

Elemental Profile of the Leafy Vegetables Commonly Consumed by Natives of North Eastern Region of India analysed using Energy Dispersive X-Ray Fluorescence

Research Article

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Abstract

There is paucity of data in respect to elemental concentration in the foodstuffs consumed by the living population of North East Region of India. Thirteen elements (Ca, K, Cl, P, S, Fe, Mn, Zn, Cu, Se, Sr, Rb and Br) were detected by using versatile Energy Dispersive X-ray Fluorescence (ED-XRF) technique in eight selected leafy vegetable foodstuffs namely *Brassica juncea*, *Colocasis esculenta* L, *Zanthoxylum rhetsa* L, *Clerodendron colebrookianum*, *Vigna unguiculata* L, *Gnetum gnemon* L, *Cucurbita pepo* and *Cucurbita maxima* commonly consumed by natives of North East State of India. The analysis of the data revealed that the selected leafy vegetables contained high and elevated concentration of K, Ca, P, Cl, S and Fe and appreciable amount of Zn, Mn, Cu, Se, Sr, Rb and Br. The contents of Fe and all major elements recorded in this study were found to be above the permissible limit as proposed by the various regulatory organizations and pose a threat to health on long term consumption. The result of the study thus provides valuable database of the nutritional and chemical quality commonly consumed leafy vegetables foodstuffs by natives of North East Region India.

Keywords: Elemental analysis; ED-XRF; Leafy vegetables; North Eastern India.

Introduction

Vegetable foodstuffs serve as an indispensable constituent of the human diet supplying the body with essential elements in addition to vitamins, protein, certain hormone precursors and energy for well being and good health [1]. Reliable database on the nutrient composition of foods including elements content are important in many areas of endeavour including health assessment, formulation of appropriate institutional and therapeutic diets, nutrition education, food and nutrition training, epidemiological research on

relationships between diet and diseases, plant breeding, nutrition labelling, food regulation and consumer protection as well as a variety of applications in agriculture research [2]. Sevenhuysen reported that food composition tables and element databases are available in most of the countries, yet the data they contain are invariably criticised as being too inaccurate for many purposes for the reasons that different workers have used different techniques of analysis and that the samples so analysed had undergone different sample preparations leading to varying degrees of losses without ignoring the inherent variable factors that affect food composition which are commonly

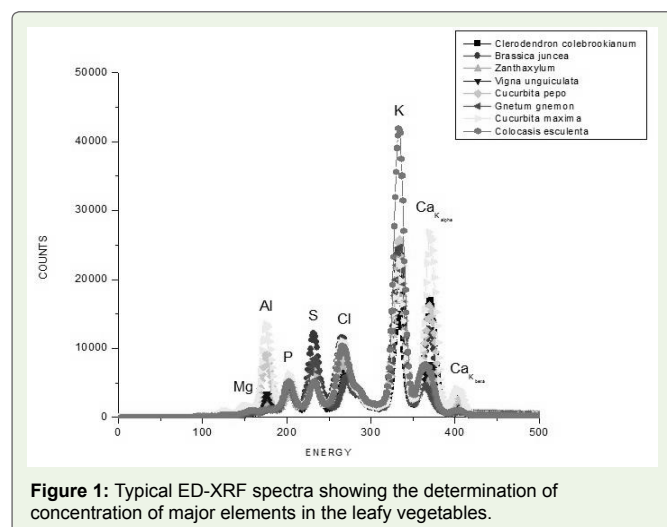


Figure 1: Typical ED-XRF spectra showing the determination of concentration of major elements in the leafy vegetables.

not specified [3]. Thus, Kirk and Sawyer published that analysis plays an important role in the assessment and maintenance of food quality and safety, both in industry and for enforcement authorities at the national and international levels [4].

Mizoram is a State in the North Eastern Region of India and is a part of the Indo-Mayammar biodiversity hot spot of the world. The State harbour variety of edible plant species including number of medicinal plants used in the traditional practices. Most commonly consumed vegetables foodstuffs of the native of this region comprises of recipes of various leafy vegetables used for culinary purposes to improve the quality of soup and also for their dietary purposes [5]. The study of the elemental content of native vegetables commonly consumed by living population are relevant and imperative because these vegetables are not only major nutrient suppliers including important essential elements acting on a metabolism of several functions in human body but also produced hazard effects on health either due to their deficiencies or their presence at an elevated level. However, there is paucity of data in respect to elemental concentration in the foodstuffs consumed by the living population of India especially North East Region of India. Only Singh and Taneja published trace elements and heavy metals concentration in the vegetables and meat foodstuffs available in the State of Manipur [6]. However, this study deals only with the analysis of two or three trace elements using conventional spectroscopic method. Here, the major and minor elements in the selected leafy vegetables foodstuffs were analyzed by using ED-XRF for the first time and their role in the human body has been discussed. The use of direct and multi-elemental analysis methods of vegetation samples with XRF has increased over the last few years. Simplicity of sample preparation, minimum need for manipulation, speed and opportunity of analyzing several different elements have promoted XRF as a useful alternative to conventional spectroscopic techniques. In addition to this, XRF's versatility stems from its rapid non-destructive multi-element determination from ppm to weight percent of elements [7]. Thus, looking at the paucity of data and detrimental role played by the elements in the human body, major and minor elements present in the leafy vegetables foodstuffs commonly consumed by the native of the North Eastern

region of India was analysed using sophisticated and versatile ED-XRF technique.

Materials and Methods

Raw materials

Eight commonly and widely consumed leafy vegetables in Mizoram, India namely *Brassica juncea*, *Colocasia esculenta* L, *Zanthaxylum rhetsa* L, *Clerodendron colebrookianum*, *Vigna unguiculata* L, *Gnetum gnemon* L, *Cucurbita pepo* and *Cucurbita maxima* were selected for analysis. The vegetables were purchased from the Central Markets of Aizawl, Mizoram, India during the month of August to December, 2015.

Sample preparation

The leaves of the collected leafy vegetables were destalked, thoroughly washed with triple distilled water to eliminate contamination due to dust and environmental pollution, air-dried and then oven dried at 60 °C and stored in plastic bags until needed. After drying, the leaves were ground into fine powder using mortar and pestle, and stored in a well labelled air tight container for analysis. The powdered samples were then formed into pellets (150mg each). The die used was 13mm and pressure of 300kg/cm² was used.

Analysis using ED-XRF Spectrometer

The elemental analysis of vegetables samples was carried out using a Xenomatrix Ex-3600 Energy dispersive X-ray fluorescence ED-XRF spectrometer installed at UGC-DAE Consortium for Scientific Research, Kolkata Center, Kolkata, India, which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV). The measurements were carried out in vacuum using different filters (between the source and sample) for optimum detection of elements. For example, for P, S, Cl, K and Ca, no filter was used, and a voltage of 6 kV and current of 200 mA were used and samples were run for 200 sec. A 0.05-mm-thick Ti filter was used in front of the source for Mn, Fe, Cu and Zn, with an applied voltage of 14 kV and a current 900 mA and samples were run for 400 sec. For higher Z elements such as Se, Br, Rb and Sr Fe filter of 0.05 mm thickness was used at a voltage of 23 kV and 200 mA current and samples were run for 600 sec. The X-rays were detected using a liquid-nitrogen-cooled 12.5 mm² Si (Li) semiconductor detector (resolution 150 eV at 5.9KeV). The X-ray fluorescence spectra were quantitatively analyzed by the software next integrated with the system. This software uses the Fundamental Parameter Method approach, which combines a theoretical basis of X-ray emission and absorption with experimental measurements for unknown sample analyses. Here, all matrix corrections etc are taken into account.

Statistical analysis of data

The experimental results were subject to statistical analysis using Excel 2007 and SPSS package v.17.0. Values are present in the standard error of mean (SEM).

Results and Discussion

The concentration of major elements (K, C, P, Cl and S) and trace elements (Fe, Zn, Cu, Mn, Se Sr, Rb and Br) in the selected leafy



Figure 2: Photographs of *Clerodendron colebrookianum*, *Brassica juncea*, *Zanthoxylum rhetsa* L., *Vigna unguiculata*, *Cucurbita pepo* L., *Gnetum gnemon* L., *Cucurbita maxima*, *Colocasis esculenta* L.

vegetables i.e *Brassica juncea*, *Colocasis esculenta* L., *Zanthoxylum rhetsa* L., *Clerodendron colebrookianum*, *Vigna unguiculata* L., *Gnetum gnemon* L., *Cucurbita pepo* and *Cucurbita maxima* is presented in the Table 1,2 and 3. Typical ED-XRF spectra of the major elements of the leafy vegetables studied is shown in the Figure 1, while the photograph of the parts of the leafy vegetables used is shown in Figure 2. Table 1,2 and 3 reveals wide variation in the major and minor elements concentration of the various leafy vegetables studied. The results of the present study reveals that K is observed generally higher in all the leafy vegetables studied as compared to other major elements recorded. The content of K, C, P, Cl and S in all the selected leafy vegetables ranged from 1436.04 ± 7.47 mg/100gm to 4288.36 ± 221.54 mg/100gm, 391.74 ± 9.56 mg/100gm to 3473.04 ± 144.39 mg/100gm, 310.01 ± 7.63 mg/100gm to 475.51 ± 30.24 mg/100gm, 336.33 ± 3.92 mg/100gm to 2043.45 ± 237.36 mg/100gm and 170.55 ± 28.71 mg/100gm to 562.50 ± 11.28 mg/100gm respectively. The highest content of K and Cl was recorded in the *Colocasis esculenta* L leaves while the highest content of Ca and P was in the *Cucurbita maxima* with *Brassica juncea* contained highest amount of S. The study reveals that the leafy vegetables contained high and elevated amount of K and Ca. Studies related to the contents of macro elements in the commonly consumed leafy vegetables from other parts of the world is limited. The values recorded in our study is in agreement with those reported by Stihl et al. [8] and Masson et al. [9] however, is more than those reported by Vincevica Gaile and Klavins [10]. As revealed from the study, all the leafy vegetable samples contain varied mean levels of elements which could be attributed to factors such as variation in genetic makeup of plant species and competitive interaction between metal ions within the lithosphere or variation in the amounts of the mineral in the soils where the vegetables grew as published by Anyawu et al. [11]. Indian Council of Medical Research [12] has recommended that the recommended dietary allowances (RDI) for Ca is 600, 1200 mg, and 800 mg per day for adult, pregnant and lactating women and 10-18 years of boys and girls respectively. On

the other hand the Recommended Nutrient Intake (RNI) for K and P is 1100 - 3750mg/day and 600-800 mg/day respectively for adults of both sexes. Similarly, the permissible limit of S in the solid food is 3100 mg/kg [13]. However, all the major elements concentration recorded in the leafy vegetables in the present study were above the permissible limit as reported by these regulatory organizations. It means that these vegetables foodstuffs were loaded with high amount of Ca, K, P, Cl and S and that may posed threat to health on long term consumption even though these elements are considered essential elements for human body. Salim et al. published that high concentration of major elements recorded in leafy vegetables in the present study may be due to high content of these metals in the soil [14]. Additionally foliar uptake of atmospheric heavy metals from emissions has also been identified as an important pathway of heavy metal contamination in vegetable crops as leafy vegetables accumulate higher metal contents than others [15]. Otten et al. [16] reported that K is one of the essential elements of human diet and play important role in vital cellular mechanisms and K deficiency in the diet can create resistance in fat and muscle cells etc from insulin, increased serum triglycerides, may lower HDL and blood supply to the vital organs and increase the chances of stroke. Like K, Ca is also an important element which plays a pivotal in signal transduction pathways, where it acts as a second messenger and contraction of all muscle cell types [17]. Cl is an element making up 0.14% of the concentration of elements in the human body and one of the important roles of Cl in the body is their electrolyte function. By combining with Na and K, Cl works well to keep all of our biological systems running smoothly [18]. P is an element that makes up 1% of a person's total body weight and present in every cell of the body. The main function of P in the human body is in the formation of bones and teeth [19]. Baker reported that as a macro element of the body, the role of S on the body is to acts as an integral part of many important compounds found in all body cells which are indispensable for life [20].

Trace element plays an important role in human health because

Table 1: K, Ca, P, Cl, and S concentration (mg/100gm) of the Selected Leafy Vegetables Foodstuffs of Mizoram. Values are mean \pm SEM, 3 observations each.

| Name of the Leafy Vegetables | Potassium (K) | Calcium (Ca) | Phosphorus (P) | Chlorine (Cl) | Sulphur (S) |
|------------------------------------|----------------------|----------------------|--------------------|----------------------|--------------------|
| <i>Clerodendron colebrookianum</i> | 1436.05 \pm 7.47 | 2053.67 \pm 31.23 | 310.02 \pm 7.63 | 336.33 \pm 3.92 | 348.12 \pm 10.31 |
| <i>Brassica juncea</i> | 2239.25 \pm 32.23 | 1438.05 \pm 14.80 | 359.94 \pm 23.03 | 1041.02 \pm 40.55 | 562.50 \pm 11.28 |
| <i>Zanthoxylum rhetsa</i> L. | 2214.34 \pm 24.77 | 541.16 \pm 23.33 | 304.81 \pm 1.89 | 685.32 \pm 13.84 | 262.60 \pm 4.63 |
| <i>Vigna unguiculata</i> L. | 2261.46 \pm 49.44 | 814.58 \pm 10.52 | 414.07 \pm 16.75 | 519.43 \pm 11.38 | 238.35 \pm 3.53 |
| <i>Cucurbita pepo</i> L. | 2505.79 \pm 39.47 | 1891.42 \pm 28.96 | 396.59 \pm 17.89 | 964.85 \pm 40.84 | 275.82 \pm 13.61 |
| <i>Gnetum gnemon</i> L. | 2415.05 \pm 43.32 | 391.74 \pm 9.56 | 396.76 \pm 47.50 | 500.42 \pm 25.86 | 418.92 \pm 11.38 |
| <i>Cucurbita maxima</i> | 2137.61 \pm 44.10 | 3473.04 \pm 144.39 | 475.51 \pm 30.24 | 996.51 \pm 25.45 | 204.14 \pm 1.63 |
| <i>Colocasis esculenta</i> L. | 4288.36 \pm 221.54 | 705.85 \pm 24.60 | 444.46 \pm 12.36 | 2043.45 \pm 237.36 | 170.55 \pm 28.71 |

Table 2: Fe, Mn, Zn and Cu concentration (mg/100mg) of the Selected Leafy Vegetables Foodstuffs of Mizoram. Values are mean \pm SEM, 3 observations each.

| Name of the Leafy Vegetables | Iron (Fe) | Manganese (Mn) | Zinc (Zn) | Copper (Cu) |
|------------------------------------|------------------|------------------|------------------|-----------------|
| <i>Clerodendron colebrookianum</i> | 24.66 \pm 4.17 | 9.63 \pm 0.61 | 3.57 \pm 0.26 | 1.45 \pm 0.09 |
| <i>Brassica juncea</i> | 31.67 \pm 1.57 | 6.79 \pm 0.29 | 4.68 \pm 0.42 | 0.43 \pm 0.03 |
| <i>Zanthoxylum rhetsa</i> L. | 16.19 \pm 1.03 | 28.59 \pm 1.87 | 2.84 \pm 0.10 | 1.34 \pm 0.07 |
| <i>Vigna unguiculata</i> L. | 15.89 \pm 1.04 | 9.08 \pm 0.24 | 3.18 \pm 0.05 | 1.06 \pm 0.1 |
| <i>Cucurbita pepo</i> L. | 27.29 \pm 1.35 | 2.40 \pm 0.1 | 3.60 \pm 0.14 | 0.54 \pm 0.08 |
| <i>Gnetum gnemon</i> L. | 16.17 \pm 1.68 | 24.17 \pm 0.41 | 4.12 \pm 0.1 | 0.56 \pm 0.06 |
| <i>Cucurbita maxima</i> | 82.15 \pm 5.59 | 2.63 \pm 0.2 | 4.08 \pm 0.03 | 0.49 \pm 0.01 |
| <i>Colocasis esculenta</i> L. | 10.97 \pm 0.74 | 9.88 \pm 1.04 | 10.44 \pm 0.17 | 0.87 \pm 0.08 |

Table 3: Se, Sr, Rb and Br concentration (mg/100mg) of the Selected Leafy Vegetables Foodstuffs of Mizoram. Values are mean \pm SEM, 3 observations each.

| Name of the Leafy Vegetables | Selenium (Se) | Strontium (Sr) | Rubidium (Rb) | Bromine (Br) |
|------------------------------------|------------------|------------------|-----------------|-----------------|
| <i>Clerodendron colebrookianum</i> | 0.03 \pm 0.01 | 6.34 \pm 0.14 | 0.51 \pm 0.04 | 1.29 \pm 0.09 |
| <i>Brassica juncea</i> | 0.015 \pm 0.00 | 12.15 \pm 0.35 | 3.54 \pm 0.12 | 3.68 \pm 0.20 |
| <i>Zanthoxylum rhetsa</i> L. | 0.01 \pm 0.00 | 3.81 \pm 0.27 | 2.17 \pm 0.17 | 1.3 \pm 0.06 |
| <i>Vigna unguiculata</i> L. | 0.08 \pm 0.04 | 5.08 \pm 0.20 | 2.42 \pm 0.08 | 1.66 \pm 0.08 |
| <i>Cucurbita pepo</i> L. | 0.02 \pm 0.00 | 6.74 \pm 0.40 | 1.38 \pm 0.02 | 0.94 \pm 0.05 |
| <i>Gnetum gnemon</i> L. | 0.012 \pm 0.00 | 0.95 \pm 0.13 | 5.12 \pm 0.1 | 0.17 \pm 0.07 |
| <i>Cucurbita maxima</i> | 0.01 \pm 0.00 | 12.25 \pm 0.37 | 1.35 \pm 0.1 | 1.05 \pm 0.06 |
| <i>Colocasis esculenta</i> L. | 0.01 \pm 0.00 | 1.85 \pm 0.1 | 3.32 \pm 0.1 | 0.15 \pm 0.06 |

they participate in biological functions that contribute to growth and good health. Analysis of data revealed that among the trace element studied, Fe concentration is found to be higher in all the leafy vegetables studied. The Fe content ranges between 10.97 \pm 0.74 mg/100gm to 82.15 \pm 5.59 mg/100gm in all the leafy vegetables studied with the highest Fe content was found in *Cucurbita maxima*. The concentration of Fe recorded in our study is less than those reported by Mohammed and Sharif [21] and Ismail et al., [22].

The high amount of Fe in the leafy vegetables may be due to the foliar absorption from the surroundings air. Codex Alimentarius Commission published that the permissible limit for Fe in food is in the range of 2.5-5.0 mg/kg depending on the foodstuff and the minimum daily requirement of Fe is ranged from about 10 to 50 mg/day [23]. Therefore, the value recorded in our study is above the recommended dietary allowances of Fe (10-15 mg/day) as per FAO/WHO [24] and NIN. Fe is a necessary nutrient element and is core

component of RBC and needed for healthy immune system and for energy production [25].

Like Fe, Mn is one of the important essential elements required in carbohydrate metabolism as well as an antioxidant in SOD enzymes. Chaturvedi et al. published that Mn is also important in regulation of immune responses of the body by breakdown of amino acids, production of energy, by regulating the metabolism of Vitamin B₁, C, E and by activation of various enzymes important for proper digestion and utilization of foods [26]. The content of the Mn recorded in the present study ranged between 2.40 ± 0.14 mg/100gm to 28.59 ± 1.87 mg/100gm and *Zanthoxylum rhetsa* L. contained the highest. The value of Mn obtained in our study is more than those reported by Singh and Taneja [6] and Gorbunov et al. [27]. As a contaminant however, no maximum permissible limit (MPL) has been fixed for Mn in vegetables. Upper tolerable limit of Mn for human is 2-11 mg/day.

In the present study, Zn concentration was recorded in the range between 3.18 ± 0.50 mg/100gm to 10.44 ± 0.17 mg/100gm and the highest Zn content was found in the *Colocasis esculenta* L. The concentration of Zn obtained in our study is in agreement with those reported by Singh and Taneja [6] but greater than the values recorded in the earlier studies from Egypt and Latin America published by Salama and Radwan [28] and Olivares et al. [29] respectively. This disparity between the results of the present study and those reported from other parts of the country could be explained by the indiscriminate use of excess Zn as micronutrients in Agricultural practices in India during the last decades [30]. Sandstead published that Zn is an important trace element involved in numerous aspects of cellular metabolism and required for the catalytic activity of more than 200 enzymes [31]. Cu is known to play an important role in human metabolism, largely because it allows many critical enzymes to function properly. As an antioxidant, Cu scavenges or neutralizes free radicals and may reduce or help prevent some of the damage they cause [32]. As expected, Cu was found to be present in appreciable amount in the selected leafy vegetables foodstuffs studied between the range from 0.43 ± 0.03 mg/100gm to 1.45 ± 0.09 mg/100gm and the highest level of the Cu was recorded from the *Clerodendron colebrookianum*. The Cu content recorded in the present study is in agreement with Singh and Taneja (2010) but less than that published by Mohammed and Sharif [21]. The analysis of data from the present study revealed that Se contents in the selected leafy vegetables foodstuffs ranged between 0.012 mg/100gm to 0.085 mg/100gm and *Vigna unguiculata* L. contained the highest. As contaminants no such limit for Se. Sunde [33] published that Se is one of the trace elements which behaves both as an antioxidant and anti-inflammatory agent.

Very interestingly, in the present study, Sr, Rb and Br were also detected in all the leafy vegetables studied with varying concentration in the range between 1.85 ± 0.10 mg/100gm to 12.250 ± 0.37 mg/100gm, 0.51 ± 0.04 mg/100gm to 5.12 ± 0.10 mg/100gm and 0.15 ± 0.06 mg/100gm to 3.68 ± 0.20 mg/100gm for Sr, Rb and Br respectively. The highest content of the Sr, Rb and Br were recorded from the *Cucurbita maxima*, *Gnetum gnemon* L and *Brassica juncea* respectively. Generally Sr, Rb and Br have no known essential role in human or mammalian health however Ohly Julius [34] published

that Rb ions are utilized by human body in a manner similar to potassium ions, being actively taken up by plants and by animal cells.

Conclusions

As revealed from the present study, ED- XRF spectrometry can be used for detailed analysis of element contents in the leafy vegetables. In addition, there was also the opportunity of analyzing some other elements such as S that can hardly be determined by other techniques. The variation of elemental content from plant to plant in this study may be mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated and preferential absorbability of the plants, use of fertilizers, irrigation water and climatic conditions. In this study, our result is well comparable with the findings of others. An elevated level of the Ca, K, S, P, S and Fe recorded in this study more than the maximum acceptable levels proposed by the regulatory organizations may pose a threat to health on long term consumption. The results of the present study thus provide a valuable database of the chemical quality of the commonly consumed leafy vegetables by natives of the North Eastern India.

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