



Determinations of the level of essential and non-essential metals in rice and soil samples Tilahun Belayneh, Zemene Atnafu, Alle Madhusudhan*

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Abstract:

The aim of the present study was to investigate the concentration of essential metals (Ca, Na, K, Mg, Cr, Cu, Fe, Mn, Ni, and Zn) and non-essential metals (Cd, Ag and Pb) in Asian rice, *Oryza sativa* and African rice, *O. glaberrimais* which are the two most cultivated species. The concentrations of essential metals (Ca, Na, K, Mg, Cr, Cu, Fe, Mn, Ni, and Zn) and non-essential metals (Cd, Ag and Pb) in rice and soil samples in South Gondar and commercially available rices in Gondar city (Ethiopia) were determined by using Flame Atomic Absorption (FAAS) and Flame Photometry (FP) techniques. The rice samples were collected from South Gondar, Fogera Woreda, Woreta town Nabega kebele and North Gondar, Arada sub city of Gondar, Ethiopia. The optimized wet digestion procedure was evaluated using standard addition (spiking) method and an acceptable percentage recovery was obtained 90-105 and 90-99 for the metals in rice and soil samples respectively. In general the concentration pattern of metals in rice was decreased as: Woreta Rice 1: Ca > Zn > K > Na > Mg > Fe > Cu > Mn > Pb > Ni > Cr > Ag > Cd and Woreta Rice 2: Ca > K > Zn > Mg > Na > Fe > Cu > Mn > Cr > Pb > Ni > Ag > Cd. The result of the study indicates that all types of rice contain considerable concentration of trace metals. The concentrations of metals in this rice were comparable to values reported in literature from different parts of the world. Except Pb and Cd the trace metals levels in the samples were above the WHO allowed limit.

Keywords: Rice (*O. sativa* and *O. glaberrima*), essential and non-essential metal, FAAS, FP

1. Introduction

Rice is a staple food crop for more than half of the world's population. The Asian rice, *Oryza sativa* and African rice *O. glaberrimais* are the two most cultivated species. In Asia alone; more than 2 billion people obtain 60-70 percent calories from rice and its products. It is also the most rapidly growing source of food in Africa, and is of significant importance to food security and food self-sufficiency in an increasing number of low-income food deficit countries^[1]. Therefore, improving the productivity of rice systems would contribute to hunger eradication, poverty alleviation, national food security and economic development^[2].

Rice is among the most important cereals and stem crops grown in different parts of Ethiopia as food crop. It is reported that the potential rice production area in Ethiopia is estimated to be about

thirty million hectares^[3-5]. Even though rice is not a traditional staple food in Ethiopia, the knowledge of its mineral nutrition is of particular interest. Although little information is available on the levels of all essential and non-essential elements present in rice from the literature, several studies have been carried out on determination of the level of toxic metals in rice, using different methods such as ICP-AES^[6] and FAAS^[7] employing dry ashing and wet digestion techniques^[8].

The human body requires a number of minerals to preserve a good health. Numbers of minerals essential to human nutrition are accumulated in different parts of plants. As it accumulates minerals essential for growth from the environment and also accumulate metals such as Cd, Co and Ag which are of known direct benefit to the plant^[9]. It has been

reported that trace of Cd and Pd can be detected in all plants and food stuffs ^[10]. Thus plants are intermediate reservoirs through which trace elements from soil and partly from water and air, transfer to man and animal ^[11].

Based on their importance elements can be essential (like K, Mg, Ca, Mn, Fe, Co, Cu, and Zn) and they are very important for growth and health, or they may be non-essential (like Cd, Ag and Pb). Based on the amount needed nutritionally minerals are grouped into macro-minerals and trace. Elements, such as Mn, Cr, Fe, Co, Cu, Zn, Se, Mo, F and I are essential trace elements, while elements like Ca, Mg and K are grouped under essential macro elements^[12].

Literature review reveals that there is a gap in knowledge on the essential and non-essential elements in rice. Therefore, this study tried to fill this gap by determining essential and non-essential minerals in soil and rice plant grown in Fogera Wereda in Northern part of Ethiopia specifically Amhara region south Gondar administrative zone, and commercially available rice in Gondar town from Arada market.

2. Materials and methods

2.1. Sampling site and description of sample area

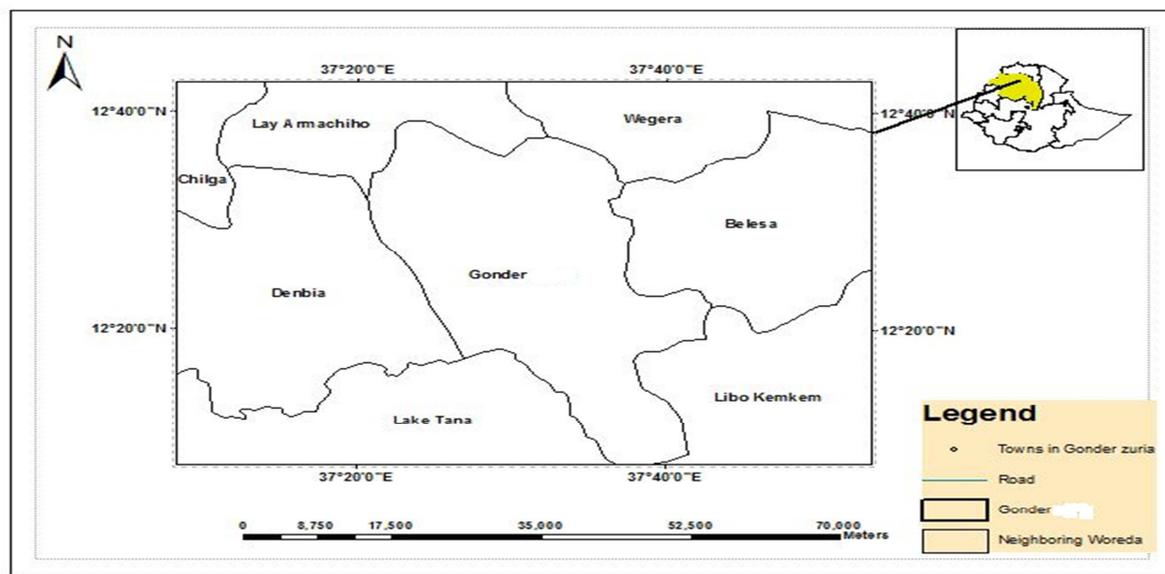
Fogera Woreda:

Fogera Woreda is one of the 151 Woredas of the Amhara Regional State found in South Gondar Zone as shown in Figure 1. It is situated at 11°46 to 11°59 latitude North and 37°33 to 37°52 longitude East. Altitude ranges from 1774 to 2410 meters above sea level and is predominantly classified as subtropical region. Based on the existing digital data, mean annual rainfall was 1216 mm, ranging from 1103 to 1336 mm from both the short (March and April) and long rains (June to September). Farmers depend on long rainy season for crop production.

Gondar city:

Gondar is a city and separate Woreda in Ethiopia which is located in the north Gondar Zone of the Amhara Region. It has a latitude and longitude of 12°36'N37°28'E with an elevation of 2133 meters above sea level.

Fig 1: Location of Fogera Woreda with major rice growing areas and sample area



2.2. Sample collection and preparation

2.2.1 Rice sampling

Rice samples were collected from Fogera Woreda in Amhara region at Wereta town from the farmers and Arada market, Gondar. To draw the representative sample from each sampling site, two samples (0.5kg) were taken from farmers and two samples (0.5kg) were taken from traders, put in a clean polyethylene bag labeled and brought to

the laboratory for further pre-treatment. Finally a sample was prepared for analysis.

2.2.2 Soil sampling

For analysis of metal levels in the soil where the sample rice has grown, the soil sample was collected from the surface horizon (15-20 cm) depth of the sampling area of the rice.

Sampling was done similar to sampling done to the rice. 0.5 kg soil samples were collected from the farmland. The three sub-samples collected from three different areas were mixed together to make homogenized and one composite sample that represent true sampling area. The soil sample collected from the sampling areas were air dried to constant weight for three days and sieved through a 2-mm sieve to remove large debris, stones, and pebbles. Then, the samples were ground using a mortar and pestle to pass through a 500- μ m sieve, then homogenized, which is ready for digestion. Finally the soil sample from the above stated area was prepared for analysis.

2.3. Optimization of digestion procedure

Wet digestion of rice and soil material with acids in a Kjeldahl apparatus (Gerhardt, made in Germany), has been well established and widely used for the determination of nutrient concentrations in rice and soil samples. Wet digestion of rice and soil material with acids in an electrical heating block, has been well established and widely used for the determination of metal concentrations in rice and soil samples. Adding the strong mineral acids or their combinations (i.e., commonly nitric and perchloric acids as they are strong oxidizing agent and forms soluble salts with metals. [13-15]

Table 1: Recovery of method optimizations (Mean \pm SD, n = 7), for rice, and soil samples

Element	Amount added(mg/l)	Amount recorded in spiked(mg/l)	%R for Rice samples	Amount recorded in spiked(mg/l)	%R for Soil samples
Cd	0.20	0.399 \pm 0.36	97 \pm 4	0.192 \pm 0.012	96 \pm 6
Pb	0.20	0.821 \pm 0.01	98 \pm 6	1.071 \pm 0.006	98 \pm 3
Cu	0.50	1.42 \pm 0.02	96 \pm 3	3.23 \pm 0.001	90 \pm 2
Ni	0.20	0.634 \pm 0.01	90 \pm 5	0.616 \pm 0.002	93 \pm 1
Zn	25.00	39.71 \pm 1.25	98 \pm 5	41.91 \pm 0.63	95 \pm 3
Mn	0.50	1.43 \pm 0.02	95 \pm 4	5.17 \pm 0.015	94 \pm 3
Fe	1.00	3.49 \pm 0.06	105 \pm 6	234.23 \pm 0.04	97 \pm 4
Ca	25.00	143.15 \pm 1.75	99 \pm 7	88.10 \pm 1.38	96 \pm 6
Mg	3.00	8.32 \pm 0.09	101 \pm 3	30.96 \pm 0.09	99 \pm 3
Cr	0.50	1.12 \pm 0.02	98 \pm 4	1.355 \pm 0.02	96 \pm 4
Ag	0.20	0.35 \pm 0.02	98 \pm 10	0.35 \pm 0.006	92 \pm 3
Na	2.00	9.17 \pm 0.10	103 \pm 6	7.83 \pm 0.04	94 \pm 2
K	4.00	7.35 \pm 4.62	105 \pm 8	6.71 \pm 0.14	95 \pm 4

%R = Percent recovery

2.3.1. Digestion of rice sample

Applying the optimized condition as above, 0.5 g of dried and homogenized rice samples were transferred into a 250 ml round bottom flask. Then 8 ml of a mixture of HNO₃ (69-72%), HClO₄ (70%) and H₂O₂ (30%) with a volume ratio of 5:2:1(v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus fitting the flask to a reflux condenser by setting the temperature first to dial at 100°C for 30 min and then increased to (270°C) for the remaining 2hrs and 30 min. The digestion was carried out in triplicate for the rice sample.

Digestion of a reagent blank was also performed in parallel with the sample keeping all digestion parameters the same. The digested samples were kept in the refrigerator, and then the total amount of metal in the sample solutions were determined by FAAS and FP.

2.1.1. Digestion of soil sample

Applying the optimized condition 0.5 g of dried and homogenized soil samples were transferred to 250 ml round bottom flasks. To this 10 ml of (5:3:2) ratio of HNO₃ (69-72%) to HClO₄

(70%) and H₂O₂ (30%) respectively were added and the mixture was digested in a Kjeldahl block digestion apparatus fitting the flask to a reflux condenser with programmed temperature at 270°C for 3:00 hrs. Rest of the steps were similar for both plant and soil sample digestion procedure. The digested samples were kept in the refrigerator, and then the total amount of metals in the sample solutions were determined by FAAS and PF^[14].

2.1.2. Validation of the method

2.1.3. Method detection limit (MDL) and limit of quantitation (LOQ)

MDL and LOQ for each metal element were determined from blank sample analysis. Seven blank samples which were similar with the composition of matrix with samples without analyte were digested.

2.1.4. Recovery

A spiking experiment was done to evaluate the accuracy of the procedure used. Known amounts of standard metal solutions were added to the rice and soil samples taking care for the dilution of the final solution. For spiking rice and soil samples, aliquots of 1 ml of 20 µg/g of Cd, Ni, Pb and Ag, 1 ml of 50 µg/g of Cu, Mn and Cr, 1 ml of 100 µg/g of Fe, 1 ml of 400 µg/g of Ca and Zn, 60 µg/g of Mg, 40 µg/g of Na and 80 µg/g of K standard solutions were added to round bottom flask (250 ml) containing 0.5 g rice and soil sample. The spiked and non-spiked samples were digested and analyzed in similar condition.

3. Results and Discussion

3.1 Optimizations of the method

In this study, to prepare a clear colorless sample solution that is suitable for the analysis using FAAS and FP, different rice digestion procedures were optimized using nitric acid (HNO₃), perchloric acid (HClO₄) acid and peroxide (H₂O₂) mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature. From the optimization procedures, the acid mixture of 5 ml of HNO₃ (69-70%), 2 ml of HClO₄ (70%), and 1 ml of H₂O₂ (30%) digestion time of 3 hrs and digestion temperature of 270°C was found the optimal condition for 0.5 g rice sample. The conventional aqua-regia digestion procedure consists of digesting soil samples is so widely used that the European Community Bureau of Reference has

certified several soil and sludge samples based on it, in addition to the total elemental concentrations. Digesting soil sample with aqua-regia produced the most accurate, efficient and reproducible results. 1:1 HNO₃: HCl, 1:3 HNO₃: HCl (aqua-regia), and 1:3:0.5 HNO₃: HCl: H₂O₂ (modified aqua-regia), and they reported that, the aqua-regia and modified aqua-regia were the most effective digestion reagents^[16]. But in this study, for the optimization procedures, the acid mixture of 5 ml of HNO₃ (69-70%) and 3 ml of HClO₄ (70%), and 2 ml of H₂O₂ (30%) digestion time of 3 hrs and digestion temperature of 270°C was found the optimal condition for 0.5 g soil sample. These optimum conditions were selected based on clarity of digests, minimum reagent volume consumption, minimum digestion time and minimum temperature applied for complete digestion of sample.

3.2. Validation of optimized procedure

3.2.1. Recovery

The mean percentage recovery of metals in the spiked rice and soil samples were found to be in the range of 90%-105 and 90%-99% respectively. This confirms that the method is of good precision and accuracy.

3.2.2. Instrumental detection limit (IDL), method detection limit (MDL) and limit of quantitation (LOQ)

For a linear calibration curve, it is assumed that the instrument response Y is linearly related to the standard concentration X for a limited range of concentration. It can be expressed in a model such as: $y = mx + b$, from this IDL can be calculated and the results are given in Table 2^[17].

3.2.3. Method blank

The method detection limit is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The procedure was undertaken in such a way that blanks or reagent and water were subjected to the same analytical procedure as the sample. Then, the mean and standard deviations of the blanks were computed. The method detection limits estimated were greater than the instrument detection limit for all metals (Fe, Zn, Cu, Cr, Mn, Ni, Pb, Mg, Ag, Cd, Ca, Na and K) in all samples (Table 2).

Table 2: IDL, LOQ and MDL (mg/L) for rice and soil samples (n = 7 for MDL and LOQ)

Element	IDL	LOQ	Rice	MDL of Soil
Cd	0.040	0.15	0.09	0.06
Pb	0.010	0.02	0.05	0.01
Cr	0.010	0.56	0.46	0.54
Fe	0.120	0.40	0.27	0.30
Zn	0.080	0.18	0.14	0.16
Mg	0.150	0.50	1.20	1.07
Cu	0.090	0.30	0.14	0.25
Ni	0.060	0.20	0.30	0.34
Mn	0.004	1.20	0.03	0.04
Ag	0.004	0.23	0.07	0.07
Na	0.060	0.20	0.16	0.18
K	0.030	0.17	0.11	0.15
Ca	0.080	0.28	0.11	0.18

IDL = Instrumental detection limit, LOQ = limit of quantification, MDL = method detection limit

3.3. Determination of metals in rice and soil samples

The levels of essential (K, Na, Ca, Mg Cr, Cu, Ni, Mn, Zn and Fe) and non-essential metals (Cd, Ag and Pb) were determined by FAAS and FP. All the rice samples contain the metals mentioned above except Cd. In all the four rice samples being analyzed, the Ca was found in appreciable amounts. This maximum availability compared with other metals indicated that it is the major components of plant nutrients. On the other hand, K and Zn are the most abundant trace metal within all the samples. The mean and standard deviation of the concentration of the metals in the rice and soil samples collected from different sites are presented in Table 3.

3.4. Distribution pattern of the metals in rice samples

Mineral uptake in plants is a function of mineral concentrations in soil, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and age of the plant^[18]. There is large difference in concentration of different metals within rice sample and slight variation in metals of

the same type along with geographical location. Rice contains higher amount of Ca (118.336-158.172µg/g). The higher levels of Ca in the rice is probably due to the fact that nutrient elements such as N, P, K, S, and Ca are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue^[19-21]. The other probable reason for higher concentration of Ca is if the soil which have been used for cultivating the plant, are highly fertilized with manure and organic residues, they will have high in available K, Ca and Mg. Hence, the plant has high amount of these metals. Zn was the most accumulated trace metal followed by K, Na, Mg and Fe in rice sample. Higher Zn levels in the rice may be attributed to the availability of this micronutrient heavy metal in relatively acidic soils of the farmland. It has been reported that Fe and Zn are the main elements that plant could accumulate and pass up in the food chain.

Therefore, the high concentration of Zn from trace metals next to Ca in rice may be because of the fact that these ions are readily transferred from the soil to plants, and accumulate in plants^[22]. The levels of other essential trace metals detected in rice were Mn, Cu, and Ni and non-essential heavy metals Pb and Ag. In general the concentration pattern of metals in rice was decreased as

Pakistan: Ca > K > Zn > Na > Mg > Fe > Mn > Cu > Pb > Ni > Cr > Ag > Cd

India: Ca > Zn > K > Na > Mg > Fe > Mn > Cu > Pb > Ni > Ag > Cr > Cd

Woreta Rice1: Ca > Zn > K > Na > Mg > Fe > Cu > Mn > Pb > Ni > Cr > Ag > Cd

Woreta Rice 2: Ca > K > Zn > Mg > Na > Fe > Cu > Mn > Cr > Pb > Ni > Ag > Cd

Table 3: Mean concentration (Mean \pm SD, n = 5, $\mu\text{g/g}$ for rice and soil) of 13 elements in rice and soil samples

Metals	Rice Samples ($\mu\text{g/g}$)				Soil Samples ($\mu\text{g/g}$)	Maximum permissible level* (WHO/FAO)
	Pakistan	India	Woreta Rice1	Woreta Rice2	Soil	
Cd	ND	ND	ND	ND	ND	0.003
Pb	0.625 \pm 0.06	0.680 \pm 0.00	0.417 \pm 0.07	0.417 \pm 0.07	0.875 \pm 0.06	0.3
Cr	0.375 \pm 0.12	0.250 \pm 0.00	0.375 \pm 0.12	1.125 \pm 0.72	0.625 \pm 0.12	0.2
Fe	2.444 \pm 0.38	2.667 \pm 0.00	4.00 \pm 0.00	4.333 \pm 0.33	223.333 \pm 0.67	15
Zn	15.206 \pm 0.25	17.706 \pm 0.00	13.172 \pm 2.83	20.739 \pm 3.62	18.085 \pm 0.09	20
Mg	5.299 \pm 0.01	5.299 \pm 0.01	5.426 \pm 0.01	16.940 \pm 0.04	27.997 \pm 0.16	350
Cu	0.935 \pm 0.10	0.898 \pm 0.12	1.152 \pm 0.10	1.406 \pm 0.12	2.783 \pm 0.57	10
Ni	0.379 \pm 0.07	0.455 \pm 0.15	0.405 \pm 0.08	0.400 \pm 0.087	0.430 \pm 0.11	1
Mn	1.166 \pm 0.00	0.958 \pm 0.04	0.833 \pm 0.00	1.139 \pm 0.04	4.708 \pm 0.04	5
Ag	0.154 \pm 0.03	0.315 \pm 0.10	0.187 \pm 0.06	0.250 \pm 0.00	0.160 \pm 0.07	0.1
Na	7.100 \pm 0.90	6.200 \pm 0.00	6.100 \pm 0.00	8.900 \pm 0.56	5.950 \pm 0.05	2500
K	15.250 \pm 0.25	11.500 \pm 0.00	10.750 \pm 0.25	25.500 \pm 1.500	29.00 \pm 0.00	3500
Ca	118.426 \pm 0.09	158.163 \pm 0.09	150.648 \pm 0.09	146.944 \pm 0.27	64.197 \pm 0.38	1000

ND = not detected, * = maximum permissible levels in mg/day for rice

Moreover, the levels of metals found in the rice of this study are compared with the maximum limit of metals in rice given by WHO guide lines. On this basis the levels of Ca, Cu, Cr, Mn, K, Mg, Fe, Ni, Zn, Ag and Cd in the analyzed rice samples were found below the guide lines, while the levels Pb which are occurred above the value given by WHO (Table 3). To the human body, lead is devastating in toxic amounts; even low concentrations of lead in the body can cause permanent damage including reduced IQ, learning disabilities, nervous system problems and shortened attention span [23-26].

3.5. Determination of metals in soil sample

The soil sample collected from one sampling areas which is located one of the most rice growing Wereda (Fogera, Nabega kebele) were found to

contain detectable metal content of Na, K, Mg, Ca, Fe, Zn, Pb, Cu, Cr, Mn, Ni and Ag in soil sample and their values are given in Table 3. Plants absorb whatever is present in the soil medium and therefore the metals are also absorbed and become bioaccumulated in the roots, stems, fruits, grains and leaves of the plant, which may finally be transferred to man in the food chain. The sorption processes of metals by plants is significantly affected by metal level in the type of soil, soil pH, the presence of competing ligands, the ionic strength of the soil solution and the simultaneous presence of competing metals [26-29]. Therefore the presence of these analyzed metals in the rice samples might be due to the high content of metals present in the soil.

4. Conclusion

The level of essential and non-essential elements in the four rice and one soil samples was determined by flame atomic absorption spectrometry and flame photometry. The optimized wet digestion method for rice and soil analysis was found effective for all of the minerals and as it was evaluated through the recovery experiment, a good percentage recovery was obtained for the minerals identified. In this study the levels of thirteen elements in the four different kinds of rice, from these the two rice's are cultivated in the farm of Amhara region typically Fogera Woreda, Woreta town and the other two rices are commercially available in Arada market Gondar city, were analyzed. The result showed that all of them contain considerable concentration of trace metals except Cd. Among the leveled metals, calcium was observed in highest level. Similarly, iron accumulated in high level among the micro metals. The results obtained in the present study were compared with international guidelines for rice. On this basis the levels of Ca, Cu, Cr, Mn, K, Na, Mg, Fe, Ni, Zn and Ag in the analyzed rice samples were found below the guide lines, while the levels of Pb was above the value given by WHO.

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