MICROELEMENTAL STUDY OF *ORYZA SATIVA* L. SEEDS

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Abstract. Agriculture is one of the most ancient occupations in the world. The demand for food grew with the growth of world's population. Over the past 30 years, the total amount of food grains in the world have increased at a faster rate than the average rate of population growth. This is made possible to increase crop production by replacing manure with chemical fertilizers, introduction of high yielding and disease resistant varieties, increased use of synthetic pesticides, herbicides and rodenticides. The crop production is increased tremendously but negative impacts are seen resulting to environmental degradation. Cultivated rice (*Oryza sativa* L.) belonging to family *Poaceae* represents the world's most important staple food, feeding more than half of the population. Asia accounts for 90 % of the world's rice production as it grows well in alluvial soil with clayey subsoil. For high yield response practice of application of chemical fertilizers along with synthetic pesticides and rodenticides is common, which, results in soil contamination and become barren. The synthetic pesticides are sprayed on the plants which contain toxic elements that are absorbed by aerial plant parts and get deposited in the grains. These toxic elements present in the grains are detected by using EDXRF spectroscopy techniques.

Key words: Energy dispersive X-ray fluorescence spectroscopy, rice grain, microelements, chemical fertilizers, pesticides.

INTRODUCTION

Rice is the most important staple food. In fact, rice is consumed in polished form (white rice) which contains starch as main component and other vitamins are in negligible amount. Thus, the low micronutrient densities are the major reason for the human micronutrients malnutrition [6, 10]. World average per capita rice consumption is 65.0 kg, European Union-27 is least with 5.7 kg, Cambodia is highest with 292 kg and India with 76.7 kg, according to Eric and Eddie [8]. Hence, in India the average daily intake of rice is around 220 g per person, and polished rice having 45.5 to 68.2 ppm (mg kg^{-1}) iron and 54.5 to 68.2 ppm zinc can only meet the RDA reported by FAO/WHO [9] without considering their assimilation (bioavailability) from the digested food in the alimentary tract. The

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upcoming era may bring innumerable health issue due to excessive use of synthetic fertilizers and pesticides. Due to ignorance about the synthetic fertilizers, pesticides and herbicides, people use excessive amounts of these chemicals creating environmental hazards. Micronutrients and vitamin deficiency diseases (hidden hunger) are most common among the rice eating population and particularly poor people who are unable to afford nutrient rich supplements. On this background, pure food with maximum use of organic fertilizers can bring change in increasing health issue of common mass.

The microelements are increasingly recognized as important factors that influence human life either as pollutants or as medicines. The microelements such as Hg, Pb and Cd are known to be toxic even at relatively low concentrations. Therefore, energy dispersive X-ray fluorescence spectroscopy (EDXRF) can provide extensive information to determine the correlation of various elements present in plants and other organisms.

In recent years EDXRF has gained worldwide acceptance due to non-destructive multi-elemental analysis of variety of samples such as food grains, blood and medical formulations over a wide range of concentrations (ppm to 100 %). The effects of chemical fertilizers, pesticides and herbicides on the elemental concentration of rice grain were also investigated by this technique [23].

In this paper, we preferred white rice seeds, Eastern Vidharbha Region local brand (*White Luchai*-112) as standards measure for its food nutrient contents. This paper deals with (1) trace elements in cereal *Orizya sativa* L., (2) As species for the preparation of calibration standards, (3) preparation of AAS containing white rice grains, (4) desired sample thickness on Boric acid powder as standard substrate, (5) microelemental analysis by EDXRF, (6) homogeneity of the standards, (7) short-term preservation (two months minimum), (8) calibration by standard techniques, and (9) the sample in powder form. As in cereal rice, samples were determined using a calibration curve drawn using the prepared s-containing white rice standards by EDXRF. The analytical values showed good agreement with the values obtained by atomic absorption spectrometry (AAS) after acid decomposition.

The work is being presented here includes micro elemental analysis of rice seeds of variety *White Luchai*-112. Concentrations of metals like K, Ca, Mn, Mg, Fe, Al, Ti, P, Na, Zn, Cu, Pb and Si are studied using EDXRF spectroscopy technique.

MATERIALS AND METHODS

Samples of dried and unpolished rice were collected from different cultivating field areas of Eastern Vidarbha Region of Maharashtra state, India. A total of 30 rice samples were collected, and all the samples were of local variety *White Luchai*-112. All of the samples (20.0 g) were stored in a polyethylene bag of thickness $\approx$ 14 μm for nearly two months before testing. For elemental analysis of rice seeds were dehusked using a roller Sheller (Model JLGJ4.5) and damaged kernels were removed with tweezers. A subset of the brown rice kernels was subsequently milled into polished rice and rice bran, the latter consists of aleurone layer plus embryo, using a JNMJ3 rice polisher. All the rice seeds-derived samples were ground to flour with a particle size in the range of 20–100 µm using a Retsch Mixer Mill MM 400 fitted with ZrO grinding jars and balls (Retsch GmbH & Co KG, Haan, Germany) and oven-dried at 65 ºC for 72 h. The pallets of 2.54 cm. diameter were prepared using hydraulic press at a pressure 10 tones/cm. a minimum of 6 pallets of samples were made to reduce the error in the analysis. Boric powder was used as plasticizer.

All XRF measurements were made with an energy dispersion EDXRF spectrometer (X-Supreme 8000, Hitachi High-Tech Analytical Science, Japan) fitted with a tungsten anode X-ray tube, Cu filter. The apparatus was operated at a voltage of 20 kV, current of 50 mA and detection of 700 s. A characteristic radiation was detected using Argon flow scintillation counter over the range of 0–110 ºC with 6 µm polypropylene window. Each sample was tested 5 times, and the results were statistically analyzed. The data was analyzed using UniQuant-2 package and evaluated using the fundamental method [1, 14].

RESULTS

To determine the detection limits of the instrument and investigate the reproducibility and accuracy further, six standard rice sample, a contaminated sample and a blank sample were chosen for testing.

The pallets of size 2.54 cm were prepared by using Boric powder as plasticizer and the EDXRF analysis results are enumerated in Table 1. The results of the analysis in using µg g^{-1} (dry weight) of the samples were collected from the different field areas of the Eastern Vidarbha Region, Maharashtra, India under study and are displayed in Table 2. The correlations between EDXRF and reference values were then examined, along with bias and standard errors of prediction (SEP), based on data from this single EDXRF scan. To investigate EDXRF precision, each validation sample was analyzed a second time, allowing standard deviations and relative standard deviation (RSD) to be calculated from the duplicate data of each sample. There are a range of sources of error in EDXRF analysis which contribute to measurement uncertainty and overall standard errors of calibration (SEC) and SEP, including errors associated with sample preparation as suggested by Blank and Eksperiandova [4], Injuk *et al*. [13] as well as less significant ‘machine errors’ (e.g. variation in sensitivity and machine drift). Since samples were analyzed as powder of whole grains in this study, sample inhomogeneity was expected to be a major source of error. However, EDXRF repeatability was found to be high produced RSD values of 3–6 %. Further, the overall SEC and SEP observed in this study were similar to those seen previously in the EDXRF analysis of pressed milk-powders where SEP were approximately 3 mg kg^{-1} for both Zn and Fe, observed by Perring and Andrey [22]. The standard deviation (SD) is high because of the variation in the elemental content. The EDXRF analysis cited in Table 1 and Table 2 shows that various trace elements exist at different levels in micrograms.

Table 1

Elemental concentration, (μg g^{-1} dry weight) for *Oryza sativa* L. variety *White Luchai*-112 (Control)

SAMPLE	K	Ca	Mn	Mg	Fe	Al	Ti	P	Na	Zn	Ni	Cu	Si
1	138.2	10.9	3.8	31.8	2.4	6.8	14.8	126.1	26.8	9.6	1.0	4.8	10.5
2	137.9	9.4	3.7	31	2.0	5.8	13.5	126.2	25.9	9.3	0.8	5.1	12.2
3	135.6	9.6	2.8	29.6	2.8	6.2	12.4	118.4	27.2	10.4	0.9	3.6	16.9
4	135.6	10.7	3.6	33.4	1.7	6.8	15.6	135.3	27.1	9.2	1.3	5.2	15.3
5	142.2	11.3	3.9	29.7	1.9	6.5	15.1	120.6	24.4	9.3	0.6	2.1	11.3
6	140.6	11.5	3.8	32.3	1.8	5.8	15	124	26.4	9.2	0.8	5	10.8
Mean	138.4	10.6	3.6	31.3	2.1	6.3	14.4	125.1	26.3	9.5	0.9	4.8	12.8
SD	2.7	0.9	0.4	1.5	0.4	0.5	1.2	5.9	1.1	0.5	0.2	0.6	2.6

Table 2

Elemental concentration, (μg g^{-1} dry weight) for *Oryza sativa* L. variety *White Luchai*-112 (Experimental)

SAMPLE	K	Ca	Mn	Mg	Fe	Al	Ti	P	Na	Zn	Ni	Cu	Si
1	117.7	11.8	3.9	34.8	2.8	5.8	16.8	102.7	27.8	10.2	1.4	5.8	9.5
2	115.3	10.7	4	35.8	2.1	5.0	15.9	100	27.9	9.9	1.3	6.3	9.1
3	119.4	10.4	4.6	35.2	2.8	4.8	15.4	102.1	26.3	9.3	1.3	4.9	10
4	118.3	12.6	4.5	30.6	2.1	6.3	16.1	100.3	27.5	9.8	1.3	5.2	8.9
5	115.9	12.3	4.6	33.1	2.0	5.3	17.8	102.1	28.6	11.5	2.4	5.0	8.8
6	116.0	10.6	4.2	33.3	2.0	5.2	15.8	108.4	26.9	9.9	1.3	5.2	10.2
Mean	117.1	11.4	4.3	33.8	2.3	5.4	16.3	102.6	27.5	10.1	1.5	5.4	9.4
SD	1.6	0.9	0.3	1.9	0.4	0.6	0.9	3.0	0.8	0.7	0.4	0.5	2.6

The percentage of NPK is above 100 μg g^{-1}. The major cause for higher percentage of NPK in comparison to other trace elements is due to excessive application of synthetic fertilizers. Elements such as iron, zinc, manganese and copper are detected in lower concentration. The chemical analysis of soil and pH measurements lead to the fact that stratified layers of limestone and blocks absorption of elements such as Zn, Cu, Fe and Mn in rice plants takes place.

To investigate any kind of correlation between elemental concentration and the dependence of these concentrations in the studied variety, the Karl Pearson correlation matrix was calculated. From the results enumerated in Table 3, it can be seen that, there is a negative correlation for Zn, Cu and Ni with other elements and weak ($r^2 \leq 0.8$), while Al, Ti, P and Na show positive significant correlation.

Table 3

Karl Pearson correlation matrix for *Oryza sativa* L. variety *White Luchai*-112, for *n* = 6

SAMPLE	**K**	**Ca**	**Mn**	**Mg**	**Fe**	**Al**	**Ti**	**P**	**Na**	**Zn**	**Ni**	**Cu**	**Si**
K	–	0.66	0.69	0.33	–0.40	–0.20	–0.10	–0.39	–0.84	–0.48	–0.77	0.45	–0.66
Ca		–	0.63	0.31	–0.64	0.29	0.92	0.69	–0.31	–0.53	–0.11	0.48	–0.45
Mn			–	0.35	0.74	0.90	0.87	0.45	0.10	–0.80	–0.12	0.91	–0.87
Mg				–	–0.64	0.16	0.33	0.91	0.44	–0.60	–0.71	0.60	0.21
Fe					–	0.04	–0.87	–0.59	0.41	0.95	–0.10	–0.90	0.38
Al						–	0.52	–0.04	0.40	0.14	0.02	0.53	0.09
Ti							–	0.28	0.07	–0.93	0.26	0.90	–0.46
P								–	0.34	0.29	0.79	0.61	–0.29
Na									–	0.45	0.78	–0.45	0.70
Zn										–	–0.07	–0.98	0.75
Ni											–	0.02	0.47
Cu												–	–0.51
Si													–

DISCUSSION

For the optimum production of agricultural products, application of chemical fertilizers and pesticides has become a fatal practice which has been creating horrific impact on the environment and human health. Heading towards material comfort in the rapid speed, the modern human being is ignoring the perpetual happiness of psychological as well as physical health [26]. Most of the rice varieties are grown in the warm and humid tropic, unfortunately tropical conditions favor the proliferation of insects on the rice plants. The insect problem is accentuated further in areas and does not undergo a distinct dispause or dormancy but occur throughout the year in overlapping generations. Therefore, pesticides and insecticides are spread on the plants during cultivation which get distributed throughout the tissues.

There are near about twenty six micro elements present in the animal body. Some of them are known to be essential for growth of human physique such as C, H, O, N, S, Ca, P, K, Na, Cl, Mn, Mg, Ti, Cu, Fe, Ni, Si, and Zn [19].

Ozturk *et al*. [21] using a staining technique, suggested not only that Zn distribution is limited to the aleurone layer in rice grains but that this element is also present in the outer parts of the endosperm which is known to be rich in proteins. The present study suggests that Zn and Cu are also present in the ventral part of the endosperm. A similar distribution pattern for Zn and Cu was observed by Lombi *et al*. [17] in rice grains. Some fertilizers are enriched by zinc [7].

As argued by Borg *et al*. [5] the evidence that ferritin is present in isolated amyloplasts, supported by Balmer *et al*. [3], may indicate that Fe localization may not be completely restricted to the aleurone layer. The present study shows that a part of the Fe is present in the ventral part of the endosperm. Therefore, it can be speculated that storage of Fe in phytate granules or in protein storage vacuoles in the aleurone may be a key process limiting its transfer to the endosperm. It is possible that the Fe transported to the endosperm from the aleurone layer is complexed by nicotianamine, as was shown in the rice endosperm by Lee *et al*. [15].

In the present piece of research work it was observed from the EDXRF analysis that the concentration of Fe element is less than that of Zn. Gregorio [11] also observed more loss of iron than zinc during polishing. This could be due to partial or complete loss of both embryo and aleurone regions during polishing, more iron is distributed in the embryo followed by aleurone layer and endosperm according to Gregorio [11] variation in the thickness of the aleurone layer or embryo size or both, etc.

There are other elements which are known to be present in animal body and are not essential which acquired in the body as environmental contaminants. Amongst these only Hg, Pb and Cd are known to be toxic even at low concentration [18]. Cadmium can also be detected by using XRF method [25]. The accuracy, repeatability and detection limits of the EDXRF can be used to detect the concentration of Cd [16]. The main cause of high concentration ($\geq$ 125 ppm) P and K, in comparison to other trace elements is due to excessive application of chemical fertilizers [2]. Iron elements play a catalytic role either in an inorganic form or combined with organic compounds as a complex of redox enzymes [20]. The results of the bulk analyses confirmed earlier reports that rice bran contains greater concentrations of Ti rice grains. We also observed a dramatic presence in the grain, which was previously observed by Signes *et al*. [24] is probably related to the way nutrients, contaminants and photosynthetates are distributed within the different parts of organs at different level of concentrations. Moreover, the presence of elements such as Mg, Al, Ti and Ni are residues of pesticides and insecticides absorbed by the plant tissues and pertain in rice grain which may be hazardous to human physique after consumption [12]. The restricted use of these artificial manure and pesticides is need of hour.

CONCLUSIONS

The accuracy, repeatability and detection limits of the energy dispersive X-ray fluorescence spectrometer were verified, and the results indicated that the apparatus is able to meet the requirements of quick screening of different elements present in rice grain. Screening can reduce the risk of the problematic rice entering the market or being stored in a grain depot.

The elements found in rice grains by EDXRF spectroscopy viz. Mg, Al, TI, Ni, and Cd are the residues of these chemical pesticides, absorbed and pertain in rice grain in various micro percents. The larger concentration of elements such as K and P is probably the result of excessive use of NPK fertilizers and accumulation in the embryo. EDXRF analysis gives out a ready reference for elemental concentration in rice cereal. Large gradients in the distribution of microelements were present in the rice grains. The information that can be generated by this technique can be utilized to optimize grain processing. Furthermore, it has the potential to provide important information in terms of grain physiology. This may indicate that some of these important micronutrients may be complexed by different ligands in rice seeds even though direct investigation of their speciation is required. So, the restricted use of these poisons needs to be practiced during the cultivation because rice seeds as whole is widely used as a food additive and as a premier health food product of Eastern Vidarbha Region of Maharashtra, India.

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