

## Determination of Seven Heavy Metals, Cadmium, Cobalt, Chromium, Nickel, Lead, Copper and Manganese in Wheat flour Samples by Flame Atomic Absorption Spectrometry

<sup>1</sup>G. Q. SHAR, <sup>1</sup>T. G. KAZI, <sup>1</sup>M. A. JAKHRANI, <sup>1</sup>S. R. SAHITO AND <sup>2</sup>M. A. MEMON

<sup>1</sup>National Centre of Excellence in Analytical Chemistry,  
University of Sindh Jamshoro, 76080, Pakistan

<sup>2</sup>Liaquat Medical University, of Health and Sciences, Jamshoro

(Received 15<sup>th</sup> January, 2002, revised 23<sup>rd</sup> September, 2002)

**Summary:** The determination of the total elemental contents using wet acid digestion method by nitric acid and hydrogen peroxide for dissolution of seven heavy metals (Cd, Co, Cr, Ni, Pb, Cu and Mn) in the wheat flour of certified and representative samples of eight wheat varieties of the Sindh, Pakistan, by atomic absorption spectrometry. The experimental study was conducted using six samples for each of eight wheat varieties of FSC&RD standard reference materials and representative samples which was collected from four agricultural research Institutes. The characteristics mean of each element for each variety of representative samples found to be in the range of 0.383-0.73, 0.50-1.82, 0.201-0.289, 0.777-1.067, 0.525-0.879, 2.42-4.12, and 23.14 -37.14 in mg/kg for Cd, Co, Cr, Ni, Pb, Cu and Mn respectively, and was compared with certified samples which is at the 95% confidence limit.

### Introduction

During recent year, the determination of trace and toxic elements in foodstuffs attracted a good deal of scientific attention. In Pakistan the wheat is the main staple food for most of the population; so it is the main source of trace and essential elements in addition to the major nutrients [1,2].

The food, due to environmental pollution [3] may also contain certain toxic elements for which body has a tolerance level and above this level these may adversely effect the biochemical system [4,5]. There fore it is necessary to assess the adequacy and safety of common diet to take daily such as wheat flour by monitoring the concentration of essential and toxic elements, and to establish their base line level. Since these elements are present in very minute quantities, highly sensitive and accurate analytical techniques are required for their measurement.

The most sensitive and accurate atomic absorption spectrophotometric technique was used for analysis of wheat flour samples [6,7]. The survey of literature revealed that the applications of Flame atomic absorption spectrophotometry for trace amounts of Cd, Co, Cr, Ni, Pb, Cu and Mn are most frequent in analytical procedures for geological materials, ores, minerals, surface and drinking water, and for biological materials, for which the speed and ease of use is appreciated [8].

### Aims and objectives

The present study was aimed to assess the safety of human diet that is consumed 70-80%

particularly by middle and low-income groups. It is therefore, important to determine the concentrations of above metals in different varieties of wheat flour in order to establish a base line level of these elements and to estimate their daily intake through this source.

Trace metal play a vital role in health maintenance of human body, and the total or partial lack may result in characteristic pathological deficiency signs and symptoms. Therefore daily intake of trace elements should be such that their lack does not lead to any disturbance [9]. On the other hand, they may be toxic in higher concentration [10].

### Results and Discussion

The up takes of elements by plants are depends on the amount of metal in soil, water and botanical factor. The great variation in uptake was obtained by same species having different varieties.

Digestion of sample with nitric acid-hydrogen peroxide was very efficiently applied to quality reference material [11].

Factors that regulate the growth of plants are depending on a number of biochemical processes involving various inorganic elements present in the soil. A large variation in mineral content of different crops [12,13,14] depends upon plant species, soil and climate. The plant species vary widely in tolerance to toxic metals and varieties within a species can also

vary three to ten folds. Variation in mineral content of different plant species grown on the same soil have also been reported [15].

Tables 3 report the most significant results of the analysis of the investigated samples. The reliability of our method is shown by the low (estimated standard deviation value) obtained (in table 3)

The detail experimental data for each element in both certified and collected samples is in such a way that the maximum concentration of Cd was observed in Sarsabaz ( $0.66 \pm 0.078$ ) and the minimum concentration of Cd was observed in Anmol i.e. ( $0.381 \pm 0.042$ )<sup>c</sup>. The level of Co was found high ( $1.22 \pm 0.131$ ) in SKD-10/9 while as low value was observed in Anmol i.e. ( $0.49 \pm 0.072$ ). The high level of Cr was found in Mehran-89 ( $0.300 \pm 0.013$ ) and the low value was observed in Soughat-90 ( $0.200 \pm 0.022$ ). Nickel is essential in very trace amount, the level of Ni in Abadgar-93 was found ( $1.077 \pm 0.223$ ) and the lower amount was detected in Mehran-89 and SKD-10/9 ( $0.840 \pm 0.112$ ). The level of Cd, Pb was found very low in all varieties and they were within recommended value.

Copper is one of the essential trace element and its concentration was found higher than the above trace and toxic elements. The level of Cu was high in Anmol ( $3.87 \pm 0.17$ ) and in Abadgar ( $3.87 \pm 0.31$ ). The Mn is essential element both for

animals and plants. The high value of Mn was observed in Abadgar-93 ( $38.10 \pm 2.88$ ) and the low value was found in SKD-10/9 ( $20.24 \pm 1.11$ ).

The all value for different trace and toxic elements in wheat flour was found to be within the permissible limit given by FAO and WHO [16, 17]. Therefore, these varieties were found safe for human consumption.

## Experimental

### Apparatus

A Hitachi Model 180-50 atomic absorption spectrometer was used, hollow-cathode lamps of cadmium, cobalt, chromium, nickel, lead, copper and manganese were used (made by Mtorika company). The spectrometer out put was connected to a Hitachi Model 180-50, (Hitachi recorder 056). The signals measured were the heights of the absorbance/division peaks. The level of toxic metals are very low, so we increase the signal out put from spectrophotometer and increase the range of chart full scale deflection to 5mV, so results are noted as number of division. The detail is given in table 1.

### Reagents

The nitric acid (65% w/v with specific gravity 1.41) and hydrogen peroxide (35% w/v) were supra pure reagents (Merck), high-purity water (electrical resistivity  $> 10 \text{ m}\Omega \text{ cm}$ ) was produced with a Milli-Q system Millipore, MA, USA).

Table 1. Instrumental conditions for the AAS measurement of Cd, Co, Cr, Ni, Pb, Cu and Mn

Elements	Wave Length (nm)	Slit width (nm)	Lamp current (mA)	Fuel flow (acetylene) (l/min)	Flow rate (Air) (l/min)	Burner Height (mm)	Oxidant (Air) kg/cm <sup>2</sup>	Fuel (Acetylene) kg/cm <sup>2</sup>	Signal out put
Cd	229.0	1.3	7.0	2.30	9.40	7.5	1.60	0.30	100%
Co	250.0	0.2	9.5	2.30	9.40	10.0	1.60	0.35	100%
Cr	358.2	1.3	6.0	2.30	9.40	7.0	1.60	0.30	100%
Ni	232.3	0.2	9.5	2.30	9.40	7.5	1.60	0.30	100%
Pb	232.3	1.3	7.0	2.30	9.40	7.5	1.60	0.30	100%
Cu	325.0	1.3	7.0	2.30	9.40	7.5	1.60	0.30	100%
Mn	279.8	0.4	7.0	2.30	9.40	7.5	1.60	0.30	100%

Table 2: Statistical data for standards of elements

Elements	Concentration range ppm (x)	Absorbance/ Division (y)	Statistical calculation $y = m x + c$		$r^2$
			m	c	
Cadmium	0 - 0.25	0 - 28 div.	110.39	0.032	0.9977
Cobalt	0 - 0.50	0 - 29 div.	58.4	-0.35	0.9994
Chromium	0 - 0.125	0 - 15 div.	121.39	-0.3977	0.9969
Nickel	0 - 0.25	0 - 14 div.	55.31	0.20	0.9984
Lead	0 - 0.25	0 - 15 div.	60.80	-0.4	0.9968
Copper	0 - 1	0 - 0.086*	0.0862	0.0004	0.9989
Manganese	0 - 1	0 - 0.196*	0.1962	0.0005	0.999

Absorbance\*, Y= absorbance, m = slope,

X = concentration in ppm c = intercept, r = correlation coefficient

*Chemical and Glasswares.*

All glassware used were of Pyrex brands. They were washed with nitric acid and chromate mixture, followed by the distilled and deionized water respectively. The statistical data for standards of elements are given in table 2.

*Sampling*

Six samples of each wheat varieties *Anmol*, *TJ-83*, *Abadgar-93*, *Mehran*, *Soughat-90*, *Sarsabaz*, *Kiran*, and *SKD10/9* which were grown in four different locations (NIA, Tandojam, Wheat Research Station Sikrand, Basic seed farm Kotdiji and Basic seed farm Ghotki) of the Sindh, Pakistan. Crops were harvested at maturity after 18-20 weeks. The grains were separated from their spikes by single heads thresher model No. AAM-8 which were randomly collected and act as the representative samples for analysis. Certified samples of same varieties was collected from the offices of the Federal Seed Certification & Registration Department Hyderabad and Sukkur act as a reference sample. These representative and reference certified samples were washed well with distilled water and dried at 105°C for 2 hour in oven. All samples were ground in a domestic grinder (Arno. Sao Paulo, SP, Brazil) separately. Subsequently, portions of the samples were dried at 60C<sup>o</sup> until constant mass. Triplicate 2gram samples of each variety of representatives and certified samples were weighed into separate 100ml flasks and treated with 5ml of nitric acid. 5ml of nitric acid was also added to empty conical flask

serving as blank. The flasks were covered with watch glasses, and their contents were heated to boil gently on an electric plate. After heating for one hour, the contents of flasks were treated with 5ml more of nitric acid, 2ml of 30% hydrogen peroxide was added [17, 18,], and the heating was continued for an other hour. 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 the flask were cooled, diluted with high purity water, and filtered through whatman # 42 paper into 25ml volumetric flasks. The contents of flasks were brought to volume with high purity water and examined by atomic absorption spectrometry for their cadmium, cobalt, chromium, nickel, lead, copper and manganese levels. The results of these measurements are presented in table 3.

*Determination of Mineral Element*

Appropriate working standard solutions of cadmium, cobalt, chromium, nickel, lead, copper and manganese were prepared from stock standard solution (1000ppm), in 2N nitric acid and the calibration curve of seven elements were obtained for concentration Vs absorbance/division data were statistically analysed using fitting of straight line by least square method using equation  $y = mx + c$

A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements.

Table 3. Determination of toxic and trace elements in flour of different wheat varieties using Atomic Absorption Spectrophotometer. (mg/kg  $\pm$  S.D, dried mass)

Elements	Sample	DIFFERENT VARIETIES OF WHEAT FLOUR							
		Anmol	TJ-83	Abadgar-93	Mehran-89	Soughat-90	Sarsabaz	Kiran	SKD-10/9
Cd	CS	0.38 $\pm$ 0.04	0.387 $\pm$ 0.044	0.441 $\pm$ 0.041	0.412 $\pm$ 0.082	0.417 $\pm$ 0.087	0.73 $\pm$ 0.091	0.614 $\pm$ 0.046	0.449 $\pm$ 0.041
	RS	0.41 $\pm$ 0.045	0.381 $\pm$ 0.042	0.449 $\pm$ 0.038	0.379 $\pm$ 0.065	0.502 $\pm$ 0.093	0.66 $\pm$ 0.078	0.487 $\pm$ 0.039	0.562 $\pm$ 0.048
Co	CS	0.56 $\pm$ 0.085	0.68 $\pm$ 0.078	0.50 $\pm$ 0.006	0.68 $\pm$ 0.085	0.56 $\pm$ 0.069	0.80 $\pm$ 0.073	1.82 $\pm$ 0.170	1.04 $\pm$ 0.097
	RS	0.49 $\pm$ 0.072	0.55 $\pm$ 0.074	0.56 $\pm$ 0.008	0.81 $\pm$ 0.073	0.58 $\pm$ 0.064	0.85 $\pm$ 0.077	0.98 $\pm$ 0.089	1.22 $\pm$ 0.131
Cr	CS	0.245 $\pm$ 0.025	0.251 $\pm$ 0.012	0.258 $\pm$ 0.008	0.289 $\pm$ 0.018	0.201 $\pm$ 0.015	0.263 $\pm$ 0.021	0.205 $\pm$ 0.026	0.223 $\pm$ 0.024
	RS	0.278 $\pm$ 0.031	0.262 $\pm$ 0.009	0.256 $\pm$ 0.005	0.300 $\pm$ 0.0130	0.200 $\pm$ 0.022	0.284 $\pm$ 0.015	0.203 $\pm$ 0.018	0.234 $\pm$ 0.025
Ni	CS	0.958 $\pm$ 0.086	1.067 $\pm$ 0.075	1.366 $\pm$ 0.096	0.777 $\pm$ 0.074	0.896 $\pm$ 0.13	1.00 $\pm$ 0.11	0.958 $\pm$ 0.076	0.847 $\pm$ 0.084
	RS	1.00 $\pm$ 0.112	1.038 $\pm$ 0.056	1.077 $\pm$ 0.223	0.840 $\pm$ 0.112	0.919 $\pm$ 0.223	0.959 $\pm$ 0.056	0.919 $\pm$ 0.068	0.84 $\pm$ 0.112
Pb	CS	0.612 $\pm$ 0.080	0.765 $\pm$ 0.075	0.632 $\pm$ 0.056	0.727 $\pm$ 0.083	0.525 $\pm$ 0.061	0.879 $\pm$ 0.052	0.792 $\pm$ 0.049	0.805 $\pm$ 0.073
	RS	0.669 $\pm$ 0.038	0.788 $\pm$ 0.049	0.781 $\pm$ 0.066	0.907 $\pm$ 0.076	0.609 $\pm$ 0.038	1.027 $\pm$ 0.059	0.788 $\pm$ 0.062	0.669 $\pm$ 0.048
Cu	CS	3.96 $\pm$ 0.23	3.82 $\pm$ 0.18	4.12 $\pm$ 0.27	3.26 $\pm$ 0.31	2.42 $\pm$ 0.19	3.77 $\pm$ 0.35	3.64 $\pm$ 0.24	3.57 $\pm$ 0.29
	RS	3.87 $\pm$ 0.17	3.69 $\pm$ 0.11	3.87 $\pm$ 0.31	3.55 $\pm$ 0.22	2.54 $\pm$ 0.24	3.75 $\pm$ 0.22	3.65 $\pm$ 0.29	3.67 $\pm$ 0.23
Mn	CS	28.23 $\pm$ 2.35	34.80 $\pm$ 2.05	37.14 $\pm$ 3.12	29.38 $\pm$ 2.31	28.87 $\pm$ 2.45	33.46 $\pm$ 1.89	27.61 $\pm$ 2.18	23.14 $\pm$ 1.47
	RS	32.58 $\pm$ 2.15	36.64 $\pm$ 1.76	38.10 $\pm$ 2.88	30.31 $\pm$ 1.23	28.36 $\pm$ 1.62	34.37 $\pm$ 1.55	28.03 $\pm$ 1.87	20.24 $\pm$ 1.11

Keys: (CS) = Certified sample

(RS) = Representative sample

### Percentage Recovery Test

The efficiency of extraction method was checked by standard addition method. The representative and reference samples were spiked with known standards and digested with nitric acid and hydrogen peroxide mixture. The matrix of standards and sample solutions was same. The percentage recovery test for different elements by digestion method adopted was 98.5 – 99% in range.

### Conclusion

The experiments carried out provides evidence that the extraction of Cd, Co, Cr, Ni Pb, Cu and Mn from wheat flour by wet acid digestion method. The results obtained from representative samples which were collected from four districts (Hyderabad, Nawab shah Khairpur (Mirs) and Ghotki) of the Sindh province, Pakistan are in good agreement with the results of FSC&RD certified samples of same varieties.

It was concluded that the up takes of elements are dependent on botanical factor.

### Acknowledgement

The author is thankful to Sha Abdul Latif University, Khairpur, Sindh for financial support and study leave.

### References

1. B. S. Hetzel, and G. F. Maberly. Trace Elements in Human and Animal Nutrition-5<sup>th</sup> edn. (ed. Mertz, W.), pp. 139, New York: Academic press. (1986).
2. K. M. Hambidge, C. Casey, and N. F. Krebs. Trace Elements in Human and Animal Nutrition-5<sup>th</sup> edn. (ed. Mertz, W.), pp. 1, New York: Academic press (1986).
3. L. Freiberg, G. F. Nordberg, and B. Vouk, Hand book on the toxicity of metals. Elsevier / North Holland Biomed. Press, Amsterdam. Eds. (1985).
4. H.W. Numberg. Pollutants and their ecotoxicological significance, John. Wiley and sons, New York. (ed. ), (1985).
5. E. Friden. Biochemistry of the essential ultratrace elements, Plenum Press New York. (ed.) (1984).
6. N. T. Crosby, Determinations of metals in foods, *The Analyst*, **102**, 225. (1977).
7. W. Bergthaller, Prog, *Plant Poly.. Carbohydr. Res.* 7<sup>th</sup> 129, (1995).
8. J. Sramkova and S. Kotrly, Sci. Pap. Univ. Pardubice, Ser. A (1999).
9. B. L. Vallee, *Physiol Rev.* **39**, 443 (1959).
10. Food and Nutrition Board, National Academy of Sciences, National Research council, U.S.A. (1980).
11. T. G. Kazi and S. A. Katz, *Spectroscopy Letters*, **20** (20), 509, (1987).
12. R. F. Keefer, D. G. Harvath., *J. Environ. Quality.* **15** (21), 26 (1986).
13. M. B. Kishkan, *Hot Science*, **21**, 24 (1986).
14. M. R. Reddy, *J. Agric. Food, Chem.*, **34** (4), 750 (1986).
15. H. W. Dougall, A. V Bogdan, *J. African, Agric.* **24**, 17. (1958).
16. World Health Organization (WHO). Recommended health based limits in occupational exposure to heavy metals. Technical report series, No. 647 (1980).
17. FAO/WHO Food standard programme.. List of maximum levels recommended for contaminants, second series, CAC/FAL-3. (1976).
18. S. A. Katz, Environmental chemistry, H. J. M; Bowen, ed., *Royal Soc. of Chem. London.*, **3**, 78 (1984).
19. H. Adelman, S. W. Jennis, S. A. Katz, *American Lab.* 31 (1981).