

## Trace Elemental Analysis of Finger Millet Samples from Kidney Effected Area using EDXRF Technique

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### ABSTRACT

Finger Millet samples are collected from different villages affected with chronic kidney disease (CKD) in Uddanam area of Srikakulam District, Andhra Pradesh, India. These samples are analyzed using Energy Dispersive X-ray Fluorescence (EDXRF). The trace elements P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se are identified and compared with NIST Apple leaf-1515. The impact of each element on human physiology and CKD is analyzed.

**Keywords:** Finger Millet, CKD, EDXRF, Trace Elements

### I. INTRODUCTION

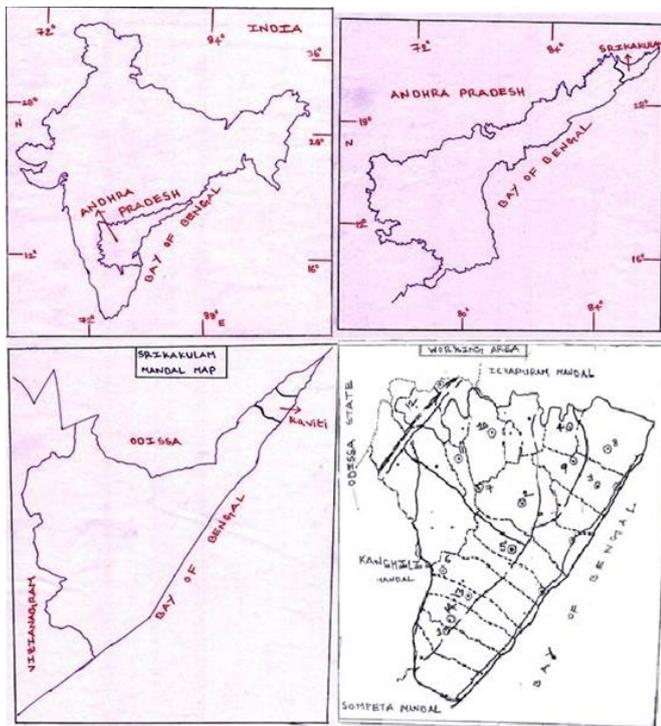
Kidneys are the two bean shaped organs that are located in the back, just under the ribs and on either side of the spine. Kidney plays an important role in the purification of blood also removal waste from the body. Chronic kidney disease (CKD) occurs due to from gradual and usually permanent loss of kidney function over time, which may take months to years.

Over a decade, a new form of kidney disease of unknown etiology has emerged in the Uddanam area in srikakulam district, Andhra Pradesh, India. Almost 50% of people suffering from kidney disease. High prevalence of chronic kidney disease (CKD) has become an environmental health issue of national concern in India. Hypertension and diabetes are known to be the main reasons for renal

failure but in areas with high prevalence of CKD, the majority of patients do not show any of these identifiable causes.

Uddanam is a lush green region in the Srikakulam District of the state of Andhra Pradesh, India (Fig:1), located on the east coast of India with rich coconut and cashew plantations. Geographically Uddanam is located at 19.0167<sup>0</sup>N, 84.6833<sup>0</sup>E. It has an average elevation of 41 meters (137 feet) above the sea level.

In the present work, Finger Millet samples are collected from fourteen villages of Uddanam area, made into pellets and are exposed to x-ray beam of the EDXRF facility available at UGC-DAE CSR Kolkata. Trace elemental concentration are evaluated using Standard Reference Material (SRM)



**Figure 1 :** Geographical location of the sampling villages

## II. METHODS AND MATERIAL

### 2.1 Study Area:

Uddanam region consists of four areas, population of each area and number of kidney patients are given in Table 1. Finger Millet samples were collected from 14 villages of Kaviti area. 1. Balli Puttuga 2. Kaviti 3. Kapasa Kuddi 4. D.Gonapa Puttuga 5. Bejji Puttuga 6. Lolla Puttuga 7. Tottidi Puttuga 8. Synasi Puttuga 9. Byreddla Puttuga 10. Rajapuram 11. Kamalai Puttuga 12. Khojjiria 13. Varakha 14. Kusumpuram. Higher prevalence of CKD is reported in kaviti area.

**Table: 1** CKD prevalence areas

SR NO	Area	Total population	No. of kidney patients	Percentage of effected patients
1	Ichchapuram	82,236	1100	1.33
2	Sompeta	79,440	1200	1.51
3	Kanchili	64,125	3200	4.99
4	Kaviti	76,019	4500	5.91

### 2.2 Sample Preparation

The samples were collected from yield crop directly from the farmer field. All the samples were collected on the same day in polythene bags. The finger millet samples were washed 2D- water removes wastage and dried at 60°C for one hour. The samples were homogenized in an agate mortar. The well homogenized powder samples were made into pellets of 13 mm diameter at EDXRF laboratory using KBR pelletiser. 0.2g of powdered samples were made into pellets.

### 2.3 Experimental procedure

Finger millet pellet samples are analyzed by Energy Dispersive X-Ray Fluorescence (EDXRF) at UGC-DAE CSR Kolkata center. X-Ray Fluorescence (XRF) spectrometry has been a technique for elemental analysis for almost 100 years based on Moseley's well known law which relates "characteristic" fluorescence radiation to the atomic number of the emitting atom [1]. Now a days composition analysis by measuring fluorescence spectra has become a routine technique utilized in a vast number of research areas ranging from material science to biomedical science. X-Ray Fluorescence analysis is non-destructive with high precision. It is a multi-elemental method to analyze most of the elements of the periodic table and more effective for elements with  $Z \geq 11$  (i.e from Sodium to Uranium). Moreover, as the fluorescence intensity is proportional to the concentration of an element present in the sample, not only qualitative but also quantitative analysis is possible.

## III. RESULTS AND DISCUSSION

The spectra are collected for sufficiently long time to ensure good counting statistics. During the irradiation of each sample, the total charge collected was noted. The x-ray spectra were recorded with Si(Li) detector. The concentrations of different elements in the finger millet samples were estimated using nEXT software.

**Table 2:** The average concentrations of trace elements in Finfer Millet samples (with standard deviation)

	Village	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se
1	Balli Puttuga	1402.38±65.07	764.67±32.97	579	4263.01±88.74	3962.2±202.40	53.25±3.06	162.58±31.69	8.72±0.71	24.57±2.13	0.41±0.50
2	Kaviti	1370.63±175.79	618.18±69.91	1082.66±32.90	5530.85±145.05	3210.59±129.93	252.55±0.70	276.06±11.03	6.64±0.67	22.66±2.94	0.7±0.95
3	Kapasa Kuddi	1494.26±138.82	974.09±51.99	579	3623.26±102.45	3998.82±177.72	152.17±5.84	62.81±40.70	9.31±1.50	32.4±0.18	0.57±0.46
4	D.Gona Puttuga	1603.61±95.55	758.07±54.71	848.57±29.85	4412.99±173.59	4620.41±306.34	143.71±31.60	149.66±10.74	8.13±1.06	26.87±0.96	0.12
5	Bejji Puttuga	1728.5±36.99	740.55±25.56	1192.35±17.42	5968.66±62.61	4264.61±175.03	77±1.23	574.5±42.19	6.76±0.51	30.05±1.47	0.35±0.39
6	Lolla Puttuga	1177.76±45.90	761.86±156.42	579	3124.8±1612.57	4424.69±137.13	162.78±5.60	134.7±8.91	7.66±0.74	19.8±3.30	0.98±0.47
7	Tottidi Puttuga	1619.43±173.99	816.37±88.37	708.2±223.78	4813.74±157.12	4835.43±819.02	246.39±147.42	100.89±12.53	9.2±0.75	25.57±0.43	1.08±0.83
8	Synasiputtuga	1608.37±18.60	782.62±6.54	858.03±241.65	5000.82±32.77	4562.6±160.07	77.56±3.56	87.76±2.49	8.62±1.09	23.63±4.26	0.57±0.73
9	Byreddlaputtuga	1681±41.18	880.64±5.70	1067.77±23.10	5215.98±40	3958.11±54.83	171.9±2.20	204.26±7.23	9.11±0.93	30.95±1.42	0.33±0.36
10	Rajapuram	1967.96±67.34	810.77±198.84	1034.04±21.44	5180.90±86.50	4446.25±61.85	81.41±3.36	347.86±65.28	10.18±1.05	33.09±3.57	0.38±0.44
11	kamalaiputtuga	1768.60±59.08	896.76±34.47	970.26±46.65	4926.13±143.56	4210.5±155.07	78.51±3	566.23±40.44	9.46±0.68	31.53±1.19	0.16±0.06
12	Khojjiria	1776.74±85.81	761.92±6.18	1131.76±32.26	5574.11±112.33	3998.76±113.55	42.15±1.39	64.12±4.11	9.18±0.97	26.19±1.01	0.5±0.34
13	Varaka	1258.67±116.90	665.12±36.70	579	2867.77±215.31	4199.93±224	154.49±5.33	134.22±11.28	8.05±0.16	20.44±2.71	0.12
14	Kusampuram	1590.66±50.95	660.42±45.18	1208.55±28.57	6044.23±65.29	4215±363.94	72.69±4.59	641.79±20.94	7.8±1.25	29.11±2.24	0.13±0.02

In the EDXRF experiment the trace elements P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se are identified. The element potassium (K) is of importance in the human body along with sodium. It regulates the water balance and acid-base balance in the blood. Potassium is found within all cells of the body and its levels are controlled by the kidneys. Potassium is low in all villages finger millet samples.

In finger millet samples, Calcium is very low in all villages with respect to standard. Low level Calcium in blood is called hypocalcaemia which can be caused by hyperparathyroidism by kidney failure, by low levels of plasma magnesium (hypo magnesias) (or) by failure to get adequate amounts of calcium (or) vitamin D in the diet.

Oxidative stress and inflammation play a major role in the progression of renal damage in chronic kidney disease (CKD). Mn is a potent anti-oxidant and co factors of the enzyme MnSOD, which is the main antioxidant enzyme in the mitochondria and responsible for protecting the cell from Reactive Oxygen Species (ROS) by scavenging mitochondrial superoxides. Manganese level was found to be equal to the standard in the Balli Puttuga village and is below the standard in

Khojjiria village. In all other villages above the standard reference value.

In CKD, the anemia that develops is frequently complex. The primary cause is inadequate production of erythropoietin by the diseased kidney [2]. Decreasing protein intake reduces iron intake and depletes iron stores. Absorption of iron from the gastro intestinal tract may also decrease [3]. Thus multiple factors can contribute to adequate total body iron stores in patients with CKD, the condition is known as “absolute iron deficiency” [4]. Iron is very high in the Bejji Puttuga, Kamalai Puttuga, Kusum Puram villages and is below the standard in Kapasa kuddi, Khojjiria villages. In all other villages the value is above the standard.

Plasma Cu was higher in the Diabetes Mellitus (DM) group when compared to the non-DM control groups. Serum urea was a positive independent determinant of plasma Zn concentration. These findings demonstrate an alteration in the distribution of Zn patients with CKD independently of the presence of DM. Also the status of Cu seems not to be influenced by CKD, but only by the metabolic derangements associated with diabetes [5]. Copper level is high in all villages Finger Millet samples.

Zinc is essential for human nutrition as it acts as a structural and functional component of several metabolic proteins and participates in cellular metabolism reactions. Zinc is also an antioxidant that reduces free radicals [6]. Lower serum concentration of zinc have been attributed to reduced food intake and intestinal absorption, uremic toxicity, interaction with Calcium, Iron and vitamin D deficiency and increased mineral loss during dialysis [7]. Zinc is very high in all other villages with respect to standard.

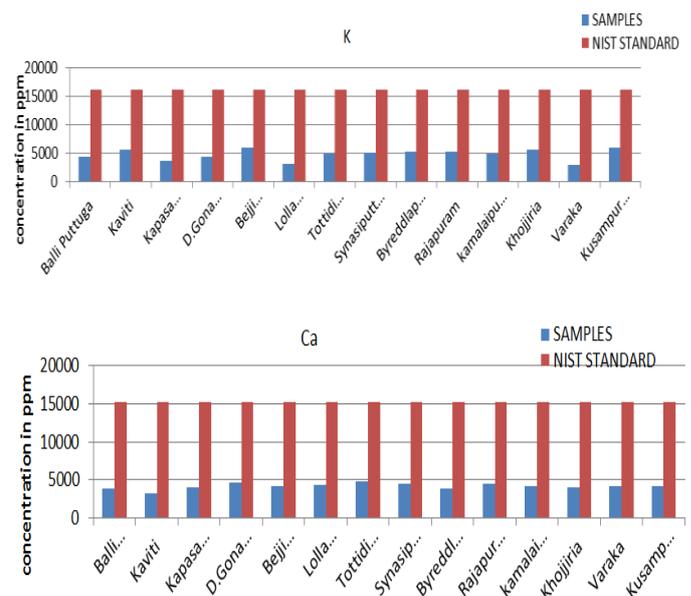
Increased serum sulfate levels are a common feature of kidney failure. In end stage renal disease, sulfate can be removed by hemodialysis and peritoneal dialysis treatment, but serum sulfate levels are often still elevated [8-10]. Increased serum sulfate concentration results in increased complication with calcium and this may in post be responsible for the parathyroid stimulation that occurs in chronic renal disease [8-11]. Sulphur has low concentration with respect to the standard.

Chlorine level the standard in Balli puttuga, Kapasa Kuddi, Lolla Puttuga, Varakha villages. Chloride makes up about 0.15% of a person's body weight and essential for maintaining acid-base electrolyte and fluid balance in the body. Hyperchloremia is an electrolyte imbalance and is indicated by a high level chloride in the blood. Chloride is an important electrolyte and works to ensure the body's metabolism to work correctly. Kidneys control the levels of chloride in blood.

Phosphorous level the standard in the Kusumpurm village and in all other villages it varies from upper to lower limit. About 900 mg of phosphorus is excreted daily in the urine and about 90% of plasma phosphates are filtered in the glomerulus. FGF-23 is produced in the bone higher phosphorus and higher calcitriol stimulate its production. As Glomerular Filtration Rate (GFR) decreases [12]. Several changes occur that effect phosphorus balance. The most important ones are a decrease in calcitriol level due to deficient 1-hydroxylation [13]. (And

with consequently lower intestinal calcium absorption hypocalcaemia and stimulation PTH production) and a decrease in the filtered amount of phosphorus with consequent hypocalcaemia, hyperphosphatemia and stimulation of PTH and FGF-23 production [14].

Selenium has high concentration with respect to standard in all areas. In patients with chronic renal failure (CRF), Se concentration in blood components is usually lower compared with healthy controls. Plasma Se-dependent GSH-PX, one of the five known forms of the enzyme, is synthesized primarily in the kidneys and it is reduced in CKD patients proportional to the progression of the disease. A significant decrease in GSH-PX activity resulting in increased Oxidative Stress (OS) is accompanied by wide spread Se-deficiency [15].



#### IV. CONCLUSION

Finger millet samples collected from the Uddanam area were analyzed for their elemental concentration using EDXRF technique. The accuracy of the obtained results was evaluated using NIST standards. In total, ten (10) elemental concentrations have been determined. The elements Cu, Se, Zn concentration is above the standard and element potassium(K) below the standard. The element Fe is below the standard in Kapasa Kuddi and

Khojjiria villages. Calcium and Sulphur were found to be low in all samples.

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## VI. REFERENCES

- [1]. R. D. Giaque, F.Asaro, F.H.Stross, T.R.Hester "High precession, Non-destructive X-Ray Fluorescence method applicable to establishing the provenance of obsidian artifacts X-Ray spectrum, 22(1) 44-53 (1993).
- [2]. Mittal S, Maesaka JK, Fishbane S, 1999. Diagnosis of Iron deficiency in end stage renal disease *semidial* 12: 231 – 234.
- [3]. Commmack R, Wriggles worth JM, Baum H 1999. Iron dependent enzymes in mammalian system. In *Iron transport and storage* 17 – 40: CRC Press Bocaraton.
- [4]. Linn.S. 1988, DNA damage by Iron and hydrogen peroxide in vitro and in vivo. *Drug Me-tab. Rev* 30:313-26.
- [5]. Maria Nazare Batista, Lilian Cuppari, Luciad Fatima campus Pedrosa, Maria das, Graces Almeida, Jose Brunode Almeida Anna Cecilia Queirozde mederos (2006). Effect of end stage renal disease and diabetes on zinc and copper status. *Biological Trace element Research* 112: 1-12.
- [6]. Prasad AS, Beck FWJ, Bao B, Fitzgerald JT, Snell DC, Stein berg JD, Zinc supplementation decreases incidence of infections in the elderly. Effect of zinc on generation of cytokines and oxidative stress. *AMJ Clin Nut* 2007:85: 837-844.
- [7]. Kiziltas H, Ekins, Trace Elemental status of chronic renal; patients undergoing Hemo dialysis, *Bio Trace Elem Res* 2008: 124: 103-109.
- [8]. Cole DEC, Evroski J.2000. The clinical chemistry of in Organic Sulfate *Crit Rev Clin Lab Sci* 37:299 – 344.
- [9]. Holmes JH, Miller ES, Hlad CJJ 1960. Serum and Urine Sulfate changes in Uremia. *Tranem, Soc Artif Intern organs* 6: 163 – 175.
- [10]. Kirschbaum B. 1998. Effect of hemodialysis on the hyper sulfatemia of chronic renal failure. *As AIO J.* 44: 314 – 318.
- [11]. Michalk D, Tschope W, Bohles HJ, Mehis possible role of inorganic sulphate in the pathogenesis of hyper parathyroidism in chronic renal failure *proc Eur. Dial Transport Assoc* 18: 561 – 566.
- [12]. Yama moto M, Igarashi T, Muramatsu M, et.al Hypocalcemia and hypercalcemia decreases the steady state level of parathyroid hormone messenger RNA in the rat *J.Clin invest* 1989: 83: 1053 – 1056.
- [13]. Portale AA, T Sai HC, Morris RC, Jo.Reduced plasma concentration of 1, 25 – dihydroxy, vitamin – D in children with moderate renal in sufficiency kidney *Int Dig.* 1987: 21: 627 – 632.
- [14]. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease *AMJ kidney dis* 2003.42 (Suppl<sub>3</sub>) 1 – 202.
- [15]. Richard MJ, Arnaud J, Jurkovitz C.et.al Trace Elements and lipid for oxidation abnormalities patients with chronic renal failure. *Nephron* 1991:57: 10-15.