Bull. Environ. Contam. Toxicol. (2006) 76:1044–1052

DOI: 10.1007/s00128-006-1023-x

Lead and Cadmium in Wheat Grain

N. Rafiq,[1] Z. T. Maqsood,[1] Z. Parveen[2]

[1] Department of Chemistry, University of Karachi, Karachi 75270, Pakistan
[2] Pesticide Research Institute, Southern-Zone Agricultural Research Centre, Pakistan Agricultural Research Council, Karachi University Campus, Old Blocks Number 09 and 10, Karachi 75270, Pakistan

Received: 21 October 2003/Accepted: 19 April 2006

Lead and cadmium are placed under the heading of toxic heavy metals (Greenwood 1984) as they have no known beneficial biological role in the human body (Hay 1987, Greenwood 1984). They adversely affect the biochemical processes and are likely to cause certain physiological disorders even at very low concentrations (Ahmad et al. 1984). These metals are the natural constituents of the earth's crust (Greenwood 1984) but are rarely available and are not toxic at the level normally encountered (Das 1990). With the rapid progress of our civilization our environment is being profusely polluted with these uncommon elements (Das 1990). The possible sources of air pollution by these metals are the power plants based on combustion of fossil fuel, combustion of petroleum products (gasoline, petrol, and diesel oil) in automobiles, incineration of PVC, Teflon and municipal solid wastes, manufacture of phosphate fertilizer, burning of tires during protest rallies, wear and tear of tires, burning of coal (Qureshi 2000), industrial operations, mining activities and metallurgical processing. The main sources of soil and water pollution by these metals are the dumping of waste water discharged by industries in the open ground near agricultural lands or into the drains leading to the rivers (Ghulam Nabi et al. 1999, Surriaya Mir et al. 1999). Plants absorb metals either via the root-system or by plain absorption and humans receive their quota of heavy metals directly through the use of vegetation (Surriaya Mir et al. 1999).

This paper describes a reliable method for wet mineralization of wheat grain to prepare samples for the determination of Cd and Pb contents and satisfactory solution of the problems faced by an analyst during this process. The main objectives of the proposed study were to get the evaluation of Cd and Pb content in different samples of wheat grain, to compare these metals' contents with their respective hygienic standards for grain and to find out the correlation coefficient between Cd and Pb. The paper also describes a detailed discussion about the factors influencing the uptake of these metals.

MATERIALS AND METHODS

Cadmium chloride monohydrate ($CdCl_2.H_2O$): BDH Laboratory Supplies (Poole, England), Lead nitrate ($PbNO_3$): BDH Laboratory Chemicals Division (Poole,

---

Correspondence to: N. Rafiq, Grain Quality Testing Laboratory, Southern-Zone Agricultural Research Centre, Pakistan Agricultural Research Council, Karachi University Campus, Old Blocks Number 09 and 10, Karachi 75270, Pakistan

England), Nitric acid (HNO_3) 65 % extra pure: Merck (Dermstadt, Germany), Perchloric acid ($HClO_4$) about 70 %: Merck (Dermstadt, Germany), Sulphuric acid (H_2SO_4) 98 %: BDH Laboratory Supplies (Poole, England) and de-ionized distilled water (DDW).

Forty-five samples of whole wheat grain were purchased from nine small flour mills situated in different local markets of Karachi during the year 2002. The samples were sealed in clean plastic bags and were stored in deep freezer at temperature below 0°C.

Before each analysis, all the required glassware were cleaned with a standard detergent thoroughly, washed with tap water and then were soaked overnight in an acid bath (30 % nitric acid). Thereafter, the glassware were rinsed firstly with tap water and finally with DDW. The beakers were further cleaned rinsing with 5 mL nitric acid before adding weighed wheat samples to them.

Prior to weighing, each sample was removed from the deep freezer and was allowed to attain the room temperature. Thereafter, all the extraneous matter was separated from it. The digestion of wheat grain sample was accomplished by wet mineralization method. This method involves the complete destruction and decomposition of the entire organic matrix in the test portion heating with nitric acid, sulphuric acid and perchloric acid (Skurikhin 1989). The procedure was carried out in two major steps i.e. first digestion and second digestion as stated below in detail.

For each sample, three 10 g sub samples of whole wheat grain were subjected to digestion; hence contamination from mills was excluded. But it provided no guarantee that contamination had not taken place during harvest, transport or storage.

In first digestion, 10 g of whole wheat grain was accurately weighed, transferred to a 500 mL beaker and 60 mL nitric acid was added to it. The beaker was covered with a watch glass and was left stand at room temperature for three hours in a fume hood to allow the sample to be reacted with nitric acid. The beaker was then placed on an electric hot plate and the reaction mixture was started to reflux at 70°C. After every ten minutes the beaker was uncovered and the reaction mixture was shaken for a little while to allow the brown fumes of nitrogen oxide gases to be evaporated from the beaker. The heating at the same temperature and the removing of fumes from the reaction mixture in the same manner were continued until the volume reduced to a ca about 5-7 mL. After the completion of first digestion, the partial digested sample became yellow in color.

In second digestion, the partial digested sample was cooled down to room temperature keeping in the fume hood and then 10 mL nitric acid, 4 mL perchloric acid and 5 mL sulphuric acid were added to it. The beaker was covered with the watch glass and was heated gently until the solution became colorless, the dense white clouds were being evolved from it and the volume reduced to a ca about 2 mL. The colorless solution was cooled down to room temperature and was checked for complete mineralization. Mineralization is considered complete if the cooled solution remains colorless (Skurikhin 1989).

To eliminate the residual acid from the completely mineralized sample solution, 10 mL DDW was added to it and was heated gently until the volume reduced to a ca about 2-3 mL. This step was repeated until the evolution of white fumes ceased. The completely digested sample was cooled down to room temperature, 15 mL DDW was added to it and was filtered with whatman 542 filter paper transferring the filtrate to a 50 mL calibrated volumetric flask containing 5 mL nitric acid. The beaker was rinsed with 7 mL portions of DDW three times and each washing was filtered collecting the filtrate in the same flask to avoid any loss of metals. Finally, the filter paper was washed with 5 mL DDW to avoid any possible loss of metals. The filtrate i.e. completely digested sample was diluted to 50 mL with DDW. The flask was stoppered; the sample solution was shaken well and was analyzed by atomic absorption spectrophotometer within 24 hours.

A blank with no wheat grain in it was also run side by side the sample using the same reagents as were used in sample digestion and following the same steps as were carried out for sample digestion.

A Perkin Elmer Model 3100 atomic absorption spectrophotometer equipped with air-acetylene flame atomizer and a hollow cathode lamp depending upon the element to be determined was used for quantitative analysis of Cd and Pb. Perkin Elmer hollow cathode lamps were operated under the manufacturer's recommended conditions. A considerably adequate and reliable sensitivity check of the instrument was found for both of the elements after setting the standard conditions given in Table 1 as recommended by the Perkin Elmer operating instructions.

Integration time: 0.1sec, replicates: 3, calibration type: nonlinear and atomic absorption technique: flame were used as instrumental parameters.

Table 1. Standard conditions for Perkin Elmer Model 3100 atomic absorption spectrophotometer.

Element	Wave Length (nm)	Slit Width (nm)	Lamp Current (mA)	Flame Gases	Slit Height
Cd	228.8	0.7	8	A-Ac^1	High
Pb	283.3	0.7	15	A-Ac	High

A-Ac^1=Air-Acetylene

RESULTS AND DISCUSSION

As far as the use of HNO_3, $HClO_4$ and H_2SO_4 in the process of wet mineralization is concerned, HNO_3 alone can not efficiently oxidize all the organic matter (Skurikhin 1989). Moreover, mixture of $HClO_4/HNO_3$ effectively eliminates organic matter and provides several other advantages. Because $HClO_4$ oxidation is more energetic, it reduces loss of elements by volatilization and effectively eliminates photometric interference produced by nitrous vapors from HNO_3 digestion (Skurikhin 1989). As stated above mineralization is considered complete if the sample solution remains colorless at room temperature after digestion. Sometimes, an analyst has to face two major problems during the first or second digestion in getting a colorless sample solution. These problems and the methods to overcome them are as follows. (1) If the solution becomes black, stop heating, cool it to room temperature, add 5 mL nitric acid and 2 mL perchloric acid to it and heat until the evolution of white fumes of sulfur trioxide occurs and the solution becomes colorless. Then heat gently to evaporate the content to a ca about 2-3 mL. (2) If the solution becomes brown in color, stop heating, cool it to room temperature, add the amount of nitric acid depending upon the intensity of the color and heat until the solution becomes yellow. If it is not, add more nitric acid again and heat until the solution becomes yellow in color. Continue to heat until the volume reduces to a ca about 2-3 mL and then follow second digestion.

Forty-five samples of whole wheat grain were analyzed to estimate Cd and Pb content. Table 2 shows Cd and Pb content in different samples of wheat grain.

Table 2. Cadmium and lead in wheat grain (mg/kg).

Sample	Cd	Pb	Sample	Cd	Pb	Sample	Cd	Pb
1	0.33	1.32	16	ND	0.67	31	0.32	1.23
2	0.31	0.85	17	0.29	1.34	32	0.21	1.17
3	0.48	1.39	18	0.13	1.07	33	0.08	ND
4	0.24	1.13	19	0.37	1.24	34	0.11	0.94
5	ND[1]	0.98	20	0.26	1.33	35	ND	0.81
6	0.17	ND	21	0.15	0.78	36	0.35	1.24
7	ND	0.53	22	0.09	ND	37	0.24	1.32
8	0.47	1.46	23	ND	0.87	38	ND	0.67
9	0.12	0.71	24	0.41	1.35	39	0.21	1.19
10	0.15	1.17	25	0.16	1.27	40	0.34	1.29
11	0.05	0.62	26	0.14	1.17	41	0.19	1.25
12	0.23	0.84	27	0.34	1.15	42	0.45	1.32
13	0.11	ND	28	0.19	ND	43	0.28	1.36
14	0.26	0.93	29	0.43	1.32	44	ND	ND
15	0.21	1.25	30	ND	0.81	45	0.23	1.13

ND[1]= Not Detected

Of forty-five samples only 8 samples (17.78 %) were found to be Cd free whereas 37 samples (82.22 %) were found to be contaminated with Cd. The content of Cd in the contaminated samples was found in the range of 0.05-0.48 mg/kg. The mean Cd content calculated was 0.25 mg/kg. The hygienic standard for Cd in grain proposed by the National Standard Bureau of Peoples Republic of China is 0.1 mg/kg (Nan and Cheng 2001). Of forty-five samples only 11 samples (24.44 %) were found to place within the hygienic standard while 34 samples (75.55 %) were found to exceed the hygienic standard (Figure 1), even the mean Cd content was also found to violate the hygienic standard.

The concentration of Cd in such a high amount may be influenced by many factors such as regional differences, annual variation and genetical variation or cultivar differences (Andersson and Petterson 1981). Atmospheric Cd deposition, fertilization practice and soil properties such as soil type, pH, origin and formation and soil Cd content may contribute to regional differences found in Cd levels (Andersson and Petterson 1981).

As far as the atmospheric deposition is concerned, Cd content of well-protected wheat grain seems to be practically unaffected by direct atmospheric deposition (Andersson and Petterson 1981). Hansen and Tjell (1978) argued that Cd in plant material like Zn to 80 or 90 % originates from the soil.

As far as the fertilization practice is concerned, Filipek-Mazur et al. (2001) investigated the effect of organic fertilizers on distribution of heavy metals among fractions in soil and found that the application of sewage sludge and its compost with peat resulted in the high mobility of Cd in soil which confirms its big share (over 50 %) in the mobile fraction and plant available and the trace amounts of this element bound in the residual fraction. More efficient use of commercial phosphorous fertilizers during a long period may contribute to high soil Cd levels (Jansson 1975). Modaihsh et al. (2001) reported that phosphorous fertilizers might bear rich content of detrimental elements typically like Cd. They found a positive significant correlation between total P and total Cd in soil and concluded that Cd accumulation in soil is associated with P accumulation.

As far as the soil type is concerned, clay and organic matter are considered as important parameters influencing bioavailability and ecotoxicity of metals in soil. As there is a large variation in the type of clay and organic matter in field soils, the quantity of these variables alone may not be good indicators of metal bioavailability (Lock and Janssen 2001). Svetina (1999) documented that soils formed from calcareous rock are more advantageous with respect to Cd uptake than sedimentary and andesitic rock, because they limit Cd accumulation in the plants growing on these types of soils. Nigam Rashmi et al. (2000) investigated the effect of various organic (carboxylic and amino) acids on the uptake and translocation of root-absorbed Cd by wheat plants grown in sand and soil culture. They found that statistically significant increase in Cd accumulation from Cd treated plants in the presence of increasing concentrations of organic acids suggest the existence of Cd-organic acid interactions in the soil-plant system resulting into the formation of organically bound Cd which is mobile and plant available. Amino acids were less effective in the mobilization of Cd as compared to carboxylic acids.

As far as the soil Cd content and soil pH are concerned, the uptake of Cd in winter wheat has been demonstrated to increase with increasing soil Cd content and with decreasing pH (Linnman et al. 1973). Okamoto (2001) found that when pH in the soil is decreased, the metals change to easily exchangeable forms thereby accelerating their mobilization and plant uptake. Csillag et al. (1998) investigated the release of Cd, Cr, Ni, Pb and Zn to the soil solution as a consequence of soil

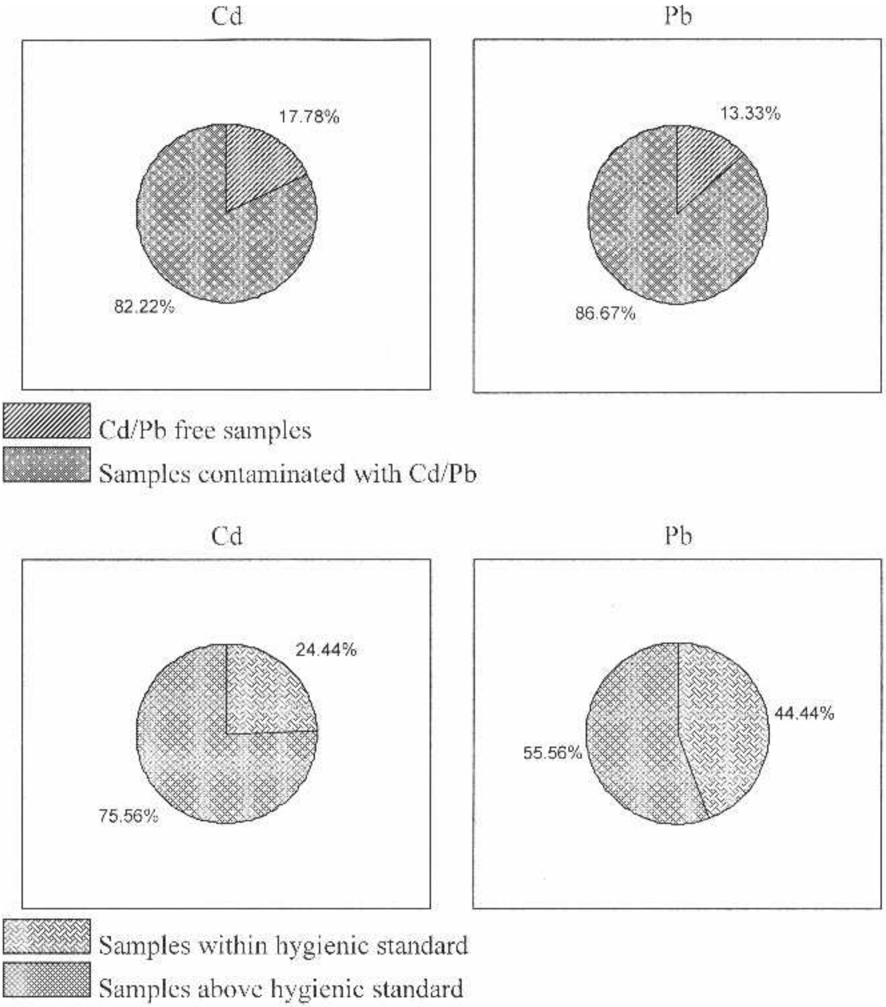

Figure 1. Comparison of Cd and Pb in wheat grain.

contamination and acidification and found that Cd, Ni and Zn enter the liquid phase in relatively higher amounts than the less mobile Cr and Pb. They also concluded that considerable release of metals into the soil liquid phase occurs only under extreme conditions (excessive metal contamination and strong acid pollution).

As far as the annual variation is concerned, Cd content of plant material may be influenced by rain via the soil and the root, when the acid rain water infiltrates the soil (Oden 1976). The acid precipitation may release some of the soil Cd and, in addition, the Cd concentration in precipitation is higher than in the normal soil solution (Andersson and Petterson 1981). Roughly high precipitation also means high Cd content in wheat grain and low precipitation means low Cd content

(Andersson and Petterson 1981). Pleigel et al. (2000) reported that the global greenhouse warming may lead to an increased accumulation of Cd, especially in those areas where both the temperature and rainfall increase. Thus, even without increasing Cd availability, the accumulation in plants may increase.

As far as genetical variation is concerned, it is known that there are differences between varieties of winter wheat as to Cd uptake (Pettersson 1977). Pleigel et al. (2000) also reported that there exists a substantial genetical variation between cultivars in the tendency to accumulate Cd in grain.

Of forty-five samples only 6 samples (13.33 %) were found to be Pb free whereas 39 samples (86.67 %) were found to be contaminated with Pb. The content of Pb in the contaminated samples was found in the range of 0.53-1.46 mg/kg. The mean Pb content calculated was 1.09 mg/kg. The hygienic standard for Pb in grain proposed by the National Standard Bureau of Peoples Republic of China is 1.0 mg/kg (Nan and Cheng 2001). Among forty-five samples only 20 samples (44.44 %) were found to place within the hygienic standard while 30 samples (55.56 %) were found to exceed the hygienic standard (Figure 1), even the mean Pb content was also found to violate the hygienic standard.

There are not such a number of factors which affect the high content of Pb in wheat grain except the absorption of Pb through air pollution. Tjell et al. (1979) reported that Pb in plant material originates to more than 90 % from atmospheric deposition. Anderrson and Pettersson (1981) reported that in the soil Pb is less available to plant than is Cd. Stopetska et al. (2001) also documented the low intensity of absorption of Pb ions by plant roots. Extra-root Pb pollution is found to occur largely in aboveground organs. Filipek-Mazur et al. (2001) reported that the greatest amount of lead was detected in the fractions attached to soil organic matter and amorphic oxides but the least amount of that element was found in the mobile and exchangeable fractions.

Despite the influence of all the above mentioned factors on the soil contamination of Cd and consequently its absorption by plant some samples were found to be Cd free which might be possible due to cultivar differences. These samples also contained less Pb content (Table 2). The correlation coefficient between Cd and Pb was found to be 0.65. Ahmad et al. (1994) alluded to an unpublished data which reported the high content of Cd in Pakistani soil. So, it is concluded that there is a great need to find out such wheat varieties having no capability to uptake Cd and then their cultivation should be preferred over the rest. In such a way we will be able to grow the wheat having no Cd and indirectly less Pb content to some extent.

Acknowledgment. The authors are thankful to Shaikh Mohiuddin, Lecturer, Department of Chemistry, University of Karachi, for providing his invaluable technical suggestions and assistance. The authors are also thankful to the staff of Southern-zone Agricultural Research Centre, Pakistan Agricultural Research Council.

REFERENCES

Ahmed S, Waheed S, Mannan A, Fatima I, Qureshi IH (1994) Evaluation of trace elements in wheat and wheat by-products. J Assoc Off Anal Chem Int 77: 11-18

Andersson A, Pettersson O (1981) Cadmium in Swedish winter wheat. Regional differences and their origin. J Agric Res 11: 49-55

Csillag J, Lukacs A, Bujtas K Nemeth T. (1998) Release of Cd, Cr, Ni, Pb, and Zn to the soil solution as a consequence of soil contamination and acidification. Adv GeoEcol 31: 673-680

Das AK (1990) Elements of life and bio-inorganic chemistry. In: First (ed) A text book on medical aspects of bio-inorganic chemistry, Satish Kumar Jain for CBS Publishers, Delhi, p 3

Filipek-Mazur Barbara, Mazur K, Gondek K (2001) The effect of organic fertilizers on distribution of heavy metals among fractions in soil. Rostl Vyroba 47: 123-128

Ghulam Nabi, Muhammad Ashraf, Muhammad Rizwan Aslam (2001) Heavy-metal contamination of agricultural soil irrigated with industrial effluents. J Sci Technol Develop 20: 32-36

Greenwood NN, Earnshaw (1984) Chemistry of the elements, Pergamon, New York

Hansen JA, Tjell JC (1978) Guidelines and sludge utilization practice in Scandinavia. Utilization of sewage sludge on land, April 10-13, 1978, Keble College, Oxford, Paper 20, Section 4

Hay RW (1987) Metals and non-metals in biology and medicine. In: First (ed) Bio-inorganic Chemistry, Ellis Horwood Limited, England, p 191

Iqbal H Qureshi (2000) Air pollution and human health. J Proc Pakistan Acad Sci 37: 109-117

Jansson SL (1975) Long-term soil fertility studies. Experiments in Malmohus county 1957-1974. J Royal Swedish Acad Agric and Forest, Supplement 10: 46-47. Stockholm

Linnman L, Andersson A, Nilsson KO, Lind B, Kjellstrom T, Friberg L (1973) Cadmium uptake by wheat from sewage sludge used as a plant nutrient source. A comparative study using flameless atomic absorption and neutron activation analysis. Arch Environ Health 27: 45-47

Lock K, Janssen CR (2001) Effect of clay and organic matter type on the ecotoxicity of zinc and cadmium to the potworm Enchytraeus albidus. Chemosphere 44: 1669-1672

Modaihsh AS, Abdallah AE, Mahjoub MO (2001) Accumulation of cadmium in arid soils as affected by intensive phosphorous fertilization. Arid Land Res Manage 15: 173-181

Nan Z, Cheng G (2001) Accumulation of Cd and Pb in spring wheat (Triticum aestivum L.) grown in calcareous soil irrigated with wastewater. Bull Environ Contam Toxicol 66: 748-754

Nigam Rashmi, Srivastava Shalini, Prakash Satya, Srivastav MM (2000). Effect of organic acids on the availability of cadmium in wheat. Chem Speciation Bioavailability12: 125-132

Oden S (1976) The acidity problem–an out line of concepts. Water Air Soil Pollut 6: 137-166

Okamoto Tamotsu (2001) Increased mobility and availability of heavy metals with acid fertilizer in soils previously amended with limed sewage sludge. Nippon Dojo Hiryogaku Zasshi 72: 1-8

Pettersson O (1977) Differences in cadmium uptake between plant species and cultivars. Swedish J Agric Res 7: 21-24

Pleijel H, Danielsson H, Gelang J, Sellden G (2000) Effects of transpiration, carbon dioxide and ozone on the content of cadmium and zinc in spring wheat grain. Environ Stress: Indic Mitigation, Eco-Conserv: 207-218

Skopetska OV, Egorova TM, Shumik SA, Musienko MM (2001) Ecologo-physiological evaluation of the condition of wheat on dark-gray podzolized soils under technogenic lead loading. Dopov Nats Akad Nauk Ukr: 187-193

Skurikhin Igor M (1989) Methods of analysis for toxic elements in food products 1. Mineralization method to determine heavy metals and arsenic according to the USSR standard. J Assoc Off Anal Chem 72: 286-290

Surriaya Mir, SM Abdul Hai (1999) Pollution due to waste-water discharge by the local industry and its control. J Sci Vision. April-June: 1-7

Svetina M (1999) The influence of different soil parent material on cadmium purification. Geochem Earth's Surf Proc Int Symp 5th: 227-229

Tjell CT, Hovmand MF, Mosbaek H (1979) Atmospheric lead pollution of grass grown in a background area in Denmark. Nature 280: 425-426