



Multi-Elemental Characterization of Edible Vegetable seeds and Leafy-vegetable seeds from Vizianagaram District, Andhra Pradesh, India Using Proton-Induced X-ray Emission (PIXE)

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Abstract

Proton-induced X-ray emission (PIXE) was applied to characterize the elemental composition of edible vegetable seeds and leafy-vegetable seeds samples collected from Vizianagaram district, Andhra Pradesh, India. The samples were oven-dried, homogenized, mixed with graphite, pelletized and irradiated with a 3 MeV proton beam generated by a 3 MV tandem Pelletron accelerator at the Institute of Physics, Bhubaneswar, India. Characteristic X-rays were detected using a Si(Li) detector and the spectra were processed with GUPIX. The analytical procedure was checked using NIST Apple Leaf certified reference material, for which the original study reports agreement between measured and certified concentrations. Across the investigated samples, the manuscript reports 22 elements: Si, P, S, Cl, K, Ca, Ti, V, Mn, Fe, Co, Ni, Zn, Ce, Cu, As, Zr, Hg, Pb, Ga, Sr and Se. Potassium was reported in every sample, whereas several other elements showed sample-dependent occurrence. The data also indicate the presence of nutritionally relevant elements such as K, Ca, Fe, Zn, Mn, Cu and Se together with potentially toxic elements including As, Hg and Pb. These findings demonstrate the utility of PIXE for rapid multi-element screening of plant-derived foods and provide baseline information for environmental and food-quality monitoring in the study region. However, quantitative dietary-risk interpretation requires confirmation of concentration units, complete sample identification, replicate measurements, analytical uncertainties and comparison with element-specific regulatory limits on an equivalent mass basis.

Keywords: PIXE technique, vegetable seeds, Leafy-vegetable seeds, major, minor and trace elements.

Introduction

Vegetables are the common man affordable and readily available sources at important proteins, vitamins, minerals and essential amino acids [1]. Most at the developing countries depend on starch-based food as the main staple food for the supply of both energy and proteins. This accounts in part for protein deficiency which prevails among the people as recognized by Food and Agricultural Organization [2]. Thus the present study on vegetable seeds and leafy-vegetable seeds reveals the presence of major, minor & trace elements required for the growth of man's metabolism, altogether the concentration of 22 elements in about 9 samples of vegetable seeds and 9 samples of leafy-vegetable seeds. Thus the study of trace, minor & major elements with respect to these medicinal plants specifies that there is a significant role in combating a variety of human ailments and diseases [3].

Indian diet is mainly vegetarian and consists of various cereals and vegetables along with spices, often used in the preparation of curries [4]. The quantitative estimation of various trace element concentrations in medicinal plants is necessary for determining their effectiveness in treating various diseases and for understanding their pharmacological benefits [5].

Trace elements play a vital role in the formation of the active chemical constituents present in medicinal plants and are therefore responsible for their medicinal as well as toxic properties [6]. Light elements such as Na, Mg, Al and P are determined by PIGE while medium Z-Elements such as K, Ca, Mn, Fe, Cu, Zn, Rb and Sr are determined by PIXE [7]. Several analyses have also revealed that most of these plants are rich in one (or) more individual elements, thereby providing a possible link to the therapeutic action of the medicines [8-11].

Plants have been used by man as medicine since time immemorial. His close observation of animals in the early days, nothing but what they use when they fall ill, led to the eventual discovery of medicinal uses of most of these plants [12]. The vegetables can generally be classified as leafy, fruit and seed vegetables. Other forms of vegetables include pulses and spices [13].

However, the present study is concentrated on leafy-vegetable seeds of Palakura, Tella gongura, Bacchali, Pudeena, Karivepaku, Munagaku, thotakura, menthikura, chukkakura and also on vegetable seeds like beans, cluster beans, carrot, TellaVanga, MokkaJonna single cross and double cross, mullangi, bendakaya & bheerakaya. Many essential

elements in diets are toxic at higher concentration, while the presence of toxic trace elements above permissible level can lead to impaired health of consumers [14]. The use of PIXE technique as an analytical tool for rapid and reliable elemental analysis of biological material has a history of several decades [15]. The Ayurveda and plant based remedies are a part of cultural heritage of India [16-17].

Plenty of researches carried on these medicinal plants after focus on the organic actions of these medicines with little attention going to the elemental contents [18]. Major, minor & trace elements present in the medicinal plants effects various metabolic activities in the human body. In recent, times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period [19].

There are many nuclear physics techniques like AAS, XRF, ICP-MS, INAA, PIGE, PIXE etc., which have been used for the trace elemental analysis but Atomic Absorption spectroscopy is followed by high bremsstrahlung, X-Ray fluorescence as highly concentrates on its structural properties, ICP-MS because of its long sample preparation procedure and only certain elemental concentrations are known at certain standard levels and as neutron reaction cross sections are different for different elements for which, we cannot generalize INAA technique for set of elements at a stretch. In fact, if we use PIXE-Proton induced X-Ray emission technique because of its multi elemental capability and easy sample preparation, we can easily find out major, minor and trace elemental concentrations for all the elements with atomic number greater than 13 simultaneously and accurately. Even AAS and ICPMS requires the total digestion of the sample using strong reactants such as HCl, H₂SO₄, HNO₃ (or) HF that can cause problems of contamination and errors due to incomplete solubilization and evaporation [20]. Thus sample preparation becomes a first crucial step in the analysis of medicinal (or) other herbs by these methods and moreover, the chemical method for matrix destruction depends on the sample chemistry and the elements to be analyzed with the results of several methodologies have been proposed for plant digestion. In this context, multi elemental non-destructive accelerator based technique like PIXE seems to be more useful.

PIXE is highly sensitive, non-destructive and multi elemental ion beam analysis technique which have wide applicability in various fields. PIXE is used for the determination of elements in the two atomic number regions $20 < Z < 35$ and $75 < Z < 85$ while PIGE is used for the determination of light Z elements [21]. On the other hand, the investigations of the trace element metabolism of human beings, animals and plants were still remaining in an interesting field of actual research. The possibility of relating trace element contents to the curative property possessed by various medicinal plants is especially attractive in this inter disciplinary field and it has been quite widely studied by different authors [22].

2. Materials and Methods

2.1 Sample Collection & Processing:

The vegetable seeds and leafy-vegetable seeds samples were collected from the Vizianagaram district of Andhra Pradesh, India. The samples were washed thoroughly to avoid possible environmental contaminations. The samples were dried by an oven at 65°C and subsequently grounded by an agate mortar. 150 mg of sample powder was mixed homogeneously with 150 mg of the graphite powder using agate mortar and then this mixture powder is pelletized into a thin pellet of uniform thickness having 10mm in diameter and 1mm thickness with 15 ton pellet machine. We can also make another graphite pellet which contains graphite alone with the same process by taking 100 mg of its powder to make necessary correction. The purpose of adding graphite powder is that it can be used as a binder of the homogenized powdered sample in addition to allowing the beam current to be monitored as graphite is a good conductor of electricity and also another reason of adding graphite is to reduce bremsstrahlung background in the PIXE spectrum [23].

2.2 Experimental procedure for PIXE

Elemental analyses of vegetable seeds and leafy-vegetable seeds were performed using the PIXE technique, which was conducted using a 3 MV Tandem Pelletron Accelerator (model: 9SDH-2, make: National Electrostatic Corporation, USA) at the Institute of Physics in Bhubaneswar, India. This accelerator has been routinely used for material analyses using the PIXE technique [24-28]. The proton beam was collimated to a diameter of 3 mm on the target under a vacuum (10^{-6} Torr) inside the PIXE chamber. The multiple-target holder was placed in the plane and normalized to the proton beam direction. The target holder was mounted on an insulated stand and was surrounded by a cylindrical electron suppressor that was held at negative potential with respect to the target. The integrated charge of the sample was measured using a current integrator, which was connected to the target holder. The proton beam of 3 MeV, which had beam current that was in the 10–15 nA range, was employed to excite the samples. The position of the proton beam on the sample was adjusted properly by viewing the sample through a view port in the scattering chamber. A 30 mm Si (Li) detector with a beryllium window, thickness of 8 μ m, was placed at $\pm 90^\circ$, with respect to the beam direction, to record the characteristic X-rays. The energy resolution of the detector was 170 eV and full-width at half maximum at 5.9 KeV. A thick filter (25 μ m aluminized Mylar) was kept in front of the detector to suppress the bremsstrahlung and the dominant low-energy X-ray peaks. Each sample was irradiated for a sufficient time to obtain good statistics and for each sample, the total charge collected and the average beam current was noted. The spectra were recorded with a PC-based multi-channel analyzer, and the system was calibrated using ⁵⁵Fe and ²⁴¹Am radioisotope standard

X-ray sources to identify the X-ray peaks. The PIXE spectrum of a vegetable seeds and leafy-vegetable seed samples were analyzed using the GUPIX software program.

2.3. Data Analysis:

X-ray spectra of edible vegetable seeds and leafy-vegetable seeds samples obtained from PIXE measurements were analyzed with the computer code GUPIX [29-30] developed by Guelph University (Guelph, Ontario, Canada) that provides nonlinear least square fitting of the spectrum. The choice to employ GUPIX was based on a number of considerations; the most important one was the good status of the databases (cross sections, fluorescence and Coster-Kronig probabilities, stopping powers and attenuation coefficients) involved [31]. GUPIX has also some helpful options, such as matrix iteration, layer thickness iteration, adding an invisible element to the fit etc., and analyzing spectra in a batch [32]. The thick target PIXE analysis was performed since the target was thick enough to stop the proton beam entirely. GUPIX utilizes the fundamental parameter method (FPM) for quantitative analysis that requires parameters of experimental geometry and the detector (H parameter), chamber window thickness, target major matrix, the energy of the incident particle, and the net charge collected. To check the adopted analysis procedure and input parameters external standard method was adopted using macrometer standard and other certified reference materials, and accordingly H values were normalized. Using this GUPIX software package, different elements present in each of the vegetable seeds and leafy-vegetable seeds were identified and their concentrations were determined. To make sure the reliability of this technique in reproducing concentrations of elements, we have checked the accuracy of this method by analyzing certified reference material NIST APPLE LEAF as an international standard and the values are given in Table-3. The measured values and the certified values are in good agreement and thus the experimental procedure adopted is reliable in analyzing the samples.

3. Results & Discussion:

In this present study PIXE technique is employed to determine a total of 22 elements including major elements Si, P, S, Cl, K, Ca, Ti, V, Mn, Fe, Co, Ni, Zn & Cu a total of 14, minor elements As, Zr, Hg and Pb a total of 5 and finally trace elements Ga, Sr and Se a total of 3 elements in various vegetable seeds and leafy-vegetable seeds samples. Mainly considered vegetable seeds namely Mullangi, Mokkaonna single cross, double cross, Beans, cluster beans (Goruchikkudu), Carrot, Tella vanga, bendakaya & bheerakaya and also leafy-vegetable seeds of Bacchali, Pudeena, Karivepaku, Munagaku. Palakura, Tella gongura, thotakura, menthikura and chukkakura.

In order to compare the elemental distribution of various vegetable seeds and leaf-vegetable seeds samples, the ranges and mean elemental concentrations were determined for each of these samples and are shown in the table-1 & table-2 respectively.

Table 1: Concentrations of the elements present in the samples in PPM of vegetable seeds.

Element	Si	P	S	Cl	K	Ca	Ti	V	Mn	Fe	Co	Ni	Cu	Zn	Ga	As	Se	Sr	Zr	Ce	Hg	Pb
BV1	21.21	9571.0	840.81	12675	787.86	2309.12	1.33	0.010	19.87	37.315	0.1065	5.67	1.6347	16.54	2.59	1.8372	0.01210	2.88	1.92	1.102	0.0133	0.10
BV3	347.84	4771.9	896.75	23672	1532.76	120.987	1.20	0.070585	8.89	31.002	0.1008	3.47	20.9	16.864	0.019	1.08	1.55	0.652	1.34	1.72	0.017	0.23
BV4	397.12	2279.1	788.5716	1818.26	2697.96	102.567	0.985	0.04	4.0832	47.15	0.942	4.86	7.34	11.09	0.055	2.18	0.564	1.471	1.67	31	0.0113	0.256
BV5	53.261	1931.9	1677.7	577.53	4954.84	220.57	0.974	0.045	23.482	39.731	0.970	6.533	2.834	16.58	0.061	0.0983	0.174	2.41	1.548	725	0.0142	0.143
BV6	187.13	940.39	1687	422.94	11936.38	3723.99	1.63	0.064	18.29	387.31	0.49731	2.56	5.31	10.12	0.198	0.731	0.0171	1.82	1.72	825	0.0151	0.158
BV7	55.613	45.218	435.2	1345.72	142.16	120.987	1.365	0.036	34.123	15.112	1.9731	4.25	20.7	13.146	0.067	0.182	0.067	2.378	1.075	895	0.0411	0.1124
BV10	88.113	117.10	3985	21456	151.44	84.72669	0.506	0.027	2.9938	39.788	0.0134	2.37	3.09	9.3	0.054	0.176	0.019	0.618	1.8	0.208	0.0167	0.114
BV14	79.654	345.62	295	14567	21358.27	797.09	1.0645	0.029	100.87	397.310	0.474	2.29	10.8	12.65	0.0213	0.21	0.007	0.93	0.936	0.567	0.01091	0.26
BV17	271.14	118.11	120	15327	284.19	18.07	0.20	0.042	26.54	38.731	0.39263	5.79	35.68	13.10130	0.0306	0.64	0.638	0.893	0.59	0.561	0.019787	0.243

Table 2: Concentrations of the elements present in the samples in PPM of leafy-vegetable seeds

Element	Si	P	S	Cl	K	Ca	Ti	V	Mn	Fe	Co	Ni	Cu	Zn	Ga	As	Se	Sr	Zr	Ce	Hg	Pb
BVL1	21.21	9571.0	840.81	12675	787.86	2309.12	1.33	0.010	19.87	37.315	0.1065	5.67	1.6347	16.34	2.59	1.8372	0.01219	2.88	1.92	1.102	0.0133	0.16
BVL2	564.32	2321.9	3997	6532.36	18730.4	2448.14	.90	0.01	60.44	86.08	0.05	5.21	33.21	17.31	0.05	0.39	0.10	0.41	0.56	0.70	0.09	0.16
BVL3	784.1	18678	2317	200.26123	834.26	10123.4	0.510	0.043	18.7055	46.653	0.133	1.9	7.5732	34.132	0.032	0.466	0.0801	0.527	1.34	0.12	0.0271	0.3
BVL4	93.713	15890	1078.34	42.167	784	630	1.04	0.037	14.567	140.53	0.064	2.87	9.71	71.242	0.045	0.1319	0.056	0.668	0.380	0.048	0.187	
BVL5	168.14	4071.0	1586.43	209.84	24947.79	17197.99	1.654	0.042	47.613	49.873	0.129	4121	6.07	6.436	0.054	0.0873	0.255	1.552	0.452	0.390	0.0856	0.246
BVL6	113.14	18610	3456	1467.24	25728.44	24918.00	1.786	0.054	20.345	94.534	0.145	1.454	7.98	53.11	0.0543	0.0436	0.073	1.567	0.376	0.074	0.386	
BVL8	123.11	5200.45	3747	140.31	3338.51	1203.45	1.056	0.09	47.89	113.65	0.433	9.85	4.356	30.7	0.0152	0.222	0.091	1.235	0.748	0.0092	0.68	
BVL9	101.14	5154.12	4147	2056.67	12211.08	1685.82	1.345	0.055	18.76	87.89	3.478	.950	8.97	76.9	0.0143	0.397	0.05	.4123	0.813	0.40	0.009	0.17
BVL10	173.14	3191.0	1546.23	2178	24147.40	2333.22	1.252	0.0455	18.88	78.747	0.087	1.95	13.498	82.31	0.0723	0.486	0.0213	0.50	0.934	0.492	0.0134	0.197

In the above table 1 the major element highest composition is K and it ranges in vegetable seeds samples as 142.16 ppm in BV7 (Carrot) and 21358.27 ppm in BV14 (Lady's finger), similarly in leafy-vegetable samples the element K ranges from 784 ppm in BVL4 (Drumstick tree leaves) to 25728.44ppm in BVL6 (Sorrel-leaf type). Next to potassium, higher concentration is associated with the element, phosphorous in vegetable seeds samples as 45.218 ppm in BV7 (Carrot) to 9571 ppm in BV1 (Radish) and similarly in leafy-vegetable seeds samples, it ranges from 2321 ppm in BVL2 (Mint) to 18678 ppm in BVL3 (Curry leaves). Next to Phosphorous, the element Calcium (Ca) in vegetable seeds is ranging from 18.07 ppm in BV17 (Ridge gourd) to 3723.99 ppm in BV6 (Cluster beans) and in leafy-vegetable seeds samples, it ranges from 630 ppm in BVL4 (Drumstick tree leaves) to 24918 ppm in BVL6 (Sorrel-leaf type). The next element after Calcium is Sulphur, which is ranging in vegetable seeds sample as 120 ppm in BV17 (Ridge gourd) to 3985 ppm in BV10 (White eggplant) and similarly in leafy-vegetable samples, it is ranging from 840.81 ppm in BVL1 (Malabar spinach) to 4147 ppm in BVL9 (Fenugreek greens).

Likewise the highest proceeding order in the concentrations of major elements is of Si, Cl, Ti, V, Mn, Fe, Co, Ni, Zn and Ce. The only element potassium consistently present in all the vegetable seeds samples and leafy-vegetable samples are very high concentrations comparatively the other elemental concentrations. Observing the elemental concentration from the table, we can select and use the particular medicinal vegetable plant as per requirement by knowing the functional values of different elements.

The minor elemental concentration in this table is present in the samples, Cu, As, Zr, Hg and Pb and the trace elemental concentration is present in the elements Ga, Sr & Se. Thus in this present study on vegetable seeds and leafy-vegetable seeds reveals the not only the presence of Vital elements required for the growth of man's metabolism like Si, P, S, Cl, K, Ca, Ti, V, Mn, Fe, Co, Ni, Zn, Ce, Cu, Zr, Ga and Se but also few toxic elements like arsenic, strontium, mercury and lead which are present in the range of trace & ultra trace elemental concentration. Among all elements, Sr and Se has low elemental concentrations in all the vegetable seeds samples. It is observed from the table1, that the results are consistent and within the permissible range of errors.

The following table-3 represents the concentrations of the elements present in the NIST (National Institute Standards and Technology) apple leaves and the same verified by the PIXE technique along with standard deviation values in µg/g are shown here for the reference.

Table:3 Mean concentration and standard deviation (µg/g) of elements present in NIST apple leaves

S.NO	ELEMENT	CERTIFIED VALUES	PIXE MEASURED VALUES
1	K(%)	1.60 ± 0.02	1.59 ± 0.02
2	Ca(%)	1.526 ± 0.015	1.532 ± 0.015
3	Mn	54 ± 3	51 ± 3
4	Fe	83 ± 5	81 ± 5
5	Cu	5.6 ± 0.24	5.2 ± 0.24
6	Zn	12.5 ± 0.03	12.3 ± 0.03
7	Rb	10.2 ± 1.5	10.2 ± 1.5
8	As	0.038 ± 0.007	0.038 ± 0.007
9	Se	0.050 ± 0.009	0.050 ± 0.009
10	Pb	0.470 ± 0.024	0.470 ± 0.024

The following table 4 and 5 portrays about the list of vegetable seeds and leafy-vegetable seeds samples along with their local name, English name and scientific names corresponding to each and every sample respectively.

Table 4. Vegetable seed sample local names along with common & scientific names

S. No.	Sample code	Telugu name	English name	Scientific name
1	BV1	Mullangi (ముల్లంగి)	Radish	Raphanus sativus
2	BV3	Mokkajonna – Single Cross (మొక్కజొన్న)	Single-cross hybrid maize	Zea mays
3	BV4	Mokkajonna – Double Cross (మొక్కజొన్న)	Double-cross hybrid maize	Zea mays
4	BV5	Beans (బీన్స్)	Common bean / French bean	Phaseolus vulgaris
5	BV6	Goruchikkudu (గోరుచిక్కుడు)	Cluster bean / Guar	Cyamopsis tetragonoloba
6	BV7	Carrot (క్యారెట్)	Carrot	Daucus carota subsp. Sativus
7	BV10	Tella vanga (తెల్ల వంకాయ)	White eggplant / White brinjal	Solanum melongena
8	BV14	Bendakaya (బెండకాయ)	Okra / Lady's finger	Abelmoschus esculentus
9	BV17	Bheerakaya (బీరకాయ)	Ridge gourd	Luffa acutangula

Table 5. Leafy-vegetable seed sample local names along with common & scientific names.

S. No.	Sample code	Telugu name	English name	Scientific name
1	BVL1	Bacchali (బచ్చలి)	Malabar spinach	Basella alba
2	BVL2	Pudeena (పుదీనా)	Mint	Mentha spp.
3	BVL3	Karivepaku (కరివేపాకు)	Curry leaf	Murraya koenigii
4	BVL4	Munagaku (మునగాకు)	Drumstick tree / Moringa leaf	Moringa oleifera
5	BVL5	Palakura (పాలకూర)	Spinach	Spinacia oleracea
6	BVL6	Tella gongura (తెల్ల గోంగూర)	White gongura / Sorrel-leaf type	Hibiscus sabdariffa
7	BVL8	Thotakura (తోటకూర)	Amaranth / Vegetable amaranth	Amaranthus spp.
8	BVL9	Menthikura (మెంతికూర)	Fenugreek greens	Trigonella foenum-graecum
9	BVL10	Chukkakura (చుక్కకూర)	Indian sorrel / Creeping sorrel	Oxalis corniculata

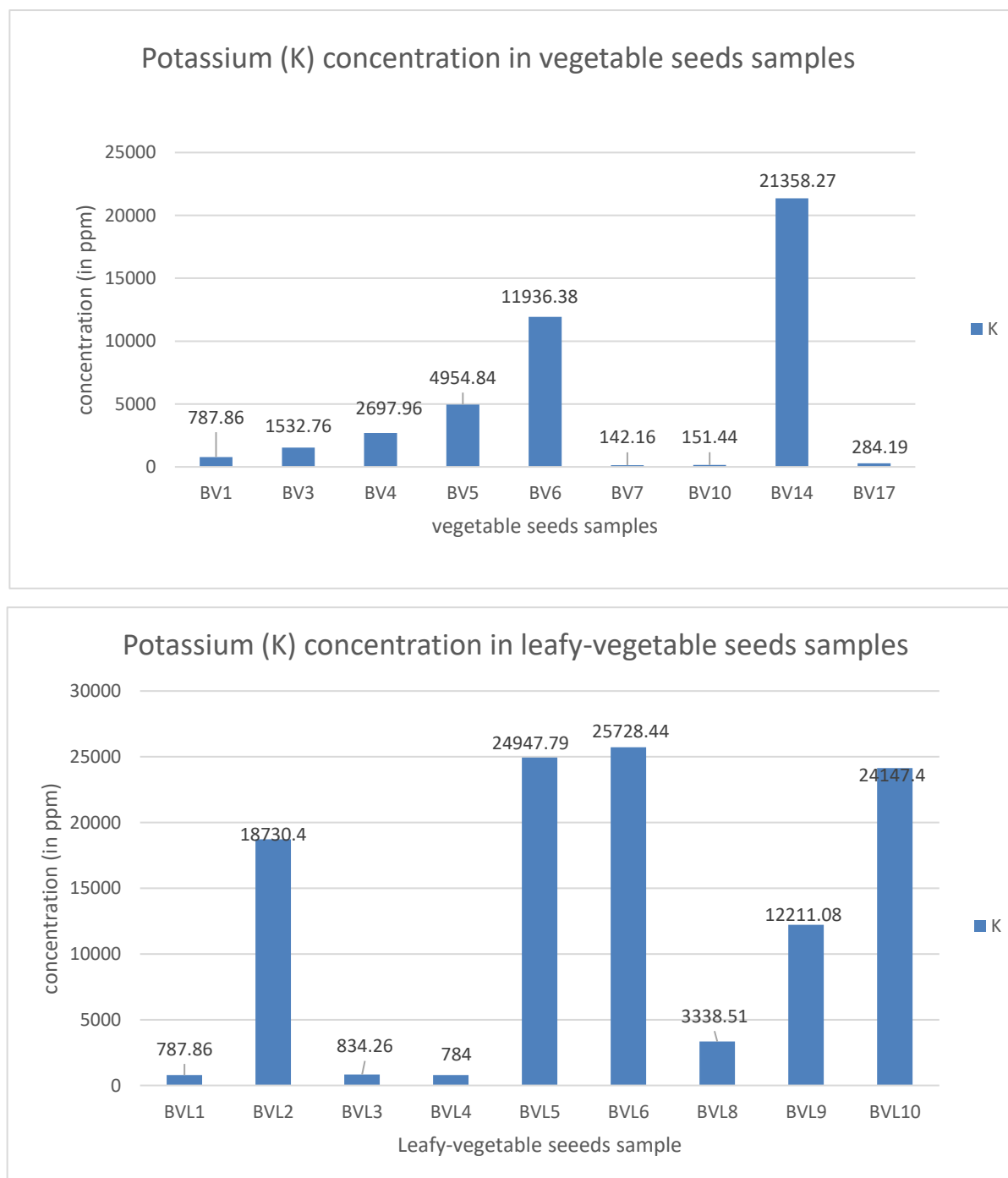


Fig-1: Bar diagram of major element potassium in vegetable & Leafy Vegetable seeds.

From the figure-1, we can conclude that potassium is present in all the vegetable seeds samples and it is especially high in BV-14 (lady's finger) and lowest concentration among the samples considered is in BV7 (carrot). In leafy-vegetable seeds samples, it is high in BVL6 (Sorrel-leaf type) and low in BVL4 (Drumstick tree leaf). The concentrations are nearly same in BVL1 (Malabar spinach), BVL3 (Curry leaf), and BVL4 (Drumstick tree leaves). Potassium is required for all living creatures with the possible exception of Blue-green algae. It is very important in nerve action and is eventually a cat ion. This cat ion within the cell functions as Sodium in extracellular fluid by influencing acid base equilibrium, osmotic pressure and water retention. When present in extra cellular fluid it influences muscle activities.

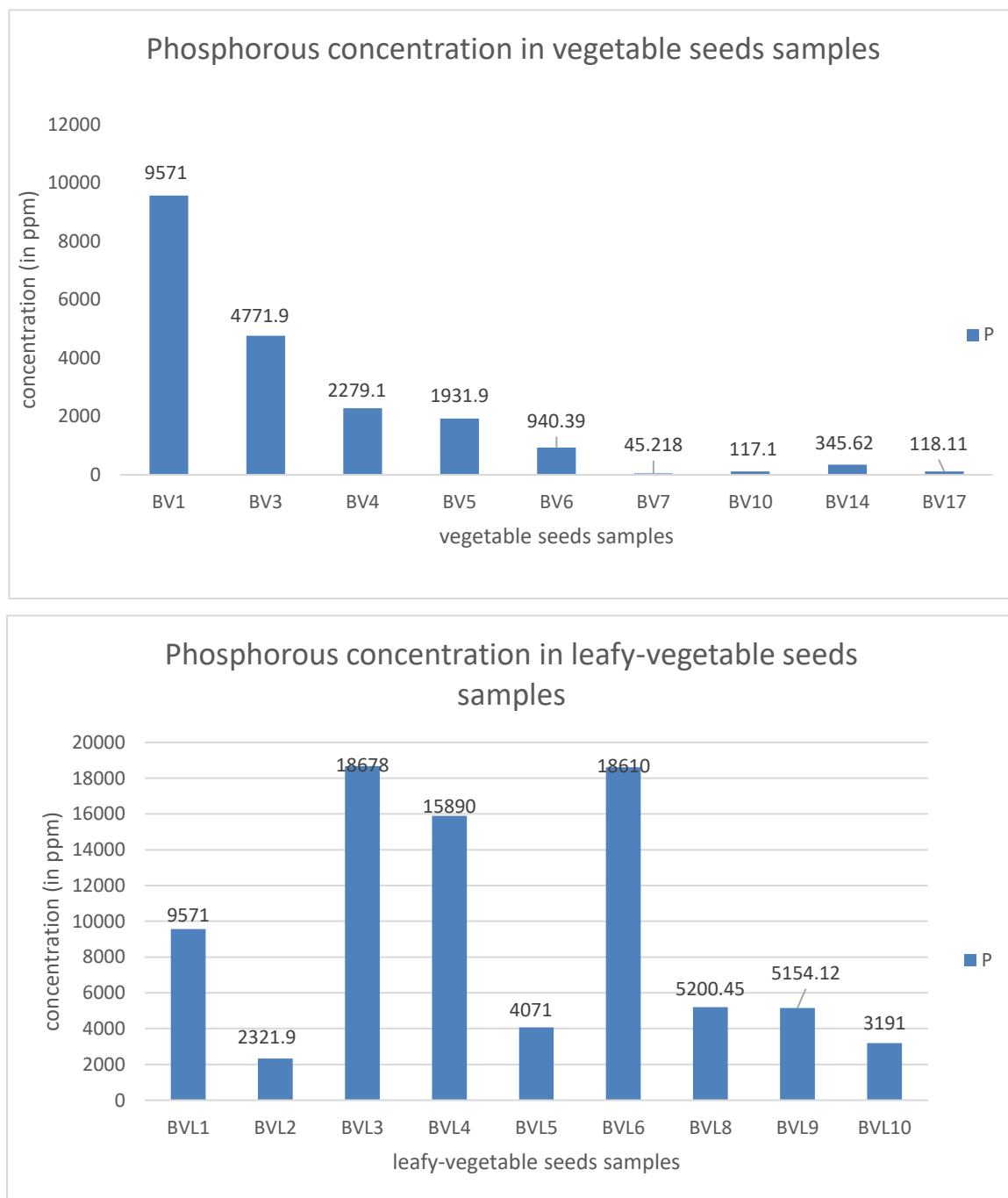


Fig-2: Bar diagram of major element phosphorus in vegetable & Leafy Vegetable seeds.

From the figure-2, it is observed that Phosphorous concentration is high in BV1 (Radish) and low in BV7 (Carrot). Where as in leafy-vegetable seeds, the highest concentration of phosphorous is in BVL3 (Curry leaves) & lowest is in BVL2 (Mint). And the concentrations in BVL8 (Amaranth) and BVL9 (Fenugreek greens) are nearly same in leafy-vegetable seeds sample that is the concentration of phosphorous is merely same in amaranth (thotakura) and leafy fenugreek seed samples.

On the other hand the calcium is existing in higher concentration in BV6 (cluster beans) of vegetable seeds & BVL6 (Sorrel-leaf type) of leafy-vegetable seeds similarly lower concentration in BV17 (Ridge gourd) of vegetable seeds & BVL4 (Drumstick leaves) of leafy-vegetable seeds respectively as shown in figure-3.

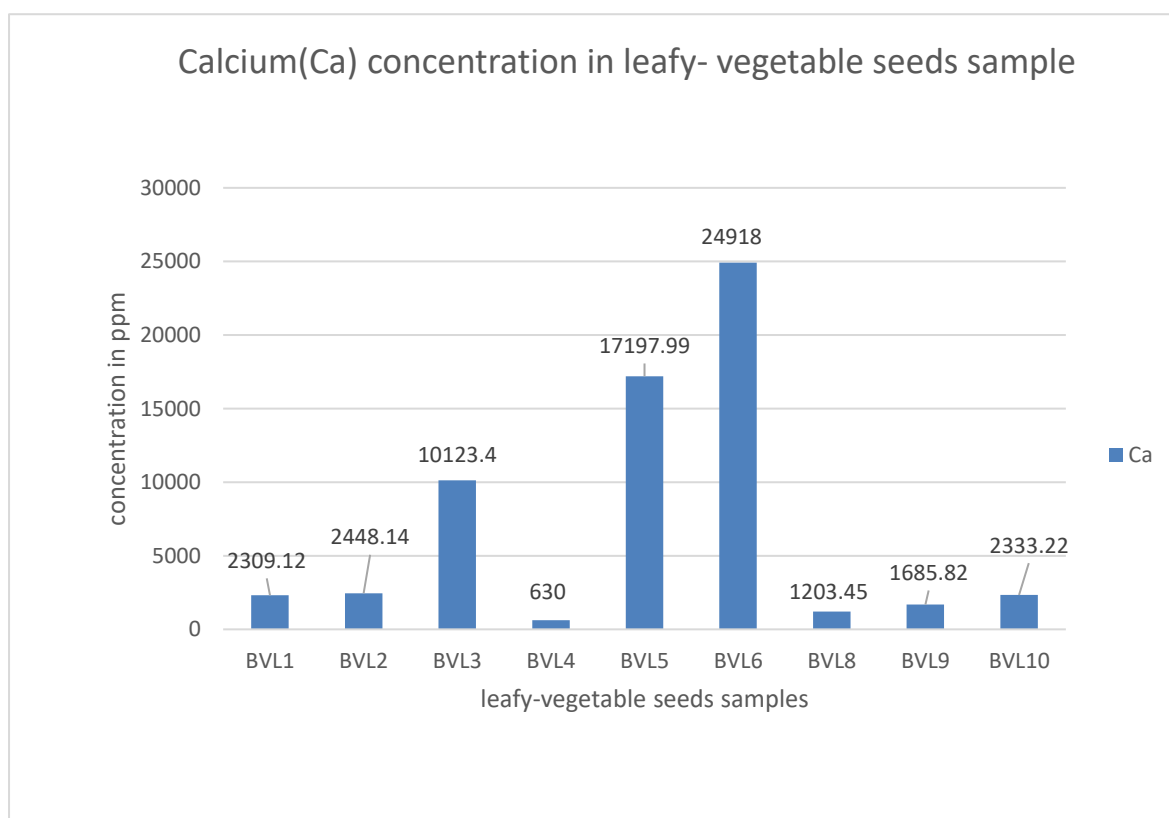
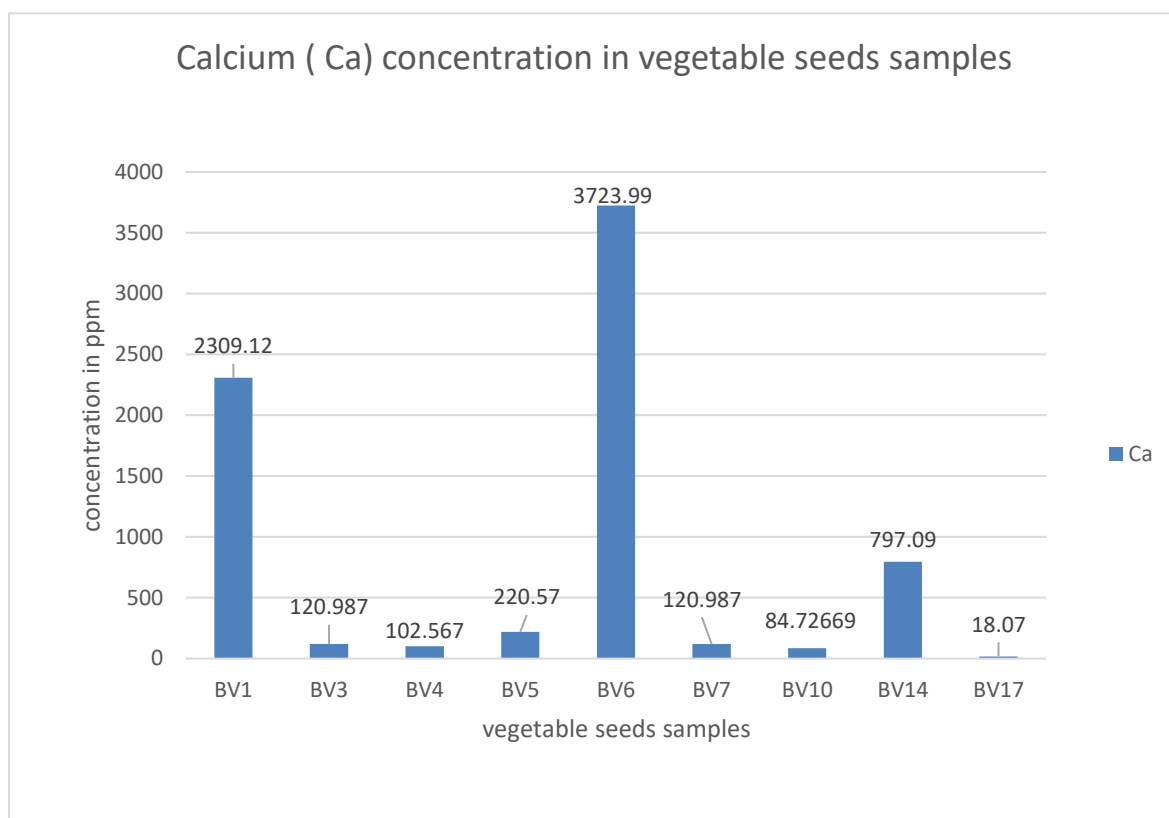


Fig-3: Bar diagram of major element calcium in vegetable & Leafy Vegetable seeds.

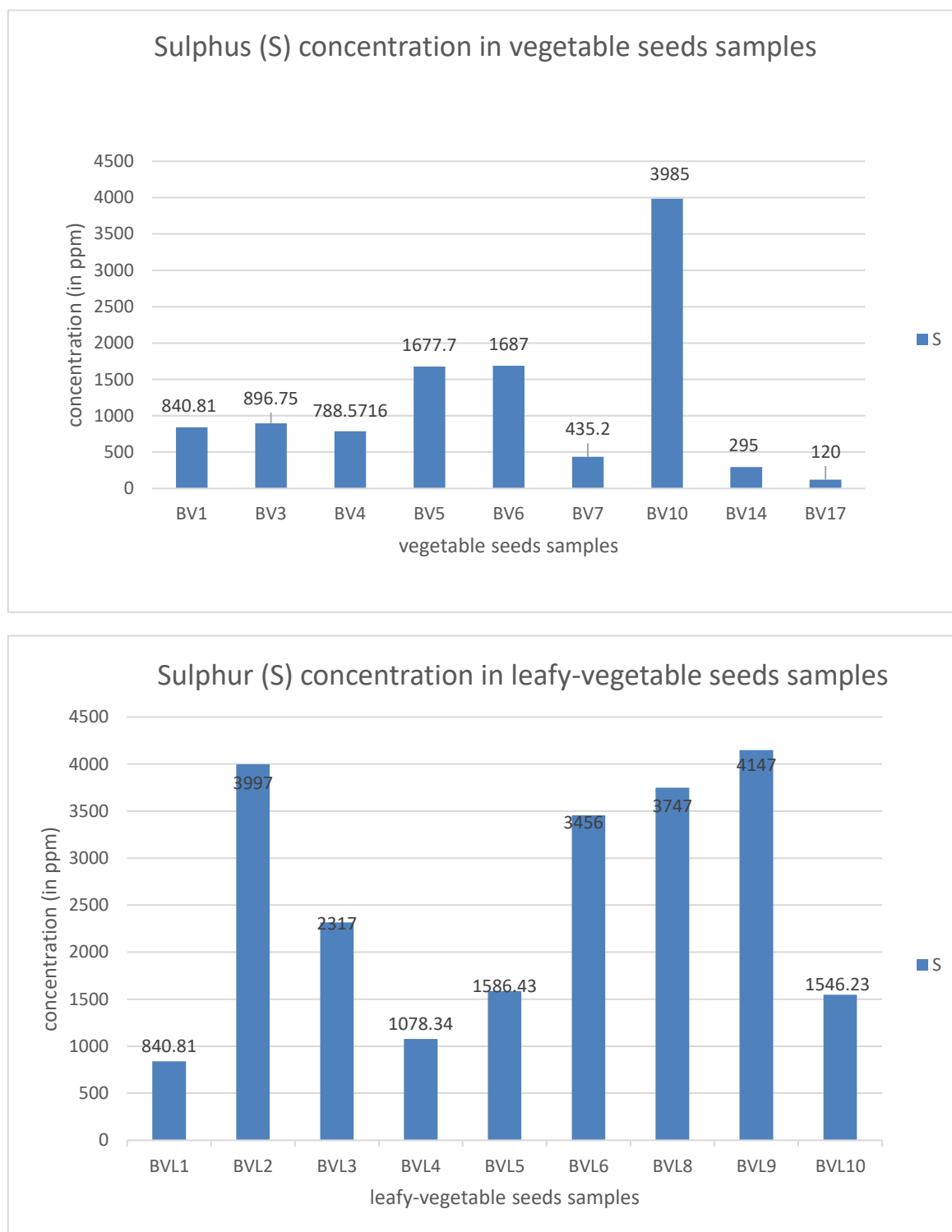


Fig-4: Bar diagram of major element sulfur in vegetable & Leafy Vegetable seeds.

The concentration of the element Sulphur is high in BV10 (white eggplant) and low is in BV17 (Ridge gourd) of vegetable seeds. In leafy-vegetable samples, it is high in BVL9 (Fenugreek greens) and low in BVL 1 (Malabar spinach). The BVL5 (Spinach) and BVL10 (Indian Sorrel) concentrations are nearly same. The element phosphorous is essential for growth and renewal of tissues. In metabolism of energy nutrients phosphorous compounds carry, store and release energy and they assist many enzymes and vitamins in extracting energy from nutrients. Sulphur is an important part of many enzymes and in antioxidant molecules like glutathione enzymes and thioredoxin, organically bonded Sulphur is a component of all proteins, as the amino acids cysteine and methionine.

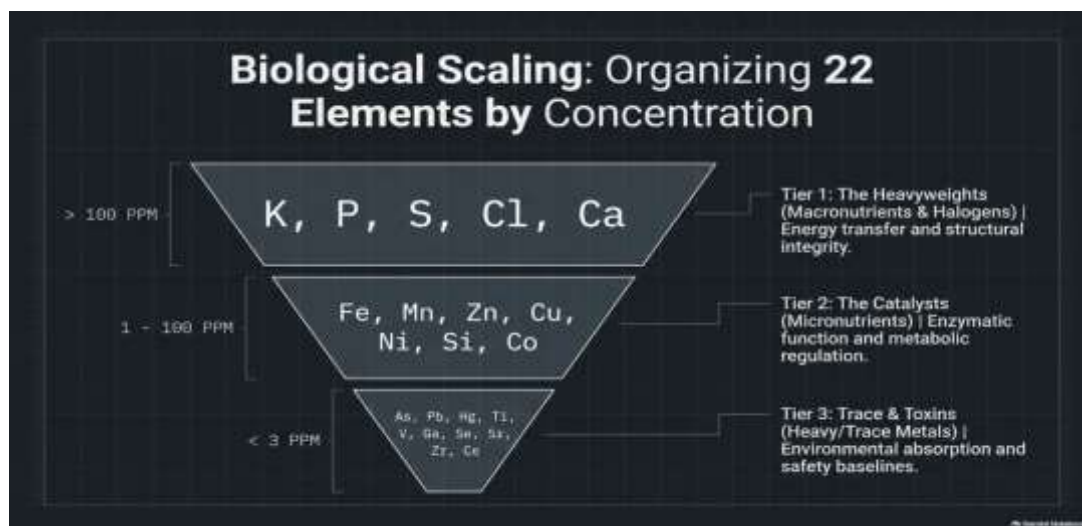


Fig-5: Classification of major, micro, trace & ultra-trace nutrient elements in all the seeds.

The figure-5 represents about biological scaling which has been done with respect to the ppm levels. The Macronutrients and Halogens (K, P, S, Cl, Ca) are present in the tier 1, whose ppm range is greater than 100. These are very useful in human system for energy transfer and structural integrity reactions. The Micronutrients (Fe, Mn, Zn, Cu, Ni, Si, Co) are present in the tier 2, whose ppm range is from 1 – 100. These are also useful in enzymatic function and metabolic regulations. The trace and toxins (As, Pb, Hg, Ti, V, Ga, Se, Sr, Zr, Ce) which are also known as heavy/trace metals present in tier 3, whose ppm range is less than 3. AS, Pb, Hg, Sr are the toxic elements which are harmful to the mankind at higher ranges.

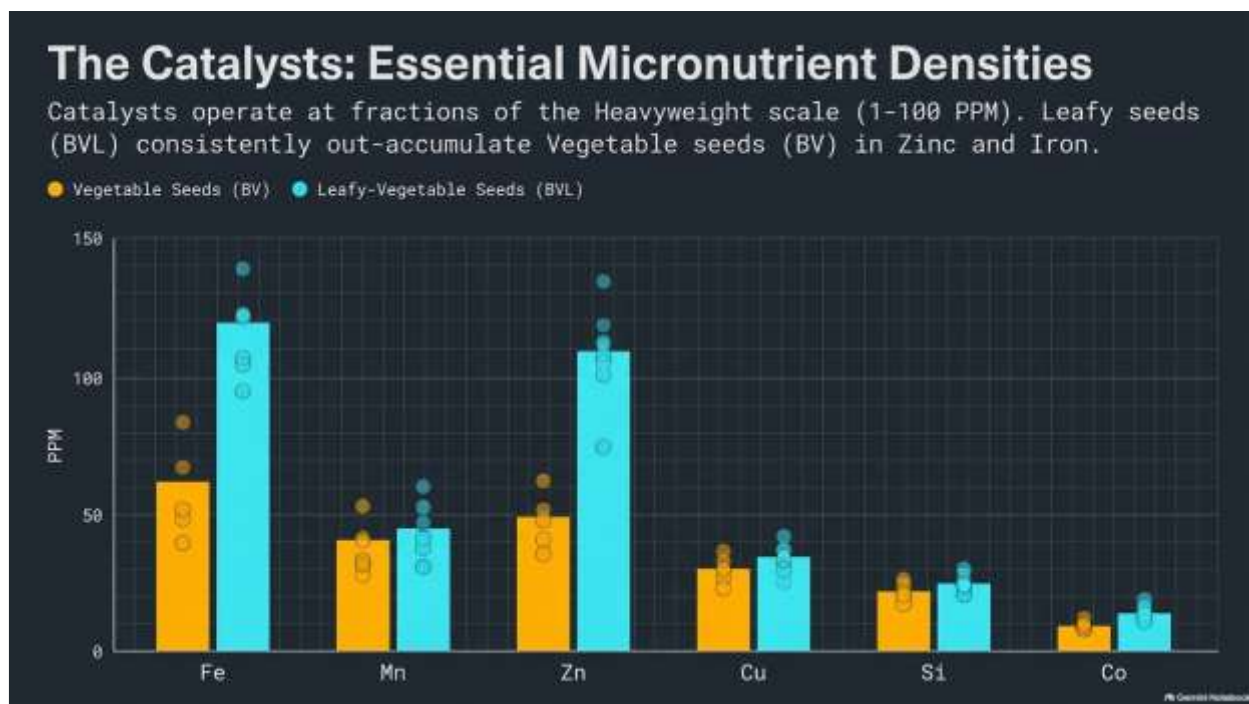


Fig-6: Bar diagram of micro nutrient elements in all the seeds.

A bar diagram has been drawn for minor nutrient elements which are present in vegetable seeds and as well as leafy-vegetable seeds and is given in figure-6. The element Iron (Fe) is more in leafy-vegetable seeds than the vegetable seeds samples. Next element Zn is high in leafy-vegetable samples and vegetable seeds samples. It is also found that the Fe, Mn, Zn, Cu, Si, Co elements are high in leafy-vegetable samples than the vegetable seeds samples. Many of the nutritionists suggests intake of leafy vegetables is good for health; our findings (figure-6) also supported it.

Silicon is non-metallic element found in nature as Silica (SiO_2) is simply one atom of the element silicon bonded to two oxygen atoms. Silicon is found in small elements in human tissues and may play a “Component” role in the ground substance, a gel like matrix composed of “glycosaminoglycans” (Carbohydrate chains and protein) that attract water silicon may also play a “component” role in connective tissues. There is also toxicity with higher amount of silicon intake for human body i.e. silicon from silica in the atmosphere from industrial process depositing in the lungs, ears,

eyes and nose causes the pathology known as silicosis.



Fig-7: Bar diagram of toxic elements in all the vegetable & leaf vegetable seeds with indication of safe thresholds.

A bar diagram has been drawn for trace and toxic elements present in all the vegetable and leafy vegetable samples as shown in figure-7. It has been found that all the trace and toxic elemental concentrations are nearly same in vegetable seeds and leafy-vegetable seeds samples and which are within the safe ranges.

Chlorine is a mineral and is considered as bulk element. The human body contains more than 50 mg chlorine per kg body weight. Chloride is of great importance for membrane potential, the electrical potential difference across a cell's membrane and it also acts as a disinfectant and kills germs contained in our food. It also lowers PH level in stomach which activates pepsinogen into pepsin, an endopeptidase.

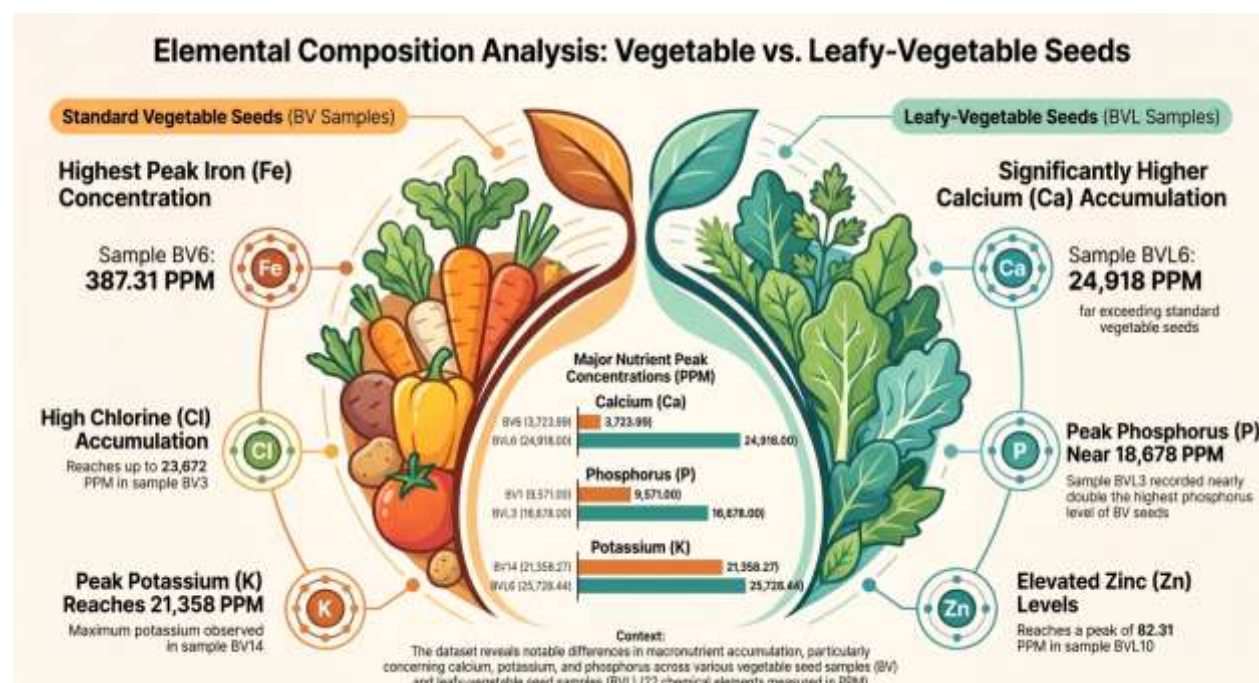


Fig-8: Elemental composition analysis in all the vegetable & leaf vegetable seeds.

The overall comparison of the major nutrient peak concentrations of Calcium, Phosphorous and Potassium in vegetable

seeds and leafy-vegetable seeds samples were represented in figure-8. Among all the vegetable seeds and leafy-vegetable seeds, the iron concentration is maximum in BV6 (Cluster beans) with 387.31 ppm, similarly the chlorine is high in BV3 (Single-cross hybrid maize) with 23,672 ppm and the Potassium is high in BV14 (Lady's finger) with 21,358 ppm. The calcium highest is in BVL6 (Sorrel- leaf type) with 24,918 ppm and the phosphorous high value in BVL3 (Curry leaf), with 18,678 ppm. The zinc is high in BVL10 (Indian Sorrel) with 82.31 ppm. Hence it is advisable to consume more vegetables and leafy vegetables for those who are having iron deficiency such as pregnant ladies, anemia patients, etc.

Calcium is essential for all organisms and used in teeth cell walls, bones systematic cardiac functions and also in some shells as structured component, important electro chemically and by co-ordination [33]. Titanium has no biological role. There is a detectable amount of titanium in the human body and it has been estimated that we can take in about 0.8mg/day, but most passes through us without being adsorbed. It is not a poison metal and the human body can tolerate in large doses. Element titanium and titanium dioxide is of a low order of toxicity. Manganese (Mn) is an important antioxidant nutrient and is important in the breakdown of fats and cholesterol and also helps in the nourishment of the nerves and the brain [34]. Vanadium functions probably part of protection system against injury of tissues and also used in the formation of enzymes. Lack of vanadium causes growth reduction, impaired reproduction and survival of young, impaired tooth and bone metabolism has a beneficial effect against tooth decay [35] and also essential for human body in the production of hemoglobin and in the oxygenation of red blood cells. Lack of iron results in anemia and red blood cells that have a low hemoglobin concentration.

In young children, Iron deficiency can manifest in behavioral abnormalities (including reduced attention), reduced cognitive performance and slow growth. In adults, severe iron deficiency anemia impairs physical work capacity. Cobalt can be found in many foods especially those that have high levels of vitamin B12, including liver, clams, oysters, lean beef, eggs, milk, yogurt, chicken and cheese. There are no recommended daily intake levels of cobalt because only it is found in trace elements. Cobalt increases body's ability to absorb and utilize vitamin B12. The Nickel containing enzyme is used for hydrolysis of urea.

Thirty five percent of the 0-5 year old children suffer from Zinc or iron [36]. It has been suggested that normal levels of Zn can prevent diarrhea [37]. Zinc is an essential component of over 200 enzymes having both catalytic and structural roles. Zinc deficiency is characterized by recurrent infections lack of immunity and poor growth [38]. Low intake of Zinc may cause coronary artery disease [39] Clinical materials prove that Zn can have good effect on eliminating ulcer and promoting healing wounds [40].

Cerium: A study of human bio-kinetics of cesium in two forms 1) incorporated in food stuff (137cs in perch and mushrooms) and 2) in ionic state (134cs in aqueous solution). Fecal excretion during first few days appears to account for a minor fraction of maximum 2-3 % of the initial gastrointestinal content of cesium [41].

From the tables-1 & 2, we can conclude that, the minor elements Cu, As, Zr, Hg and Pb are present in all the sample with very minute levels. Cu is highest in BV17 with 35.68 ppm in vegetable seeds ample and it is high in leafy-vegetable sample BVL10. Among all these minor elements lead and Arsenic are toxic elements to the human metabolism and their values are found very very low range in vegetable seeds samples and as well as leafy-vegetable seeds samples. Similarly Zr values are also found at very minute levels in all the seeds samples.

Copper is an element necessary for the absorption and use of Fe in the formation of hemoglobin, part of many enzymes, and helps in the formation of protective covering around nerves. It acts as a catalyst for oxidation. Copper [42] deficiency may cause cardiovascular disease and over copper deficiency occurs, symptoms include, neutropenia, cardiac disorders, osteoporosis and anemia [43]. Excess of copper is toxic. Arsenic takes a role in the metabolism of methyl compounds and the deficiency of it will lead to impairment of growth reproduction and heart function.

Mercury poisoning (also known as hydrargyria or mercurialism) is a disease caused by exposure to Hg or its compounds. In high enough doses mercury produces toxic effects. Toxic effects include damage to the brain, kidney and lungs [44]. Mercury poisoning can result in several diseases, including acrodynia (pink disease) [45], Hunter-Russell syndrome, and Minamata disease [46]. Symptoms typically include sensory impairment (vision, hearing, and speech), disturbed sensation and a lack of coordination. Exposure to mercury can occur from breathing contaminated air [47], eating foods that have acquired mercury residues during processing [48]. Exposure to mercury vapour in mercury may cause amalgam dental restorations [49] and from improper use or disposal of mercury and mercury containing objects, for example, after spills of elemental mercury or improper disposal of fluorescent lamps [50].

Lead absorption is due to contamination from the atmosphere and soil. While it is true that the detected level of Pb fall below toxic level of 1mg/day. Zirconium, it is ubiquitous, being present in nature in amounts higher than most trace elements. It is taken up by plants from soil and water and accumulated in certain tissues. Apparently, the metal is neither an essential nor toxic element in the conventional sense. In this present study, the trace elements are Ga, Se and Sr present in all the samples. From the above Fig. we can conclude that the element Ga range is as 0.019 in BV3 (Single-cross hybrid maize) to 2.59 in BV1 (Radish), Se range is as 0.012 in BV1 (Radish) to 1.55 in BV3 (Single-

cross hybrid maize), Sr range 0.618 in BV10 (White eggplant) to 2.88 in BV1 (Radish). From the tables-1 & 2 we can conclude that in this present study Ga, Se and Sr are trace elements and their range as in Ga with 0.0143 in BVL9 (Fenugreek greens) to 2.59 in BVL1 (Malabar spinach), Se with 0.01 in BVL1 (Malabar spinach) to 0.25 in BVL5 (Spinach), Sr with 0.41 in BVL2 (Mint) to 2.88 in BVL1 (Malabar spinach). Gallium apparently has no biological role but is said to stimulate the metabolism. Gallium compounds appear not to be particularly toxic.

Selenium is essential for the thyroid gland functioning and thyroid hormone biosynthesis and metabolism. Selenium is also required for the function at a small number of selenocysteine (Sec)-containing selenoproteins implicated in thyroid hormone metabolism and gland function. These findings enforce the notion that many people might profit from improving their Se status, both as a means to reduce the individual health risk as well as to balance a Se deficiency which often develops during the course of illness leads to muscle and pancreas degeneration hemolysis.

Strontium chemically resembles calcium, but it is a white silvery metal softer than calcium and even more reactive towards water due to this reason, the human body does a poor job of distinguishing and absorbed by the body and stored in the skeleton in places where calcium should be. Strontium chloride is sometimes used in tooth pastes for sensitive teeth. Strontium ranelate is used in treatment of osteoporosis.

Thus over all view of these results from vegetables seeds and leafy-vegetable seeds show that many of the medicinal plants contain elements which have cardinal importance. Many major, Minor and trace elements required for the growth and development of human metabolism in the human body. Results also show that some of the vegetables and vegetable leaves contain toxic elements like Arsenic, Cesium, Mercury and lead but they are below the accepted toxic levels [51].

4. Conclusion

The major, minor and trace elements in vegetable seed samples of Mokkaionna single and double cross, Beans, Cluster beans, carrot, Tellavanga, bendakaya & bheerakaya and also leafy-vegetable seed samples of Bacchali, Pudeena, Karivepaku, Munagaku. Palakura, Tella gongura, thotakura, menthikura and chukkakura have been detected by PIXE technique, as it is one of the most powerful techniques for its quick multi-elemental trace analysis capability and high sensitivity. As the different vegetables are having different concentrations of different elements, we can conclude that these vegetable & leafy vegetable seeds are having their own vital roles in the treatment of diseases. In the present study, the concentrations of K, P, Ca and S were present highly in all the samples followed by the remaining elements in next major, minor and trace levels correspondingly.

The Macronutrients and Halogens (K, P, S, Cl, Ca) are present in the tier 1 of figure-5, whose ppm range is greater than 100. These are very useful in human system for energy transfer and structural integrity reactions. The Micronutrients (Fe, Mn, Zn, Cu, Ni, Si, Co) are present in the tier 2, whose ppm range is from 1 – 100. These are also useful in enzymatic function and metabolic regulations. The trace and toxins (As, Pb, Hg, Ti, V, Ga, Se, Sr, Zr, Ce) which are also known as heavy/trace metals present in tier 3, whose ppm range is less than 3. As, Pb, Hg, Sr are the toxic elements which are harmful to the mankind at higher ranges. The element Iron (Fe) is more in leafy-vegetable seeds than the vegetable seeds samples. Next element Zn is high in leafy-vegetable samples and vegetable seeds samples. It is also found that the Fe, Mn, Zn, Cu, Si, Co elements are high in leafy-vegetable samples than the vegetable seeds samples. Many of the nutritionist suggests intake of leafy vegetables is good for health; our findings (figure-6) also supported it.

It has been found that all the trace and toxic elemental concentrations are nearly same in vegetable seeds and leafy-vegetable seeds samples and which are within the safe ranges. Significantly, Potassium, Calcium, Phosphorous, zinc are found as high in leafy-vegetable seeds, hence it is advisable to consume more leafy vegetables for better health. Similarly, in the present study the iron concentration is also observed as high in leafy-vegetables. So at low cost, it is very useful to overcome the iron deficiency. It is also use full to the pregnant ladies, anemia patients, etc. Observing the elemental concentration from the table, we can select and use the particular medicinal vegetable plant as per requirement by knowing the functional values of different elements. Hence the present study establishes the multi elemental analysis capability of PIXE and its use in biological field.

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References

1. Thompson, H.C. & Kelly, W.C. (1990). Vegetable Crops. 5th Ed. New Delhi: Mac Graw Hill Publishing Company Ltd, 120-125.
2. Ladeji, O.Okoye, Z.S. & Ojobe, T. (1995). Chemical evaluation of the nutritive value of leaf of fluted pumpkin (*Telferia occidentalis*). Food Chem. 53, 353-355.
3. Shirin.K, Imad.S, Shafiq.S, Fatima.K Determination of major and trace elements in the indigenous medicinal plant

- with ania somnifera and their responsible correlation with therapeutic activity. J.Saudi chem. Soc 2010;14:97-100.
4. Singh.V, Garg.A.N Availability of essential trace elements in Indian cereals, vegetables and spices using INAA and the contribution of spices to daily dietary intake.Food Chem 2006;94:81-89. [5]
 5. K.Nomita Devi, H.Nandakumar Sarma,PIXE-PIGE analysis of some Indian medicinal plants .Nuclear Instruments and methods in physics research. B 268(2010)2144-2147.
 6. N.S. Rajurkar, M.M. Damame, Appl. Radiat. Isotopes 49 (1998) 773.
 7. K.Nomita Devi, H.Nandakumar sarma, Sanjiv Kumar.Estimation of essential and Trace elements by PIXE and PIGE techniques.Nuclear Instruments and Metods in physics research B 266(2008)1605-1610.
 8. G.J. Naga Raju, P. Sarita, G.A.V. Ramana Murty, M. Ravi Kumar, B. Seetharami Reddy, M. John Charles, S. Lakshminarayana, T. Seshi Reddy, S. Bhuloka Reddy, V. Vijayan, Appl. Radiat. Isotopes 64 (2006) 893.
 9. D.K. Ray, P.K. Nayak, S.R. Panda, T.R. Rautray, V. Vijayan, C.C. Christopher, S. Jena, IJPIXE 16 (2006) 47.
 10. N. Ekinci, R. Ekinci, R. Polat, G. Budak, J. Radioanal. Nucl. Chem. 258 (2004) 127.
 11. B. Mohanta, A. Chakraborty, M. Sudarshan, R.K. Dutta, M.Baruah, J. Radioanal. Nucl. Chem. 258 (2003) 175.
 12. M. Rombi, Phytotherapy, Herbal Health Publisher, Surrey, 1982.
 13. Van C.W. Epehuijsen, Growing Native Vegetables in Nigeria, FAO of United Nations, Rome, 1974.
 14. E.J.Underwood, Trace Elements in Human and Animal Nutrition, 4TM Ed. Accademic Press, New York, 1977.
 15. W.Maenhaut and Meth.B, Nucl.Instr.35 (1988)388, K.G.Malmquist and Meth.B Nucl.Instr.49 (1990)183.
 16. Mukherjee PK, Kumar NS and Heinrich M, Plant made pharmaceuticals(PMPs) Development of Natural Health Products from Bio-Diversity,Indian J pharm Edu Res ,2008 42(2),113-121.
 17. Mukherjee PK and Wahile A,Integrated approaches towards Drug Development from Ayurveda and other Indian systems of Medicine, J.Ethnopharmacol,2006,103,25-35.
 18. Garg AN, Kumar A, Nair AGC, Reddy AVR, (2007) J. Radioanal. Nucl. Chem. 271: 611–619.
 19. S.A.Dahanukar, R.A.Kulkarni,N.N.Rege(2000).Indian Journal Of Pharmacology 2000;32: S 81-S 118.
 20. I.Queralt, M.Ovejero, M.L.carvalho, A.F.Marques,J.M.Liabres,X-ray spectrum 34(2005)231.
 21. S.A.E.Johansson, J.L.campbell,PIXE:A Novel Technique for elemental analysis,John Wiley and Sons Ltd., 1988.
 22. G.J. Naga Raju, P. Sarita, G.A.V. Ramana Murty, M. Ravi Kumar, B. Seetharami Reddy, M. John Charles, S. Lakshminarayana, T. Seshi Reddy, S. Bhuloka Reddy, V. Vijayan, Appl. Radiat. Isotopes 64 (2006) 893
 23. Vijayan,V.,Behera,S.N.,Ramamurthy,V.S.,Puri,S.,Shahi,J.S.,Singh,N.,1997.Elemental composition of flyash from a coal-fires Thermal power plant:a study using PIXE and EDXRF,X-ray spectrum.26,65.
 24. Sekar K., Satyam P.V., Kuri G., Mahapatra D.P. and Dev B.N., An RBS study of inter diffusion across a brominated Si(111)/Cu interface with and without a barrier layer, Nucl. Instrum. Methods. B 73, 1993, 6.
 25. Vijayan V., Ramamurty V.S. and Behera S.N., Particle induced X-ray emission (PIXE) analysis of coal fly ash, Int. J. PIXE 5, 1995, 211.
 26. Dev B.N., Materials modifications in heavy ion interactions with single crystals and their ion beam characterization, Nucl. Instrum. Methods. B 156, 1999, 258.
 27. Rabiprasad G.V., Mahapatra D.P., Punithvelu A.M., Somayajulu B.L.K. and Gopalan K., Nucl. Instrum. Methods Instr. and Meth. B 172, 2000, 66.
 28. Rout B., Ghose S.K., Mahapatra D.P., Dev B.N., Bhakru H. and Haberl A.W., Status of ion micro beam facility at the Institute of Physics, Bhubaneswar, India. Nucl. Instrum. Methods. B 181, 2001, 110–115.
 29. Maxwell J.A., Campbell J.L. and Teesdale W.J., The Guelph PIXE software package, Nucl. Instrum. Methods B 43, 1989, 218.
 30. Campbell J.L., Hopman T.L., Maxwell J.A. and Nejedly Z., Nucl. Instrum. Methods B 70, 2000, 193.
 31. Maxwell J.A., Campbell J.L. and Teesdale W.J., The Guelph PIXE software package, Nucl. Instrum. Methods B 43, 1989, 218.
 32. Sariff A., Bulow K., Elfman M., Kristiansson P., Malmqvist K. and Pallon J., 2002 Calibration of a new chamber using GUPIX software package for PIXE analysis, Nucl. Instum. Methods B.189,131.
 33. N.K.Sharat Singh, Ch.Bino Devi, Th.Sony Singh and N. Rajmuhon Singh; Trace elements of some selected medicinal plants of Manipur.Indian Journal of Natural products and Resources.Vol.1(2),June 2010,pp.227-331.
 34. U.C. Chaturvedi, Risha Shrivastava, R.K. Upreti, Curr. Sci. 87 (2004) 1536.
 35. N.K.Sharat Singh, Ch.Bino Devi, Th.Sony Singh and N. Rajmuhon Singh; Trace elements of some selected medicinal plants of Manipur.Indian Journal of Natural products and Resources.Vol.1(2),June 2010,pp.227-331.
 36. Xiaoe Yang, J. Trace Elem. Med. Biol. 18 (2005) 293.
 37. E.M.N. Hamilton, E.N. Whitney, F.S. Sizer, Nutrition: Concepts and Controversies, fourth ed., West Publishing Co., St. Paul, MN, USA, 1988.
 38. Singh V and Garg A N ,Availability of essential trace elements in Ayurvedic Indian Medicinal Herbs using INAA,Applied Radiation Isotopes,1997,48(1),97-101.
 39. Singh R B ,Gupta UC, Mittal N,Niaz, MA,Ghosh S and Rastogi V,Epidemologic study of trace elements and Mg on the risk of coronary artery disease in rural and urban Indian Population ,J AM Coll Nutr,1997,16(1),62-67.
 40. Agram MS, Studies on Zinc in wound healing, Acta Darm Venereol Suppl (Stockh),1990,154,1-36.
 41. Christopher L .Raaf (Editor) Lund University,Aprial 2006.Sweden.Human metabolism of caesium.NKS-120 . ISBN 87-7893-181-9.

42. E.M.N. Hamilton, E.N. Whitney, F.S.Sizer, Nutrition: Concepts and Controversies, fourth ed., West Publishing Co., St. Paul, MN, USA, 1988.
43. Wiliam DM, Copper Deficiency in humans, *Semin Hematol*, 1983, 20, 118-128.
44. Clifton JC 2nd (2007). "Mercury exposure and public health". *Pediatr Clin North Am* 54 (2): 237–69, viii. doi:10.1016/j.pcl.2007.02.005. PMID 17448359.
45. Bjørklund G (1995). "Mercury and Acrodynia". *Journal of Orthomolecular Medicine* 10 (3 & 4): 145–146.
46. Davidson PW, Myers GJ, Weiss B (2004). "Mercury exposure and child development outcomes". *Pediatrics* 113 (4 Suppl): 1023–9. doi:10.1542/peds.113.4.S1.1023. PMID 15060195.
47. ATSDR Mercury ToxFAQ (April 1999). "ToxFAQs: Mercury". Agency for Toxic Substances and Disease Registry. Retrieved 2007-07-25.
48. Dufault R, LeBlanc B, Schnoll R et al. (2009). "Mercury from chlor-alkali plants: measured concentrations in food product sugar". *Environ Health* 8 (1): 2. doi:10.1186/1476-069X-8-2. PMC 2637263. PMID 19171026. Lay summary – Medscape Today (2009-01-27).
49. Levy M. (1995). "Dental Amalgam: toxicological evaluation and health risk assessment". *J Cdn Dent Assoc* 61: 667–8, 671–4.
50. Goldman LR, Shannon MW; American Academy of Pediatrics:Committee on Environmental Health (July 2001). "Technical report: mercury in the environment: implications for pediatricians". *Pediatrics* 108 (1): 197–205. doi:10.1542/peds.108.1.197. PMID 11433078. Retrieved 2007-07-25.
51. H.J.M.Bowen, *Environmental chemistry of the elements*, Academic Press Inc. London(1979).144.