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As Report

Evaluation of Eleven Macro and Micro Elements Present in Various Hybrids of Millet (*Pennisetum glaucum, or P. Americanum*)

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Abstract

Maize and Millet Research Institute (MMRI) situated in Yousuf wala, District Sahiwal, Punjab, Pakistan was selected to grow nine different hybrids/cultivars of millet for study to comprehend the variable concentration of macro, micro and trace and toxic elements in their grains. Wet digestion method was used for the preparation of samples and flame atomic absorption spectrophotometer for analysis of eleven major and minor elements. High values of macro-elements i.e. sodium and potassium was found in ICMP-451 and magnesium in ICMP-53506. The high value of essential micro-elements i.e.zinc (50mg/kg), manganese (8mg/kg), and copper (8mg/kg) was calculated in ICMP-53506, Bullo-94-1, and ICMP-83720 respectively. In case of trace and toxic micro-elements, high concentration of nickel, cobalt, chromium and cadmium was found in O.B.V, Bullo-7704, ICMP-83401, and ICMP-83720 in the edible part of millet plants (grains) cultivars respectively.

Keywords: Millet cultivars; Trace and Toxic elements; Atomic Absorption Spectrophotometer.

Introduction

Millet is one of the important cereal for live stock and is a chief cereal in the definite areas of Asia and Africa where people like and eat as a delicious food. However, in United States millet is used for poultry feeding and cage birds. The seed or grains of millets have been used as food for human and domestic animals. The macro and micro element had very important role in animals as well as plants for the development and maintenances of the cell as well as can cure many diseases. However, excess or deficiency of these nutrients in environment may cause health hazard, therefore the monitoring to these nutrients are essential in all kind of environment, which is concerned with human life. It has been proved that the iron and iodine deficiency causes health problems affecting more than 30% of global population [1]. The existence of iron has been found in all kind of cells and having vital functions but its deficiency causes anemia [2]. In most of cereals, which grown in underdeveloped countries contain large quantity of oxalate, phosphate, fiber, and other inhibitors, which prevent the uptake of iron there fore, utilization of these cereals may causes iron deficiency diseases [3]. The concentration of heavy metals such as cadmium, copper, iron, nickel, chromium, lead, and zinc present in scalp hair of hypertensive patients is reported [4]. Level of heavy metals in biological samples had an important factor for the safety, as well as quality of food and the value of toxic metals [5]. Breeding is one of the important tools for food commodities to reduce the level of highly toxic elements such as arsenic, cadmium, chromium, cobalt, nickel and

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lead [6]. Deposition of heavy metals in food plants depends on environmental factors as well as soil and water, however, major source of toxic metals are closely related to their soil. Waste water or solid material may cause deposition of heavy metals in grains [7, 8].

Last few years, researchers have focused their study to the concentration levels of trace and toxic elements that are transported through roots to various parts of plants [9]. The gene and species of plant plays an important role to absorb and accumulate minerals to various parts of plant variably [10]. It was observed that the movement of heavy metals can be restricted with improvement of genetic characteristic such as cadmium uptake was reduced in rice [11, 12]. As, Cd, Cr, Cu, Ni, Pb and Zn as well as the role of environment (soil types induced by contaminated sludge and water) on uptake of various metal ions in different types of cereals is reported [13-18]. Metals migrate from the polluted areas, may be from soil, water and atmospheric environment to plant based food and finally enter in to animals and human body. Similar type of study was also carried out to six maize (*Zea mays* L.) hybrids for the investigation and accumulation of heavy trace and toxic elements in their most important part (grains), which is used as staple food for human [19]. The interest of major and minor elements has gradually increased on the basis of their importance for life. Major elements need relatively large quantity to regulate many biochemical process where as minor elements needs at low level and both have dietary benefits, however some are very toxic for human [20-22]. It has been reported by the researchers that deficiency or excess of certain trace elements may associate with incurable diseases such as diabetes, hepatitis and cancer [23]. The study of heavy metals such as lead, cadmium and chromium in diabetes, and cancer patients were found as different compared to normal persons [24]. Analytical work was also carried out on polluted and non-polluted Marsilea minuta plants for the biological activity of cadmium and chromium [25].

The aim of our study was to evaluate the variable uptake of elements (Na, K, Mg, Ca, Zn, Mn, Cu, Ni, Co, Cr and Cd) in different genotypes of millets while as plants grows in the same soil. This study would be beneficial medically for those patients who are suffering due to lack or high level of elements.

Experimental

Materials and Methods

Sample collection

Nine samples of millets cultivars; Bullo-94-1, Bullo-94-2, Bullo-7704, O.B.V, IC-8206, ICMP-451, ICMP-53506, ICMP-83401, and ICMP-83720 were collected from Maize and Millets Research Institutes, Yusafwala, District Sahiwal, Punjab Pakistan during the month of September 2005 at the time of harvesting. A 5kg of cultivar were mixed randomly taken from various lots and made representative sample of 200 grams of each genotypes, packed in air tight polythene bags without chemical treatment. All the genotypes were handled similarly. Finally, brought to the Department of Chemistry, Shah Abdul Latif University, Khairpur for further process.

Sample preparation

All samples of millets were washed with distilled water followed by deionised water to remove all foreign particles. The millet samples of different genotypes were dried in oven at 70 ºC, 80 ºC, 90 ºC, 100 ºC, 110 ºC and 120 ºC with the interval of 8 hours to obtain the constant weight. Three replicate samples were prepared in which each contain 2 gram of each cultivar and these were weighed separately on paper and transferred in to 100 ml conical flask. Then 5ml of nitric acid were added to each flask, parallel to sample flasks, certified reference material of millets and blank were also prepared. Sample flasks, certified reference material of millets and blank were capped with watch glasses, placed on temperature controlled hot plate, heated at 70-75 ºC round about 1 to 1.30 hour till semi-dried content obtained. Removed the flasks from hot plate, added more 5ml of nitric acid and repeated heating similarly to obtained semi-dried content. Again removed flasks and leave them to cool down for the period of 10 minutes. In the next step, added 3 ml of hydrogen peroxide put them on hot plate for further heating until semi-dried content was obtained. Flasks were removed from hot plate, added 50 ml of deionised water, heated flasks without watch glasses until half of the water was removed from flasks, removed the flasks, leave them to cool down, filtered contents on whatman # 42 papers into 25 ml volumetric flasks. The color of the digesting mixture was light yellow and had very low viscosity. The contents of the flasks were brought to volume with high purity water and 2N nitric acid [29].

Reagents and glassware

Ultra-pure water obtained from ELGA lab water system (Bucks, UK) was used throughout the work. Chemicals and reagents that were used during the preparation and analysis of the samples, blanks and reference materials were of analytical grade. Hydrochloric acid (37%, s.g. 1.19), nitric acid (65%, s.g. 1.4), hydrogen peroxide (30%), were analytical reagent-grade E. Merck (Darmstadt, Germany). The internal standards as well as external standards were prepared and run during the analysis. Standard solutions of Na, K, Mg, Ca, Fe, Zn, Mn, Cu, Co, Ni, Cr, and Cd were prepared by dilution of 1000 µg/ml certified standard solutions, Fluka Kamica (Buchs, Switzerland) of corresponding metal ions. The chemical modifiers, $Mg(NO_3)_2$ 5.00 g/L and palladium 3.00 g / L, stock standard solutions were prepared from $Mg(NO_3)_2$ Merck (Darmstadt, Germany) and Pd 99.999% Sigma–Aldrich (Milwaukee, WI, USA). All glassware and plastic material used previously treated for a 24 h in 2M nitric acid and rinsed with double distilled water and then with ultra-pure water.

Determination of macro and micro elements

A series of standard solutions of cation, i.e. sodium, potassium, calcium, iron, zinc, manganese, copper, lead, nickel, cadmium, chromium and cobalt were simultaneously run in the concentration range of un known samples on atomic absorption spectrophotometer. The calibration curves of each atom for concentration versus absorbance were obtained and concentration of unknown calculated from calibration curves for each atom [29, 30].

Instrumentation

All samples of nine millet cultivars in triplicate as well as blank, after digestions were carried out to Perkin Elmer model AA Analyst-100, S. N0. 040SI090409, Atomic absorbance / flame spectrophotometer with a deuterium lamp back corrector equipped with 10cm burner head. Hollow cathode lamps of respective metals manufactured by Perkin Elmer Company under the licence No. 110901-93022 Perkin Elmer Ltd. (Singapore) was used which provided narrow spectral lines of moderate intensity with air-acetylene and air-acetylene nitrous oxide system. The instrument was coupled with Fujitech personal computer of model AK-455L, serial No. MA 44EO 1802218, Registered Trade Mark Ocean Office Automation U. A. N: 111-111-662 from which the commands were given to operate the equipment as well as to store the data by giving the three variable reading and other necessary calculations of analyst. All parameters of AA Analyst-100 spectrophotometer such as lamp intensity and band pass width and other measurement conditions were used according to the manufacturer's recommendations and hollow cathode lamps were used as radiation sources for analyst. Total metals of our study were analysed under optimized operating conditions by FAAS with air–acetylene flame [31].

Results calculation

Results were calculated from the calibration curve obtained by statistical analyses of concentration versus absorbance data for each metal ion using the fitting of straight line by the least squares method. The validity of the acid digestion method was checked by employing the certified reference millet sample. The measured values of the elements were in close agreement with the certified values provided by the Seed Certification and Registration Department (the percentage recovery of all elements was 98.99%). Mean values for all elements differed by less than 1% from the certified values. The results were statistically evaluated with the help of the MINITAB II statistical software computer program (Minitab Inc**.,** State College, PA).

Results and Discussion

Dietary intake of essential and trace metals from millet food sources have been estimated by analysing different cultivars of millets in order to evaluate the safety of food and its consumers. The analysis was carried out of nine millet cultivars to estimate the exact concentration of eleven elements i.e. sodium, potassium, magnesium, calcium, zinc, manganese, copper, cobalt, nickel, chromium and cadmium. The data of these elements are given in table 1-3, which shows that the mean value (366.409±1.968mg/kg) of sodium was higher in ICMP-451 than rest of eight cultivars, the precise value of same metal was found in Bullo-94-2, Bullo-7704 and ICMP-83720 cultivars. Lowest value (149.485±10.228mg/kg) of sodium was found in IC-8206 that didn't match /close to any other value of millet cultivar. Highest up take of potassium (4114.77±143.24mg/kg) and lowest (2391.08±19.655mg/kg) were calculated in ICMP-451 and IC-8206 respectively.

The results of magnesium in (Table 1) shows that the total mean value (1070.43± 6.063mg/kg) of magnesium is higher in ICMP-53506 and lower value (611.318± 4.787mg/kg) in IC-8206. However, insignificant difference in the level (791.045±13.57 and 789.646±6.275) of magnesium was analysed in Bullo-94-1 and O.B.V cultivars respectively. Similarly, trend of magnesium absorption was also detected in other pair of cultivars with a slight margin i.e. Bullo-94-2 (853.389±10.391 mg/kg) and ICMP-53506 (856.188±12.793mg/kg). Calcium is one of the essential macronutrient for the formation of bones but its unexpected results have been obtained in all nine millet cultivars; none of the cultivar shown positive result, which means either calcium is not present in any hybrid or it exist below the detection limit.

The fruits and vegetables containing beta-carotene, which converted into vitamin "A" within the body, which fulfill the requirement of vitamin "A" deficiency and this role is played in the presence of elements such as iron, zinc and copper [26]. Iron, zinc, manganese and copper are included in micronutrients and its concentration is lower than that of macronutrients and almost the order of concentration of these heavy metals have been calculated in such a way; iron > zinc > manganese > copper. When we discuss the variety wise, it was found that the result of iron in Bullo-7704 on top of its value (87.500±5.050mg/kg) and Bullo-94-1 on bottom of iron value (28.03±1.430mg/kg) while four cultivars i.e. ICMP-83401, ICMP-53506, ICMP-84122 and Bullo-94-2 had close values 39.394±4.832, 40.530±1.994, 42.614 ±0.986 and 43.750±3.548 respectively. Zinc is another important element and functions for the normal metabolism of vitamin A, its deficiency may interfere with vitamin A metabolism in various ways. Zinc is required for the enzymes that convert retinol into retinal and its absence in enzymes, which prevent night blindness, is inactive. Zinc deficiency produces night blindness because it interacts with vitamin A deficiency [27, 28]. The analytical results of zinc in mentioned (Table 2) shows that none of millet genotype possesses more than 50.376mg/kg and not below than 30.108mg/kg which were detected in ICMP-53506 and Bullo-94-1 respectively, where as the uptake rate of zinc in Bullo-94-2 (40.699±0.723mg/kg) and ICMP-84122 (39.758±1.816mg/kg) genotypes were almost same.

Copper deficiency impairs the formation of connective tissue proteins, collagen, and elastin. The important antioxidant enzyme superoxide dismutase (SOD) requires copper, together with copper and manganese, to function. Reduced activity of SOD has been linked to Alzheimer's disease, rheumatoid arthritis, vision problems, atherosclerosis, and lung injury, especially in the elderly. The concentration of manganese and copper as mentioned in (Table 2), are very close to each other but the rate of absorption varies all genotypes; the maximum concentration (7.732±0.095mg/kg) of manganese was observed in Bullo-94-1, copper (7.685±0.048mg/kg) in ICMP-83720 millet cultivar. Both of these values are identical when we roundup the decimal digits. Lowest level of manganese (4.001±0.287mg/kg) and copper (4.691±0.063mg/kg) were detected in IC-8206 and Bullo-7704 cultivars respectively. Most of the genotypes had more or less same capability to uptake manganese and copper such as Bullo-94-2, O.B.V, IC-8206, ICMP-451, ICMP-53506 and ICMP-83401 except Bullo-94-1, Bullo-7704 and ICMP-83720, which shows significant variation in absorbing manganese and copper.

Nickel, cobalt, chromium and cadmium are micro but trace and toxic elements, which are required at trace level to play an important functions in many metabolic systems of the body, without which systems of the body paralysis. When we look to (Table 3), we find that the level of nickel in majority of the genotypes is higher as compared to cobalt, chromium and cadmium. In case of nickel highest value (4.264±0.37mg/kg) is found in O.B.V and minimum value (0.716±0.056mg/kg) to its next IC-8206 cultivar. Most of genotypes possess close values of nickel except three genotypes. Cobalt is another trace element in which the maximum value (2.5±0.107) is found in Bullo-7704, minimum (0.82±0.071 mg/kg) in ICMP-83720 and values of cobalt in Bullo-94-1 & IC-8206 are alike (1.31mg/kg) with variation of standard division while as the value of ICMP-451 and ICMP-53506 is not detected. The level of chromium was found to be higher (5.972±0.481mg/kg) in ICMP-83401and lower (0.222±0.024mg/kg) in Bullo-94-2 but it is not detected in three cultivars; Bullo-7704, O.B.V and ICMP-451. Cadmium is one of the last an element in this study that contains the maximum concentration (1.253±0.07mg/kg) in ICMP-83720, minimum (0.483±0.03mg/kg) in Bullo-84-2 and not detected in Bullo7704 genotypes.

Table 1. **Determination the concentration of essential macronutrients from millets cultivars in mg/kg (Na. K, Mg and Ca).**

Names of Cultivar	Na		K		Mg		Ca
	Mean ± S.D.	% Recovery S.D.	Mean ± S.D.	% Recovery S.D.	Mean ± S.D.	% Recovery S.D.	Mean ± S.D.
Bullo-94-1	214±26	12.1	3683±13	0.35	791±14	1.77	ND
Bullo-94-2	194±13	6.7	3999±172	4.3	853±10.0	1.17	ND
Bullo-7704	198±11	5.56	3708±8.0	0.22	886±7.0	0.79	ND
O.B.V	182±2.0	1.1	3390±50	1.47	790±6.0	0.76	ND
IC-8206	150±10	6.67	2391±20.0	0.84	611±5.0	0.82	ND
ICMP-451	366±2.0	0.55	4115±143	3.48	856±13.0	1.52	ND
ICMP-53506	278±27	9.71	3942±35	0.89	1070±6.0	0.56	ND
ICMP-83401	189±4.0	2.12	4026±13	0.32	966±5.0	0.52	ND
ICMP-83720	313±8.0	2.56	3621±37	1.02	938±6.0	0.64	ND

Table 2. **Determination the concentration of essential micronutrients from millets cultivars in mg/kg (Fe, Zn, Mn and Cu).**

Names of Cultivar	Fe		Zn		Mn		Cu	
	Mean	% Recovery S.D.	Mean	% Recovery S.D.	Mean	% Recovery S.D.	Mean	% Recovery S.D.
Bullo-94-1	28.0±1.0	3.57	30.0±0.3	1.0	8.0±0.1	1.25	5.0±0.1	2.0
Bullo-94-2	44.0±4.0	9.09	41.0±0.7	1.71	7.0±0.4	5.71	6.0±0.02	0.33
Bullo-7704	88.0±5.0	5.68	30.0±1.0	3.33	7.0±0.3	4.29	5.0±0.1	2.0
O.B.V	70.0±3.0	4.29	45.0±1.0	2.22	6.0±0.2	3.33	6.0±0.1	1.67
IC-8206	83.0±6.0	7.23	34.0±0.8	2.35	4.0±0.3	7.5	5.0±0.05	1.0
ICMP-451	33.0±2.0	6.06	38.0±1.0	2.63	6.0±0.3	5.0	7.0±0.1	1.43
ICMP-53506	41.0±2.0	4.88	50.0±2.0	4.0	7.0±0.4	5.71	7.0±0.3	4.29
ICMP-83401	39.0±5.0	12.8	46.0±2.0	4.35	7.3±0.1	1.37	6.0±0.1	1.67
ICMP-83720	43.0±1.0	2.33	40.0±2.0	5.0	6.0±0.3	5.0	8.0±0.1	1.25

Table 3. Determination the concentration of trace and toxic micronutrients from millets cultivars in mg/kg (Co, Ni, Cr and Cd).

Names of Cultivar	Ni		Co		Cr		Cd	
	Mean	% Recovery S.D.	Mean	%Recovery S.D.	Mean	% Recovery S.D.	Mean	% Recovery S.D.
Bullo-94-1	1.7±0.1	5.88	1.3±0.1	7.69	1.3±0.1	7.69	0.6±0.1	16.7
Bullo-94-2	2.5±0.2	8	2.1±0.2	9.52	0.2±0.02	10.0	0.5±0.03	6.0
Bullo-7704	2.93±0.3	10.2	2.5±0.1	4.0	N. D	-	N. D	-
O.B.V	4.3±0.4	9.3	1. 7±0.1	-	N. D	-	0.8±0.1	12.5
IC-8206	0.7±0.1	14.3	1.3±0.1	7.69	1.0±0.04	4.0	1. 2±0.03	2.5
ICMP-451	2.0±0.2	10	N. D	-	N. D		0.9±0.03	3.33
ICMP-53506	2.5±0.2	8	N D	-	3.8±0.4	10.5	1.04±0.1	9.62
ICMP-83401	2.8±0.2	7.14	1.0±0.1	10.0	6.0±0.5	8.33	1.2±0.1	8.33
ICMP-83720	2.2±0.2	9.09	0.8±0.1	12.5	3.6±0.2	5.56	1. 3±0.1	7.69

Conclusion

It has been concluded that the highest concentration of sodium and potassium were found in ICMP-451, magnesium and zinc in ICMP-53506, manganese in Bullo-94-1, iron and cobalt in Bullo-7704, copper and cadmium inICMP-83720, nickel in O.B.V and chromium in ICMP-83401. However, cobalt was not detected in ICMP-451 and ICMP-53506, chromium in Bullo-7704, O.B.V and ICMP-451, and cadmium in Bullo-7704. The case of calcium was found exceptional in which none of genotype contains calcium concentration.

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