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Title: Determination of selected metals in pumpkin peel, flesh and seed samples from three different areas in Ethiopia by microwave plasma-atomic emission spectrometry

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July, 2021

Addis Ababa, Ethiopia

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A thesis submitted to the Chemistry Department of Addis Ababa University in partial fulfilment of the requirements for the Degree of Master of Science in Chemistry.

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Declaration

I declare that this thesis entitled “Determination of selected metals in pumpkin peel, flesh and seed samples from three different areas in Ethiopia by microwave plasma-atomic emission spectrometry" has not been submitted in any form for another degree, diploma or an award at any University or other institution. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published work of others has been acknowledged in the text and a list of references is given.

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Abbreviations and Acronyms

ANOVA	Analysis of variance
ADI	Average daily intake
CCV	Calibration curve verification
CRM	Certified reference material
CR	Cancer risk
CR_t	Total cancer risk
DNA	Deoxyribo nucleic acid
FAO	Food and Agriculture Organization
HI	Hazard index
HQ	Hazard quotient
HRA	Health risk assessment
LOD	Limit of detection
LOQ	Limit of quantification
MP- AES	Microwave plasma-atomic emission spectrometry
QC	Quality control
RSD	Relative standard deviation
RNA	Ribo nucleic acid
SD	Standard deviation
SF	Slope factor
UV	Ultra-Violet
WHO	World Health Organization

Abstract

In this study the level of selected metals (Na, Mg, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Cd) and health risk assessment of heavy metals (Mn, Ni, Cu, Zn, Cr, Cd) in pumpkin peel, flesh and seed were determined using microwave plasma-atomic emission spectrometry. Samples were collected from three different parts of Ethiopia (Woliso, Oromiya Region; Minjar Shenkora, Amhara Region and Arbaminch, South Nations and Nationality People Region). A 0.5 g dried and powdered samples were wet acid digested (69% HNO_3 and 30% H_2O_2) in three stages for each sample by microwave digester with optimized conditions. The calibration curves were linear with correlation coefficient (R^2) value ranging from 0.996 to 0.9999 which indicates a very good linearity of curves. The calculated percentage recovery for different parts of pumpkin samples digestion method were found in the range 90-106% for peel; 92-110% for flesh and 90-108% for seed which are within the acceptable range for all metals, indicating the efficiency of the optimized procedure. The results show the mean concentration of studied metals in pumpkin peel sample from three areas were K (27806), Ca (5268), Mg (4716), Fe (157), Na (167), Mn (28.9), Zn (24.2), Cu (8.14), Ni (3.79), Cr (0.28), Cd (0.24) mg/kg. The average concentration of metals determined in pumpkin flesh 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. Pumpkin seed average metal concentration from the three sites 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. The concentration levels of K, Cr, Fe, Ni, Cu and Cd obtained are higher than daily recommended values of guidelines (FAO/WHO, 2015). All calculated values of HQ from three sites were less than the permissible limit (1.0). This indicates no adverse non carcinogenic health risk due to consumption of the pumpkin from selected areas. The HI for pumpkin flesh from three areas were found to be less than unity, with HI values = 0.54, 0.42, 0.54 for Woliso, Minjar Shenkora and Arbaminch, respectively. HI values from pumpkin seed were = 0.73, 0.61 and 0.62 from Woliso, Minjar Shenkora and Arbaminch, respectively. HI values from pumpkin edible part in all selected areas are less than one and thus have no adverse health impact on consumers. This indicates ingestion of pumpkin from selected areas is safe from non-carcinogenic risk. The total cancer risk (TCR) due to the consumption of pumpkin flesh and seed through Cr and Cd from all sampling areas are between 1.1×10^{-4} to 1.8×10^{-4} which is approximately equal to the maximum limit value of 1 x 10^{-4} indicating the non-risk of exposure to cancer due to the consumption of pumpkin from the selected areas. ANOVA result shows that for most of the determined metals there was a significant variation within and between sample sites which could be ascribed to 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. The Pearson correlation coefficients of most metals from three sites have shown strong relationship. Pearson correlation coefficient between metal concentrations of pumpkin peel sample was 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; pumpkin flesh samples have a perfect positive relationship between K/Cu and Fe/Ni (r = +1). The perfect positive correlation is detected in between Na/Fe, Mg/Mn and a negative perfect correlation is in between Mg/Cd and Ca/Cr from the pumpkin seed samples.

Keywords: pumpkin, metals, health risk, Ethiopia, microwave digester, microwave plasma-atomic emission spectrometry.

1 Introduction

1.1 Background

The origin of pumpkin has been ascribed to Guatemala, Central Mexico or Columbia. The name pumpkin originated from a Greek word Pepon which suggests large melon. French converted the Pepon to Pompon and English adapted the word Pompion. Lastly, the American colonists replaced the ion with kin giving rise to pumpkin (Dhiman et al., 2009). In Ethiopia, this plant is locally known as Dubba. Botanically speaking, pumpkins are fruits, as they contain seeds and develop from the flower-producing part of a plant. However, despite notable exceptions, usually prepared and served as vegetables because it is not sweet as other fruits.

Pumpkin is a temperate and subtropical region growing plant. It grows best in areas where annual day time temperatures range 20 to 30 ^{o}C, but can tolerate 10 to 40 ^{o}C. It prefers a mean annual rainfall within the range of 600 to 1,600 mm, but tolerates 300 to 2,800 mm. It belongs to the family of *Cucurbitaceae*. *Cucurbitaceae* is a wide family with about 130 types and 800 species. Pumpkin grows easily from either seeds or cuttings with roots. It can grow as a creeping or climbing plant if given a support and its stem can grow up to a length of 10 m. It can be grown as a monocrop or intercropped with maize and other field crops. It is widely cultivated as garden and farm vegetable (Oyeleke et al., 2019).

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 (*Cucurbita*) are *Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata*. These three species are cultivated worldwide and have high production yields compared with other vegetables and they are evaluated for the simple production technology. The color of flesh ranges from pale yellow to crimson. The miniature pumpkins are *C. pepo* and the giant type is *C. maxima* varieties.

Pumpkins are widely used in the food arts. Many different parts of the plant are edible, like, flowers, leaves, shoots, roots, immature and mature fruits, peel and seeds. Pumpkin is eaten raw and in the form of preserves, such as soups, smoothies and juices. Pumpkin flesh is additionally used as an additive to bread, cakes, cookies, chocolates and candies (Kim et al., 2012). Ethiopia has a comparative advantage in a number of horticultural commodities due to its favorable climate, and edaphic conditions for the production of tropical, sub-tropical and temperate vegetables in the lowlands (2200), respectively, proximity to European and Middle Eastern markets and almost all types of fruits and vegetables can be grown in Ethiopia. Presently, the most export products are fresh beans, strawberries, tomatoes, courgettis, peppers and fresh herbs. A potential new crop is table grapes. However, the assembly of horticultural crops is far less developed than the assembly of food grains within the country. On average 2,399,566 tons of vegetables and fruits are produced by public and personal commercial farms, this is often estimated to be about 2 percent of the total crop production in 2017/2018. In most regions of Ethiopia, Cucurbita had been cultivated since several years ago that matured fruit of the plant is used as edible parts (Zinash, 2013). However, the production, use and export trends of pumpkin are not satisfactory. According to Ethiopian export data, Ethiopian pumpkin exporters' data base in January 2020, 1,880 tons are exported to Djibouti.

1.2 Objectives

1.2.1 General objective

- ❖ To determine selected metals in pumpkin peel, flesh and seed samples from three different areas by microwave plasma-atomic emission spectrometry.

1.2.2 Specific objectives

- ❖ To develop an optimum working procedure for digestion to determine selected metal contents in pumpkin peel, flesh and seed by microwave digester.
- ❖ To detect the macro, micro and toxic metals in pumpkin (peel, flesh and seed) parts.

- To determine the amount of minerals that found in different parts of pumpkin.
- To compare minerals found in different parts of pumpkin from three different areas of Ethiopia.
- To compare the results of this study with reported results in the literature.
- To compare the results of this study with other vegetables.
- To estimate non carcinogenic and carcinogenic risk due to life time ingestion of trace metals from pumpkin.

1.3 Statement of the problem

Vegetable crops of economic importance that are largely produced in Ethiopia include pepper, kale (Ethiopian cabbage), onion, tomato, chilies, carrot, garlic and cabbages. Green beans and peas, okra, asparagus, cauliflower, broccoli, celery, eggplant, paprika and cucumbers have now adays emerged as important export vegetables (Assefa et al., 2013). Ethiopians practice mainly cereal based food habit is largely affecting children in most part of the country. Research reports indicated that many people are suffering from lack of vitamins and essential minerals. In general, 60 to 80% of health problems in Ethiopia are communicable diseases and nutritional problem. Because of social and cultural habits of the population like dietary preferences for meat and other animal products, distaste for vegetable crops, lack of consumer awareness, vegetable and fruit production and consumption is relatively limited (Habtamu, 2016).

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. It has huge concentration of β-carotene which protect against certain cancers and is a powerful ally against degeneration aspect of aging. Pumpkin has no cholesterol, low in fat and sodium and rich in vitamins. Pumpkin seed used as medicinal purpose for internal as well as external treatment problems and also used for production of soap, perfumes and lotions, food flavorings, food preservation, nutraceuticals, pharmaceuticals and cosmeceuticals.

In most regions of Ethiopia, mostly in Gambiella, Jijiga, Arbaminch, Dire-Dawa, Kulubi, Harar, Gurage Zone and other low land area pumpkin had been cultivated more since several years ago that 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 (Meseret, 2018). It has always been very popular for its high yield, good storage life, longer periods of consumption, high nutritive value, and fitness in transport. However, the production, use and export trends of pumpkin are not satisfactory. Most Ethiopians assumed even in their movies and films, vegetables like pumpkin are considered as food of low-income peoples. So, this study aimed to create awareness about the high nutritional and medicinal values of pumpkin and to determine the selected metals from pumpkin peel, flesh and seeds samples and estimate the health risk assessment from three different areas of Ethiopia.

1.4 Significance of the study

Nowadays scientists are exploring unnoticed crops, otherwise a rich source 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 (Elinge et al., 2012). 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. Minerals such as Zn, Cu, Mn, and Fe act as antioxidant. The low Na and high K content in the pumpkin is used to a significant clinical implication for improving cardiovascular health. Zn is essential in male reproduction and cellular protection (Dotto and Chacha, 2020). Since there is limitation of research information about pumpkin and no work has been done on metal contents and its health risk assessment of pumpkin different parts in Ethiopia, the findings of this study will be helpful to have deeper knowledge about the nutritional and medicinal inputs of pumpkin in Ethiopia.

2 Literature review

2.1 Minerals

In the context of nutrition, minerals are chemical element inorganic substances required as essential nutrients by all body tissues organisms to perform functions necessary for the maintenance of life. Minerals are considered to be essential in human nutrition and that they are important constituents of bones, teeth, tissues, muscles, blood, and nerve cells. Generally, the minerals help in the maintenance of acid-base balance, the response of nerves to physiological stimulation, blood clotting, structural, physiological, catalytic, and regulatory (Hashash et al., 2017). They are chemical constituents employed by the body in some ways. Minerals may be broadly classified as macro (major), micro (minor), or trace and toxic. The macro-minerals required in amounts of 100 milligrams or more per day include calcium, magnesium, phosphorus, sodium, sulfur, and chloride.

Calcium is the foremost abundant mineral within the body. It is used structurally, to create bones and teeth, and also as a messenger in cell signaling. It helps muscles relax and contract, bone health, cardiovascular system, blood pressure regulation, immune health system. Inadequate intake of calcium increases the danger of osteoporosis (bone loss with no apparent cause). Excess intake of calcium may cause kidney stone formation gas bloating, constipation, and reduces mineral absorption in general.

Magnesium is widely employed by the body for metabolic processes. Some of its main functions include energy production, synthesis of biomolecules, and as a structural component of cell membranes and chromosomes. Magnesium is required for creating protein, contraction, nerve transmission, and system health.

Magnesium is important to take care of both the acid-alkaline balance within the body and healthy functioning of nerves and muscles (including the heart), also on activate enzymes to metabolize blood sugars, proteins and carbohydrates. Muscle twitches, nervousness, abnormal heartbeat and disorientation may be indications of a magnesium deficiency.

Excess intake of magnesium causes weakness in people with kidney failure (Minaleshewa, 2007).

Phosphorus forms a neighborhood of the bones within the sort of the mineral hydroxyapatite. It is also utilized in cell membranes and is a component of the energy molecules, ATP (ATP) and ADP (ADP). DNA and RNA also contain phosphate. Phosphorus is vital for healthy bones and teeth; found in every cell; a part of the system that maintains acid-base equilibrium.

Potassium is the most abundant mineral found within the physical body next to P and Ca. It is of great physiological importance, contributing to the transmission of nerve impulses, the control of striated muscle contractility, and therefore the maintenance of a normal vital sign. It is obviously essential for plants and cannot be entirely replaced by any other elements. Efficiency symptom includes irregular heartbeat, loss of appetite and muscle cramps.

Sodium helps to take care of proper blood volume and vital signs. Most adults require between 1.5 and 3.8 grams of common salt per day. Sodium and chloride are critical life-sustaining minerals. Sodium chloride (salt) may be a required part of the diet. Chloride is required for correct fluid balance and stomach acid.

Micro-elements or trace elements are present at low levels in the body or required in smaller amounts in the diet, including iron, copper, cobalt, dine, zinc, manganese, molybdenum, fluoride, chromium and selenium. The micro-minerals are required in amounts less than 100 milligrams per day (Soetan et al., 2010).

Iron is employed in red blood cells to hold oxygen to the tissues and is additionally a critical component of the many metabolic proteins and enzymes. It is necessary for the production of energy, the synthesis of collagen, and the functioning of the system. Iron deficiency is common only among children and premenopausal women. Great care must be taken to not take an excessive amount of iron, as excess amounts are stored within the body's tissues and adversely affect the body's immune function, cell growth and heart health. Iron absorption is often blocked by calcium, magnesium, manganese, zinc, antiacids and tetracycline (a common antibiotic). Deficiency of iron results in anemia which is recognized by its symptoms such as low blood iron level, small and red blood cells and low blood hemoglobin values. Iron toxicity usually results from a genetic disease called hemochromatosis. This disease causes over absorption and accumulation of iron, which can result in liver and heart damage.

Copper may be a cofactor in enzymes involved in energy production, animal tissue formation, and iron metabolism. Copper is required with iron for the synthesis of hemoglobin. It works with many enzymes like those involved in protein metabolism and hormone synthesis. The deficiency of copper causes low white blood corpuscle count and poor growth. Excess intake of copper can cause vomiting, systema nervosum disorder.

Cobalt is present in the body as a part of vitamin B_{12}, which has 4% cobalt in its chemical structure. This means that a cobalt deficiency is basically a vitamin B_{12} deficiency. A loss of appetite, emaciation, weakness, anemia, and decreased productivity are some of cobalt deficiency symptoms. Excessive amounts of cobalt may cause cardiomyopathy with high mortality risk.

Iodine is a critical mineral in the body. It is a component of the hormone and is required for normal thyroid function.

Zinc plays multiple roles in the body. It is involved in many cellular metabolic processes and is employed in growth and development, the immune system, neurological function, and reproduction. Zinc is the most universal of all trace elements involved in human metabolism. More than 100 specific enzymes require zinc for his or her catalytic function. If zinc is far away from the catalytic site, activity is lost.

Manganese is an important element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to take care of normal nerve, brain and thyroid function. The deficiency of this mineral is not common. it is often lost by food processing. A deficiency of manganese affects brain health, glucose tolerance, normal reproduction, and skeletal and cartilage formation. The best food sources of manganese are grains and cereal products, while animal products are the poorest. Toxicity from manganese is uncommon. Exposure to a high level of Mn can cause both mental and affective disorder, alongside increased slowness and clumsiness of the body movements.

Fluoride hardens enamel and stabilizes the mineral in bones. Natural sources of fluoride include tea, fish consumed with the bones, and a few fruit juices.

Chromium exists in two main forms: trivalent and hexavalent forms. Hexavalent chromium is recognized as an industrial toxin linked to lung cancer; trivalent chromium is acknowledged as an essential nutrient. The latter is known to improve insulin sensitivity and therefore, to influence carbohydrate, fat and protein metabolism. Diabetes and coronary heart disease are associated with low chromium concentration in human tissue.

Toxic elements such as mercury, arsenic, cadmium, and lead, can diminish mental and central nervous system function; cause damage to blood composition as well as the kidneys, lungs, and liver; and reduce energy levels (Hajeb, et al., 2014).

Cadmium has no known nutritional value, and it's highly toxic to both plants and animals. The biochemical effects of Cd in humans include interference with enzymatic activity, the ability to interact with nucleic acids and damaging kidneys, hypertension and anosmia (absence of smell). Cadmium is understood to accumulate within the kidney. This kidney damage leads to calcium deficiency in the rest of the body, particularly in the skeleton.

2.2 Pumpkin

Pumpkin belongs to the family of *Cucurbitaceae*. *Cucurbitaceae* is a wide family of plants with about 130 types and as many as 800 species. According to the Food and Agriculture Organization (FAO) of the United Nations, the world production of pumpkins in 2018 was over 27,643,932 tons harvested from 2,042,955 hectares (FAOSTAT, 2020). Figure 1 shows the production share of pumpkins by region. China is the World's leading pumpkin producer with 7,838,809 tons yearly production followed by India, Russia, Ukraine and U.S.A. Egypt is the first from Africa followed by South Africa. The production statistics (Table 1) show the world's top 15 pumpkin producing countries by total production, production per capita, harvested area and yield (FAOSTAT, 2020).

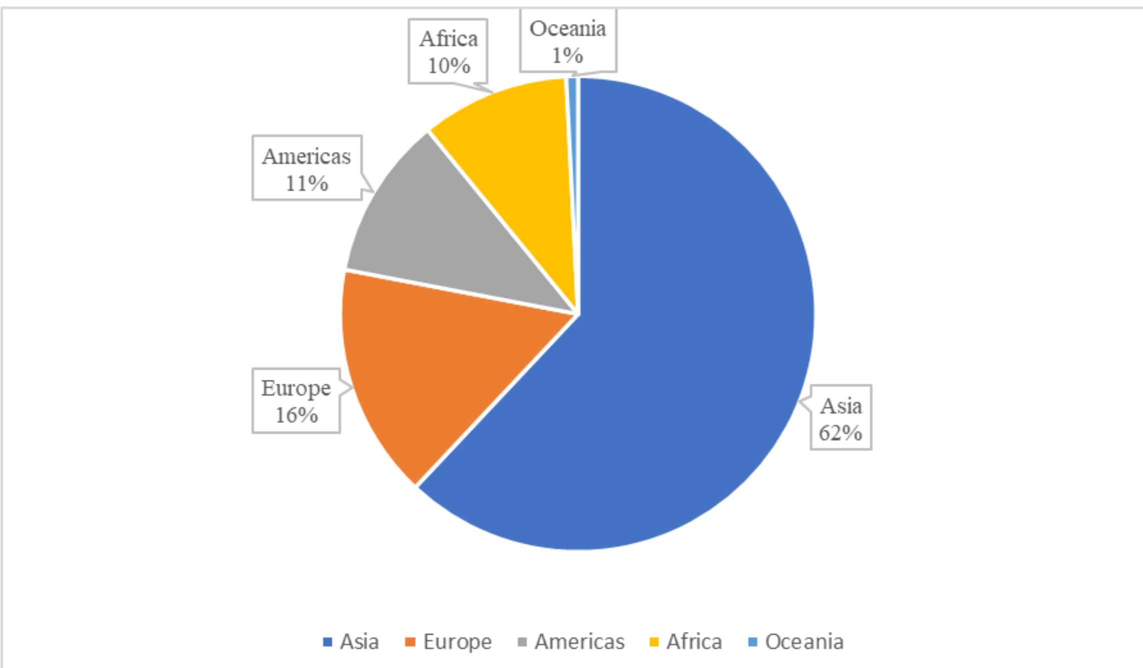


Figure 1. Production share of pumpkins in continent, 2018 (FAOSTAT, 2020)

Table 1. World's top 15 pumpkin producing countries by total production, production per capita, harvested area and yield.

Country	Production (tons)	Production per person (kg)	Hectare	Yield (ton/hectare)

China	7,838,809	5.62	425,230	18,434
India	5,073,678	3.40	528,753	9,595
Russia	1,224,711	8.34	57,012	21,481
Ukraine	1,209,810	28.6	58,600	20,645
USA	1,005,150	3.07	41,640	24,139
Mexico	677,048	5.43	36,721	18,437
Indonesia	603,325	2.28	8,828	68,342
Italy	580,188	9.60	18,486	31,385
Cuba	518,862	46.2	57,018	9,100
Turkey	489,999	6.06	106,697	4,592
Spain	476,396	10.2	9,857	48,331
Egypt	463,451	4.75	25,399	18,247
South Africa	419,791	7.27	40,731	10,306
South Korea	371,391	7.19	10,915	34,026
Philippines	294,125	2.76	20,203	14,558

Cucurbita, includes five 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 (*Cucurbita*) are *Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata*. The leaves, flowers and fruit shape and color rarely help in identification. The only reliable feature for identification is that the stalk of mature fruit, especially for the purpose of attachment to the fruit base. The main pointers are *Cucurbita maxima*: Stalk soft and spongy, not ridged, not enlarged at the apex. *Cucurbita moschata*: Stalk hard, angled, expanded at apex, but angles not individually attached to fruit base and *Cucurbita pepo*: stalk hard, strongly angled, especially near apex with each finger attached to the fruit base. These three species are cultivated worldwide and have high production yields compared with other vegetables and they are rated for the simple production technology.

There is a large variation in the size and shape of pumpkin fruits and the average fruit weight fluctuates between 8 and 10 kg, sometimes even up to 20 kg have been noticed. The color of flesh ranges from pale yellow to crimson. The miniature pumpkins are *C. pepo* and the giant type is *C. maxima* varieties. The three popular types of *Cucurbita* (pumpkin) are shown in Figure 2.

A B C

Figure 2. Images of three major types of pumpkins, A: *Cucurbita maxima*, B: *Cucurbita pepo* and C: *Cucurbita moschata*. (Image was taken from google image).

C. moschata is a leading crop cultivated since pre-historic time and currently most common variety of pumpkin in Asia, Europe and the USA. *C. moschata* is grown in almost all the regions of India while *C. maxima* mostly grown in the hills and subtropical regions. In Nigeria, fluted pumpkin is primarily grown as leafy vegetable and is used for human consumption. The main growing season is summer and rainy seasons in most countries.

Pumpkins contain about 90% of water and 2% of seed. Pumpkin seeds contain 52% of oil (Sito and Barc, 2005). Pumpkin flesh characterized by a low content of fat (2.3%), carbohydrates (66%), proteins (3%), and by a high-carotenoids content with values of 172 to 462 µg. g^{-1} (Noseworthy and Brent, 2016).

2.2.1 Parts of pumpkin

Pumpkin plant contains several components which give us a perfect fruit once it has matured. Such as stem, tendril, leaves, pumpkin shell, pulp, ribs, blossom end, cavity, brains, seeds, seed coat and nut. Stem is brown to brownish green in color and slightly curved located on the very top of the pumpkin; it is attached to the vine and provides nutrients to grow the fruit. Tendrils are green, thin and hairlike structure; while the plant is growing, the tendrils twist around objects on the ground to protect it from the elements, like wind. Leaves of the pumpkin absorb energy from the sun to allow the plant and fruit to grow. Pumpkin shells refer to both the skin and the pulp of the fruit. The skin is the external layer of the pumpkin. This is a protective layer that keeps insects and disease out of the fruit. Pulp is also known as the meat of the fruit which is used to cook. Ribs are the external shape of the pumpkin which made up of indented edges running from top to bottom. When the fruit is young a flower blossom is at the end of the fruit. This is known as the blossom end, which becomes the bottom of the fruit. Fibrous strands better known as brains, this part of the fruit consists of its fibrous strings and seeds. Once the fibrous strings and seeds are removed, the empty cavity of the fruit is left. Seeds are located in the center of pumpkin and attached to the fibrous strings. The seeds can be separated, dried and eaten, or used for the next harvest. Seed coat is the outer layer of the seed which helps to protect the nut inside that will eventually grow into a pumpkin plant. It is also known as the seed jacket. Nut is located inside of the seed coat. When a seed is planted the moisture and warmth triggers the nut to begin to grow into a new plant (Agriculture, Forestry, 2009). Figure 3 shows the major parts of pumpkin fruits which is the main concern of this study.

Figure 3. Major parts of pumpkin fruit

2.2.2 Nutritional values of pumpkin

A healthy diet mainly consists of fruits and vegetables and the consumption can prevent a wide range of diseases. A minimum of 400 g of fruits and vegetables per day is recommended by WHO/FAO (excluding potatoes and other starchy tubers) for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (Mintesnot, 2016).

Fruits and vegetables are providing an abundant, cheap source of fiber, vitamins and minerals. They have the highest nutritional value when eaten fresh, although an exception may be fermented foods, in which the process of fermentation can increase the content of B-vitamins. Vegetables and fruits are excellent sources of vitamins A and C, iron, calcium, potassium, carbohydrates and proteins.

Pumpkin is the most economically important vegetables which are widely used in the culinary arts. Many different parts of the plant are edible, e.g., flowers, leaves, fruits 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 (Kim et al., 2012).

In Middle East, pumpkin is used for the preparation of sweet dish called *halwa*. In South Asian countries, pumpkin is converted into sweet dish called *kaddu ka halwa* eaten during fasting as a delicacy. Pumpkin can also be used to flavor alcoholic and non-alcoholic beverages (Dhiman et al. 2009).

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 (Meseret, 2018).

2.2.3 Medicinal value of pumpkin

The fruits of pumpkins have a lot of biologically active compounds: vitamin C, vitamin A, vitamin E, minerals, pectin and carotenoids. It has huge concentration of β-carotene which protect against certain cancers and is a powerful ally against degeneration aspect of aging. Pumpkin has no cholesterol, low in fat and sodium and rich in vitamins. In the human body carotenoids keep same chemical reactivity as in plants - catching free radicals and active atomic oxygen (Han et al., 2012). Carotenoids potentially play an important role inhuman health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen. Free radicals are a natural byproduct of energy metabolism and are also generated by ultraviolet rays, tobacco smoke, and air pollution. They lack a full complement of electrons, which makes them unstable, so they steal electrons from other molecules, in the process they damage those molecules (Lakshminarayana et al., 2008).

Pumpkin seeds have many health benefits as they are lower in cholesterol and have antidepressant qualities due to the presence of tryptophan which can elevate mood. Pumpkin seeds are regarded as a remedy for depression. One gram of pumpkin seed protein contains as much of tryptophan as a full glass of milk. Seeds also contain Omega-3 and Omega-6 essential fatty acids (Dhiman et al., 2009) and have a broad range of health functions in the body. Its oil is used in giving quick relief in scalding of urine, spasmodic infection of the urinary passage and has been reported to cure gonorrhea. Pumpkin seeds provide high phosphorous levels and can be used as a potential agent in lowering the risk of bladder stone disease. Pumpkin seeds also contain cucurbitacin which rid the body off intestinal parasites and are also traditional remedy for tape worm and safe for children and pregnant woman. The seeds have anti-inflammatory properties which help in the treatment of arthritis. Pumpkin seed oil is useful in promoting wellness in HIV/AIDS patients (Younis et al., 2000).

The fiber plays an important role in the prevention and cure of diabetes, obesity, atherosclerosis, heart diseases and colon cancer. Pumpkins have long been used for traditional medicine in many countries, such as China, Argentina, India, Mexico, Brazil, and Korea, since pumpkin flesh and seeds are rich in proteins, minerals, antioxidant vitamins such as carotenoids and tocopherols, low in fat and calories (Stevenson, 2007). β-Carotene reduces skin damage from the sun and acts as an anti-inflammatory agent. Pumpkins have been used traditionally to relieve edema during pregnancy and after delivery. In China, former Yugoslavia, Argentina, India, Mexico, Brazil, and America pumpkins are utilized in the pharmaceutical industry (Saha et al., 2011).

2.2.4 Cosmeceutical value of pumpkin

Pumpkin is a beauty-boosting superstar (Figure 4). It used for body and face scrubs, lip balms, hair masks and shower jell. Pumpkin is made up of alpha hydroxy acids (similar to glycolic acid and lactic acid), which are fruit acids that slough dead skin cells, increase cell turnover and keep the skin brighten, smooth and glowing. Pumpkin is a good source of vitamin C which is a powerful antioxidant and also contains beta-carotene which helps to reverse UV damage and improve skin texture. It protects the skin from radical damage which is responsible for causing wrinkles and even skin cancer. Pumpkin is packed with vitamins including vitamin, A, C, and E and essential fatty acids which help with anti-aging and moisturizing. Because of the vitamin C found in pumpkin, it can also be used as a spot treatment or as a brightener, which can be helpful during colder months when sun exposure is minimal, and our skin tone can seem a little dull.

A stable makeup remover was formulated using 5–15% pumpkin seed oil. The remover containing 5% pumpkin seed oil was able to remove 89.3%, 67.7%, and 41.3% of foundation, liquid, and pen eyeliners, respectively, while those of the remover containing 10% pumpkin seed oil were 78.3%, 66.9%, and 38.4%, and those of the remover containing 15% pumpkin seed oil were 84.4%, 69.8%, and 41.9%, respectively.

Figure 4. Different types of cosmetics produced from pumpkins.

2.2.5 Physical characteristics of pumpkin fruit

There is a large variation in the size and shape of pumpkin fruits and the average fruit weight fluctuates between 8 and 10 kg, sometimes even up to 20 kg have been noticed depending on the variety. The miniature pumpkins are *C. pepo* and the giant type is *C. maxima* varieties. The color of fruit peel can vary from green to orange. The color of flesh ranges from pale yellow to crimson. Pumpkin seeds, are flat, asymmetrically oval, light green or brown in color and usually covered by a white husk and used as edible.

2.2.6 Chemical composition of pumpkin

The chemical composition of pumpkin varies from one cultivar or species to other. Proximate composition of the pumpkin pulp varied between 75.8 and 91.3% moisture, 0.20 and 2.70% crude protein, 0.47 and 2.10% crude ash and 3.1 and 13% carbohydrate content. Pumpkin fruits have many nutritional components including polysaccharides, proteins, essential amino acids, valuable antioxidants, carotenoids and minerals. Seeds of pumpkin are rich in oil and the variability in the oil content is due to its broad genetic diversity (Dar et al., 2017).

2.2.7 Sample decomposition

Most available instruments for quantitative analyses are designed for the analysis of liquid samples. Because of this solid samples must be dissolved in suitable solvents. Eliminating interfering substances from the matrix and obtaining the element in a homogeneous and easily accessible matrix are the main purposes of sample decomposition. Organic components are Dry and wet decomposition are basic procedures used for sample preparation.

2.2.7.1 Dry decomposition

Dry decomposition by fluxes (fusion processes) is normally used for silicates, refractory materials, some mineral oxides and iron alloys. The sample is mixed with a flux and then fused to form products that can be dissolved in water or dilute acid. High temperature (300 to 1000 ^{o}C) is required and it is achieved by flame, conductive or microwave assisted heating. The limitations of this method are the impurities in the fluxes, risk of contamination and losses by volatilization (Braz, 2003).

2.2.7.2 Wet decomposition

Oxidizing agents are decompose the organic samples before metal content determination or to extract metals from inorganic matrices. Concentrated acid plus heating is used for decomposition. The strength of the acid, its oxidizing power, its boiling point, the solubility of the resulting salts, safety in manipulation and purity are the important aspects to consider. Nitric acid, vitriol, orthophosphoric acid, hydrogen peroxide and also as mixtures of such reagents, are used for organic samples, metallic alloys, common minerals, soils, rocks, clays and silicates (Braz, 2003). Thermal, ultrasonic and radiant (infrared, ultraviolet and microwave) forms of energy can be used for performing wet decomposition procedures.

Wet decomposition-thermal energy is an open vessel decomposition and is often performed using heat from a bunsen, hot plate, digestion block, oven or muffle. Time consumption (hours), the use of large amounts of reagents, contamination from the environment, pre-concentration of reagent impurities and the need of constant supervision are the main limitations of this method (Braz, 2003).

Wet decomposition/extraction-ultrasonic energy. The extraction effect is caused by acoustic cavitation, that is, bubble formation and subsequent disruptive action. The collapse of bubbles, created by sonication of solutions, leads to the generation of extremely high local temperature (5000 K) and pressure (10 GPa) gradients. A diluted acid medium is normally used to decrease blank values and reducing both reagent and time consumption.

Wet dissolution-radiant energy-ultraviolet radiation. Ultraviolet radiation decomposition devices are used for complete removal of organic materials. Examples are heavy metal determination in waste-waters or liquid foodstuffs like beer and wine. Ultraviolet radiation decomposition requires the addition of a little amount of acid plus peroxide to the sample, without a big increase within the temperature. This allows low contamination levels from reagents and avoids losses of volatile elements (Braz, 2003).

Wet decomposition-radiant energy-microwave radiation. Microwave-assisted sample preparation is employed for a good range of applications, including decomposition of inorganic and organic materials. The interaction of microwave radiation (2450 MHz, 12.2 cm) with sample and reagents causes both ionic migration and dipole rotation. This leads to fast heating of the mixture with consequent decomposition (Braz, 2003). The advantages of this system are the short time needed (minutes) to perform decomposition of sample, direct heating of samples and reagents (the vessels are only indirectly heated by the hot solution), minimal contamination and no loss of volatile elements. The use of small amounts of reagents decreases the blank signal (Braz, 2003). From these alternatives in this study, wet decomposition microwave assisted sample preparation was used.

3 Experimental

3.1 Equipment and apparatus

In this study a plastic film was used as carpet for drying pumpkin samples, ceramic mortar and pestle were used to grind the dried pumpkin samples, 300 μm sieve (Chicago, ILL. 60656 USA) was used for sieving the ground sample powder, polyethylene bottles were used to pack the dried and sieved parts of pumpkin samples, digital analytical balance (Mettler Tolledo) with ± 0.0001 g precision to weigh pumpkin samples, Ethos up microwave digester were used to decompose pumpkin samples, beakers, measuring cylinders, pipettes, filler, desiccator, plastic weighing pan, volumetric flask (50 mL), fast filter paper, plastic funnel, wash bottle, detergent, safety gloves, spatula were used during digestion process. Refrigerator was used to keep the digested samples.

3.2 Instrument

Milestone Ethos up microwave digester with maxi 44 rotor (Milestone Inc, USA) was used for the digestion of different parts of pumpkin samples. For the determination of selected metals from pumpkin peel, flesh, and seed samples, microwave plasma-atomic emission spectroscopy (MP-AES) (Agilent Technologies, model 4200, USA) was used. The system is safer, cost-efficient, sensitive, lower detection limit to sub ppb level, run on air instead of combustible gases and faster than flame atomic absorption. Table 2 shows the operating conditions of MP-AES during analysis of selected metals.

The MP-AES instrument utilizes a microwave plasma. It runs on air rather than combustible gases. It is often installed in either a centralized laboratory or a foreign location. By using nitrogen because the source gas for the plasma, running costs are greatly reduced, and safety is greatly increased by removing the need for hazardous laughing gas and acetylene. Additionally, the upper temperature of nitrogen plasma atomization/ionization source improves detection limits, linear range, and long-term stability.

The emission intensities for various elements studied depends on gas flow, sample flow, and microwave power. The plasma gas flow, nebulizer gas flow, observation height, and other operating parameters were optimized for obtaining higher stability of the plasma and maximum emission intensity.

The basic principle is that when an atom of a selected element is excited by providing an external energy, it emits radiation (light) during a characteristic pattern of wavelengths forming a spectrum, because it returns to the bottom state (Figure 5).

MP-AES is employed for simultaneous multi-analyte determination of elements. The technique provides better linear dynamic range, detection limits, and analysis speed as compared to standard flame atomic absorption spectrometers.

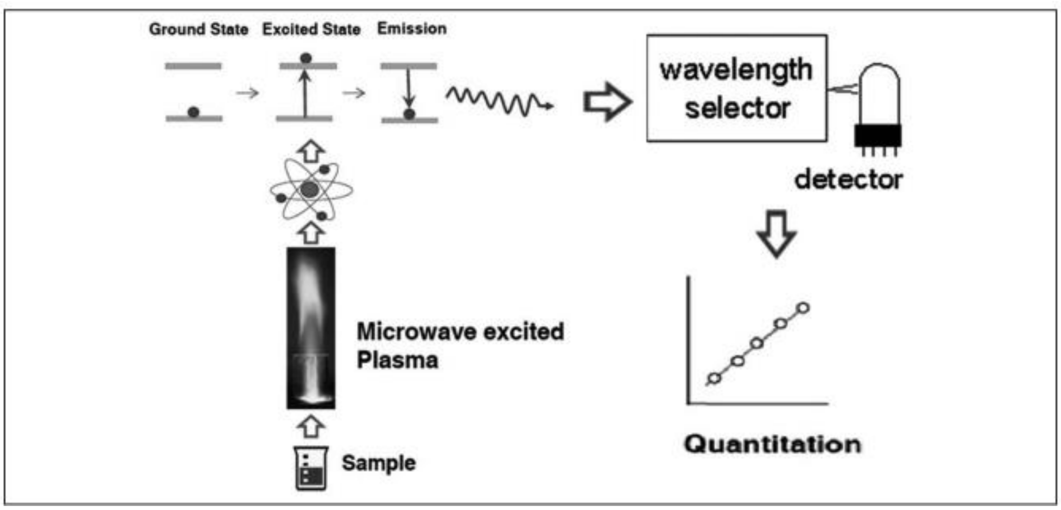


Figure 5. Schematic diagram of MP-AES elemental analysis set up (Balaram et al., 2014).

Table 2. Operating condition of Agilent 4200 MP-AES during metal analysis of pumpkin samples.

Parameters	Na	Mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Wavelength (nm)	588.995	285.213	766.491	393.366	425.433	403.076	371.993	352.454	324.752	213.857	228.802
Background correction	Auto	Auto	Auto	Auto	Auto	Auto	Auto	Auto	Auto	Auto	Auto
Replicates	3	3	3	3	3	3	3	3	3	3	3
Pump speed(rpm)	15	15	15	15	15	15	15	15	15	15	15
Blank subtraction	On	On	On	On	On	On	On	On	On	On	On
Sample introduction	Manual	Manual	Manual	Manual	Manual	Manual	Manual	Manual	Manual	Manual	Manual
Stabilization time (s)	15	15	15	15	15	15	15	15	15	15	15
Sample uptake time(s)	15	15	15	15	15	15	15	15	15	15	15
Sample uptake fast pump	On	On	On	On	On	On	On	On	On	On	On
Rinse time	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Read time(s)	3	3	3	3	3	3	3	3	3	3	3
Nebulizer flow(L/min)	0.95	0.6	0.75	0.6	0.9	0.9	0.65	0.7	0.7	0.45	0.5

N/A: not applicable

3.3 Reagents and chemicals

All chemicals and reagents used during analysis were high purity analytical grade reagents. Nitric acid (69% HNO_3, MRS scientific, UK) and hydrogen peroxide (30% 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 standard and sample preparation.

3.4 Softwares

ArcGIS10.7 software was used to draw the sample site map, MP-expert software was used for the interpretation of data in microwave plasma atomic emission spectroscopy, Microsoft excel 2016 were used for data analysis.

3.5 Description of the study area

Pumpkin samples were collected from Amhara region (North Shewa, Minjar Shenkora Wereda), Oromiya region (West Shewa, Woliso) and South region (Gamo Gofa, Arbaminch Zuriya Wereda) which was grown in 2020/2021 harvesting season. The reason for the selection of Woliso and Arbaminch is the production, commercial availability in urban markets specially in Addis Ababa (capital city of Ethiopia) and high popularity in consumption of pumpkin compared to other parts of Ethiopia and Minjar Shenkora is selected to compare the composition of minerals with that of high consumption areas (Woliso and Arbaminch) because even if pumpkin is grown in the area (Minjar Shenkora) people do not use it as an important vegetable.

Minjar Shenkora is located at the southern end of North Shewa Zone of the Amhara region. It is located 9° 0' 0'' N latitude and 39° 25' 59'' E longitude and an elevation of 1804 meters above sea level. It is approximately 130 km far apart from Addis Ababa in East direction.

Woliso is located in the West Shewa Zone of the Oromia region. It has a latitude and longitude of 8°32′N 37°58′E with an elevation of 2063 meters above sea level. It is far by 114 km southwest of Addis Ababa.

Arbaminch is located 6°1'60" N latitude and 37°32'60" E longitude in the Gamo Gofa Zone of the Southern Nations, Nationalities, and People's Region about 500 kilometers south of Addis Ababa, at an elevation of 1285 meters above sea level. Figure 6 shows sample site map of this study.

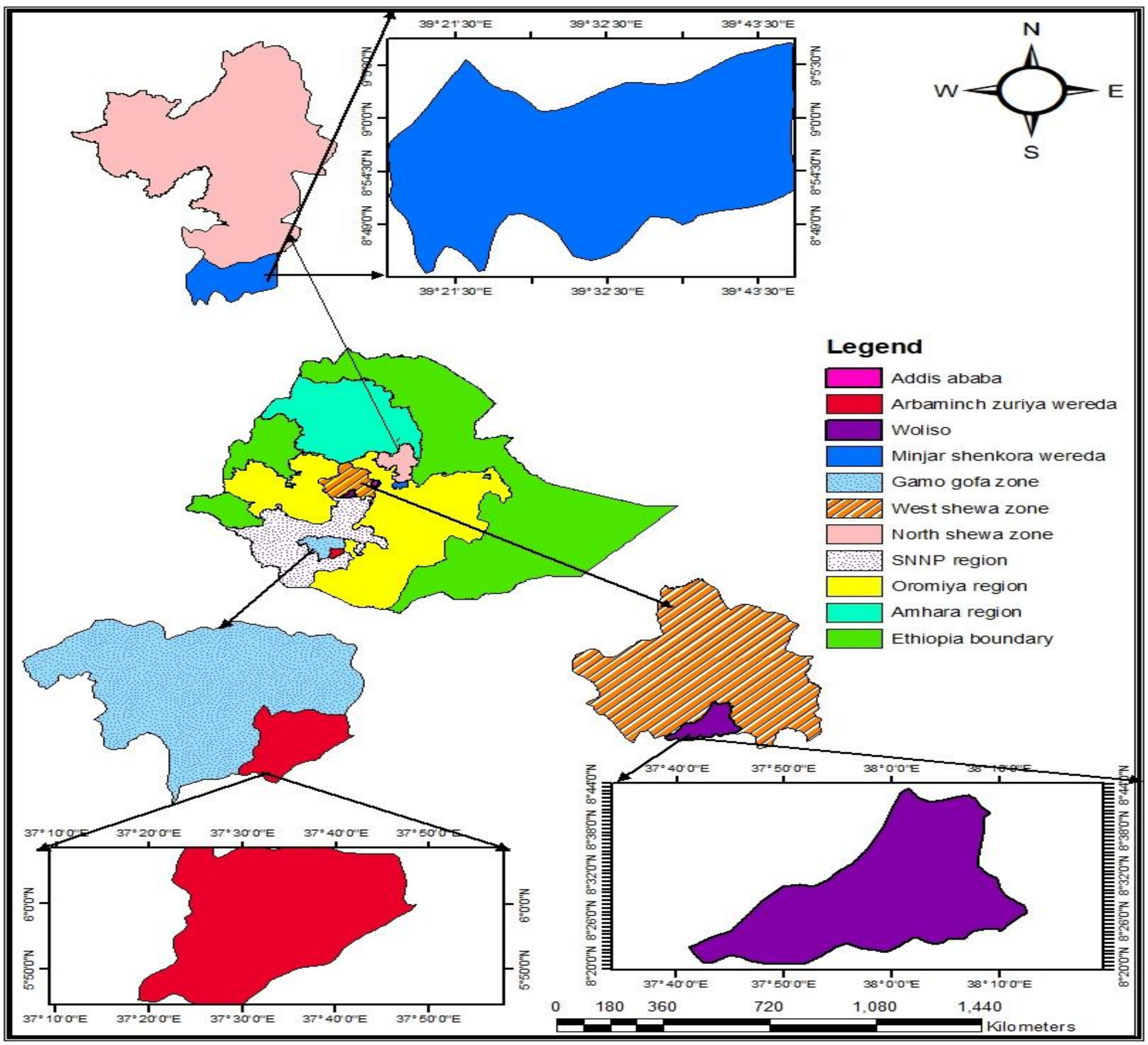


Figure 6. Map showing sites from which samples were collected.

3.6 Collection and preparation of pumpkin samples

Sample preparation is a fundamental step towards a successful analysis and its importance cannot be under estimated. Pieces of pumpkin sample (each weighing 4 to 10 kg) were collected from each site (Minjar Shenkora, Woliso and Arbaminch) in December 2020, which is harvesting time of 2020/2021 production season of ripe pumpkin fruit in Ethiopia. The sample was transported to Addis Ababa University, Chemistry Department analytical chemistry laboratory and washed with tap water followed by distilled water to remove adsorbed dust. The fruit sample was then cut in to half. The seeds were detached from the flesh using bare hands. The seed sample was then washed with tape water to remove staked flesh and rinsed by distilled water. The fibrous strands removed properly from the flesh. The peel was separated from flesh and cut in to small pieces by knife in order to facilitate drying. Since the flesh of pumpkin is highly watery instead of chopping shaving was done by knife to facilitate drying. The three separated parts (peel, flesh and seed) of pumpkin were air-dried for one month at room temperature on clear plastic film at analytical laboratory working bench. The dried sample were ground and homogenized in to powder using mortar and pestle and sieved by using 300 microns mesh size sieve. The sieved sample was stored in polyethylene bottles and kept in desiccator until the time of digestion. Finally, the prepared powder samples were transported to Ethiopian Conformity Assessment Enterprise chemical analysis laboratory for digestion and analysis.

3.7 Optimization of digestion procedure

The optimum working procedure should be determined before carrying out any experimental activities. The aim of optimization procedure in this study is to determine the usage of smaller reagent volume, smaller voltage, shorter time and lower temperature to decompose different parts of pumpkin samples completely using wet acid digestion method carried out by microwave digester in three stages. Organic components are assumed to decompose in the form of different gaseous forms like CO_2, NO_2 and H_2O. The complete decomposition is measured by visualizing clear and colorless solutions. For each part of pumpkin samples 0.5 g dried and powdered sample was weighed and put in to clean nitric acid-soaked digestion vessels and different volumes of 69% nitric acid and 30% 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 in this study for pumpkin peel sample was 7 mL HNO_3:1 mL H_2O_2 volume ratio of reagents, 1800 W power, 200 ^oC digestion temperature, 25 min at 1st stage; 1800 W, 200 ^oC, 20 min 2nd stage and 1800 W, 200 ^oC, 15 min for 3rd stage. For pumpkin flesh sample the optimized condition was 6 mL HNO_3: 2 mL 30% H_2O_2 reagent ratio, 1200 W, 200 ^oC, 25 min 1st stage; 1200 W, 200 ^oC, 20 min 2nd stage and 1200 W, 200 ^oC, 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 ^oC, 25 min 1st stage; 1600 W, 200 ^oC, 20 min 2nd stage and 1600 W, 200 ^oC, 0 min for the 3rd stage.

The optimization conditions for the digestion of pumpkin peel, flesh and seed samples of this study are listed in Table 3a, 3b and 3c.

Table 3a. Different trialed conditions for optimization of digestion procedure for 0.5 g pumpkin peel samples.

Trial	Reagent volume ratio (HNO_3: H_2O_2)	Power (W)			Temperature (^oC)			Digestion time (min)		
		1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage
1	7:2	1800	1800	1800	200	200	200	25	20	15
2	**7:1**									
3	6:2									
4	6:1									
5	5:2									
6	5:1									
1	7:1	1000	1000	1000	200	200	200	25	20	15

2		1200	1200	1200						
3		1400	1400	1400						
4		1600	1600	1600						
5		**1800**	**1800**	**1800**						
1					180	180	180			
2	7:1	1800	1800	1800	190	190	190	25	20	15
3					**200**	**200**	**200**			
1								155	10	5
2	7:1	1800	1800	1800	200	200	200	20	15	10
3								**25**	**20**	**15**

The bold numbers indicate the selected point at which clear and colorless solution is observed during optimization process.

Table 3b. Different trialed conditions for optimization of digestion procedure for 0.5 g pumpkin flesh samples.

Trial	Reagent volume ratio (HNO_3: H_2O_2)	Power (W)			Temperature (°C)			Digestion time (min)		
		1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage
1	7:2									
2	7:1									
3	**6:2**	1800	1800	1800	200	200	200	25	20	15
4	6:1									
5	5:2									
6	5:1									
1		1800	1800	1800						
2	6:2	1600	1600	1600	200	200	200	25	20	15
3		1400	1400	1400						

4		**1200**	**1200**	**1200**						
5		1000	1000	1000						
1					180	180	180			
2	6:2	1600	1600	1600	190	190	190	25	20	15
3					**200**	**200**	**200**			
1								25	25	10
2	6:2	1800	1800	1800	200	200	200	**25**	**20**	**15**
3								20	15	10

The bold numbers indicate the selected point at which clear and colorless solution is observed during optimization process.

Table 3c. Different trialed conditions for optimization of digestion procedure for 0.5 g pumpkin seed samples.

Trial	Reagent volume ratio (HNO_3: H_2O_2)	Power (W)			Temperature (°C)			Digestion time (min)		
		1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage
1	7:2									
2	7:1									
3	6:2	1800	1800	1800	200	200	200	25	20	15
4	**6:1**									
5	5:2									
6	5:1									
1		1000	1000	1000						
2		1200	1200	1200						
3	6:1	1400	1400	1400	200	200	200	25	20	15
4		**1600**	**1600**	**1600**						

5		1800	1800	1800						
1					180	180	180			
2	6:1	1600	1600	1600	190	190	190	25	20	15
3					**200**	**200**	**200**			
1								25	20	5
2	6:1	1600	1600	1600	200	200	200	**20**	**20**	**5**
3								20	15	5

The bold numbers indicate the selected point at which clear and colorless solution is observed during optimization process.

3.8 Digestion of samples

A 0.5 g of dried, powdered and sieved pumpkin peel sample was put in to weighing pan and measured by Mettler Tolledo 0.0001g resolution analytical balance and transferred to 100 mL vessels. Then optimized volume of 7 mL 69% HNO_3 and 1mL of 30% H_2O_2 was added to pumpkin peel sample in the vessel. The mixture was shaken and the vessel was properly closed. The vessel was placed in to Maxi 44 Rotor 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 to 50 mL volumetric flask by using funnel and fast flow filter paper. The filtrate solution was filled up to the mark of volumetric flasks. The flask was closed appropriately and kept in to refrigerator for analysis. Pumpkin flesh and pumpkin seed are also digested in the same procedure as pumpkin peel sample. The difference was the optimized condition (volume ratio used, power, temperature and time adjusted) during digestion. The optimized condition for pumpkin flesh sample 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 samples 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.

3.9 Preparation of standard solution

The intermediate and working standards of each metal were prepared from 1000 mg/L standard stock solution which is 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 intermediate standards for Cr, Cd, Mn and Ni; 100 mg/L for Na, K, Ca, Mg, Cu, Fe and Zn was prepared by diluting stoke 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 and the instrument was calibrated.

3.10 Determination of metals in pumpkin samples

After calibrating the instrument, the prepared clear sample solutions of different parts of pumpkin were taken out from refrigerator and the concentration of selected metals was analyzed 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 to be analyzed and the read out was recorded. 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.

3.11 Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) of metals of interest in this study are obtained by determining metals from nine replicate blank solutions. LOD is not necessarily quantified as a particular value. It is the lowest analyte concentration that produces a response detectable but not quantifiable above the noise level of the system. It can be calculated by multiplying the pooled standard deviation of the reagent blank (SD_{blank})

by three (LOD = 3 x SD_{blank}). Less values of LOD indicate that the presence of trace amounts of metals of interest in the sample can be detected by the method. Limit of quantification (LOQ) is the smallest quantity of analyte that can be measured with acceptable accuracy and precision. It is calculated as ten times the standard deviation of blank solution (LOQ = 10 x SD_{blank}). The limit of detection of all eleven metals in this study was smaller than the obtained sample concentration and given in Table 4.

Table 4. Wavelength, LOD, LOQ, correlation coefficient and calibration curve equation for determination of metals using MP-AES instrument.

Metals	Wave length (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	0.009	0.996	I = 14653C-1652
Ca	393.366	0.026	0.088	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	0.086	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

3.12 Method validation

Method validation may be a procedure of performing numerous assessments designed to verify that an analytical test system is suitable for its intended reason and is capable of providing beneficial and legitimate analytical data. Analytical Parameters for the determination of metals were evaluated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and linearity.

3.12.1 Precision

Precision of a way is that the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. From the measured mean values and standard deviation (SD), precision as relative standard deviation (% RSD) is calculated as shown in equation (1).

$$\%RSD = \frac{SD}{mean} * 100 \quad (1)$$

3.12.2 Accuracy

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value. It is measured 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 from 10mg/L of 2.5 mL of Mn, Ni, Cr, Cd were added to 0.5 g of each pumpkin peel, flesh and seed sample parts. Then spiked and non-spiked samples were digested and analyzed in the same condition using the method being validated. Accuracy is calculated as percent recovery as shown in equation 2.

$$\%Recovery = \frac{spiked\ sample - unspiked}{amount\ added} * 100 \quad (2)$$

Table 5 displays the calculated percentage recovery for different parts of pumpkin samples and it was found in the range 90–106% for peel; 92–110% for flesh and 90–108% for seed which are within the acceptable range for all metals indicating the efficiency of the optimized procedure.

Table 5. Recovery results for pumpkin peel, flesh and seed samples.

	Peel				Flesh				Seed			
Metal	Un spiked (mg/kg)	Added (mg/kg)	Spiked (mg/kg)	Recovery (%)	Un spiked (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

3.13 Instrument validation

The efficiency of instrument was confirmed through calibration curve verification (CCV) and quality control (QC) method running by one point from the linear range of calibration curve of known concentration standard solutions for each metal of interest. The percent recovery of calibration curve verification was ranges from 96–104% and 97–105% for quality control recovery. It indicates the instrument is efficient to analyze the respective metals.

Table 6. The CCV and QC recovery values for respective metals.

	CCV			QC		
Metals	Run (mg/L)	Instrument read (mg/L)	Recovery (%)	Run (mg/L)	Instrument read (mg/L)	Recovery (%)
Na	3.0	3.08	103	4.0	4.0	100
Mg	2.0	2.06	103	1.0	1.1	105
K	3.0	3.02	101	4.0	4.0	99
Ca	3.0	3.06	102	4.0	4.1	103
Cr	0.2	0.20	98	0.4	0.4	99
Mn	0.2	0.19	96	0.4	0.4	97
Fe	2.0	1.98	99	3.0	2.9	97
Ni	0.2	0.20	98	0.4	0.4	98
Cu	2.0	1.99	99	3.0	3.0	100
Zn	2.0	2.07	104	3.0	3.1	103
Cd	0.2	0.20	99	0.4	0.4	101

3.14 Health risk assessment

Health risk assessment (HRA) is the method used to determine the magnitude and probability of an adverse health effect from exposure. It is very important to safe guard the public health (Taiwo, 2019). Toxic metals enter to human body through ingestion (food, water), inhalation (air) and absorption through dermal contact (Beetseh and Onum 2013). From the analysis of this study the edible part of pumpkin contains Cd and Cr exceeding the recommended level. The none carcinogenic and carcinogenic health risk assessment of metals ingested from pumpkin edible parts are calculated based on the assumption that an adult of 61 kg (African adult average body weight) (Sarah et al., 2012) ingested 0.2 kg fresh weight of pumpkin flesh 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 is converted in to dry weight by multiplying with conversion factor 0.085 (Khanum et al., 2017).

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.

$$HQ = \frac{ADI}{RFD} \quad (3)$$

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.

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:

$$ADI = \frac{C*Cf*IR*ED*EF}{BW*AT} \quad (4)$$

where C – metal concentration (mg/kg); Cf – conversion factor (0.085), 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 7.

Table 7. values of parameters for ADI input.

Factor	Value	Unit
IR	0.2/0.1	kg/day
ED	67	Year
EF	96	day/year
AT	6432	Day
BW	61	Kg

The hazard index (HI) is 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):

$$HI = \sum HQ \tag{5}$$

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.

$$CR = ADI * SF \tag{6}$$

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

$$TCR = \sum CR \tag{7}$$

Total cancer risk from 1.0 x 10^{-6} to 1.0 x 10^{-4} is the acceptable range for cancer risk (Zeng et al., 2015). Table 8 shows the oral RFD and SF value for minor and trace metals in food.

Table 8. Reference doses (RfD) and slope factors (SF) of selected metals.

Metals	RFD (mg/kg/day)	SF $(mg/kg.d)^{-1}$
Cr	1.50	0.50
Mn	0.01	_
Ni	0.02	_
Cu	0.04	_
Zn	0.30	_
Cd	0.001	0.64

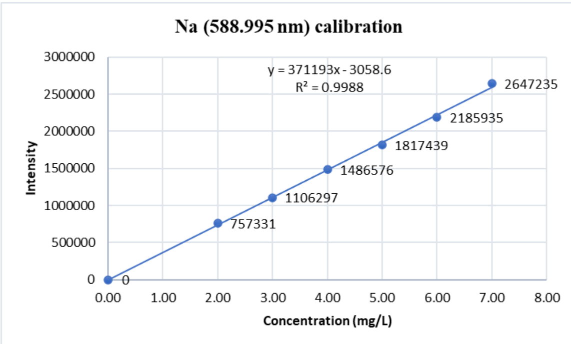
4 Results and Discussion

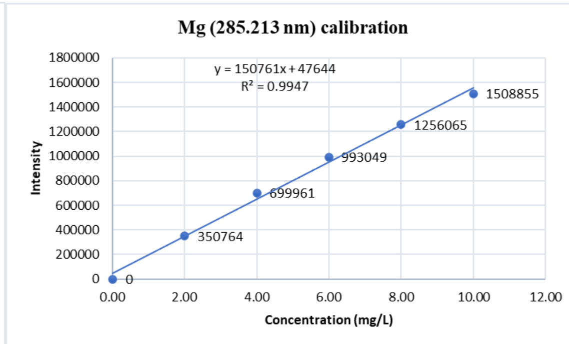
4.1 Calibration curve of standards

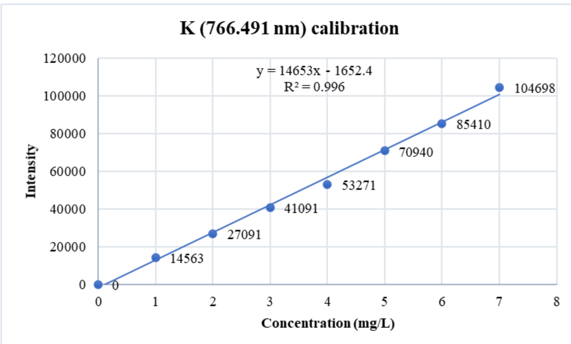
The intermediate and working standards of each metal were prepared freshly from 1000 mg/L standard stock solution by serial dilution with deionized water. Calibration curve was obtained by running series of the prepared working standards for each corresponding metals as mentioned under section 3.9. The correlation coefficient was from 0.996 to 0.9999 which indicates a very good linearity of curves. Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed in Table 9. The calibration graph of each of metals of interest is shown in Figure 7.

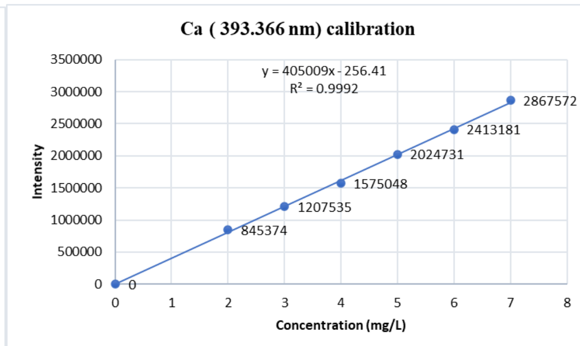
Table 9. Intermediate, working standards and correlation coefficients of the calibration curves for determinations of metals using microwave plasma atomic emission spectrometry.

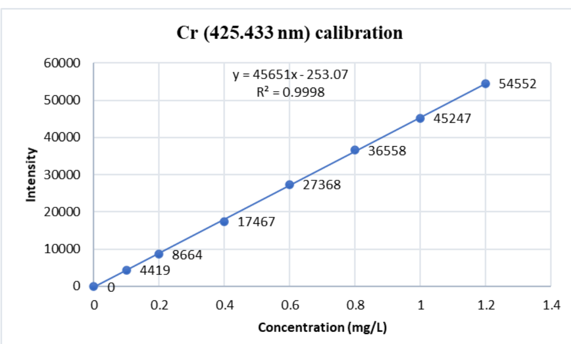
Metal	Conc. of intermediate standard (mg/L)	Conc. of working standard (mg/L)	Correlation coefficient
Na	100	2, 3, 4, 5, 6, 7	0.9988
Mg	100	2, 4, 6, 8, 10	0.9974
K	100	1, 2, 3, 4, 5, 6, 7	0.996
Ca	100	2, 3, 4, 5, 6, 7	0.9992
Cr	10	0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2	0.9998
Mn	10	0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2	0.9999
Fe	100	1, 2, 3, 4, 5, 6, 7	0.9999
Ni	10	0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2	0.9999
Cu	100	1, 2, 3, 4, 5, 6, 7	0.9998
Zn	100	1, 2, 3, 4, 5, 6, 7	0.9971
Cd	10	0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2	0.9996

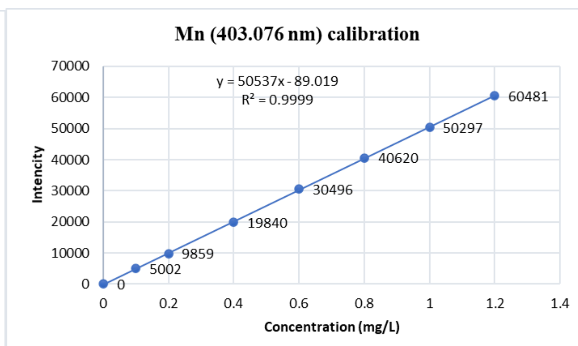

Na (588.995 nm) calibration
y = 371193x - 3058.6
R² = 0.9988
Intensity
Concentration (mg/L)
0
757331
1106297
1486576
1817439
2185935
2647235

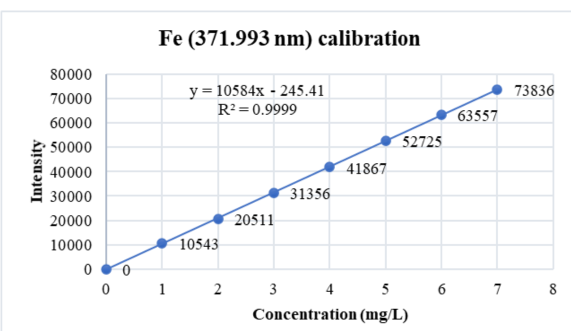

Mg (285.213 nm) calibration
y = 150761x + 47644
R² = 0.9947
Intensity
Concentration (mg/L)
0
350764
699961
993049
1256065
1508855

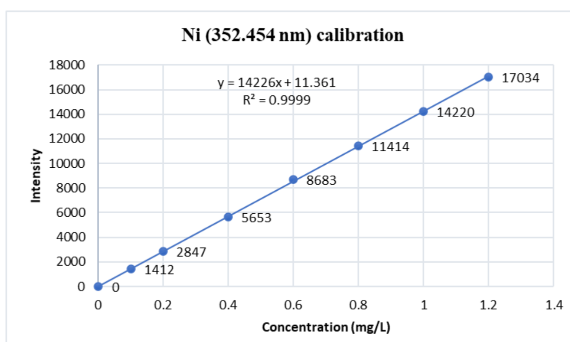

K (766.491 nm) calibration
y = 14653x - 1652.4
R² = 0.996
Intensity
Concentration (mg/L)
0
14563
27091
41091
53271
70940
85410
104698


Ca (393.366 nm) calibration
y = 405009x - 256.41
R² = 0.9992
Intensity
Concentration (mg/L)
0
845374
1207535
1575048
2024731
2413181
2867572


Cr (425.433 nm) calibration
y = 45651x - 253.07
R² = 0.9998
Intensity
Concentration (mg/L)
0
4419
8664
17467
27368
36558
45247
54552


Mn (403.076 nm) calibration
y = 50537x - 89.019
R² = 0.9999
Intencity
Concentration (mg/L)
0
5002
9859
19840
30496
40620
50297
60481


Fe (371.993 nm) calibration
y = 10584x - 245.41
R² = 0.9999
Intensity
Concentration (mg/L)
0
10543
20511
31356
41867
52725
63557
73836


Ni (352.454 nm) calibration
y = 14226x + 11.361
R² = 0.9999
Intensity
Concentration (mg/L)
0
1412
2847
5653
8683
11414
14220
17034

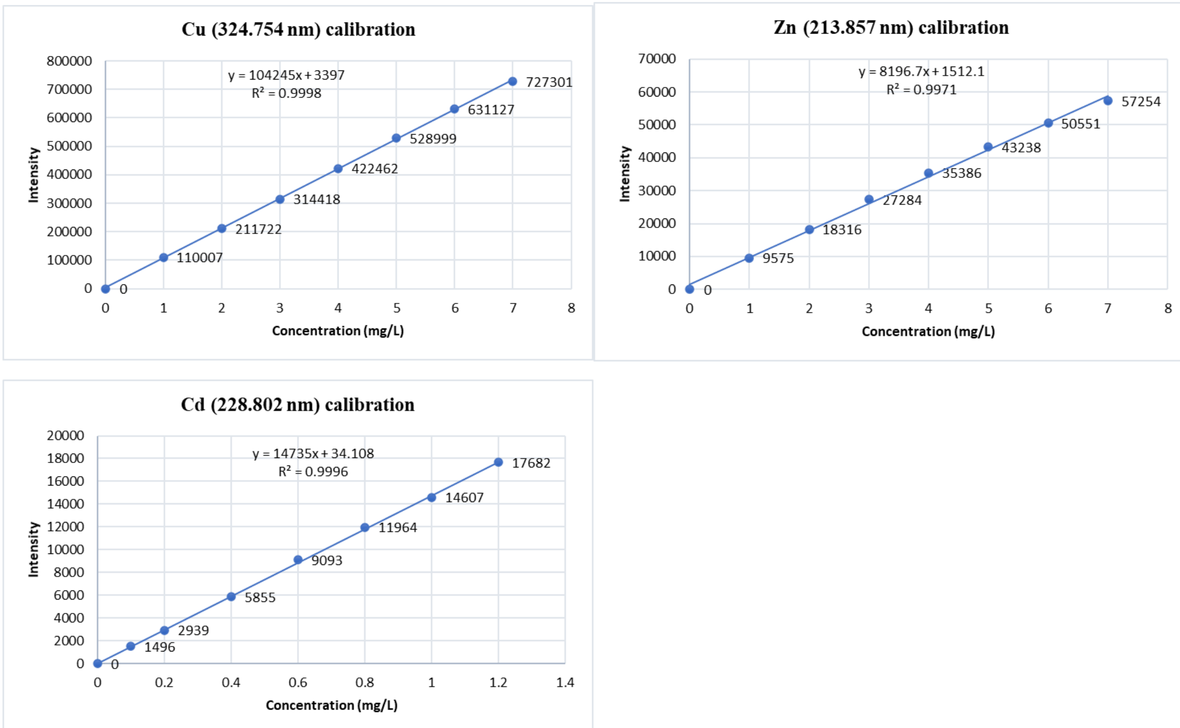


Figure 7. calibration curves of standard metal solution.

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

A total of 11 metals were determined by using microwave plasma-atomic emission spectroscopy (MP-AES). The results obtained from MP-AES are in terms of mg/L and were converted in to mg/kg by using equation (8):

$$\frac{mg}{gk} = \left(\frac{Cs - Cb}{Mo}\right) * df \quad (8)$$

where Cs – sample concentration in mg/L; Cb – concentration of blank; Mo – mass of test portion; df – dilution factor.

The most abundant elements were K, Ca, Mg, Na and Fe. From the overall mean concentration pumpkin peel has relatively higher concentration in Mg (4716 mg/kg), Ca (5268 mg/kg) and Ni

(3.79 mg/kg). Pumpkin seed has higher in Na (171 mg/kg), Cr (0.69 mg/kg), Mn (33.4 mg/kg), Fe (225 mg/kg), Zn (67.8 mg/kg), Cd (1.39 mg/kg) and pumpkin flesh has higher concentration only in K (29531 mg/kg) and Cu (15.1 mg/kg) than pumpkin seed and peel. Figure 8 shows the percent contribution of selected metals in each part of pumpkin sample.

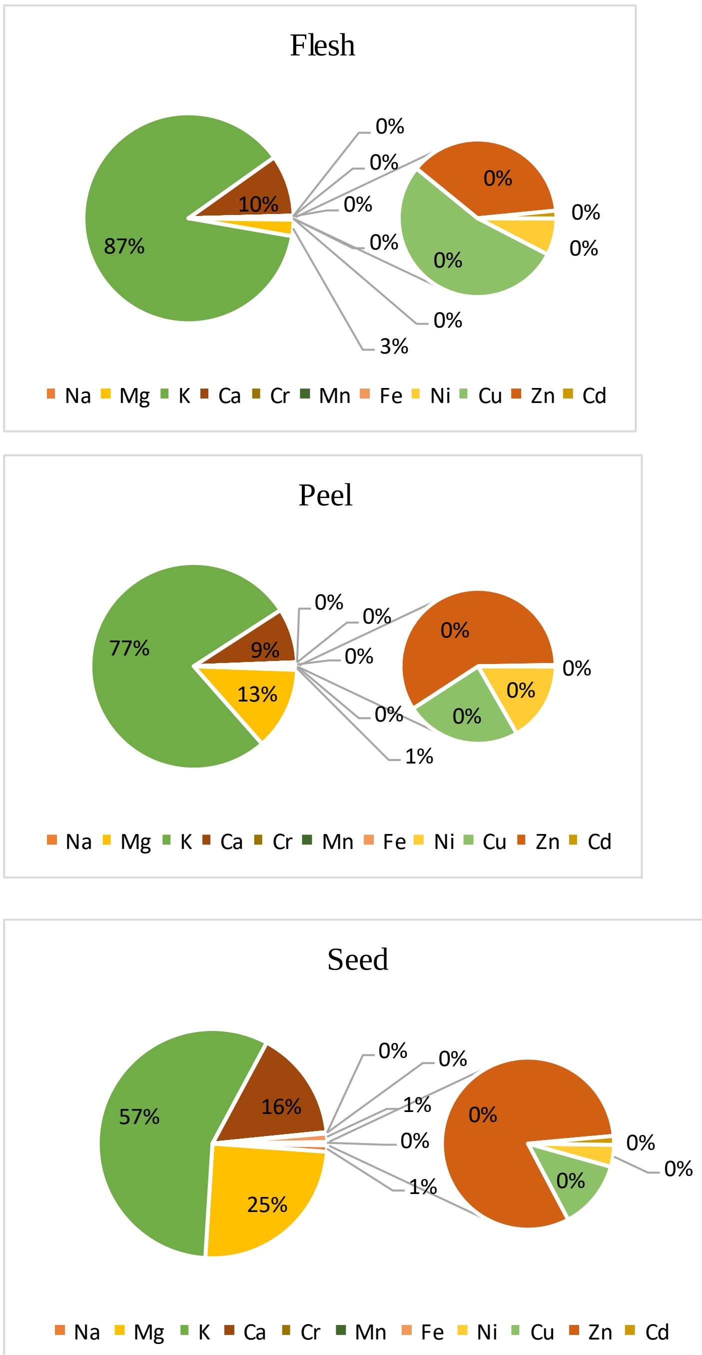


Figure 8. Graph of the overall average concentration in different pumpkin parts.

Table 10 shows that there is a variation in concentration of metals with in pumpkin parts (peel, flesh seed) and along topographical location of samples (Woliso, Minjar Shenkora and Arbaminch). This variation mainly ascribed to the stage of maturity (age) of the pumpkin fruit when collected, the mineral composition of soil and water since it collected from the compound of farmers residence and environmental factors. All selected elements are successfully determined except Cr, Ni and Cd which have low precision (%RSD >10).

4.2.1 Concentration of metals in pumpkin peel sample

The concentration pattern of metals in pumpkin peel collected from Woliso declined as K > Mg > Ca > Na > Fe > Mn > Zn > Cu > Ni > Cr > Cd. The pattern of the average concentration of metals in pumpkin peel from Minjar Shenkora was decreased as K > Ca > Mg > Fe > Na > Zn > Mn > Cu > Ni > Cr > Cd and the pattern of concentration of elements in pumpkin peel collected from Arbaminch was decreased as K > Ca > Mg > Fe > Na > Mn > Zn > Cu > Ni > Cr > Cd. Concentration of K is the highest in pumpkin peel for all places followed by Ca, Mg, Fe and Na in Minjar Shenkora and Arbaminch site where as in Woliso pumpkin peel Mg is 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 were K (27806), Ca (5268), Mg (4716), Fe (157), Na (167), Mn (28.9), Zn (24.2), Cu (814), Ni (3.79), Cr (0.28), Cd (0.24) mg/kg mg/kg (Table 10).

4.2.2 Concentration of metals in pumpkin flesh sample

As shown in Table 10, the metal concentration pattern in Woliso pumpkin flesh was K > Ca > Mg > Fe > Na > Mn > Zn > Cu > Ni > Cd > Cr; Minjar Shenkora pumpkin flesh metal concentration pattern was decreased as K > Ca > Mg > Na > Fe > Zn > Cu > Mn > Ni > Cr > Cd and for Arbaminch pumpkin flesh the pattern was K > Ca > Mg > Fe > Na > Cu > Mn > Zn > Ni > Cd > Cr. The average concentration of determined pumpkin flesh metals 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.

4.2.3 Concentration of metals in pumpkin seed sample

Table 10. Mean concentration and standard deviation of each sample

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

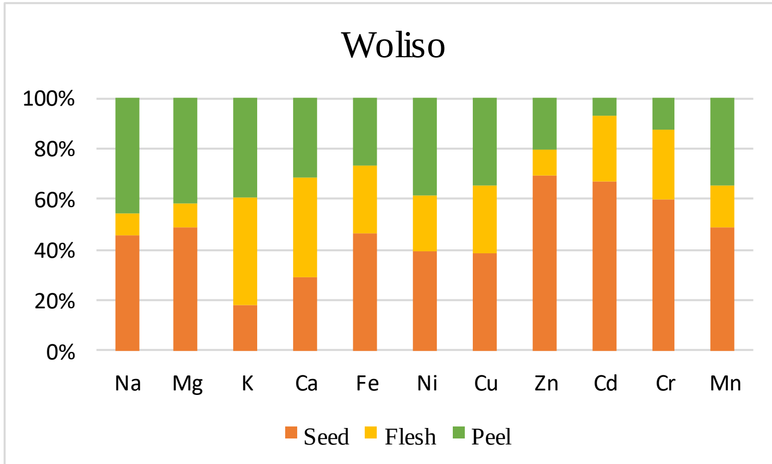
Woliso pumpkin seed concentration pattern was K > Mg > Ca > Na > Fe > Zn > Mn > Cu > Ni > Cd > Cr. Minjar Shenkora pumpkin seed metal concentration pattern is K > Mg > Ca > Fe > Zn > Na > Mn > Cu > Ni > Cd > Cr in addition Arbaminch pumpkin seed metal concentration pattern was decreased as K > Mg > Ca > Fe > Na > Zn > Mn > Cu > Ni > Cd > Cr. Pumpkin seed average metal concentration from the three sites Woliso, Minjar Shenkora and Arbaminch 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 potassium and also a good source of major, minor and trace metals that are in the order of essentiality to human beings.

4.3 Comparisons of the metal concentration of different parts of pumpkin

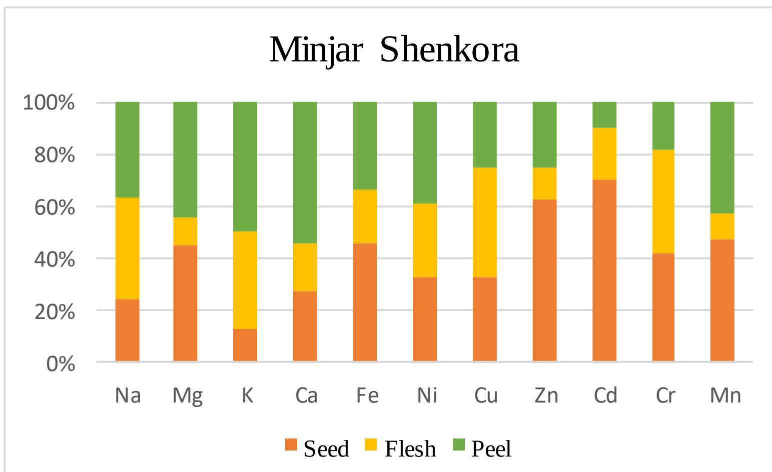
Metals are compared in the three parts of Woliso pumpkin, most metals (Cu, Zn, Fe, Mg, Na, Mn, Ni, Cr and Cd) are present in higher amounts in the pumpkin seed than in the flesh and peel. Ca and K are higher in Woliso pumpkin flesh. Figure 9a shows the concentration comparison in percentage of different parts of Woliso pumpkin samples.

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. Figure 9b shows the concentration comparition of minjar shenkora pumpkin sample.

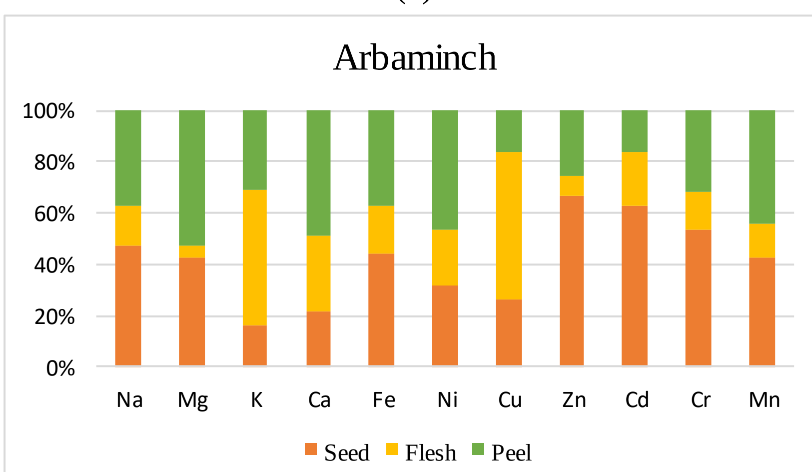
Na, Fe, Zn, Cd and Cr are in high concentration in Arbaminch pumpkin seed; Pumpkin peel of Arbaminch sample is high in Mg, Ca, Mn and Ni concentration whereas K and Cu are higher concentration in Arbaminch pumpkin flesh. Figure 9c displays the comparison of three parts of Arbaminch pumpkin sample.



(a)



(b)



(c)

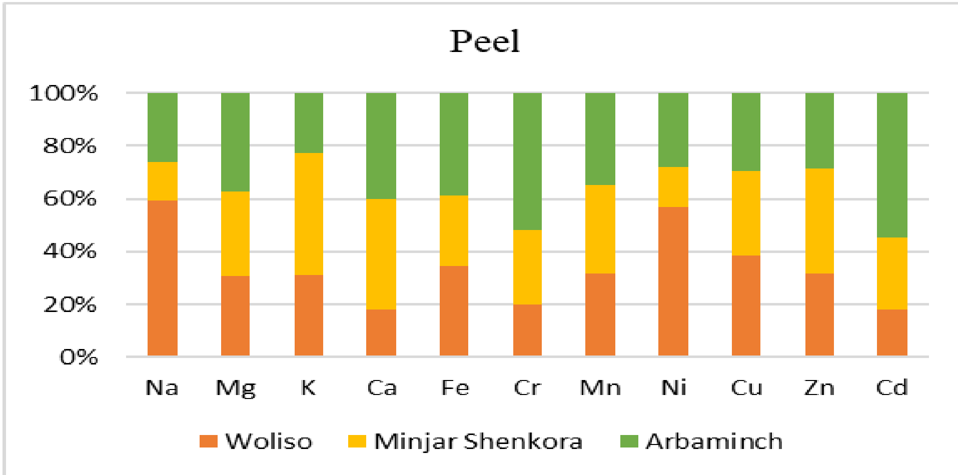
Figure 9. Comparison of concentration of metals in pumpkin parts (a) from Woliso, (b) Minjar shenkora and (c) Arbaminch.

4.4 Comparisons of metal concentration of pumpkin in different areas

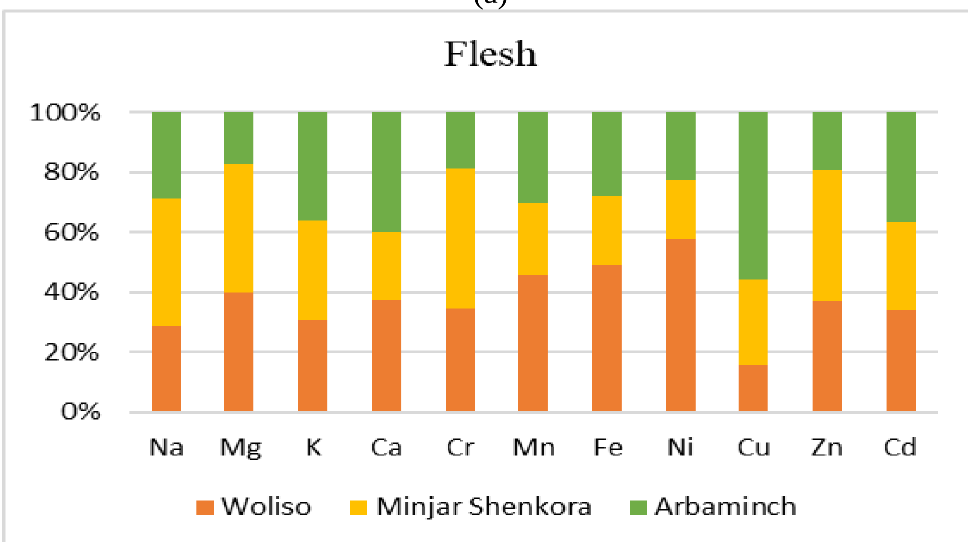
The concentration of Mg, Fe, Cr, Mn, and Cd are the highest in pumpkin peel from Arbaminch sample than Minjar Shenkora and Woliso pumpkin peel. Na, Ni and Cu are found in high concentration in Woliso pumpkin peel, while K, Ca, and Zn are in higher concentration in Minjar Shenkora pumpkin peel. In general, most minor and trace metals are in higher concentration in Arbaminch pumpkin peel sample. Figure 10a displays the comparison of pumpkin peel metal concentration from woliso, Minjar Shenkora and Arbaminch sampling areas.

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 has higher concentration in Fe, Mn and Ni than Arbaminch and Minjar Shenkora. Figure 10b shows the pumpkin flesh metal concentration comparison from three sampling areas.

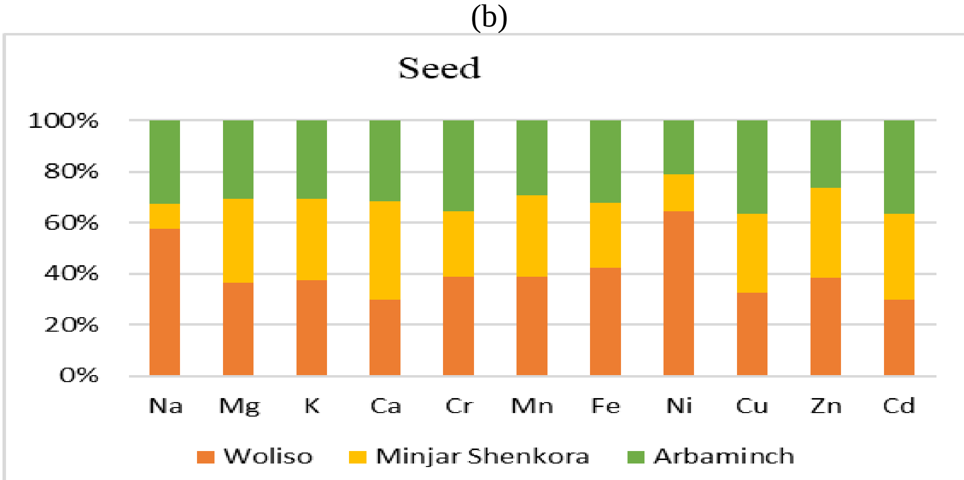
Except Ca, Cu and Cd, all determined metal concentrations in pumpkin seed from Woliso is higher than Minjar Shenkora and Arbaminch pumpkin seed. Ca is higher in Minjar Shenkora pumpkin seed while Cu and Cd are in higher concentration in Arbaminch pumpkin seed. Comparison of pumpkin seed metal concentration from three sampling areas is shown in Figure 10c.



(a)



(b)



(c)

Figure 10. Comparison of metal concentration in pumpkin parts from different area. (a) Peel, (b) flesh and (c) seed.

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

The levels of macro, micro and trace metals 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 11. From the reported data potassium is the most abundant and has highest concentration in all parts of pumpkin sample.

4.5.1 Pumpkin peel

The mean levels of Na in this study are comparable to those reported in Bangladesh indigenous pumpkin peel (Amin et al., 2019) and lower than those reported in Egypt (Hashash et al., 2017) and Bangladesh hybrid pumpkin peel (Amin et al., 2019). 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., 2017 and Hashash et al., 2019, respectively.

4.5.2 Pumpkin flesh

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. (2017), while lower than other reports from Ethiopia (Belete et al., 2018), Egypt (Hashash et al., 2017) and Bangladesh (Amin et al., 2019). Higher concentration of Mg is reported by Belete et al. (2018) 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.

4.5.3 Pumpkin seed

The concentration of Na (Table 11) in this study is lower than reported from Egypt (Hashash et al., 2017), Nigeria (Elinge et al., 2012) and Austria (Martinec et al., 2019) and comparable with other Ethiopian (Weldesenbet, 2020) and Croatian (Martinec et al., 2019) results. Mg, K, Cr, Mn, Cu and Zn concentration in this study are comparable with other report from Ethiopia (Weldesenbet, 2020). Ca is higher in this study from all other reports from Ethiopia, Egypt, Nigeria, Zimbabwe, Croatia and Slovenia.

Table 11. 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	Hashash et al., 2017
	9.65	3.35	687	1.36	_	AAS, FP (Na)	Bangladesh indigenous	Amin et al., 2019
	60.6	3.66	1233	0.96	_	AAS, FP(Na)	Bangladesh hybrid	Amin et al., 2019
	16.7	**472**	**2781**	**527**	**0.03**	**MP-AES**	**Ethiopia**	**This study**
Flesh	13.3	221	3869	439	_	AAS	Ethiopia	Belete et al., 2018
	76.5	25.1	189	5.5	_	ICP-AES, EFP	Egypt	Hashash et al., 2017
	20.8	5.64	1616	0.82	_	AAS, FP (Na)	Bangladesh indigenous	Amin et al., 2019
	24.8	4.77	1518	0.74	_	AAS, FP (Na)	Bangladesh hybrid	Amin et al., 2019
	5.6	38	139	10	_		India	Dhiman et al., 2009
	6.31	**84.8**	**2953**	**319**	**0.04**	**MP-AES**	**Ethiopia**	**This study**
Seed	7	592	809	46	_		Ethiopia	Weldesenbet, 2020
	155	60.5	224	6.65	_	ICP-AES, EFP	Egypt	Hashash et al., 2017
	1.35	4.34	435	4	_	AAS, FP (Na)	Bangladesh indigenous	Amin et al., 2019
	0.98	3.69	558	3.76	_	AAS, FP (Na)	Bangladesh hybrid	Amin et al., 2019
	170	67.4	237	9.78	_	AAS, AES	Nigeria	Elinge et al., 2012
	68	345	_	141	_	ICP-AES	Zimbabwe	Kwiri et al., 2014
	7.97	35.5	22.5	13.4	0.01	ICP-OES	Croatian	Martinec et al., 2019
	45.8	32.1	42.4	9.48	0.01	ICP-OES	Slovenia	Martinec et al., 2019
	149	115	85.5	33.6	_	ICP-OES	Austria	Martinec et al., 2019
	17.1	**467**	**1067**	**292**	**0.07**	**MP-AES**	**Ethiopia**	**This study**

Sample part	Concentration (mg/100 g)						Method	Country	Reference
	Mn	Fe	Ni	Cu	Zn	Cd			
Peel	1.25	4.95	_	0.49	0.05		ICP-AES, EFP	Egypt	Hashash et al., 2017
	0.36	4		0.03	0.15		AAS, FP (Na)	Bangladesh indigenous	Amin et al., 2019
	0.38	15.7	_	0.02	18.8	_	AAS, FP(Na)	Bangladesh hybrid	Amin et al., 2019
	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	Belete et al., 2018
	1.35	6.48	_	0.55	0.65		ICP-AES, EFP	Egypt	Hashash et al., 2017
	0.45	42.1		0.06	0.23		AAS, FP (Na)	Bangladesh indigenous	Amin et al., 2019
	0.43	4.79	_	0.06	0.21	_	AAS, FP (Na)	Bangladesh hybrid	Amin et al., 2019
	0.05	0.44	_	0.05	0.26	_		India	Dhiman et al., 2009
	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	Weldesenbet, 2020
	4.55	12.4	_	0.85	0.85		ICP-AES, EFP	Egypt	Hashash et al., 2017
	1.35	6.02		0.31	18.8		AAS, FP (Na)	Bangladesh indigenous	Amin et al., 2019
	0.98	5.51	_	0.26	16.4	_	AAS, FP (Na)	Bangladesh hybrid	Amin et al., 2019
	0.06	3.75	_	_	14.1	_	AAS, AES	Nigeria	Elinge et al., 2012
	_	12	_	_	1.24	_	ICP-AES	Zimbabwe	Kwiri et al., 2014
	0.19	2.7	_	0.74	6.77	0.14	ICP-OES	Croatian	Martinec et al., 2019
	0.18	2.66	_	0.01	0.01		ICP-OES	Slovenia	Martinec et al., 2019
	0.56	2.65	_	_	_		ICP-OES	Austria	Martinec et al., 2019
	3.34	**22.5**	**0.34**	**1.07**	**0.07**		**MP-AES**	**Ethiopia**	**This study**

4.6 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 vegetable. Concerning to this, the comparison in Table 12 is between pumpkin flesh with other vegetable types which are more consumed in Ethiopia. The comparison is done in minor and toxic metal concentration depending upon the available data. The data in Table 12 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 from all the studied sites than other vegetables.

Table 12. Comparison of pumpkin flesh metal concentrations with other vegetables those reported in the literature from Ethiopia.

Sample	Concentration (mg/100 g)							
	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Reference
Cabbage	6.20	22.6	101	1.5	10.4	40.1	0.04	Gezahegn, et al., 2017
Potato	6.66	0.9		20.4	11.3	56.7	6.01	Berihun, et al., 2021
Tomato	5.80	2.42	_	13.9	2.01	24.6	2.43	Berihun, et al., 2021
Ethiopian kale	13.3	30.6		11.1	2.41	88.3	6.25	Berihun, et al., 2021
Swiss chard	2.20	869	159	11.6	11.6	219	0.10	Gezahegn, et al., 2017
Carrot	0.82	53	403	2.44	7.68	44.9	0.06	Yeshiwas and Tadele, 2017
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

4.7 Daily intake of metals from pumpkin edible parts

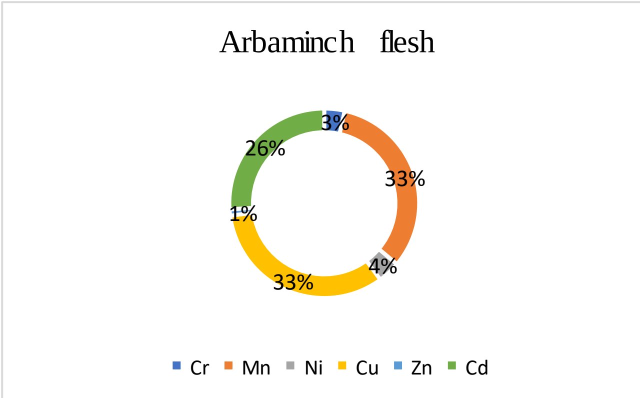
Table 13 shows the metal concentration, recommended daily intake (mg/day) for different age group and the upper limit concentration established by FAO/WHO jointly. A 0.4 kg of vegetable intake per day is recommended by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO, 2012). Assuming if 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, indicating pumpkin alone does not satisfy the daily requirement. K, Cr, Fe, Ni, Cu, Cd are higher than daily recommended values but still below the upper limit, indicating pumpkin in the selected areas is as well much sources of these elements. Mg is above the upper limit and Mn is within the recommended range.

Table 13. The average metal concentration (mg/200 g) in flesh and (mg/100 g) in seed, recommended daily intake (mg/day) and upper limit values which is recommended by FAO/WHO (2012) jointly, IOM (1997), NIHN (2013), Nordic Council of Ministers (2014).

Metals			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	17.1	1500-1900	2300	1500	1500	1500	2300	2300
Mg	170	467	60-100	220-230	310-320	400-420	350-400	65-110	350
K	5906	1067	2000-2300	2500-3000	3500-4700	3500-4700	2900	ND	ND
Ca	638	292	500-800	1300	1000-1300	1000-1300	1200	2500	3000
Cr	0.07	0.07	0.05-0.07	0.06-0.08	0.10	0.03-0.1	0.03	0.25	0.25
Mn	1.92	3.34	1.80	1.80	2.10	3.00	2-2.6	11.0	11.0
Fe	22.2	22.5	3.9-17.8	8-18	8-18	8.00	10.0	40.0	45.0
Ni	0.43	0.34	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	1.08	0.34-0.44	0.7-0.9	0.90	0.90	1.00	8.00	10.0
Zn	2.13	6.78	8-11	8-11	8.00	11.0	3.4-20	23-28	45.0
Cd	0.09	0.14	0.002-0.0031	0.004	0.01	0.06	0.01	0.03	0.20

4.8 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 Tables 14. 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 from three area were found less than unity, with HI = 0.54, 0.42, 0.54 for Woliso, Minjar Shenkora and Arbaminch flesh, respectively. HI values from pumpkin seed were = 0.73, 0.61 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 areas 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 11 shows the percentage contribution of each metal to the total hazard index value in different parts of pumpkin from three sampling sites.



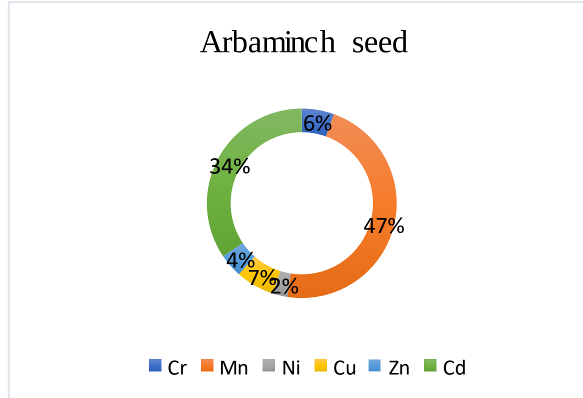


Figure 11. Percentage contribution of each metal to the total HI

Table 14. 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

	Minjar Shenkora				
Metals	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

	Arbaminch				
Metals	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 Equations (4) and (6). 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} 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. Table 15 shows the TCR due to the consumption of pumpkin flesh and seed from all sampling areas which is approximately equal to the maximum limit value (1 x 10^{-4}), indicating no risk of exposure to cancer due to the consumption of pumpkin from the selected areas. 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 (Zeng et al., 2015). Gebeyehu and Bayissa (2020) has 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 to the data obtained 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.

Table 15. Cancer risk and total cancer risk values from edible parts of pumpkin.

		Woliso	Minjar Shenkora	Arbaminch
	Edible part	CR	CR	CR
Cr	Flesh	5.1 x 10^{-5}	6.9 x 10^{-5}	2.7 x 10^{-5}
	Seed	5.5 x 10^{-5}	3.7 x 10^{-5}	5.1 x 10^{-5}
	Flesh	8.3 x 10^{-5}	7.1 x 10^{-5}	8.9 x 10^{-5}
Cd	Seed	1.1 x 10^{-4}	1.2 x 10^{-4}	1.3 x 10^{-4}
TCR	Flesh	1.3 x 10^{-4}	1.4 x 10^{-4}	1.1 x 10^{-4}
	Seed	1.6 x 10^{-4}	1.6 x 10^{-4}	1.8 x 10^{-4}

4.9 Statistical analysis

4.9.1 Analysis of variance (ANOVA)

One way ANOVA was used to compare the mean value of the metals of each part of pumpkin samples between different sampling sites. It was performed by using Microsoft excel 2016 and the results are shown in Table 16a, 16b and 16c.

Table 16a. ANOVA between and within pumpkin peel sample

Parameter	Metals										
	Na	Mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
F_{cal}	10146	340	197	1806	2.89	28.1	4.35	66.7	3.64	33.4	5.2
F_{crit}	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14
p-value	$2.6E^{-11}$	$6.7E^{-07}$	$3.4E^{-06}$	$4.6E^{-09}$	$1.3E^{-01}$	$9.0E^{-04}$	$6.8E^{-02}$	$8.0E^{-05}$	$9.2E^{-02}$	$5.6E^{-04}$	$4.9E^{-02}$

The statistical analysis in Table 16a 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.

Table 16b. ANOVA within and between samples of pumpkin flesh from different sampling areas.

Parameter	Metals										
	Na	Mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Fcal	300	13417	75	1097	61	9873	371	985	545	103	1.00
Fcrit	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14
p-value	$9.7E^{-07}$	$1.1E^{-11}$	$5.8E^{-05}$	$2.0E^{-08}$	$1.0E^{-04}$	$2.8E^{-11}$	$5.2E^{-07}$	$2.8E^{-08}$	$1.6E^{-07}$	$2.3E^{-05}$	$4.2E^{-01}$

It is shown in Table 16b that, except Cd all determined metals in this study (Na, Mg, K, Ca, Cr, Mn, Fe, Ni, Cu and Zn) are significant difference between mean of pumpkin flesh sample from three different areas.

Table 16c. ANOVA within and between samples of pumpkin seed from different sampling areas.

Parameter	Metals										
	Na	Mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Fcal	24242	263	105	427	0.98	43.9	140	345	25.2	106	7.63
Fcrit	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14	5.14
p-value	$1.9E^{-12}$	$1.4E^{-06}$	$2.1E^{-05}$	$3.4E^{-07}$	$4.3E^{-01}$	$2.6E^{-04}$	$9.2E^{-06}$	$6.4E^{-07}$	$1.2E^{-03}$	$2.5E^{-05}$	2.25E

The result of the analysis in Table 16c shows 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 the three areas.

Significant difference in the metal mean concentration of different parts of pumpkin may be due to the maturity of fruit, type of soil and the level of traditional fertilizer (dung) used, since the samples were collected from the residence compound of farmers.

4.9.2 Pearson correlation coefficient of metals

The Pearson product-moment correlation coefficient or Pearson correlation coefficient (r) is a measure of the strength of a linear association between two variables. It attempts to draw a line of best fit through the data of two variables, and indicates how far away all these data points are to this line of best fit. The Pearson correlation coefficient, *r*, covers a range of values from +1 to -1. A value of 0 indicates that there is no association between the two variables. A value greater than 0 indicates a positive association that is, as the value of one variable increases, so does the value of the other variable. A value less than 0 indicates a negative association; that is, as the value of one variable increases, the value of the other variable decreases. A value of +1 indicates a perfect positive relationship. A correlation coefficient value -1 indicates a perfect negative relationship. The high correlation coefficient near to +1 and -1 means a strong relationship between two variables.

In this study a Pearson correlation coefficient between metal concentrations of pumpkin peel samples is shown in Table 17. A perfect positive relationship (+1 correlation value) between Na/Ni, Mg/Cr, Mg/Cd, Cr/Cd and 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.

Table 17a. Pearson correlation coefficient between metal concentration of pumpkin peel sample.

	Na	Mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Na	1.00										
Mg	-0.48	1.00									
K	-0.41	-0.60	1.00								
Ca	-0.98	0.65	0.21	1.00							
Cr	-0.49	1.00	-0.59	0.66	1.00						
Mn	-0.75	0.94	-0.29	0.87	0.94	1.00					
Fe	0.40	0.61	-1.00	-0.21	0.60	0.30	1.00				
Ni	1.00	-0.43	-0.47	-0.96	-0.44	-0.71	0.46	1.00			
Cu	0.86	-0.86	0.11	-0.95	-0.87	-0.98	-0.12	0.83	1.00		
Zn	-0.50	-0.52	0.99	0.31	-0.51	-0.19	-0.99	-0.55	0.01	1.00	
Cd	-0.49	1.00	-0.59	0.66	1.00	0.94	0.60	-0.44	-0.87	-0.51	1.00

Table 17b shows the Pearson correlation coefficient between metal concentrations of pumpkin flesh samples. 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 is 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.

Table 17b. Pearson correlation coefficient between metal concentration of pumpkin flesh samples

	Na	mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Na	1.00										
Mg	0.60	1.00									
K	-0.19	-0.90	1.00								
Ca	-0.99	-0.71	0.33	1.00							
Cr	0.84	0.94	-0.69	-0.91	1.00						
Mn	-0.72	0.13	-0.55	0.61	-0.22	1.00					
Fe	-0.63	0.25	-0.65	0.51	-0.10	0.99	1.00				
Ni	-0.55	0.33	-0.71	0.43	-0.01	0.98	1.00	1.00			
Cu	-0.22	-0.91	1.00	0.36	-0.72	-0.52	-0.62	-0.69	1.00		
Zn	0.71	0.99	-0.82	-0.81	0.98	-0.02	0.10	0.19	-0.84	1.00	
Cd	-0.95	-0.82	0.49	0.98	-0.97	0.46	0.35	0.26	0.52	-0.90	1.00

The correlation depicted in Table 17c shows the Pearson correlation coefficient between metal concentration of pumpkin seed samples which is collected from Woliso, Minjar Shenkora and Arbaminch areas. The 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.

Table 17c. Pearson correlation coefficient between metal concentration of pumpkin seed samples.

	Na	mg	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd
Na	1.00										
Mg	0.65	1.00									
K	0.78	0.98	1.00								
Ca	-0.96	-0.40	-0.57	1.00							
Cr	0.95	0.38	0.56	-1.00	1.00						
Mn	0.70	1.00	0.99	-0.46	0.45	1.00					
Fe	1.00	0.70	0.83	-0.93	0.93	0.75	1.00				
Ni	0.93	0.88	0.95	-0.79	0.78	0.91	0.96	1.00			
Cu	0.20	-0.62	-0.46	-0.48	0.49	-0.56	0.12	-0.17	1.00		
Zn	0.26	0.90	0.81	0.03	-0.05	0.87	0.34	0.59	-0.89	1.00	
Cd	-0.58	-1.00	-0.96	0.31	-0.30	-0.99	-0.64	-0.83	0.69	-0.94	1.00

5 Conclusion

This study evaluated the levels of selected macro, micro and toxic metals and the non-carcinogenic and carcinogenic health risk assessment of minor and toxic metals in different parts (peel, flesh and seed) of pumpkin collected from Woliso (Oromiya, West Shewa), Minjar Shenkora (Amhara, North Shewa) and Arbaminch (SNNP, Gamo Gofa) administrative regions of Ethiopia.

The optimized wet digestion method for different parts of pumpkin was evaluated through the recovery experiment by spiking known concentration standards to solid sample and a good percentage recovery was obtained 90–106, 92–110, 90–108% for peel, flesh and seed, respectively. The overall selected 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 in higher concentration for all parts of pumpkin and the studied areas as well. The concentration levels of K, Cr, Fe, Ni, Cu and Cd obtained are higher than daily recommended values of guidelines (FAO/WHO, 2015) and this may expose to the health risk for people living around the area. The calculated non-carcinogenic risks factor (HI) for flesh and seed from the three sampling areas was below or equal to one and thus it is safe from a potential health influence for non-carcinogenic health risk. The total cancer risk (TCR) due to the consumption of pumpkin flesh and seed through Cr and Cd from all sampling areas are between 1.1×10^{-4} to 1.8×10^{-4} which is equivalent to the maximum limit value of 1×10^{-4} indicates the non-risk of exposure to cancer due to the consumption of pumpkin from the selected areas. According to ANOVA results, the level of some metals between and within the sampling sites showed significant difference which could be ascribed to 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 showed strong relationship.

6 References

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