Comparative Study of Important Macro and Micro-nutrient Elements between Two Varieties of Rice (*Oryza sativa* L.) and its Soil

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Summary: Comparative study of important macro and micronutrient elements between two varieties of rice, i.e. Kashmir Nafis and NIAB IRRI-99 as well as its soil have been carried out using Atomic Absorption Spectrometry. Maximum concentration (mg/kg) of macro-elements Na and K were found in Kashmir Nafis rice variety (i.e. 478, 3668) respectively, whereas highest concentration (2542) of Mg was found in NIAB IRRI-99 rice variety. Highest amount (mg/kg) of micro-elements Zn, Mn, and Cu were found in NIAB IRRI-99 rice variety (i.e. 43.09, 56.31and 6.48) respectively, while the highest concentration of Fe and Ca (1043 and 77.68) respectively, were found in Kashmir Nafis rice variety. Maximum concentration (mg/kg) of Al and Ba were found in NIAB IRRI-99 rice variety (i.e. 17.02 and 5.77) respectively. The high level (mg/kg) of Cd, Cr and Ni were found (i.e. 0.392, 1.01 and 1.90) in Kashmir Nafis rice variety and the maximum concentration of Co and Pb were found (i.e. 4.70 and 1.20) respectively in NIAB IRRI-99 rice variety. The concentrations (mg/kg) of the soil was determined for Na, K, Ca, Mg, Zn, Fe, Mn, Cu, Al, Ba, Ni, Pb, Co, Cd and Cr (3284, 2457, 3204, 41519, 91, 5731, 536, 25.65, 35964, 111, 16.66, 5.64, 13.52, 1.95 and 13.63) respectively from the same place where both varieties were grown.

Introduction

Many studies have recognized the role of macro and microelements in human metabolism [1-3]. Macroelements, e.g., Na, K, Mg, Ca, and microessential elements, e.g., Fe, Zn, Mn, are vitally important for certain biochemical systems, whereas trace and toxic elements, e.g., Cd, Cr, Co, Ni, Pb if present in relatively high amounts, adversely affect these systems [4,5]. The concentrations of essential, non-essential and trace and toxic elements in the human body are affected by a number of factors such as nutrition, sex, age, retention, chemical form of the trace elements and binding sites available to them. The intake of these elements is related to our environment. However, food is the main source. It is thus significant to assess the adequacy and safety of the diet by monitoring the concentration of essential and toxic trace elements in various food articles and integrated human diets [6-14]. Dietary inadequacies of trace elements are estimated to affect the health, mental and physical function and survival of more than two billion people worldwide. Women of child-bearing age and children are mostly at risk [15]. Inorganic nutrient metabolism traditionally has been investigated by the chemical balance technique. A controlled diet is administered over a period of time and the inorganic content of the diet and of fecal and urinary samples is then measured in order to estimate the absorption and retention of the element of interest [16]. The harmful effects of Pb and Cd have been well-documented [17-19]. Soil status and response of micronutrient have always been studied [20]. Analytical results on trace elements have been reported on one sample for given species in many of the earlier reports. However it would be difficult to assign to a simple value as the representative one for that species, since the variability of the concentrations seem to exist among samples originating in different localities, depending on elemental characteristics. Trace element distributions among different parts of plants and their physiological roles are also of other interest. Cereal especially rice is staple food in most of the developing countries including Pakistan. It also constitutes a major source of animal feed. Although it contributes most significantly as a source of carbohydrates and proteins. Its potential contribution of minerals including trace elements is often overlooked. In the absence of recent systematic data on elements, it would be very difficult to determine the adequacy of our diet with respect to minerals, especially trace elements. Keeping in view their importance in human diet, prevalent varieties and new genotypes of rice were analysed in our laboratory for important macro- and microelements.

The present investigation was undertaken to analyse and assess the amount of various macro and micronutrient elements in two rice varieties. These were collected from Mutation Breeding Division, NIAB Faisalabad as a representative sample and the reference sample of the same was obtained from Seed Certification & Registration Department Islamabad.

Results and Discussion

Table1 indicates the list of fifteen macro and micro-nutrients, which were analysed from two rice varieties and their soil. These varieties and their soil were collected from experimental field of Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Analytical results of rice and soil samples shows that sodium, potassium, calcium and magnesium are macro nutrient elements present in a higher concentration and are essential for human nutrition. The highest concentration (mg/kg) in case of sodium, potassium and calcium was found in certified samples of Kashmir Nafis varieties i.e. (480 ± 53.5, 3681 ± 433.3, and 78.3 ± 9.8) and the soil of the same plot possess concentration (3284 ± 248, 5457 ± 249 and 3204 ± 231) respectively. In case of magnesium high content i.e. (2535 ± 124) was determined in certified sample of NIAB IRRI-99 variety and its soil contain concentration (41519 ± 4042) mg/kg.

Iron, manganese, zinc and copper are essential micronutrients present in rice varieties at different concentrations. Highest value of iron was detected among these four elements in two varieties. The maximum concentration (1043 ± 77.60) of iron was found in representative sample of Kashmir Nafis variety whereas its soil contains (5732 ± 111.5) concentration in mg/kg. Manganese, zinc and copper were found high (56.3 ± 5.4, 43.1 ± 6.2 and 6.8 ± 0.76 mg/kg) respectively in representative samples of NIAB IRRI-99 variety.

Aluminum and barium are an other micronutrients. The highest level (mg/kg) of aluminum is present in certified sample of NIAB IRRI-99 (i.e.20.64 ± 2.22) and its concentration in soil was found (35964 ± 4301) mg/kg. Similarly, maximum barium (mg/kg) was detected in the representative sample of NIAB IRRI-99 (i.e.5.77 ± 0.53) whereas the soil of the same place contains (111 ± 16) mg/kg concentration.

Cadmium, chromium, cobalt, nickel and lead are trace and toxic elements and present in a very minute quantity in rice and other food commodities. The concentration of cadmium and nickel was found to be high (0.399±0.042 and1.93±0.30 mg/kg) respectively in certified samples of Kashmir Nafis. High content of chromium and lead (1.09±0.15 and1.29±0.13 mg/kg) respectively was determined in certified samples of NIAB IRRI-99. The concentration (4.70±0.27mg/kg) of cobalt was found in representative samples of NIAB IRRI-99, which is recorded concentration as compared to other trace and toxic elements.

Experimental

Sample Collection

The clay loam soil was collected from experimental fields of Mutation Breeding Division Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. The soil was air-dried and sieved (<0.5mm).

Table 1. Concentration of macro and microelement elements in two varieties of rice and soil samples. (mg/kg)

	Kashmir Nafis Variety		NIAB IRRI-99 Variety		Soil of NIAB
Elements	Rep. sample	Cert. sample	Rep. sample	Cert. sample	
Na	478 ± 67	480 ± 54	358 ± 20	356 ± 19.5	3284 ± 248
K	3668 ± 596	3681 ± 434	3391 ± 644	3382 ± 594.5	5457 ± 249
Mg	2253 ± 262	2259 ± 282	2542 ± 129	2535 ± 123	41519 ± 4042
Ca	78 ± 11	78 ± 10	70 ± 10	70.5 ± 10.4	3204 ± 231
Fe	1043 ± 78	1040 ± 77	884.22 ± 74	889.02 ± 67.43	5731 ± 111
Mn	42 ± 3.13	41.25 ± 3.59	56.31 ± 5.36	54.89 ± 2.78	536 ± 39
Zn	29.74 ± 3.61	28.48 ± 2.55	43.09 ± 6.18	41.83 ± 4.49	90.8 ± 9.6
Cu	5.89 ± 0.62	5.6 ± 0.48	6.80 ± 0.76	6.62 ± 0.55	25.7 ± 3.1
Al	14.98 ± 1.33	13.72 ± 1.33	17.02 ± 1.56	20.64 ± 2.22	35964 ± 4301
Ba	4.34 ± 0.79	4.90 ± 0.88	5.77 ± 0.53	5.05 ± 0.66	111 ±16
Cd	0.392 ± 0.05	0.399 ± 0.042	0.329 ± 0.035	0.350 ± 0.058	1.95 ± 0.17
Cr	1.01 ± 0.14	0.87 ± 0.11	0.95 ± 0.00	1.09 ± 0.15	13.6±1.38
Co	2.06 ± 0.07	2.26 ± 0.34	4.70 ± 0.27	3.69 ± 0.34	13.5 ± 1.39
Ni	1.90 ± 0.31	1.93 ± 0.30	0.94 ± 0.11	1.01 ± 0.13	16.7 ± 2.3
Pb	0.82 ± 0.09	0.91 ± 0.05	1.20 ± 0.09	1.29 ± 0.13	5.64 ± 0.51

Rep. = Representative
Cert. = Certified

Samples of paddy (Kashmir Nafis and NIAB IRRI-99) varieties were collected from Mutation Breeding Division, NIAB, Faisalabad as a representative sample and the reference sample of the same was obtained from Seed Certification & Registration Department. Both paddy genotypes were dehusked by dehusker machine at Nuclear Institute of Agriculture (NIA) Tandojam. The seeds were cleaned and stored in polyethylene bags at ambient temperature.

Contamination Control

All glassware were soaked in chromic acid for 24 hours and washed with water oven dried and stored in dust and fume free atmosphere without touching their insides.

Pre-treated filter papers were used to filter digested rice samples. For this purpose, filter papers were soaked in 0.1 M disodium salt of EDTA and washed with water to remove suspected metal impurities. The process was continued until washings were free from traces of sodium

Procedure

Two rice varieties and soil samples were dried at 105 °C in oven. Replicate 1g sample of dried soil and 2g sample of each rice variety were weighed in to 100ml conical flasks and treated with 5ml of nitric acid. 5ml of nitric acid were also added to empty conical flask serving as a blank. The flasks were covered with watch glasses, and their contents were heated to reflux gently on an electric plate. After refluxing for one hour the contents of flasks were treated with 5ml more of nitric acid, 2ml of 35% hydrogen peroxide was added, and the heating at gentle reflux was continued for another hour [21, 22]. The watch glasses were removed from the flasks, and the heating was continued until the volumes of their contents were reduced to 2-3ml. The contents of flask were cooled, diluted with high purity water, and filtered through Whatman # 42 paper into 25ml volumetric flasks. The contents of the flasks were brought to volume with high purity water and analysed by atomic absorption spectrometry for their sodium, potassium, calcium, magnesium, iron, zinc, manganese, copper, aluminum, barium, nickel, lead, cobalt, cadmium and chromium levels.

Instrumentation

A Hitachi Model 180-50 atomic absorption spectrophotometer Japan equipped with standard burner and air-acetylene flame was used for the determination of elements such as, sodium, potassium, calcium, magnesium, iron, zinc, manganese, copper, aluminum, barium, nickel, lead, cobalt, cadmium and chromium. Nitrous oxide was used for the determination of aluminum and barium elements. The hollow-cathode lamps (made by Mtiorika company) of all above elements were operated at lamps current 9.5, 9.5, 7.3, 7.0, 9.5, 9.5, 7.0, 7.0, 9.5, 9.5, 9.5, 7.0, 9.5, 7.0, and 6.0mA respectively. The flow-rate for fuel 2.30 1min^{-1} and air 9.40 1min^{-1} was used respectively to obtain a clear yellow flame (reducing condition). The spectrophotometer out put was connected to a Hitachi recorder 056 with a range of 5mV. The signals measured were the heights of the absorbance/division peaks. All instrumental parameters are given in Table 2.

Table 2. Instrumental conditions for metal analysis

Elements	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Burner height (mm)	Oxidant (Air) kg/cm^2	Fuel (Acetylene) kg/cm^2
Na	590	0.4	9.5	7.5	1.60	0.25
K	766.8	2.6	9.5	7.5	1.60	0.3
Ca	422.2	2.6	7.3	12.5	1.60	0.4
Mg	285.5	2.6	7.0	7.5	1.60	0.2
Fe	248.3	0.2	9.5	7.5	1.60	0.3
Zn	214.0	1.3	9.5	7.5	1.60	0.2
Mn	279.8	0.4	9.5	7.5	1.60	0.2
Cu	325.0	1.3	9.5	7.5	1.60	0.2
Al	309.5	1.3	9.5	12.5	1.60(N_2O)	0.45
Ba	553.8	1.3	9.5	7.5	1.60(N_2O)	0.45
Ni	232.3	0.2	9.5	7.5	1.60	0.45
Pb	232.3	1.3	7.0	7.5	1.60	0.45
Co	250.0	0.2	9.5	10.0	1.60	0.35
Cd	229.0	1.3	7.0	7.5	1.60	0.30
Cr	358.2	1.3	6.0	7.0	1.60	0.30

Reagents and Calibration

The supra pure nitric acid (65% w/v) and hydrogen peroxide (35% w/v) reagents (Merck), high-purity water (electrical resistivity $\rangle$ 10mΩ cm) was produced with a Milli-Q system Millipore, MA, USA).

Calibration was obtained with external standards. The standards solutions were prepared by diluting a 1000mg/l multi element solution (ICP Multi element standard iv, Merck, Darmstadt, FRG) with the same acid mixture used for sample dissolution. Glassware were cleaned by soaking with the contact over night in a 10% (w/v) nitric acid solution and then rinsed with deionized water.

Solutions were aspirated into atomic absorption spectrophotometer and absorbance / divisions' measurements were made for each element using optimum instrumental conditions for flame atomization mode.

Reference standards were also run in parallel for inter calibration of our own standards. Elemental concentrations were computed on an IBM compatible PC using Microsoft Excel program.

Conclusions

It was observed that the certified samples of Kashmir Nafis variety contains the maximum concentration of macro-nutrient elements (i.e. Na, K and Ca) whereas the maximum concentration of Mg was found in certified samples of NIAB IRRI-99 variety. Kashmir Nafis rice variety possess highest concentration of the iron which indicates that it is a good source of iron. High contents of remaining essential micro-elements were present in representative samples of the NIAB IRRI-99 variety.

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