

ASSESSMENT OF THE ELEMENTAL PROFILE OF LEAFY VEGETABLES BY SYNCHROTRON-RADIATION-INDUCED ENERGY DISPERSIVE X-RAY FLUORESCENCE SPECTROSCOPY

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The nutrient profiling of green leafy vegetables is largely concentrated on biochemical assays and their elemental composition is often overlooked. At the same time, the investigation of the elemental composition of plants is essential, as they are required in several metabolic processes for the normal growth and development of the human body and their deficiency can lead to several clinical disorders. In this paper, we consider the potential of the synchrotron-radiation-induced energy dispersive X-ray fluorescence spectroscopy technique as a rapid, sensitive, and simultaneous multielemental detection tool to investigate the elemental composition of different elements present in some leafy vegetables: dill, fenugreek, mustard, and chenopodium. The X-ray fluorescence spectra of the leaves of dill, fenugreek, mustard, and chenopodium were excited by synchrotron X-ray radiation having an energy of 15 keV and recorded in the energy range <20 keV. The recorded spectrum shows the presence of potassium, calcium, manganese, iron, nickel, copper, zinc, arsenic, and selenium with varying concentrations in different leafy vegetables. PyMca software was applied to determine the concentration of the various detected elements. The relative quantitative comparison of the detected elements shows that chenopodium leaves are a rich source of potassium among all the leafy vegetables studied. The leaves of mustard and chenopodium are abundant in calcium, while the leaves of dill and fenugreek have a higher content of trace elements like manganese, iron, copper, nickel, selenium and zinc. Herein, the role of the detected elements in human and plant health is also described.

Keywords: synchrotron radiation, X-ray fluorescence, leafy vegetables, elemental analysis.

Introduction. Green leafy vegetables are widely present in Indian cuisine, as they are readily available, affordable, and provide a range of dietary benefits. Therefore, they are consumed by the inhabitants of the low and middle income group of countries because at the consumer level these are a source of low caloric food, fibers, antioxidants, phytochemicals, vitamins, minerals, and trace metals that provide immense health benefits beyond basic nutrition and are also a cheap source of energy [1]. Being rich in health protecting compounds and elements, they have garnered immense attention for its consumption in daily diets in order to alleviate the problem of malnutrition [2]. The phytochemicals present in the leaves of green vegetables are reported to be effective in the treatment of cancer, diabetes, cardiovascular diseases, and hypertension [3]. The antioxidant compounds present in the green leaves have the capability to prevent oxidative degradation [4, 5]. Green leafy vegetables such as dill (soya), fenugreek (methi), mustard (sarson), and chenopodium (bathua) are available seasonally and often incorporated in the diet as they are a rich source of several nutrients, vitamins, β -carotene, ascorbic acid, riboflavin, folic acid, and minerals [6]. The dill plant is a glabrous aromatic plant rich in nutritive mineral components. It has different applications in biological and pharmacological studies because of its antimicrobial, antioxidative, and anti-spasmodic activities [7]. The mustard leaf is one of the most nutritious green leafy vegetables and an excellent source of dietary fiber and vitamins A, B, C, E, and K [8, 9]. Further, fenugreek leaves exhibit hypoglycemic, hypercholesterolemic, antioxidative, laxative, and fungicide effects [10]. Similarly, chenopodium is a fast-growing plant with numerous health benefits such as diuretic, laxative, sedative, anthelmintic, hepatoprotective, and antiparasitic properties [9, 11, 12].

Minerals and phytonutrients, like calcium, potassium, magnesium, sodium, iron, copper, cobalt, zinc, manganese, chromium, and selenium, are required for the growth and maintenance of the human body. These minerals are part of the enzymatic reaction and hormones. The insufficient intake of these minerals might be responsible for their nutritional deficiency.

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Several factors directly or indirectly influence the levels of mineral intake from place to place. [13]. Due to changing environmental conditions and anthropogenic activities over the years, the agricultural soil faces the risk of heavy metal, organic, and inorganic contamination. The contaminants can change the composition of elements and compounds in the plant as well as lead to the accumulation of heavy metals in them [14]. These issues raise serious concerns regarding the quality control of food products [15]. Therefore, it becomes a prime necessity to identify the possible sources of these phytonutrients and minerals and screen their mineral and nutrient content using appropriate techniques and experimental protocols.

The biological samples have high moisture content and spatially heterogeneous composition. So, a number of experimental problems exist during the elemental investigation of biological materials [16]. It is difficult to analyze trace elements in a matrix that has major concentration of hydrogen, carbon, and oxygen. Also, biological systems are not readily compatible with the vacuum system and require plenty of sample preparation during the metal and trace element analysis [17]. The established methods of the elemental analysis of biological samples like atomic absorption spectroscopy (AAS), inductively coupled plasma spectroscopy (ICPS), and graphite furnace atomic absorption spectroscopy have several drawbacks. AAS and ICPS require a relatively large amount of samples in solid form, which have to be brought in liquid form by complex and expensive techniques. Also, sample digestion procedures dilute the elements of interest below the detection limits of instruments [17–19]. Thus, there is a need to analyze the potential of non-destructive techniques for the investigation of the elemental composition of biological samples. The developments in the field of X-ray fluorescence (XRF) spectroscopy can be utilized for overcoming these limitations, as it offers nondestructive, simultaneous multi-elemental detection without the need to digest the sample and change its physical state [16]. X-ray fluorescence uses a high energy X-ray that is irradiated onto the sample to create vacancies in the inner shells of atoms of the sample. The filling of these vacancies by the jump of electrons from the outer shells leads to the emission of characteristic X-rays that can be detected and analyzed to get the qualitative and quantitative analysis of the elemental concentration of the sample with high sensitivity [20]. Despite being a versatile technique for elemental detection, XRF using conventional sources has some limitations such as poor elemental sensitivity. These limitations can be largely resolved by replacing the conventional X-ray sources by a synchrotron radiation source [16]. Synchrotron radiation is highly polarized, collimated, bright, and intense. These properties of synchrotron radiation have revolutionized the quality of XRF to detect very small numbers of atoms with great sensitivity [21]. Synchrotron-radiation-induced XRF allows rapid analysis of the plant material with adequate sensitivity (its limit lies in the femtogram range), requiring only minimal sample preparation and a relatively short analysis time [16, 21, 22].

The aim of the present study is to demonstrate the potential of synchrotron-radiation-induced X-ray fluorescence for the elemental investigation of the commonly used leafy vegetables: dill, fenugreek, mustard, and chenopodium. For this, synchrotron-radiation-induced energy dispersive XRF spectra (SR-EDXRF) of the pellets of the powder of dill, fenugreek, mustard, and chenopodium leaves were recorded using a micro XRF BL-16 beamline at Indus-2, RRCAT, Indore with an incident beam energy of 15 keV. The findings about leafy vegetables are reported in the present account by acquiring XRF spectra in the energy region <20 keV.

Experimental Detail. The leaves of dill, fenugreek, mustard, and chenopodium were collected from a local farm at Allahabad, India. The leaf samples were washed thoroughly with double distilled water to remove surface impurities and then dried at room temperature. In order to remove water and other volatile materials having low temperature thresholds, the leaves were heated at 80°C in a hot air oven and ground to a fine powder. The oven drying of biosamples results in the oxidation of the organic material present in them. During this process, the carbon, hydrogen, nitrogen, and sulfur present in the leaves are converted into CO₂, H₂O, NH₃, and SO₂, respectively, and liberated. Further, 250 mg of the sample fine powder was pressed in a 2-ton semi-automatic hydraulic pellet press to obtain thick pellets having 1.5-cm diameter. The pellet samples were placed on the sample stage for the SR-XRF analysis. The XRF measurements were made on a synchrotron radiation microfocus XRF facility at the beam line 16 (BL-16), Indus-2, RRCAT, Indore. The geometry of the experimental setup used for the XRF measurements is shown in Fig. 1.

A combination of water cooled (S1) and uncooled (S2) four blade slits was used to reduce the scattering of the X-ray background. The double crystal monochromator (DCM) and a pair of symmetric and asymmetric Si (111) crystals (mounted side by side) were used for the monochromatization of the incident beam, while the beam spot was micro-focused on the sample by employing a pair of Kirkpatrick–Baez focusing optics consisting of Pt coated bendable mirrors. The sample was mounted on a sample holder and placed on the sample stage at an angle of 45° with respect to the incident X-ray beam direction, while the detector was placed at 90° angle with respect to the incident beam in the perpendicular horizontal

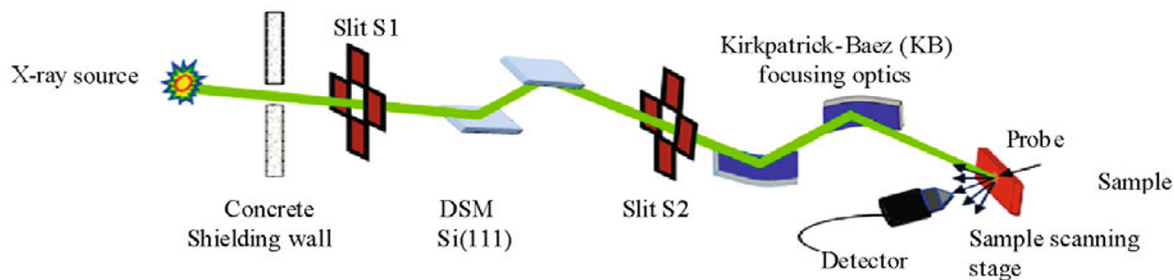


Fig. 1. Schematic of experimental setup used for the synchrotron induced X-ray fluorescence measurement.

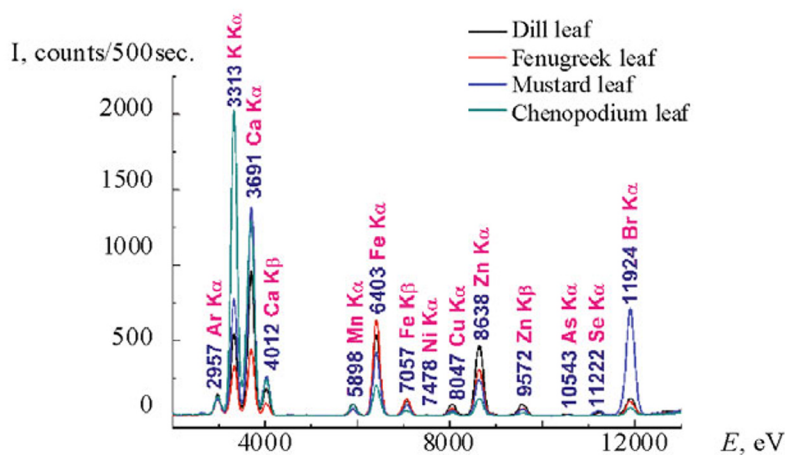


Fig. 2. Recorded synchrotron radiation induced X-ray fluorescence spectra of different leafy vegetables.

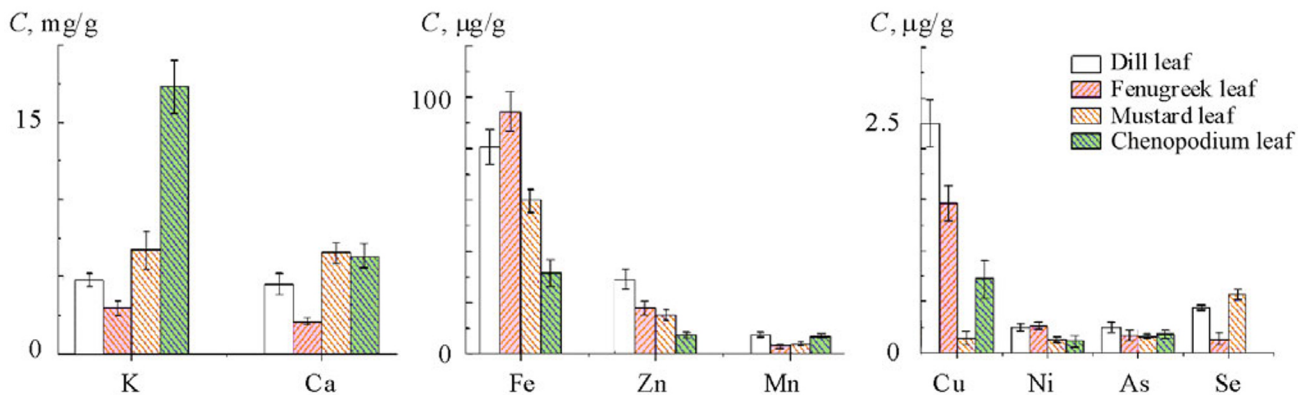


Fig. 3. Variation of the concentration of elements in the leafy vegetables.

plane. The detector was placed at a distance of 31 cm from the sample. An incident X-ray energy of 15 keV having incident photon flux (I_0) $\sim 10^7$ photon s^{-1} mm^{-2} was allowed to fall on the sample and used for exciting the XRF. The emitted XRF was collected by a Vortex (SII, Nano, USA) energy dispersive spectroscopy detector at an energy resolution of ~ 138 eV at 5.898 keV (Mn- K_α X-ray) energy and a 550 s acquisition live time. The energy of the X-ray lines of elements and their concentration were obtained by analyzing the XRF spectra by PyMca 5.1.2 open source software program. The background of the spectra was corrected, and the peaks were fitted in the Gaussian mode by the least square fitting algorithm. The primary

beam parameters were used for the normalization of the area of the X-ray lines. The information about the geometry of the setup, detector parameters, acquisition live time, active detector area, sample to detector distance, and absorbers between the sample and the detector (air and beryllium window, etc.) were used for calculating the concentration of the detected elements. "Short tail," "Stripping," "Escape peaks," "Sum peaks," and "Scattering peaks" were also included in the analysis of the concentration [23]. The concentration of elements was estimated by including the intensities of both K_{α} and K_{β} lines. The results of this study are expressed in the form of the mean value \pm and standard deviation of the 27 samples.

Results and Discussion. Figure 2 presents the recorded energy dispersive XRF spectrum of the pellets of the leaves of dill, fenugreek, mustard, and chenopodium covering the energy range <20 keV. It contains various strong and weak characteristic X-ray lines of the elements present in the leafy vegetables. The strong emission lines belong to the major constituents, whereas the weak lines come from the trace species. The detected elements along with the observed energy of the X-ray lines are marked in Fig. 2. The spectrum shows the K_{α} lines of potassium, calcium, manganese, iron, nickel, copper, zinc, and arsenic, which confirms the presence of these elements in the leaf of dill, fenugreek, mustard, and chenopodium. In addition to these lines, the spectra also display the presence of the X-ray line of bromine. The appearance of the X-ray line of bromine in the spectra of leafy vegetables might be due to the interference caused by the atmosphere and has no relationship with the elemental composition of the sample. The XRF measurements in this study are limited by the excitation energy. The fluorescence of the leafy vegetable samples was excited by the incident X-ray beam having an energy of 15 keV, and the samples excited by this energy do not contain the X-ray lines of elements having atomic number <13 . Elements with low atomic number have low fluorescence yield as X-rays from these elements are absorbed strongly on the surface of the sample due to the high attenuation coefficient, making the analysis of low atomic number elements virtually impossible [16].

The determined concentration of the elements present in the dill, fenugreek, mustard, and chenopodium leaves is listed in the Table 1, and the variation of concentration of elements in these leafy vegetables is shown in Fig. 3. The data depict the variation in the concentration of the detected elements in different leafy vegetables. The SR-EDXRF spectrum of the leaves of fenugreek, dill, chenopodium, and mustard shows the presence of the K_{α} line of calcium at 3691 eV with its intensity varying in each sample. A comparison of the concentration of calcium in all the investigated pellets reveals that calcium is found in maximum quantity in the mustard and chenopodium leaves (6.53 and 6.33 mg/g, respectively), while it is observed to be in a smaller quantity in the fenugreek and dill leaves (4.49 and 2.09 mg/g). Calcium is an important structural component of the cell wall and cell membrane in plants. It is essential for the maintenance of the integrity of the cell wall, as it is required for the formation of calcium pectate. It acts as a counter cation for organic and inorganic anions in the vacuole. It is required for the development of meristematic regions of roots and shoots. It also acts as an intracellular messenger in the cytosol and promotes pollen tube growth and plant elongation. Calcium is also involved in the enzymatic activities of α -amylase, ATPase, phospholipase, esterase, pyruvate kinase, glucose-6-phosphate, and arginine kinase. Moreover, it is also involved in maintaining the structure of chromosomes as well as for the regulation of the anaphasic movement of chromosomes [24–28]. Calcium is a key component in the bones, teeth, and extracellular serum. There are reports that describe the involvement of calcium in vascular contraction, muscle functions, conduction of nerve impulses, vasodilation, intracellular signalling, and hormonal secretion. It also plays an important role in the cation–anion balance in the osmoregulation of cells and acts as an activator of several enzyme systems in protein synthesis and carbohydrate transfer [29]. Calcium also aids in regulating heartbeat and triggers the formation of blood clots.

The SREDXRF spectra of leafy vegetables depict another strong peak at 3313 eV, which is a characteristic K_{α} line of potassium. Its intensity also varies in the spectrum of different leaves. A quantitative comparison shows that the mustard and chenopodium leaves contain 6.72 and 17.33 mg/g of potassium while the fenugreek and dill leaves contain 4.74 and 2.94 mg/g of potassium. Potassium is an indispensable macronutrient of the plant that plays important regulatory roles in the physiological processes like assisting in the germination of seeds and seedling emergence by initiating the rapid imbibition of water, cation–anion balance, synthesis of proteins, carbohydrate translocation and metabolism, phloem transport, and osmoregulation. In addition, potassium also helps in the transport of water and minerals through the xylem. It plays an important role in activating the ATP synthase enzyme during the process of photosynthesis. It helps in the regulation of the stomatal activity and balances the CO_2 entry and the removal of water vapor from the intercellular spaces of plants. It also helps in balancing the nutrients in plants as it helps in activating nitrate reductase and starch synthase enzymes. Potassium plays a key role in mitigating various stresses like metal toxicity, chilling, drought, and salinity [30–34]. Potassium is one of the most important and abundant macronutrients in the human body after calcium and phosphorus. It assists in a range of important body functions such as maintaining blood pressure, body fluid, and electrolyte balance. It is also essential for

TABLE 1. Observed X-ray K_{α} Lines, Detected Elements, and their Concentration in the Leafy Vegetables

Observed X-ray K_{α} line, eV	Detected element	Concentration, $\mu\text{g/g}$			
		Dill leaf	Fenugreek leaf	Mustard leaf	Chenopodium leaf
3313	K	$4.74 \pm 0.51^*$	$2.94 \pm 0.44^*$	$6.72 \pm 1.22^*$	$17.33 \pm 2.75^*$
3691	Ca	$4.49 \pm 0.71^*$	$2.09 \pm 0.24^*$	$6.53 \pm 0.65^*$	$6.33 \pm 0.74^*$
5898	Mn	7.27 ± 1.12	3.2 ± 0.78	3.99 ± 0.70	7.17 ± 0.82
6403	Fe	80.58 ± 6.73	94.5 ± 7.69	60.03 ± 4.45	31.64 ± 5.16
7478	Ni	0.24 ± 0.04	0.26 ± 0.03	0.13 ± 0.03	0.11 ± 0.06
8047	Cu	2.25 ± 0.73	1.47 ± 0.27	0.14 ± 0.06	0.72 ± 0.29
8638	Zn	28.93 ± 4.34	18 ± 2.54	15.23 ± 2.26	7.55 ± 1.15
10543	As	0.24 ± 0.05	0.17 ± 0.05	0.16 ± 0.02	0.18 ± 0.04
11222	Se	0.44 ± 0.02	0.13 ± 0.06	0.57 ± 0.05	–

*Concentration in milligrams.

normal nerve impulse conduction and muscle contraction. Electrolytes like potassium are very important for the normal functioning of the cardiac muscle fiber, and any imbalance in the electrolyte concentration in the human body can increase the chances of a heart attack. The intestinal muscles rely on potassium along with other minerals like sodium, calcium, and magnesium for normal gastrointestinal motility.

Trace metals have garnered attention during the recent years as they play an important role in chemical, biological, biochemical, metabolic, catabolic, and enzymatic reactions in the living cells of plants, animals, and human beings. The trace elements such as Mg, Zn, Fe, Cu, Ni, and Se were detected in the leafy vegetables under consideration. The concentration of manganese is observed to be 7.27, 3.2, 3.99, and 7.17 $\mu\text{g/g}$ in the dill, fenugreek, mustard, and chenopodium leaves, respectively. Manganese is an important micronutrient in both plants and animals. Manganese plays an important role in respiration, photosynthesis, pathogen defense, scavenging reactive oxygen species, and hormone signalling. It also plays important roles in histidine synthesis, DNA repair, chloroplast development, phospholipid synthesis, Ca^{2+} signalling purine, and urea catabolism. It is an important element of the metalloenzyme cluster of the oxygen-evolving complex (OEC) in photosystem II (PSII) and is involved in the water-splitting reaction in PSII. Manganese is also required for protein glycosylation and pectin and hemicellulose biosynthesis. It plays an active role in activating NAD malic decarboxylases, phosphoenolpyruvate carboxylase, PP2C phosphatases, and IAA-amino acid conjugate hydrolases. Manganese is also a cofactor of phenylalanine ammonia-lyase (PAL), which is involved in the synthesis of lignin. It also helps in the detoxification of reactive oxygen species as a cofactor of manganese superoxide dismutase [35–39]. In human beings, manganese is an essential component of enzymes and antioxidants that help in controlling the nervous system functions, blood sugar, and the cholesterol level [40]. It has been established that the permissible limit of the manganese daily intake should be 0.2 $\mu\text{g/g}$. In order to fulfill these requirements, green leafy vegetables can serve as a good source in comparison with other vegetables [41].

The result also shows that dill leaves are rich in zinc as compared to fenugreek, mustard, and chenopodium leaves. The concentrations of zinc in the leaf of dill, fenugreek, mustard, and chenopodium are 28.93, 18, 15.23, and 7.55 $\mu\text{g/g}$, respectively. Zinc is one of the essential micronutrients required by both plants and humans. Zinc is an important component of carbonic anhydrase (CA) and is involved in carbohydrate metabolism. It is also involved in protein metabolism, which is related to the stability and function of genetic materials. It is essential for chromatin proteins like TFIIIA protein and g32p protein, which are linked to transcription and replication. It also acts as an activator of Zn-activated kinase, capable of chemically modifying histone and nonhistone proteins. Zinc is required for maintaining auxin in an active state and is also involved in maintaining the integrity and stabilization of cellular membranes by interaction with phospholipids and the sulfhydryl groups of the membrane protein. In addition, it also exerts an inhibitory action on the membrane damage catalyzed by O_2^- -generating NADPH oxidase [42–44]. In humans, zinc is involved in various enzymatic activities and the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids [45]. It also plays an important role in polynucleotide transcription, translation, and genetic expression. The literature shows that the daily intake of zinc for

humans should not be less than nor more than 2.5 $\mu\text{g/g}$. In fact, zinc is absorbed more readily from animal sources than plant sources due to the phytic acid content [46]. Despite this, green leafy vegetables can also be a modest source of zinc.

Dill leaves also show the rich presence of copper, as the copper content in the dill leaves is found to be 2.25 $\mu\text{g/g}$. The copper content of the other leafy vegetables is relatively low in comparison to dill leaves. For instance, fenugreek, mustard, and chenopodium leaves contain 1.47, 0.14, and 0.72 $\mu\text{g/g}$ copper, respectively. Copper is crucial for both plants and animals. It is an important structural element in regulatory proteins. Copper also participates in mitochondrial respiration, cell wall metabolism, photosynthetic electron transport, oxidative stress responses, and hormone signaling. It is a cofactor of enzymes like amino oxidase, Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, laccase, plastocyanin, and polyphenol oxidase. It is also a redox-active transition metal and plays an important role in iron mobilization, oxidative phosphorylation, signaling of transcription, and protein trafficking [47–50]. In humans, copper is important for the functioning of many copper-containing metalloenzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasmin, superoxide dismutase, and dopamine. The daily intake of copper by humans should not be less than 2.8 $\mu\text{g/g}$. Copper is also involved in protein metabolism, hemopoiesis, erythropoiesis, connective tissue metabolism, nerve conduction, immune functions, and hormone synthesis [51].

Further spectral observations show that iron is observed to be in a higher concentration in the leaves of fenugreek (94.5 $\mu\text{g/g}$) than in the leaves of dill (80.56 $\mu\text{g/g}$), mustard (60.03 $\mu\text{g/g}$), and chenopodium (31.64 $\mu\text{g/g}$). Iron is an essential micronutrient of plants, required during chlorophyll synthesis, maintenance of the chloroplast structure and function, nitrogen fixation, DNA synthesis, respiration, and photosynthesis. It is a prosthetic group constituent of many enzymes like cytochromes, catalases, and peroxidases and is required for the activation of the metabolic pathways of plants. Due to its physicochemical properties, it has high affinity to active metalloprotein sites and acts as a cofactor in redox reactions, required for oxygen production and use. It is a cofactor of many enzymes, required during plant hormone synthesis like lipoxygenase, 1-aminocyclopropane acid-1-carboxylic oxidase, abscisic acid, and ethylene [52–55]. It also participates in a number of metabolic processes including oxygen transport, DNA synthesis, and electron transport. It has been recommended that the daily intake of iron should be up to 5 $\mu\text{g/g}$ [56, 57]. Iron is an indispensable part of hematopoiesis. It is also bound in hemoglobin and other proteins such as transferrin and ferritin. The enzymes participating in the production of new cells, amino acids, hormones, and neurotransmitters are also dependent upon the availability of iron. Green vegetables are a good source of iron and is available at a low cost, and cooking in an iron utensil would be an effective strategy to increase the iron intake as recommended for anemia convalescence [58].

The results also show the presence of selenium in the leaves under investigation. The quantitative comparison reveals that selenium is seen to be in a higher concentration in the leaves of dill and mustard (0.44 and 0.57 $\mu\text{g/g}$, respectively) and in a lower concentration in the fenugreek leaf (0.13 $\mu\text{g/g}$). However, its presence is not detected in chenopodium leaves. Selenium is a biologically active trace metal that exerts a positive effect on the plant growth and stress tolerance at a low concentration [59]. It has been reported that selenium should not be less than 0.14 $\mu\text{g/g}$ in the human daily diet. The dietary intake of a low level of selenium induces oxidative stress-related conditions. Selenium is necessary for the maintenance of the normal growth and development of human. It is a major structural component of enzymes like glutathione peroxidase, thioredoxin reductase, and deiodinases that play an important role in cellular function, signal transduction, reproduction, tumor prevention, and muscle and brain function [60]. The results of this study show that dill and mustard leaves are a significant means for selenium supplementation in the human body [61].

The spectral analysis also shows the presence of nickel in the leaves. The amount of nickel is observed to be higher in the fenugreek leaf (0.26 $\mu\text{g/g}$) than in the dill leaf (0.24 $\mu\text{g/g}$). The leaves of fenugreek and chenopodium have a very low concentration of nickel. The concentration of nickel in the fenugreek leaf is 0.13 $\mu\text{g/g}$, while that in the chenopodium leaf is 0.11 $\mu\text{g/g}$. Nickel is regarded as an essential plant micro-nutrient. It is an activator of urease enzyme and plays an important role in hormonal activities and lipid metabolism. It is also reported that nickel also activates an isoform of glyoxalase I enzyme that helps in the degradation of the cytotoxic compound methylglyoxal (MG). In addition, it also plays an important role in phytoalexin synthesis and plant disease resistance [62, 63]. Despite its utility as a micronutrient, nickel has been identified as a pollutant and toxicant beyond the threshold level. Excessive doses of nickel have been reported to cause hypersensitivity, genotoxicity, teratogenicity, immunotoxicity, and hematotoxicity [62, 63]. Therefore, it is recommended that the concentration of nickel in the human diet should not exceed 0.02 $\mu\text{g/g}$. At elevated concentrations, nickel exposure may induce free radical generation in the human body, which leads to oxidative damage and affects kidney and liver functions. The accumulation of this heavy metal in the human body also induces the risk of lung cancer, cardiovascular diseases, neurological disorder, and high blood pressure [64, 51].

The result of this study also shows that the leaves of dill, fenugreek, mustard and chenopodium accumulate arsenic at a low concentration. The leaves of dill display the maximum concentration of arsenic (0.24 µg/g), while those of dill, fenugreek, and mustard have a relatively smaller concentration of it (0.17, 0.16, and 0.18 µg/g, respectively). Arsenic is an extremely toxic metalloid that causes nausea, vomiting, diarrhea, weakness, loss of appetite, cough, and headache when inhaled for a relatively short period of time, while the long-term intake is responsible for cardiovascular diseases and diabetes. Arsenic poisoning affects the bone marrow and the cellular elements of blood [64]. The recommended permissible limit for it is below 3 µg/g in the human diet [65]. One of the possible sources of arsenic in the human diet is plants and their derived products. Arsenic is absorbed by the plant tissues, and its uptake upsets the plant metabolism and interferes with the normal growth of the plant [66]. It is released in the environment by the smelting of arsenic containing ores, coal burning, and various applications such as fungicides, insecticides, herbicides, pesticide, and preservative [7, 9, 10, 67]. The results of the present study show that the synchrotron-radiation-induced X-ray fluorescence technique proves to be a promising probe for simultaneous multielemental analysis in different species of leafy vegetables, and this technique can be a better alternative to other destructive analytical techniques.

Conclusions. The results of the present study demonstrate the applicability of synchrotron-radiation-induced X-ray fluorescence as a rapid data generation, sensitive, and multielement detection technique for the analysis of biological samples. This technique provides information about the mineral, trace metal, and heavy metal compositions of the plant sample in any physical state. The results also give important insights into the elemental profile of dill, fenugreek, mustard, and chenopodium leaves. The data show that the leaves of chenopodium are abundant sources of macronutrients like potassium, while the leaves of mustard and chenopodium are a good source of calcium in the human diet. In contrast, the leaves of dill and fenugreek are rich in trace elements like manganese, iron, copper, zinc, nickel, and selenium. The detected elemental profile of leafy vegetables using an experimental approach involving SRXRF can add to the existing knowledge on nutrients.

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REFERENCES

1. S. K. Chang, K. Nagendra Prasad, and I. Amin, *Int. Food Res. J.*, **20**, 457–465 (2013).
2. K. Shridhar, P. K. Dhillon, L. Bowen, S. Kinra, A. V. Bharathi, D. Prabhakaran, K. S. Reddy, and S. Ebrahim, *Nutr. J.*, **13**, 1–9 (2014).
3. R. K. Yadav P. Kalia, R. Kumar, and V. Jain, *Int. J. Agric. Food Sci. Technol.*, **4**, 707–712 (2013).
4. J. A. Morales-Gonzalez, *Maria de Lourdes Reis Giada*, **4**, 87–112 (2013).
5. R. L. Pollock, *Global J. Med. Res.*, **16**, 31–39 (2016).
6. S. Gupta and J. Prakash, *Plant Foods Hum. Nutr.*, **64**, 39–45 (2009).
7. M. M. Saleh-e-in, A. Sultana, M. A. Hossain, M. Ahsan, and S. K. Roy, *J. Sci. Ind. Res.* **43**, 483–494 (2008).
8. N. A. Anjum, I. Ahmad, M. E. Pereira, A. C. Duarte, S. Umar, and N. A. Khan, The plant family Brassicaceae: contribution towards phytoremediation, *Environ. Pollut.*, **21**, Springer Science (2012).
9. L. Renthlei, K. B. Singh, M. Sudarshan, S. S. Ram, and N. M. Singh, *Indian J. Nutr.*, **3**, 1–6 (2016).
10. M. Al-Habori and A. Raman, *Pharmacological properties of Fenugreek — The Genus Trigonella*, Ed. G. A. Petropoulos, Taylor and Francis, London–New York, **10** (2002), pp. 163–182.
11. M. G. Bomkazi, C. Njume, N. I. Goduka, and G. George, *2nd Int. Conf. Nutrition and Food Sciences*, **53**, 97–102 (2013).
12. S. Ramteke, B. L. Sahu, N. S. Dahariya, K. S. Patel, B. Blazhev, and L. Matini, *J. Environ. Protect.*, **7**, 996–1004 (2016).
13. J. Nouri, N. Khorasani, B. Lorestani, M. Karami, A. H. Hassani, and N. Yousefi, *Environ. Earth Sci.*, **59**, 315–323 (2009).
14. M. Intawongse and J. R. Dean, *Food Addit. Contam.*, **23**, 36–48 (2006).
15. J. O. Olowoyo, O. O. Okedeyi, N. M. Mkolo, G. N. Lion, and S. T. R. Mdakane, *S. Afr. J. Bot.*, **78**, 116–121 (2012).
16. A. S. Bharti, S. Sharma, N. Shukla, M. K. Tiwari, and K. N. Uttam, *Natl. Acad. Sci. Lett.*, **40**, 373–377 (2017).
17. K. W. Jones, B. M. Gordon, A. L. Hanson, and W. M. Kwiatek, J. G. Pounds, *Ultramicroscopy*, **24**, 113–328 (1988).

18. S. Sharma, N. Shukla, A. S. Bharti, and K. N. Uttam, *Natl. Acad. Sci. Lett.*, **41**, 65–68 (2018).
19. N. Shukla, A. S. Bharti, S. Srivastava, and K. N. Uttam, *Natl. Acad. Sci. Lett.*, **40**, 47–51 (2017).
20. H. R. Verma, In: *Atomic and Nuclear Analytical Methods*, Springer, Berlin, Heidelberg (2007).
21. G. M. Hettiarachchi, E. Donner, and E. Doelsch, *J. Environ. Qual.*, **46**, 1139–1145 (2017).
22. M. K. Tiwari, P. Gupta, A. K. Sinha, S. R. Kane, A. K. Singh, S. R. Garg, C. K. Garg, G. S. Lodha, and S. K. Deb, *J. Synchrotron Radiat.*, **20**, 386–389 (2013).
23. V. A. Sole, E. Papillon, M. Cotte, Ph. Walter, and J. Susini, *Spectrochim. Acta B*, **62**, 63–68 (2007).
24. P. J. White and M. R. Broadley, *Ann. Bot.*, **92**, 487–511 (2003).
25. K. Thor, *Front. Plant Sci.* (2019), <https://doi.org/10.3389/fpls.2019.00440>.
26. R. G. W. Jones and O. R. Lunt, *Bot. Rev.*, **33**, 407–426 (1967).
27. W. A. Albrecht, *Plant Soil.*, **33**, 361–382 (1970).
28. H. G. Burstrom, *Biol. Rev.*, **43**, 287–316 (1968).
29. R. S. Uchida, In: *Plant Nutrient Management in Hawaii Soils*, Manoa College of Tropical Agriculture and Human Resources, eds. J. A. Silva, R. S. Uchida, University of Hawaii at Manoa (2000), pp. 31–55.
30. M. Hasanuzzaman, M. H. M. B. Bhuyan, K. Nahar, M. S. Hossain, J. A. Mahmud, M. S. Hossen, A. A. C. Masud, Moumita, and M. Fujita, *Agronomy*, **8**, 31 (2018), doi:10.3390/agronomy8030031.
31. M. Wang, Q. Zheng, Q. Shen, and S. Guo, *Int. J. Mol. Sci.*, **14**, 7370–7390 (2013).
32. M. K. Ashley, M. Grant, and A. Grabov, *J. Exp. Bot.*, **57**, 425–436 (2006).
33. U. R. Malvi, *J. Agric. Sci.*, **24**, 106–109 (2011).
34. K. Prajapati and H. A. Modi, *Indian J. Plant Sci.*, **1**, 177–186 (2012).
35. S. Alejandro, S. Höller, B. Meier, and E. Peiter, *Front. Plant Sci.* (2020), <https://doi.org/10.3389/fpls.2020.00300>.
36. L. C. Campbell and R. O. Nable, In: *Manganese in Soils and Plants. Developments in Plant and Soil Sciences*, eds. R. D. Graham, R. J. Hannam, and N. C. Uren, Springer, Dordrecht, 33 (1988).
37. R. Millaleo, M. Reyes-Díaz, A. G. Ivanov, M. L. Mora, and M. Alberdi, *J. Soil Sci. Plant Nutr.*, **10**, 470–481 (2010).
38. W. P. Kelley, *Bot. Gaz.*, **57**, 213–227 (1914).
39. V. K. Yachandra, K. Sauer, and M. P. Klein, *Chem. Rev.*, **96**, 2927–2950 (1996).
40. W. W. Gezahegn, A. Srinivasulu, B. Aruna, S. Banerjee, M. Sudarshan, P. V. L. Narayana, and A. D. P. Rao, *IOSR J. Environ. Sci., Toxicol. Food Technol.*, **11**, 57–68 (2007).
41. G. Singh, A. Kawatra, and S. Sehgal, *Plant Food Hum. Nutr.*, **56**, 359–364 (2001).
42. P. H. Brown, I. Cakmak, and Q. Zhang, In: *Zinc in Soils and Plants. Developments in Plant and Soil Sciences*, ed. A. D. Robson, Springer, Dordrecht (1993), p. 55.
43. M. R. Broadley, P. J. White, J. P. Hammond, I. Zelko, and A. Lux, *New Phytol.*, **173**, 677–702 (2007).
44. C. Cabot, S. Martos, M. Llugany, B. Gallego, R. Tolrà, and C. Poschenrieder, *Front. Plant Sci.* (2019), <https://doi.org/10.3389/fpls.2019.01171>.
45. H. Marschner, *Mineral Nutrition of Higher Plants*, 2nd ed., Academic Press, London (1995).
46. J. R. Hunt, *Am. J. Clin. Nutr.*, **78**, 633S–639S (2003).
47. I. Yruela, *Funct. Plant Biol.*, **36**, 409–430 (2009).
48. B. Printz, S. Lutts, J. Hausman, and K. Sergeant, *Front. Plant Sci.* (2016), <https://doi.org/10.3389/fpls.2016.00601>.
49. M. Droppa and G. Horváth, *Crit. Rev. Plant Sci.*, **9**, 111–123 (1990).
50. C. E. Plantas, *Braz. J. Plant. Physiol.*, **17**, 145–156 (2005).
51. M. Singh, *Indian J. Pediatr.*, **71**, 59–62 (2004).
52. G. W. Miller, I. J. Huang, G. W. Welkie, and J. C. Pushnik, In: *Iron Nutrition in Soils and Plants. Developments in Plant and Soil Sciences*, ed. J. Abadía, Springer, Dordrecht (1995), p. 59.
53. J. Morrissey and M. L. Guerinot, *Chem. Rev.*, **109**, 4553–4567 (2009).
54. C. Curie and J. Briat, *Annu. Rev. Plant Biol.*, **54**, 183–206 (2003).
55. H. V. Marsh, H. J. Evans, and G. Matrone, *Plant Physiol.*, 632–638 (1963), doi: <https://doi.org/10.1104/pp.38.6.632>.
56. Food Safety and Standards Regulations (2009), www.indiaenvironmentalportal.org.in/files/FSSAI_regulations
57. G. R. Rout and S. Sahoo, *Rev. Agric. Sci.*, **3**, 1–24 (2015).
58. M. Kumari, S. Gupta, A. J. Lakshmi, and J. Prakash, *Food Chem.*, **86**, 217–222 (2014).
59. M. Hasanuzzaman, M. A. Hossain, and M. Fujita, *J. Plant Sci.*, **5**, 354–375 (2010).
60. Y. Mehdi, J. L. Hornick, L. Istasse, and I. Dufrasne, *Molecules*, **18**, 3292–3311 (2013).

61. P. E. Mabeyo, M. L. K. Manoko, A. Gruhonjic, P. A. Fitzpatrick, G. Landberg, M. Erdelyi, and S. S. Nyandoro, *Int. J. Food Sci.*, **549676**, 1–8 (2015).
62. P. H. Brown, R. M. Welch, and E. E. Cary, *Plant Physiol.*, **85**, 801–803 (1987).
63. C. C. Fabiano, T. Tezotto, J. L. Favarin, J. C. Polacco, and P. Mazzafera, *Front. Plant Sci.*, **6** (2015), doi: 10.3389/fpls.2015.00754.
64. E. M. Alissa and G. A. Ferns, *J. Toxicol.*, **870125**, 1–21 (2011).
65. K. Jomova, Z. Jenisova, M. Feszterova, S. Baros, J. Liska, D. Hudecova, C. J. Rhodes, and M. Valko, *J. Appl. Toxicol.*, **31**, 95–107 (2011).
66. P. M. Finnegan and W. Chen, *Front. Physiol.*, **3**, 1–18 (2012).
67. V. Pasricha and R. K. Gupta, *J. Pharm. Phytochem.*, **3**, 47–57 (2014).