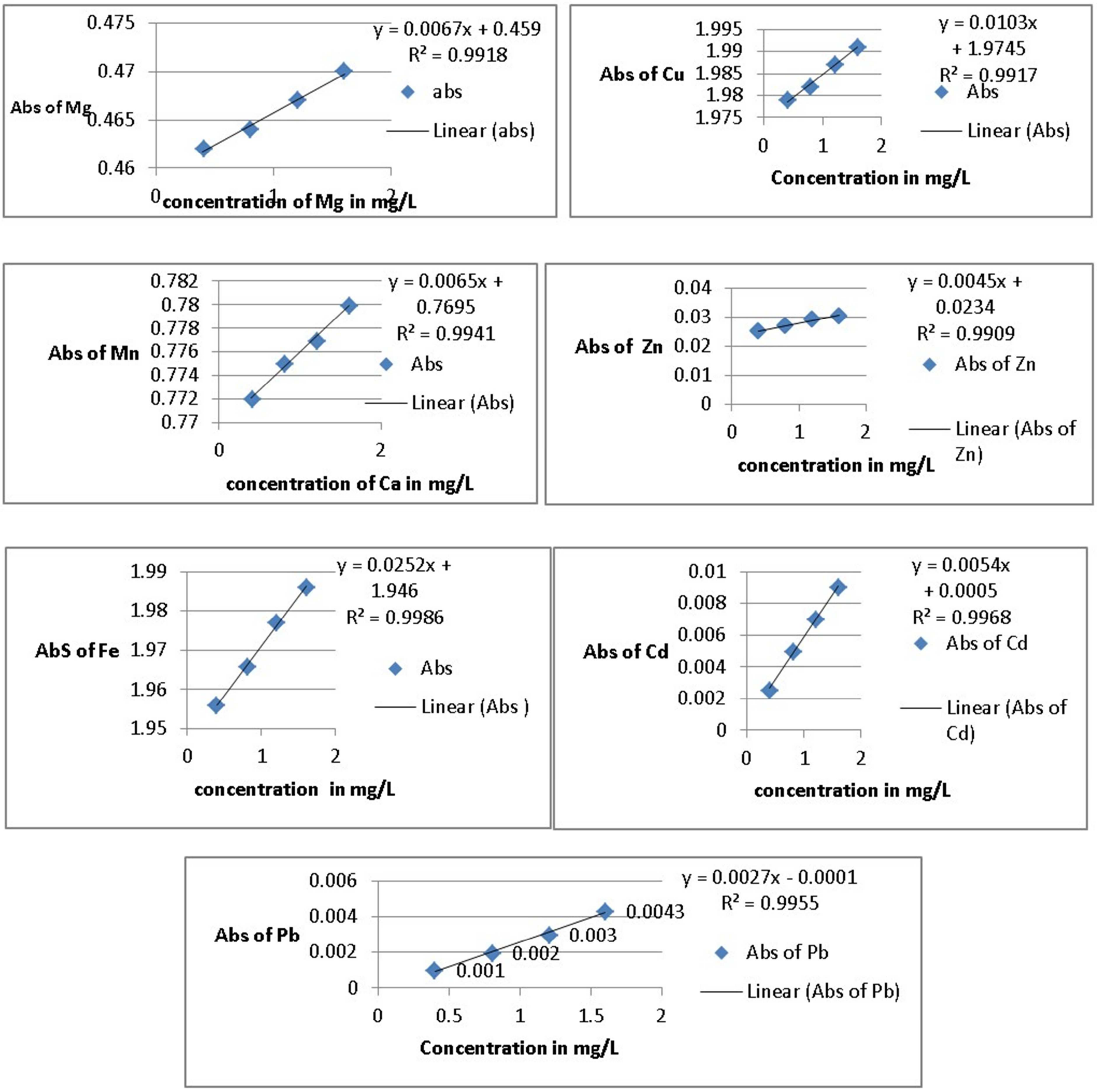

Figure 4. *a-g: Calibration curves for standard solutions.*

Based on the above data, the absorbance of each standard solution for each metal was measured by using flam atomic spectroscopy and the calibration curves were plotted (in appendices). As indicated by the correlation coefficient (R 1], the performance of flam atomic spectroscopy used in this study, therefore, good and reliable to warrant its use in the analysis of the selected essential and non-essential metals in pumpkin seed and fruit investigated in this study.

3.7 Comparison of Metal Contents of Pumpkinleaf, Seed and Fruit Samples with Other Journals and WHO or FAO Recommended Values

Comparison of the values for pumpkin's leaf, seed and fruit with other reported data of pumpkins leaf, seeds and fruits are very essential to know the dietary mineral intake of individuals who use pumpkins plants in their diets. However, there is no standard reference material to do so and the determined results should be compared with the investigations made in other countries by other investigators. Many researchers have reported the concentrations of mineral nutrients in pumpkin as shown in table 5. But in Ethiopian there is no studies were made on the concentrations of essential and toxic metals on pumpkin leaf, fruit and seeds. From the table, it is possible to see that the concentration of all metals in pumpkin leaf, seed and fruit lied between on that of other reported data of pumpkin plant. For the purpose of comparison the results of this study with the results of different literatures in table 5.

Table 5 Comparison of mineral concentrations of pumpkinleaf, seed and fruit with other studies in mg/kg and FAO or WHO recommended values.

Table 5. *Comparison of metal contents of pumpkin with FAWO/WHO and other literatures (mg/kg).*

Elements (leaf)	Pb	Cd	Zn	Fe	Cu	Mg	Mn
Analyzed mg/kg	0.014	0.012	8.62	34.62	20.01	524.6	190.22
Reported mg/kg	0.033-0.058	0.09-0.406	0.08-19.92	0.87-35.65	0.3–28.12	35 – 592	0.138-341
FAO/WHO mg/day	<0.03	<0.02	2-11	0.2- 18	200-900	30-420	0.003-2.3
Reference	[42]						

Leaf values in mg/kg.

Elements (seed)	Pb	Cd	Zn	Fe	Cu	Mg	Mn
Analyzed mg/kg	0.013	0.147	7.673	28.568	23.600	454.280	202.222
Reported mg/kg	0.033-0.058	0.09-0.406	0.96-19.92	13.71-35.65	12.06–25.793	198 – 592	190-341
FAO/WHO mg/day	<0.03	<0.02	2-11	0.2- 18	200-900	30-420	0.003-2.3
Reference	[32, 34]	[9]	[23]	[26]	[12]	[26], [10], [19]	

Seed values in mg/kg.

Elements (fruit)	Pb	Mg	Zn	Fe	Cu	Cd	Mn
Analyzed (mg/kg)	0.014	255.2	11.930	31.467	8.200	0.088	187.36
Reported (mg/kg)	0.001	108.43-278	0.086-14.2	13.7-35.7	0.01-9.4	0.49	165-305.81
FAO/WHO mg/day	<0.03	26-270	2.8-10	7.7-24.5	200-900	<0.02	0.003-2.3
Reference	[32, 42]	[42]	[23]	[26] [42]	[12]	[26], [10], [19]	

fruit values in mg/kg.

As shows from above (table 5), it is possible to see that metal concentration of pumpkin leaf, seed and fruit were compared to some other studies of pumpkin *leaf,* seeds and fruits done in other places [10, 23, 32, 42]. Generally, the concentration of all minerals, analyzed in this study lied between on the range of other reported data concentration. This is might be in the cause of relationships between soil fertilities, PH values, climatic condition of pumpkin was cultivated and the capacities of pumpkin's plant to accumulate minerals were nearly similar.

The mean concentrations of those two toxic metals, concentration of Cd and Pb in this study lied on the range of the reported concentration of other reported data in all the leaf, seed and fruit samples, but in some exceptions concentrations of Pb was vary in a very smallest deviation in the leaf and fruit sample from that of the reported ranges of other pumpkin fruits. On the other hand concentration of Pb was low in the seed sample in that of the reported ranges of other pumpkin seeds. This is might the richness or poorness of pumpkins due to soil fertility, P^H values and climate conditions of surrounding that the pumpkin cultivated were different in that of the reported data. In the two samples the concentration of two toxic metals (Cd and Pb) were below the reported concentrations of WHO or FAO recommended values, as shown from the table above.

3.8. Statistical Analyses

Differences between the mean values of the various samples obtained in this study were evaluated by ANOVA, which tells us if there are any statistical differences between the means of three or more independent groups. whether the sources for variations were from experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, P^H of soil, water, the capacities to stored, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides etc or other variations in cultivation procedures). Linear regression statistical test and correlation analysis were performed for the calculation of the slope (m), and correlation coefficient (R) of the regression line as shown in table 4. Statistical analysis was based on triplicate measurements of all samples. There are three means (avarage concentration) of each metals in leaf, fruit and seed samples of pumpkin. The grand mean can be calculated as the weighted average of the individual group means as:

$$\ddot{X} = \left(\frac{N1}{N}\right)\dot{x}_1 + \left(\frac{N2}{N}\right)\dot{x}_2 + \left(\frac{N3}{N}\right)\dot{x}_3 \quad (1)$$

where N_1- number of measurements seed

N_2- number of measurements in leaf, and N_3 in fruit.

To calculate the variation ratio needed in the ANOVA, it is necessary to obtain several other quantities called sums of squares due to factor (SSF) and the sum of the squares due to error (SSE)

$$SSF = N_1(\dot{x}_1 - \ddot{X})^2 + N_2(\dot{x}_2 - \ddot{X})^2 + N_3(\dot{x}_3 - \ddot{X})^2 \quad (2)$$

Where; $\dot{x}1$, $\dot{x}_2$ and $\dot{x}_3$ are individual mean concentrations of metals in seed, leaf and fruit respectively. $\ddot{X}$-grand mean concentration of metals in pumpkin as well as N_1, N_2 & N_3 are number of measurements in seed, leaf and fruit respectively.

These two sums of squares are used to obtain the between-groups variation and the within-groups variation. The error sum of the squares is related to the individual sample variances by:

$$SSE = (N_1 -1)S_1^2 + (N_2 -1)S_2^2 + (N_3-1)S_3^2 \quad (3)$$

S_1^2, S_2^2 and S_3^2 are standard deviations of metal concentration in seed, leaf and fruit respectively.

Then, The total sum of the squares SST is obtained as the sum of SSF and SSE:

$$SST = SSF + SSE \quad (4)$$

The number of degrees of freedom for each of the sum of squares is as follows.

SST has N -1 degrees of freedom. Each metal has 3 trials in 3 samples (leaf, seed and seed) 3x3=9-degree of freedom (DF).

SSF has n -1 degrees of freedom. Each metals has 3-trial in 3-samples, then 3-1 = 2 DF.

SSE has N - n degrees of freedom. Each metals has 3-trial in 3-samples, then 9-3= 6 DF. By dividing the sums of squares by their corresponding degrees of freedom, we can obtain quantities that are estimates of the between-groups and within-groups variations (mean square MSF & MSE).

$$\text{Mean square due to factor levels} = MSF = \frac{SSF}{n-1} \quad (5)$$

$$\text{Mean square due to error} = MSE = \frac{SSE}{N-1} \quad (6)$$

The quantity MSE is an estimate of the variation due to error while MSF is an estimate of the error variation plus the between-groups variation. If the factor effect is significant, MSF is greater than MSE. Then the test statistic is the F value, calculated as:

$$F = \frac{MSF}{MSE} \quad (7)$$

Table 6. *Using equation 7 Pairwise comparison between the critical value of F at the 95% confidence level for 2 and 6 degrees of freedom by* F_{test}.

Samples	Parameters	Metals						
	metals	**Mg**	**Mn**	**Fe**	**Cu**	**Pd**	**Zn**	**Cd**
seed, Leaf& fruit	Obtained F-vale	199.047	14.862	2.899	15.4	0.001	4.257	0.059
	Critical F-value	12.861	11.306	0.802	0.021	0.020	0.086	0.105
	conclusion	S	S	S	S	S	NS	NS

*D (m):differences between means, $F_{ritical} = \frac{MSF}{MSE}$ for 2&6 degree of freedom at 95% confidence level (N1=N2=N3=3), S: significant, NS: not significant.

As shown in (table 6) ANOVA test at 95% confidence level indicated that there were significant differences between the mean values of seed and fruit sample for metals Mg, Mn, Fe, Zn, and Cu. Significant differences were observed between seed and fruit samples in most metals except Cd and Pb. From the table 6 the calculated f-value is greater than F- critical value indicates there is no significant difference concentration of Cd & Pb between leaf, seed and fruit pumpkin sample. The source for this significant difference between sample means may be the difference capacity or accumulations of seed, ledf and fruit of pumpkin minerals absorption by plants from the soil.

4. Conclusions

This work is to contribute the understanding of mineral concentrations in pumpkin leaf, seeds and fruits. The levels of five essential and two toxic metals were determined in pumpkin leaf, seed and fruit samples collected from sheka zone, in four kebeles of yeki woreda by using FAAS method. The results revealed that collected pumpkin from this wereda can be considered as a source of valuable components. The leaf has higher concentration of minerals such as Mg, Mn, Cu from essential and Cd & Pb from toxic metals than seeds and fruit except Mn from essential metal is less in it. But the storages or accumulations of Fe, Zn and Pb in the fruit sample were higher than the accumulation of the seed sample. All parts of pumpkin (leaf, seeds and fruit) are rich in minerals and recommended to be taken for healthiness. The values are more or less comparable with the data reported by different researchers in different countries in different times. Concentrations of essential metals in the pumpkin seeds and fruit samples were Mg (454.280) > Mn (202.222) > Fe (28.568) > Cu (23,600) > Zn (7.673) mg/ kg in seed sample, concentration of Mg (255.233) > Mn (187.360) > Fe (31.467) > Zn (11.930) > Cu (8.200) mg/kg in fruit sample and concentration of Mg (54.6) > Mn (190.22) > Fe (34.62) > Zn (8.62) > Cu (20.01) mg/kg in leaf sample respectively. On the other hand the concentration of the two toxic metals Cd was (0.147) > Pb (0.013) mg/kg in the seed sample, concentration of Cd (0.088) > Pb (0.014) mg/kg in the fruit samples and concentration of Cd (0.012) < Pb (0.014) mg/kg in respectively. Whereas concentrations of all minerals, Mg, Mn, Fe, Cu, Zn, Cd and Pb in pumpkin samples were in the range of the detection limit of FAAS. The levels of the two toxic metals, such as Cd and Pb were below in the WHO/FAO recommended values.

Higher metal concentration of the pumpkin samples are indicated that, it is an interested edible food which can be included in our daily diet that can give various health benefits to improve our overall heaths. It has various beneficial effects to health's, such as anti-diabetic, anti-carcinogenic, antioxidant and anti-microbial potential. There are other various health beneficial effects of pumpkin also reported such as inhibition of kidney stone formation, and hypotensive, anti-inflammatory and blood-coagulatory effects and hypertensive.

A pairwise student's *t*-test at the 95%confidence level and Pearson's correlation for all the pumpkin leaf, seed and fruit samples indicated that there were significant differences between the mean values of the minerals contents of pumpkin leaf, seeds and fruit samples except Cd and Pb. The correlation coefficient (r) in most minerals was positive which indicates that a strong correlation between the concentrations of the same metal in the two samples and no interference of the element on itself in the seed and fruit samples respectively. Same way between seed and leaf as well as leaf and fruit.

Declarations

The authors declare that, it is original work and has not been published for any journal or any other conferences and that all sources of materials used in this work have been duly acknowledged.

Conflicts of Interest

There are no conflicts to declare.

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