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Evaluation of toxic elements in rice (*Oryza sativa*) commercially available in Pakistan; multivariate study

[2]Jalbani, N., [1,*]Aftab, A. K., [2]Bhutto, S., [2]Ahmed, F. and [3]Mahroze, A. K.

[1]*Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Sindh, Pakistan*
[2]*Pakistan Council of Scientific and Industrial Research Laboratories Complex, Karachi Pakistan*
[3]*Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore Pakistan*

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Abstract

The aim of this study was to determine the level of toxic elements in rice samples. Microwave-assisted digestion method was used for sample preparation to determine the cadmium (Cd) and lead (Pb). A Plackett–Burman experimental design (PBD) was used as a multivariate strategy for the evaluation of the effects of some important variables at once. Furthermore, certain variable showed up as significant, and they were optimized by using Plackett–Burman, whereas most significant variables were optimized by 2^3+ star central composite designs (CCD). The accuracy of the optimized procedure ensured by the analysis of certified reference material (CRM). Outcomes are that the highest values of Cd found in brown rice (0.0889 ± 0.0056 mg/Kg), whilst the lowest concentration was found in white rice samples 0.001 ± 0.0001 mg/Kg. There is a relatively significant differences were found between the two categories of rice samples at the p-values (P = 0.05).

Introduction

Heavy metals accumulation in the environment is a health hazard due to their persistence bioaccumulation and toxicity to the plants, animals and human beings (Sharma *et al.*, 2005). Trace metal analysis in food matrixes is always an important task either from a toxicological or nutritional standpoint (Uluozlu *et al*., 2009). An excessive accumulation of some pollutant will occur in inhabi tants who have tendency to high intakes of certain foods. In addition Cd and Pb are non essential elements in metabolic process and may cause a lethal affects on human health (Lowik, 1996; Goyer, 1997). The toxic elements are introduced into the environment from natural sources as well as anthropogenic activity. These toxic elements (especially Cd and Pb) transported from aquatic media to foodstuff, to create health vulnerability due to the ingestion of rice, fruits and vegetables grown in contaminated soils (Dudka *et al.*, 1996; Samoe-Peterse *et al.*, 2002; Aftab *et al*., 2011). It was intensively investigated that uptake of metals by plants depends on the physico-chemical makeup of the plant species and soil (Carbonell-Barrachina *et al*., 2002).

It has been reported that population are extensively vulnerable to exposure of these toxic elements because of their immature renal systems and exhibit a narrow tolerance to these non essential elements (Tuzen and Soylak, 2007). The determination of elements in food samples traditionally been performed by digestion with acid (or acid mixtures), which is time consuming and involves some potential drawbacks such as analyte losses and sample contaminations due to contaminated environment. The microwave-assisted digestion is also an adequate determinative technique, but it demands skilled sample handling, much time to cool the reactor before opening (Soylak *et al*., 2005).

The main advantage of microwave assisted samples pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors (Mesko *et al*., 2006).

Analysis of metals in food samples has conventionally been performed by atomic absorption spectrometry (AAS) (McCarthy and Ellis, 1996; Jalbani *et al.*, 2007). Procedures involving optimization by multivariate techniques have been increasingly used as they are faster, more economical and effective, and allow more than one variable to be optimized simultaneously (Lavilla *et al*., 1999; Martinez *et al.*, 2002; Yebra-Biurrun *et al*., 2005).

The Plackett-Burman designs (PBDs), introduced in 1946 by Plackett and Burman, allows discovering the most significant variables for a certain system with only few experiments (Plackett and Burman, 1946). The PBDs design is one of the most frequently applied chemometric techniques in multivariate optimization (Massart *et al*., 2003). It has been used for preliminary evaluation of the significance of variables (Jalbani *et al.*, 2006; Jalbani *et al*., 2010).

The main idea of this study was to design microwave-assisted digestion method for determination of toxic elements from rice samples

*Corresponding author.
Email: *chemist_afi@yahoo.com*
Tel: +92 3332690504

i.e white and brown rice which are widely consumed by the local population of country. Plackett-Burman experimental design was also used as a multivariate strategy for the evaluation of the effects of several important variables at once. Parameters influencing microwave assisted digestion, such as irradiation time, acid mixtures, and temperature are fully investigated. From these studies, certain variables showed up as significant, and they were optimized by a 2^3+ star central composite design (CCD). Optimum values of the variables were selected for the development of acid digestion method to extract TEs (Cd and Pb) from rice samples.

In this article, we have described the current knowledge of rice Cd and Pb transporters and their (possible) roles in metal accumulation.

Materials and Methods

Reagents and glassware

All reagents were used of analytical grade Merck, (Darmstadt, Germany). De-ionized water was used through out the work. Concentrated (65%) nitric acid and hydrogen peroxide (30%) were of analytical grade (Merck, Darmstadt, Germany) and were checked for possible trace element contamination. Standard solutions of Cd and Pb were prepared from stock solutions, (1000 μgmL^{-1}) Fluka Kamica (Switzerland) of corresponding metal ions. Standard reference materials Soya Flour (SRM 07148) were purchased from FAPAS. Glassware was kept overnight in 10% (v/v HNO3), rinsed with deionized water before use. After rinsing the glassware was dried in an oven at 80°C for one hour.

Instrumentation

A Hitachi Model 5000 Z Graphite Furnace atomic absorption spectrophotometer was used for determination of Cd and Pb in rice samples. The instrumental parameters are given in Table 1. The concentrations were obtained directly from calibration graphs after correction of the absorbance for the signal from an appropriate reagent blank. The calibration curves for Cd (1-3 μgL^{-1}) and Pb (10 - 30 μgL^{-1}) were established with solutions prepared from a 1000 μgmL^{-1} stock solution. The energy sources, Hollow cathode lamps (Perkin-Elmer) were operated at recommended current. A milestone microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900W, was used for acid digestion of rice samples.

Sampling

Thirty samples of white rice and twenty brown rice samples were purchased from local super markets of different areas of Sindh, Pakistan, during the year of 2011 - 2012. The collected samples were packed on different dates to observe the variation in the elemental contamination levels of the products. The dried samples were ground with an agate ball mixer mill (MM 2000; Retsch, Haan, Germany). The powdered samples were sieved through nylon sieve to obtain particle size [ø] 30 - 65 µm and then digested with the help of acids using microwave digestion and conventional digestion procedure used for comparative purpose and than analyzed by AAS.

Table 1. Measurement conditions for electro-thermal atomic absorption spectrometry (ETAAS)

Parameter		**Cd**			**Pb**	
Lamp current (mA)		7.5			7.5	
Wavelength		228.8			283.3	
Slit width (nm)		1.3			1.3	
Cuvette		Tube			Tube	
Temperature Programming						
Process		**Cd**			**Pb**	
Drying	80°C	120°C	30 Sec	80°C	120°C	30 Sec
Ashing	300°C	300°C	30 Sec	400°C	400°C	30 Sec
Atomization	1500°C	1500°C	10 Sec	2000°C	2000°C	10 Sec
Cleaning	1800°C	1800°C	3.0 Sec	2400°C	2400°C	3.0 Sec
Common Parameters						
Sample volume		10 µL analyte				
Backgrund Correction		D^2 Lamp				
Carrier gas Argon		200 mL/mint				

Digestion procedures

Microwave assisted-acid digestion method (MAD)

For microwave digestion optimization, three replicates of CRM, finely homogenized powdered of white and brown (1.0 g each) rice samples were weighed into a dry, clean teflon digestion vessel, and the concentrated nitric acid and hydrogen peroxide (HNO_3–H_2O_2) 5 mL and 2 mL respectively were added at minimum (-) and maximum (+) levels separately. The vessel was closed and than placed in the rotor and tightened. The loaded rotor was placed in microwave oven, after digestion and cooling for 30 min, the vessels were opened carefully. Each digested solution was filtered through a Whatmann 42 filter paper in 50 mL volumetric flasks and than filled with distilled water up to the mark. The standard reference material and blank solution were digested under the same conditions.

Conventional wet acid digestion method (CDM)

Triplicate of 1.0 g of sub samples of rice were placed into 250 mL Pyrex conical flask. Added 10 mL of a concentrated HNO_3 (65%) and 5 mL of H_2O_2 (30%) to each flask and solutions were heated on electric hot plate at 80°C, for 2 - 3 h, till the clear transparent solutions were obtained. The final solutions were

filtered through Whatman 42 filter paper. The final solutions were collected in polyethylene flask, for the determinations of Cd and Pb by ETAAS.

Statistical analysis

All analyses were carried out at least in triplicate. Experimental data were analyzed by the analysis of paired t-test at p values of (0.05). The obtained data was subjected to PBD and CCD using the computer program Minitab 13.2 (Minitab Inc., State College, PA) and Excel. Central composite design (CCD) was performed on the basis of the significant correlation among the factors.

Calibration and sensitivity

Calibration and standard addition graphs were obtained for Cd and Pb. However, as the acid composition of acid mixture and different matrixes of samples, the certified reference material was used throughout the development of method.

The detection and quantification limits, given by

$$\mathrm{LOD} = 3\times\frac{s}{m} \; and \; \mathrm{LOQ} = 10\times\frac{s}{m}$$

Respectively, where "s" is the standard deviation of ten measurements of reagent blank and m is the slope of the calibration or standard addition graph was obtained for Cd and Pb. The limit of quantification (LOD) of studied elements, being reached, for Cd 0.10 μgL^{-1} and for Pb 2.25 μgL^{-1} was calculated.

Experimental design

Plackett–Burman design (PBD)

The PBD was used as a screening approach with the aim of establishing the significant factors that influence the proposed method and selecting suitable digestion conditions. The application of this experimental design reduced the development time of the methods and provided less ambiguous digestion conditions, hence facilitating the data interpretation. For the evaluation of five factors at two levels PBDs with only twelve experiments is described instead of the 27 = 128 required for a full factorial design. This data were visualized by Minitab (Release 13 of MINITAB) Version 5.1 (Box et al., 1978; Montgomery, 1991). This optimization method permits the estimation of the principal effects of the variables studied as well as the values for each (+) representing the maximum and (−) the minimum level.

Twelve experiments were carried out for completing the design matrix, the resulting values (1 - 12) are being the % recovery of Cd and Pb average value of six replicates as represented in Table 3 on the basis of interaction effects. In addition, the evaluations of interactions among factors are also important, while the effects of some factors are less significant. Such interactions are not allowed by a design of this type, and to evaluate them, the effects of some variables were omitted, although the effects of the most significant factors were evaluated by CCD.

Central 2^3+ star orthogonal composite designs

Having screened out the variables that did not have a significant effect on the response, the remaining three factors were optimized to provide the maximum metal recovery. A CCD with 6 degrees of freedom and involving 16 experiments was performed to optimize the most significant variables i.e, AM, t and T for the determination of Cd and Pb in rice as shown in Table 4.

Results and Discussion

Optimization of the microwave assisted acid digestion procedure

To optimize MAD, efforts were focused on using the minimum amount of acid mixture and time of sample digestion in order to obtain complete and reproducible recovery of both elements (Cd and Pb) from complex matrixes. This would offer an important practical advantage due to shorter acid digestion time. It was observed that the variable temperature (T) provided a significantly higher recovery of both elements from white and brown rice matrixes.

Table 2 describes the five factors which were selected to be examined, and their levels represented as low (−) and high (+). One factor is related to the acid mixture, AM (HNO_3:H_2O_2), and other factors correspond to the temperature treated samples with acid mixture, and the exposure time to microwave irradiation which are symbolized by P, and sample weight (0.5 - 1.0 gm) for all experiment, respectively. The effect of changing factor from low level to high level value in MAD was examined on a selected response such as percentage recovery, according to the following equation:

$$\%\,\mathrm{Recovery} = \frac{\text{Microwave assisted digestion method}}{\text{Convention al wet acid digestion method}}\times 100$$

A recovery close to 100% would show quantitative extraction efficiency for both elements. The optimized five variables by PBCDs after MAD procedure are represented in Table 3, among the five variables the three selected as most significant variables and were further optimized by CCD as shown in Table 4.

Table 2. Factors and levels used for the Plackett-Burman and Central Composite designs in the factorial design

Variables	Unit	Low (-)	High (+)	Optimum conditions
HNO_3: H_2O_2 (AM) (1: 1)	mL	5.0	8.0	8.0
Time (t)	min.	5.0	10	10
Temperature (T)	°C	100	180	180
Microwave power (P)	W	250	900	900
Sample wt (S.wt)	gm	0.5	1.0	1.0

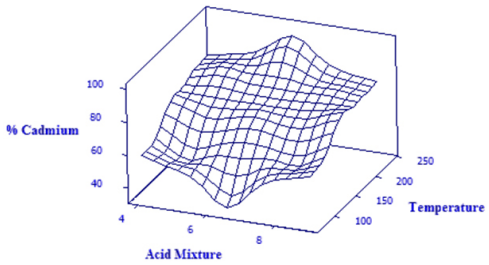

Figure 1. Plot for surface response % recovery of Cadmium vs temperature and acid mixture

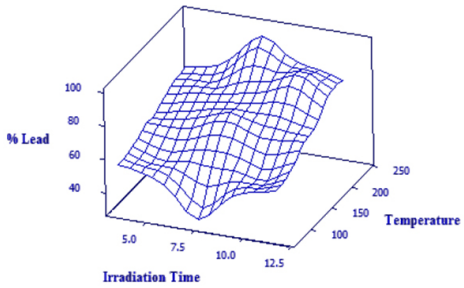

Figure 2. Plot for surface response % of Lead vs temperature and irradiation time

Optimization by central composite design

Results of the factorial design demonstrated that the variables acid mixture (AM), time (t), and temperature (T) at the studied levels required a final optimization. Thus, a CCD involving these variables and their interaction was studied by surface response methodology. The optimization recovery of Cd and Pb with these 3 most significant variables was performed with a design response surface experiment (Table 4). Sixteen experiments were performed, and 3 levels were assigned to each variable AM at 4, 5, and 9 mL; t of 6 and 23 min; and T of 65, and 234 °C for estimation of the response surface. The study of estimated response surface methodology (RSM) for the most significant variables i.e. AM, T and t for Cd and Pb showed the optimium values as shown in figure 1 and 2. The experimental results obtained for the recovery of Cd and Pb is given in Table 4.

The accumulation of trace and TEs and their subsequent uptake by rice grains represent a direct pathway of these elements into the human food chain, which is a major concern. For this purpose, certified reference materials, Soya Flour (07148), which matched the rice grain matrix as closely as possible, were analyzed. Recoveries of trace and TEs were computed by comparison of MAD data against values of certified CRM values. A recovery 99.6

Table 3. Plackett-Burman design

Experiment Number	A (AM)	B (T)	C (t)	D (P)	E (S.wt)	% Recovery* Cd	Pb
1	+	-	+	-	-	69.1	65.3
2	+	+	-	+	-	90.3	84.5
3	-	+	+	-	+	47.4	43.2
4	+	-	+	+	-	78.6	73.8
5	+	+	-	+	+	62.5	68.5
6	+	+	+	-	+	64.4	58.4
7	-	+	+	+	-	97.2	93.6
8	-	-	+	+	+	66.4	61.5
9	-	-	-	+	+	45.6	52.1
10	+	-	-	-	+	63.3	59.2
11	-	+	-	-	-	71.2	75.6
12	-	-	-	-	-	58.8	53.4

* Mean values of triplicate results'

Table 4. Central 2^3+ star composite design (n = 16) for the set of (AM), (T) and (t) for determination of Cd, and Pb

Run	A (AM)	B (T)	C (t)	% recovery Cd	Pb
1	-	-	-	42.4	38.3
2	+	-	-	50.2	45.8
3	-	+	-	77.1	70.5
4	+	+	-	85.7	85.3
5	-	-	+	82.3	81.6
6	+	-	+	55.3	51.4
7	-	+	+	77.2	74.3
8	+	+	+	98.3	97.8
9	$-J^a$	0.0	0.0	80.2	78.5
10	$+J^b$	0.0	0.0	95.3	93.2
11	0.0	$-K^a$	0.0	50.4	50.1
12	0.0	$+K^b$	0.0	95.5	92.1
13	0.0	0.0	$-a^a$	77.8	74.2
14	0.0	0.0	$+a^b$	75.3	70.3
15	0.0	0.0	0.0	34.7	32.8
16	0.0	0.0	0.0	34.3	30.2

$+J^a$ = 9.022689 mL, $-J^b$ = 3.97731 mL, $+K^b$ = 234.090°C, $-Ka^b$ = 65.910°C, -aa = 3.2955 min, $+a^b$ = 11.705 min

Table 5. Determination of Cd and Pb in standard reference Materials Soya Flour (07148) (µg/Kg dried basis)

Toxic Elements	Certified Values	CAD	MAD	% Recovery
Cadmiun (Cd)	235	234±12.6	233±11.5	99.6
Lead (Pb)	375	379±24.4	374±21.7	98.7

and 98.7% would show quantitative of Cd and Pb obtained by proposed method. The results of white rice are presented in Table 5, showed that relatively significant difference was observed at the p-value of 0.05, when comparing the average TEs values obtained from MAD and CAD, indicating that MAD is applicable for different matrixes.

The relative standard deviations for analytes under study in Soya Flour were found for both elements by MAD and CAD in the range of 5.38 - 6.44% and 4.94 - 5.80%. The concentrations of Cd and Pb in different rice samples are summarized in Table 6 and 7. The element contents in rice grain are in fair according with those reported values.

Estimated effects of variables

For both elements, the most significant effect was found for variable of temperature, in the order of Cd >Pb, while reverse the case for AM and S.wt. The maximum recoveries of both elements were observed at 97.2 and 93.6°C. It can be seen in experiment 2 of Table 2 that at (−) level of S.wt and t with optimum values of other variables such as AM, T and P the % recovery of Cd and Pb were 90.3 and 84.5, respectively. The influence of microwave irradiation

Table 6. Analytical results obtained for Cd and Pb in white rice (mg/Kg dried basis)

Samples	Cd	Pb
1	0.0889±0.0054	0.0555±0.0027
2	0.018±0.0012	0.12±0.011
3	0.011±0.001	0.074±0.0045
4	0.041±0.0022	0.081±0.0056
5	0.025±0.001	0.08±0.0035
6	0.025±0.0011	0.068±0.0023
7	0.029±0.001	0.207±0.01
8	0.08±0.0042	0.28±0.011
9	0.037±0.0019	0.12±0.011
10	0.034±0.0022	0.123±0.01
11	0.021±0.0013	0.282±0.013
12	0.045±0.0021	0.26±0.011
13	0.003±0.0001	0.081±0.006
14	0.005±0.0002	0.062±0.004
15	0.009±0.0006	0.26±0.015
16	0.006±0.0004	0.124±0.011
17	0.001±0.0001	0.079±0.005
18	0.007±0.0005	0.144±0.012
19	0.013±0.0011	0.136±0.011
20	0.05±0.0023	0.086±0.006
21	0.018±0.0013	0.071±0.005
22	0.021±0.0011	0.215±0.011
23	0.046±0.0031	0.065±0.005
24	0.033±0.0026	0.085±0.006
25	ND	0.13±0.011
26	0.008±0.0005	0.191±0.012
27	0.078±0.0057	0.012±0.001
28	0.008±0.0007	0.091±0.0045
29	0.007±0.0004	0.056±0.0035
30	0.0124±0.0011	0.074±0.0055

Table 7. Analytical results obtained for Cd and Pb in in Brown rice (mg/kg dried basis)

S.No.	Cd	Pb
1	0.082±0.0062	0.502±0.033
2	0.053±0.0033	0.396±0.012
3	0.023±0.001	0.431±0.022
4	0.019±0.001	0.426±0.032
5	0.008±0.0005	0.395±0.021
6	0.061±0.0046	0.368±0.012
7	0.031±0.0022	0.426±0.021
8	0.075±0.0061	0.331±0.011
9	0.092±0.0054	0.687±0.043
10	0.089±0.0065	0.081±0.006
11	0.045±0.0031	0.062±0.041
12	0.195±0.005	0.26±0.011
13	0.015±0.003	0.426±0.025
14	0.024±0.001	0.079±0.0051
15	0.158±0.005	0.144±0.003
16	0.415±0.012	0.331±0.011
17	0.009±0.0006	0.086±0.005
18	0.018±0.0021	0.396±0.012
19	0.041±0.0021	0.691±0.041
20	0.037±0.0017	0.713±0.058

power (P) was not significant, it was observed that at low level of P, both elements were quantitatively extracted (experiment 3) when other parameters were at optimum level. The maximum recoveries of both elements (97.2 for Cd and 93.6 for Pb) were achieved at the temperature of 180°C of microwave, as shown in Table 2.

From the results of the PBDs (Table 2), it is clearly observed that high amounts (+) of HNO_3:H_2O_2 mixture AM, T and t provided a significantly high recovery for Cd and Pb as compared to other factor i.e. sample weight and microwave irradiation power. There was significant difference between 0.5 and 1.0 gm sample mass for both metals at the p-value of (0.05). The results indicated that 1.0 gm of sample wt was sufficient for optimum recovery of both metals.

Evaluation of metals in rice (Oryza sativa)

The proposed methodology was applied to the triplicate of each raw, processed and branded sample, to extract Cd and Pb. The mean elemental concentration for both brown and white rice expressed as:

$$\bar{x} \pm ts/\sqrt{n}$$

Table 6 represents the average level of TEs were found to be high in some of brown rice as compared to white rice, a significant differences were observed between both type of rice samples ($p > 0.05$). These variations may represent the change in the composition of soil, water and fertilizers or associated with peculiarity of rice varieties and rice processing. Whilst Table 6 and 7 provide an important indication regarding rice quality and risks posed, it is very important that they are viewed in light of two other key factors one industrial processing and second cultivated areas. Metal absorption for a variety of agricultural products has been shown to be dependent on geographical origin (Schwartz and Hecking, 1991; Anderson *et al.*, 1999).

The high level of TEs in air, water and food may cause detrimental effects on human health and children are more sensitive to these metals than adults (Divrikli *et al.*, 2006). It was reported in literature that Cd and Pb have long biological half lives; they accumulate in bone, blood, lung, kidney, and various other organs (Gairola and Wagner, 1991). Consequently, the results of this present study on Cd and Pb levels in the rice allowed us to clarify some issues relating to the actual contents of these elements in such an important staple food. However, the contribution of rice-based products to the total intake of these elements is significant and turned out to be particularly high in the case of Cd and Pb.

The levels of Cd, and Pb were high in brown rice. They were toxic to plants because they would cause oxidative stress and displace other essential metals in plant pigments or enzymes, leading to disruption of function of these molecules and of many metabolic processes, and finally reduction of growth and yield (Wang *et al.*, 2003). Moreover, TEs may enter into the food chain because of uptake and accumulation by crops, posing a potential threat to human health (Sponza and Karaoglu, 2002). It has also been reported that Cd toxicity induced oxidative damage characterized by an accumulation of lipid peroxides and oxidized proteins, as a result of the inhibition of antioxidant systems, in plants (Sandalio *et al.*, 2001; Vitoria *et al*., 2001).

Conclusion

The microwave-assisted digestion method is inexpensive, rapid, minimum amount of acid and shorter time is required for digestion of samples as compared to conventional wet acid digestion. In the view of results, an elevated level of Cd and Pb were found in brown rice samples, whilst the lower levels of both elements are found in white rice, thus white rice appear to be good for human consumption because of low content of TEs and high content of nutrients. Finally, notable differences were observed between white rice and brown rice, which may affect the likelihood of exposure and the risk posed to human consumers.

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