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MULTIELEMENT ANALYSIS OF PAKISTANI BARLEY (*HORDEUM VULGARE* L.) VARIETIES BY FLAME ATOMIC ABSORPTION SPECTROMETRY

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Abstract

Barley (*Hordeum Vulgare* L.) is widely known due to its nutritional potential, in Pakistan it is only grown at a few agricultural experimental stations. There is no published data about the chemical composition of Pakistani barley varieties; however, research laboratories have studied their agronomical characteristics. The mineral concentration of five Pakistani barley varieties and their soil were studied, namely Sadabahar, Bajawar -2000, Frontier-87, Sonober-96, Soorab-96 and the soil of two agricultural plots, namely Wheat Research Institute Faisalabad and National Agriculture Research Centre Islamabad. The analysis included: 15 macro and micronutrients and they varied in concentration from 0.22-8285.1 mg kg^{-1} in barley seed and soil concentration in both of above plots varies from 1.63-14719.8 mg kg^{-1}. Among all the varieties; Sadabahar showed the highest Mg, Zn and Cr content (2686.6, 39.19, 1.22 mg kg^{-1}) respectively. Bajawar -2000, showed the highest Na, K, Ca, Fe, and Ni content (1768.0, 8285.1, 891.0, 937.02, and 4.56 mg kg^{-1}) respectively. Frontier-87 showed the highest Ba content (18.58 mg kg^{-1}), Sonober-96 showed the highest Co, Pb and Cd content (4.41, 1.45, 0.29 mg kg^{-1}), Sorab-96 showed the highest Al content 39.50 mg/kg. Since all varieties were grown under two environmental conditions i.e. Wheat Research Institute, Faisalabad and National Agriculture Research Centre Islamabad. These results may be useful in the food industry for the selection of hull barley varieties for human consumption and to produce substantially mineral contents

Keywords: Atomic absorption spectrometry, barley, livestock feed.

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INTRODUCTION

Archeological evidence has shown that barley was one of the earliest cultivated cereal grains. Directions on how to successfully grow barley have been found inscribed on clay tablets dating from 1700 B.C. [Harland 1979]. Today barley is ranked third among feed grains cultivated in the U.S. [Evans and Davenport 1984]. In Europe barley is the most widely cultivated cereal grain used in animal feed [Todorov 1988]. Barley is a cool season plant and has been adapted throughout temperate growing regions by development of specific cultivars that matched local growing conditions. Extremes in climatic conditions have been observed to alter the nutritional composition of barley, but with careful management all barley can be used as livestock feed [Hoppner *et al.* 1968]. Barley contains a large proportion of starch it is used primarily as an energy source [NRC 1989]. Protein content of most barley grain ranges from 7.5 to 17% on a dry matter basis with 75% of that protein being digestible. However, protein levels in excess of approximately 13.6% dry matter may not substantially increase the value of barley to livestock feeders [Neuman 1985] if other feed ingredient costs are held constant.

Barley, corn and grain sorghum are low in calcium content. However, barley contains more phosphorus than corn or milo [NRC 1984]. The primary need for supplemental macro minerals will be calcium. Potassium levels should also be evaluated. Barley is low in several trace minerals, but requirements can usually be met by providing trace mineral salt. Trace mineral deficiency is principally a geographical problem and consultation with soils experts and nutritionists in specific areas are necessary to determine if supplementation is necessary and what type of supplementation is needed. Fortifying diets with vitamin E, choline, zinc and sulfur did not improve the performance of cattle finished on high-energy rations based on dry rolled barley and a standard supplement [Dinusson 1968]. However, cattle requirements for these nutrients have changed since this study was done due to livestock genetic selection for growth. Information supplied from one area to another may not be applicable because geographic variation is more pronounced in barley than in corn [Dinusson 1960].

Barley (*Hordeum vulgare* L.) is used mainly for brewing and as animal feed but there is a growing interest in it for human food and industrial uses [Oscarsson *et al.* 1996]. It contributes significantly to the human food supply as malt products, and to animal livestock feed [Elfverson *et al.* 1999]. Many spontaneous and induced barley varieties occur, and this offers the potential to select particular genotypes for specific uses [Nilan *et al.* 1993]. Hulless (HB) or naked barley is a genetically improved variety that allows easier removal of the hull and a fairly new industry has developed around uses of selected HB in order to increase the digestible energy of the grain, especially for swine and poultry [Bhatty 1999]. HB has been investigated for several potential new applications as whole grain, and for its value-added products. These include bran and flour for multiple food applications [Bhatty 1999].

Interest in hulless barley has increased due to its soluble dietary fiber, glucan, and high protein contents. Even when compared to oats, a glucan-rich cereal, barley presents higher amounts [Lapvetelainen and Aro 1994, Marconi *et al.* 2000, Francisco and De Sa 2001]. Glucan is particularly interesting for human consumers because it decreases blood cholesterol and glucose levels [Newman *et al.* 1998].

The objectives of this study were to investigate the different uptake of mineral contents of five Pakistani barley varieties, namely Sadabahar, Bajawar -2000, Frontier-87, Sonober-96, and Soorab-96 at two different environmental conditions.

MATERIALS AND METHODS

BARLEY SAMPLING

We selected two research institutes/centres of the Pakistan; Wheat research station (WRI), Faisalabad and National agricultural research center (NARC) Islamabad, Pakistan, where Sadabahar, Bajawar -2000, Frontier-87, Sonober-96, Soorab-96 varieties were grown without any chemical fertilizers and collected the samples randomly at the maturity stage to make a representative sample.

The sowing season of the barley varieties/cross/lines Pedigree begin on 1^{st} November on ward to 10^{th} January and are harvested in the end of March to May. All varieties were grown in experimental fields in 2002 at Wheat research station (WRI), Faisalabad and National agricultural research center (NARC) Islamabad, Pakistan (23º49'21"S; 51º14'35"W).

SOIL SAMPLES

Composite soil samples (0-15 cm depth) were collected from both sites of the barley growing areas. A kg of the soils from each site was taken in the plastic bags. The samples were air-dried on ground with a wooden pestle in a mortar to a fine powder and were stored for the purpose of conduct of various analyses.

All soil samples were obtained from the jurisdiction of above agricultural research institutes / centers. Soil samples from the plots of the WRI, and NARC were collected in the sunny days of the month of April and May 2002, with the help of a wooden tool to avoid contamination of the soils with any of the element being studied. All the soil samples were collected in sample bags and brought to the laboratory of the Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro (Pakistan).

PREPARATION OF THE SOIL SAMPLES

The soil samples were air dried ground with a wooden mortor and sieved through 2mm nylon mesh size sieve. The samples were stoppered tightly in plastic bottles and labeled. These samples were then used for further investigation.

The study on up-takes of fifteen macro and micronutrients by barley from the soil was carried out. Two agricultural research institutes / centers were selected for five varieties.

CHEMICAL ANALYSIS

All barley varieties and soil samples were dried at 105 ºC in oven for 12 hours. Five replicate 1.0 g samples of dried soils and 2.0 g of barley varieties were weighed in to 100 ml conical flasks and treated with 5 ml of nitric acid; 5 ml 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 more of nitric, 2 ml of 35% hydrogen peroxide was added, and the heating at gentle reflux was continued for another hour. The watch glasses were removed from the flasks, and the heating was continued until the volumes of their contents were reduced to 2-3 ml. The contents of flask were cooled, diluted with high purity water, and filtered through Whatman # 42 paper in to 25ml volumetric flasks. The contents of the flasks were brought to volume with high purity water and examined by atomic absorption spectrometry for their sodium, potassium, calcium, magnesium, iron, zinc, manganese, copper, cobalt, chromium, nickel, barium, aluminum, lead and cadmium levels. Total mineral content was determined by the wet digestion method. The above-mentioned analyses were performed according to the standard Official Methods of Analysis of the Association of Official Analytical Chemists [AOAC 1995]. Total insoluble minerals were determined using Flame Atomic Absorption Spectrophotometer (FAAS), according to Approved Methods of the American Association of Cereal Chemists [AACC 2000]. Data were reported on a dry weight basis. The results of these measurements are presented in Table 1.

INSTRUMENTATION

A Hitachi Model 180-50 atomic absorption spectrophotometer was used for the determination of elements such as, sodium, potassium, calcium, magnesium, iron, zinc, manganese, copper, cobalt, chromium, nickel, barium, aluminum, lead and cadmium. Hollow cathode lamps (made by Meltorika Company) of sodium, potassium, calcium, magnesium, iron, zinc, manganese, copper, cobalt, chromium, nickel, barium, aluminum, lead and cadmium were used as radiation source. Air- acetylene gas was used as a fuel during atomization step of Na, K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Ni, Ba, Al, Pb, and Cd. whereas for Al and Ba, air-acetylene-nitrous oxide was used. 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, 9.5, 9.5, 9.5, 6.0, 9.5, 9.5, 9.5, 7.0, and 7.0mA respectively. The flow-rate for fuel 2.21 $Lmin^{-1}$ for Na; 2.3 $Lmin^{-1}$ for K; 2.6 $Lmin^{-1}$ for Ca, 2.0 $Lmin^{-1}$ for Mg; 2.30 $Lmin^{-1}$ for Fe; and Cd; 2.0 $Lmin^{-1}$ for Zn, Mn, Cu, Co, Cr, Ni and Pb; 5.61 $Lmin^{-1}$ for Ba and Al respectively. Flow-rate for air 9.40 $Lmin^{-1}$ for Na, K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Ni, Pb, and Cd, and 5.91 $Lmin^{-1}$ for Al and Ba were 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.

REAGENTS AND CALIBRATION

The supra pure nitric acid (65% w/v) and hydrogen peroxide (35% w/v) reagents (Merck), high-purity water (electrical resistivity >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 1000 mg L^{-1} 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.

Table1: Spectroscopic Study of macro and micronutrients in five Barley (Hordeum Vulgare L.) cultivars collected from two different locations of Pakistan (Wheat Research Institute Faisalabad and NARC Islamabad)

Element	Barley varieties / cultivars											
	Sadabahar		Bajawar-2000		Frontier-87		Sonober-96		Soorab-96		Soil	
	WRI	NARC	WRI	NARC	WRI	NARC	WRI	NARC	WRI	NARC	WRI	NARC
Na	1327.5± 42.8	1323.9± 50.49	1768.0± 68.5	1764.46± 69.27	948.37± 39.97	951.32± 24.19	660.19± 87.23	664.92± 109.60	891.68± 46.19	886.95± 47.14	8283.9± 248.27	3892. 0± 480.01
K	7884.0± 375.0	7892.7± 474.4	8285.1± 883.4	8276.37± 790.5	7448.0± 149.0	7455.0± 85.50	7334.68± 162.3	7343.10± 115.2	7107.98± 65.0	7090.5± 649.7	12456.9± 248.9	14719.8± 412.7
Ca	826.1±1 11.68	831.53± 94.06	891.0± 55.84	885.6± 73.6	674.74± 55.84	679.31± 32.44	682.31± 92.86	677.98± 109.04	674.79± 55.84	680.0± 47.95	3204.1± 231.2	2403.1± 231.2
Mg	2686.6± 54.8	2684.88± 78.7	2253.4± 251.7	2256.85± 262.7	1911.66± 45.5	1916.84± 68.7	2177.45± 183.6	2174.0± 212.64	1613.07± 106	1609.6± 79.6	41518.6± 4042	50513.4± 3398
Fe	504.99± 50.8	505.95± 64.67	937.02± 99.15	933.66± 86.28	327.38± 32.79	331.22± 26.30	428.19± 59.70	425.79± 48.79	466.59± 59.70	464.2± 35.6	5731.5± 111.5	5974.0± 376.7
Zn	39.19±4.86	38.4±3.87	28.60±1.04	28.23±2.76	27.09±1.39	27.72±1.23	24.82±2.86	25.71±1.48	32.76±3.68	33.77±2.70	90.78±9.64	95.31±8.58
Mn	23.92±2.99	23.07±2.45	15.40±1.43	15.68±1.47	15.40±1.43	15.68±1.47	17.67±1.09	17.10±1.09	17.39±1.61	16.53±1.43	536.25± 39.31	611.78± 54.51
Cu	7.72±0.81	7.54±0.65	7.17±0.43	7.32±0.27	6.29±0.60	6.44±0.46	7.57±0.79	7.68±0.51	8.05±0.84	8.12±0.73	25.65±3.10	35.03±2.99
Co	3.60±0.48	4.05±0.51	4.34±0.58	3.14±0.44	3.14±0.44	3.12±0.43	4.41±0.68	3.62±0.44	3.17±0.41	3.14±0.44	13.52±1.39	15.64±0.98
Cr	1.22±0.09	1.19±0.11	1.05±0.08	1.08±0.05	0.75±0.05	1.17±0.14	0.87±0.11	0.87±0.11	0.98±0.05	0.67±0.06	13.63±1.38	13.63±1.64
Ni	2.89±0.33	2.81±0.23	4.56±0.41	4.45±0.28	1.31±0.24	1.42±0.24	2.44±0.16	2.39±0.15	3.29±0.39	3.15±0.37	16.66±2.32	18.01±2.21
Ba	15.16±2.20	14.23±1.76	4.90±0.88	5.15±0.53	18.58±1.76	17.02±1.32	7.17±0.75	8.75±0.70	10.49±1.76	8.4±0.88	111.08±16.03	111.08± 16.03
Al	14.35±2.22	14.82±0.67	15.13±1.11	13.01±1.45	17.49±2.22	16.31±2.78	14.35±1.12	20.64±2.23	39.50±4.45	34.79±4.45	35964.3± 4301	42035.7± 4379
Pb	1.27±0.02	1.30±0.03	0.86±0.04	0.90±0.02	0.82±0.09	0.85±0.05	1.45±0.09	1.44±0.11	1.44±0.11	1.39±0.12	5.64±0.51	5.25±0.51
Cd	0.23±0.04	0.24±0.02	0.23±0.04	0.24±0.02	0.22±0.02	0.22±0.01	0.29±0.03	0.26±0.03	0.24±0.03	0.25±0.03	1.95±0.17	1.63±0.12

WRI= Wheat Research Institute, NARC= National Agriculture Research Centre.

Table 2: Instrumental conditions for the AAS measurement of Na, K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Ni, Ba, Al, Pb, and Cd.

Elements	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Fuel flow (acetylene) (l min^{-1})	Flow rate (Air) (l min^{-1})	Burner height (mm)	Oxidant (Air) kg cm^{-2}	Fuel (Acetylene) kg cm^{-2}	Signal out put
Na	590	0.4	9.5	2.21	9.4	7.5	1.60	0.25	100%
K	766.8	2.6	9.5	2.3	9.4	7.5	1.60	0.3	100%
Ca	422.2	2.6	7.3	2.6	9.4	12.5	1.60	0.4	100%
Mg	285.5	2.6	7.0	2.0	9.4	7.5	1.60	0.2	100%
Fe	248.3	0.2	9.5	2.30	9.4	7.5	1.60	0.3	100%
Zn	214.0	1.3	9.5	2.0	9.4	7.5	1.60	0.2	100%
Mn	279.8	0.4	9.5	2.0	9.4	7.5	1.60	0.2	100%
Cu	325.0	1.3	9.5	2.0	9.4	7.5	1.60	0.2	100%
Co	250.0	0.2	9.5	2.0	9.4	10.0	1.60	0.35	100%
Cr	358.2	1.3	6.0	2.0	9.4	7.0	1.60	0.30	100%
Ni	232.3	0.2	9.5	2.0	9.4	7.0	1.60	0.30	100%
Ba	553.8	1.3	9.5	5.61	5.91(N_2O)	7.5	1.60(N_2O)	0.45	100%
Al	309.5	1.3	9.5	5.61	5.91(N_2O)	12.5	1.60(N_2O)	0.45	100%
Pb	232.3	1.3	7.0	2.0	9.4	7.5	1.60	0.2	100%
Cd	229.0	1.3	7.0	2.30	9.4	7.5	1.60	0.30	100%

Reference standards were also run in parallel for inter calibration of our own standards. Elemental concentrations were computed on an IBM compatible PC using excel computer program. The statistical calculations for standards are given in Table 3.

Table 3: Statistical data for standards of elements.

Elements	Concentration range mg kg^{-1} (x)	Absorbance/ Division (y)	Statistical calculation y = m x + c		
			m	c	r^2
Na	0 – 0.5	0 – 0.205	0.4111	-0.0018	0.9996
K	0 – 0.0156	0 – 0.0041	0.29	-0.0002	0.9995
Ca	0 – 0.016	0 - 0.0045	0.3121	-0.0005	0.9983
Mg	0 – 2	0 - 0.638	0.3165	0.0051	0.9995
Fe	0 – 3	0 - 910	03016	-0.0022	0.9996
Zn	0 – 1	0 - 0.235	0.2349	-0.0027	0.9989
Mn	0 – 1	0 – 0.134	0.1324	0.0021	0.9985
Cu	0 – 2	0 –0.138	0.0693	-0.0001	0.999
Co	0 – 1	0 – 0.053	0.0529	0.0004	0.9997
Cr	0 – 1	0 – 0.070	0.0698	0.0002	0.999
Ni	0 – 1	0 – 0.111	0.1104	0.0022	0.998
Ba	0 – 1	0 – 22 div.	22.057	-0.0001	0.9974
Al	0 – 25	0 – 0.0358	0.0014	-0.086	0.9999
Pb	0 – 1	0 – 0.063	0.0638	-0.0009	0.9992
Cd	0 – 0.125	0 – 25 div.	356.52	0.5074	0.999

Absorbance*, div. =Divisions

RESULTS AND DISCUSSION

The mineral content of the five Pakistani barley varieties can be summarized in Table 1. The highest contents of Na, Mg, Zn, Mn, Cu, Cr, Ni, and Ba (1327.5± 42.8, 2686.6±54.8, 39.19±4.86, 7.72±0.81, 1.22±0.09, 2.89±0.33, 15.16±2.20 mg kg^{-1} respectively), were found in Sadabahar variety grown in plot of WRI, Faisalabad where as same variety grown on the plot of NARC, Islamabad, which contains highest level of K, Ca, Fe, Co, Al, Pb and Cd (7892.7±474.4, 831.53± 94.06, 505.95±64.67, 4.05±0.51, 14.82±0.67, 1.30±0.03, 0.24±0.02 mg kg^{-1} respectively). Bajawar-2000 is an other variety, which was also grown in both of above agricultural research institute/centre, in which maximum concentration of Na, K, Ca, Fe, Zn, Co, Ni, and Al (1768.0±68.5, 8285.1±883.4, 891.0±55.84, 937.02±99.15, 28.60±1.04, 4.34±0.58, 4.56±0.41, 15.13±1.11 mg kg^{-1}) was detected in the plot of WRI, where as the maximum concentration of Mg, Mn, Cu, Cr, Ba, Pb and Cd (2256.85±262.7, 15.68±1.47, 7.32±0.27, 1.08±0.05, 5.15± 0.53, 0.90±0.02, 0.24±0.02 mg kg^{-1} respectively) was detected from the plot of NARC.

Similarly highest level of Co, Ba and Al (3.14±0.44, 18.58±1.76 and 17.49±2.22 mg kg^{-1} respectively) were observed from Frontier-87 variety that was grown in WRI. However in case of same variety that was grown in the plot of NARC, the highest level of Na, K, Ca, Mg, Fe, Zn, Mn, Cu, Cr, Ni, and Pb (951.32±24.19, 7455.0±85.50, 679.31±32.44, 1916.84±68.7, 331.22±26.30, 27.72±1.23, 15.68±1.47, 6.44±0.46, 1.17±0.14, 1.42±0.24, 0.85±0.05 mg kg^{-1} respectively) were determined and the uptake of the Cd in both plots is same (0.22±0.02 mg kg^{-1}). Third variety, Sonober-96 absorbed maximum concentration of the Ca, Mg, Fe, Mn, Co, Ni, Pb and Cd (682.31±92.86, 2177.45±183.6, 428.19±59.70, 17.67± 1.09, 4.41±0.68, 2.44±0.16, 1.45±0.09 and 0.29±0.03 mg kg^{-1} respectively) which were grown in the field of WRI. The highest uptake of Na, K, Zn, Cu, Ba and Al (664.92±1.90, 7343.10±115.24, 25.71±1.48, 7.68±0.51, 8.75±0.70, and 20.64± 2.23 mg kg^{-1} respectively) were found from the said variety but uptake of Cr was found exactly same (0.87±0.11 mg kg^{-1}) in both plots.

Soorab-96 variety, absorbed maximum concentration of Na, K, Mg, Fe, Mn, Co, Cr, Ni, Ba, Al, and Pb (891.68±46.19, 7107.98±644.99, 1613.07±105.96, 466.59± 59.70, 17.39±1.6, 3.17±0.41, 0.98±0.05, 3.29±0.39, 10.49±1.76, 39.50±4.45, and 1.44±0.11 mg kg^{-1} respectively) from the plot of WRI, Faisalabad while as maximum absorption of Ca, Zn, Cu, and Cd (680.0±47.95, 33.77±2.70, 8.12±0.73, and 0.25±0.03 mg kg^{-1} respectively) took place from the plot of NARC, Islamabad.

In case of soil samples, highest concentration of Na, Ca, Pb, and Cd (8283.9± 248.27, 3204.1±231.2, 5.64±0.51 and 1.95±0.17 mg kg^{-1} respectively) was detected from the soil of the Wheat Research Institute (WRI), Faisalabad and the highest concentration of the K, Mg, Fe, Zn, Mn, Cu, Co, and Al (14719.8±412.7, 50513.4±3397.4, 5974.0±376.7, 95.31±8.58, 611.78±54.51, 35.03±2.99, 15.64± 0.98, 42035.7±4378.9 mg kg^{-1} respectively) were determined from the NARC, Islamabad.

CONCLUSIONS

The difference in concentration of mineral contents of same barley varieties grown in two different agricultural research plots were compared to each other, since all varieties were grown under two different environmental conditions. It is important to consider these differences in mineral concentration when comparing results with the same varieties grown in two different locations i.e. WRI, Faisalabad and NARC, Islamabad. The mineral contents of barley of the Pakistani varieties studied were found to be the maximum concentration of Na, K, Ca and Fe in the Bajawar-2000 variety, highest level of Mg, Zn, Mn and Cr detected in Sadabahar variety, only maximum uptake of Cu was determined in Soorab-96 variety. Maximum absorption of Co, Pb and Cd was observed in

Sonobar-96 variety, highest level of Cr detected in Sadabahar. Similarly highest uptake of Ba and Al were detected in Frontier-87 and Soorab-96 varieties respectively. It has been noted from Table 1. that highest absorption of all these elements were found to be in all of varieties that were grown on the plot of Wheat Research Institute (WRI), Faisalabad except of only Cu, which was found in National Agriculture Research Centre (NARC) Islamabad in Soorab-96 variety.
The concentrations of these nutrients in cereal tissue were at the low end of the plant sufficiency range and those in the grain were well within the maximum tolerable limit for livestock feed. Thus, it is imperative that these plots be maintained and monitored to further assess the benefits and risks of using source-separated municipal solid waste compost and other chemicals.

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