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Determination of lead and Cadmium Contents in (*oryza sativa*) rice samples of agricultural areas in Gillan- Iran

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ABSTRACT: The major source of Cadmium intake for rice eating countries in Asian countries like Iran is rice. Due to the accumulation of heavy metal compounds formed in plants for food could be an important factor in human toxicity; we have been investigating the Cadmium and Lead contents in rice as the high daily intake in the north Iranian diet and high incidence of diseases among people in the north provinces in Iran. To assess the levels of Cadmium and Lead in Hashemi raw rice (*Oryza Sativa*), 100 samples after harvesting and 50 samples from one-year stored were randomly collected from 10 major rice production areas in 2012 .All samples were collected in during harvesting of rice in Gillan fields in two consequent years. Samples were analyzed by Atomic Absorption Spectrophotometer in 4 stages of : raw , rinsing , boiling & draining and cooking rice . Results were determined as mean ± SD of dry weight from three replicates in each test. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Atomic Absorption Spectrophotometer.The mean content of Cadmium and Lead in all samples had Cadmium and lead contents above maximum level which is recommended by FAO/WHO Expert Committee on Food Additives (JECFA) .Average concentrations of lead and cadmium in cooking rice is lower than boiling and draining rice and the results show that washing and rinsing rice has the significant role) in decreasing the cadmium and lead contents. Repetitive washing of the rice can greatly reduce level of heavy metal contents. The total dietary exposure levels of Lead and Cadmium determined in this study were compared with the provisional tolerable weekly intakes (PTWLS) by the JECFA and showed higher than it. Our results reveal that weekly Cadmium and lead intake from rice were higher than the maximum weekly intake recommended by WHO/FAO.

***Keywords*:** Hashemi rice, Cadmium, Lead, Contaminant, Gillan

INTRODUCTION

Several factors may influence contaminant accumulation such as spices, level and duration of contaminant exposure, topography, agricultural field conditions, amending soil with compost made from municipal sewage sludge and potential bioaccumulation. Due to the accumulation of heavy metal compounds formed in plants for food could be an important factor in human toxicity **.**Rice is one of the most important and widespread species, being the staple food crop for the majority of the world population especially in Asia and is the second food in high consumption among Iranian people. Daily consumption of rice in Asia countries ranges between 158-178 g/ person-day (Nogawa and Ishizaki, 1979; Rivai et al., 1990).We have been determining the Cadmium and Lead contents in rice as the high daily intake in the north Iranian diet and high incidence of diseases among people in Gillan province. This study was aimed to assess the levels of selected heavy metals in Hashemi rice (oryza sativa) from different agricultural areas in the Gillan province in the north of Iran based on weekly Cadmium and lead intake from rice , associate with determining the lead and cadmium contents in different forms of rice (raw- rinsing – boiling and draining – cooked). Rice samples from the most important agricultural areas in the Gillan province were collected from two different years of harvesting (2011 and 2012) due to comparison the heavy metal contents in different age of samples in order to find if increasing of environmental factors have significant role of contaminating crops. The major source of Cadmium intake for rice eating countries in Asian countries like Iran is rice. It is known that, those who take rice (oryza sativa) as staple food for daily energy, exposed to significant amounts of cadmium contents via rice, as rice cropped even from non-polluted areas probably contain Cadmium because of using fertilizers in the farms which have Cadmium(Watanabe et al., 1989).

MATERIALS AND METHODS

To assess the levels of Cadmium and Lead in Hashemi (*oryza sativa*) raw rice 100 samples after harvesting and 50 samples from one-year stored were randomly collected from 10 major rice production areas in 2012 .All samples were collected in during harvesting of rice in Gillan fields in two consequent years. For heavy metal analyses 50 gram of each sample was weighed and oven-dried at 60^0c to a constant weight. Each oven-dried sample was ground in a mortar until it could pass through a 60 mesh sieve. The samples were stored in clean, dry, high density polyethylene bottles of 100 ml capacity with screw caps. All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid for 24hrs, cleaned thoroughly with distilled water and dried in such a manner to ensure that any contamination does not occur. Ten gram of powdered sample was weighed precisely on electronic balance (Shimadzu LIBROR AEX 200G) The samples were put in a 100 ml digestion flask and 20 ml of mixture was added to it and heated on a hot plate in the fuming chamber. A digestion mixture comprising of concentrated HNO3 and hydrochloric acid in the ratio of 3:1 was used for wet digestion of the samples. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). This method has been followed in 4 stages for raw rice, rinsing (4 times washing) rice, Boiling and the draining rice, cooked rice. All draining and cooking rice samples have rinsed 4 times then followed by the procedure. Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents. The flasks were firstly heated slowly and then vigorously till a white residue is obtained. The residue was dissolved and made up to 10 ml with 0.1 N HNO3 in a volumetric flask. The samples were analyzed by an Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals: Pb and Cd, using at least four standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines (AOAC, 1998). Concentrations were expressed in terms of mg/Kg on a dry weight basis. Analysis of variance (ANOVA) followed by multiple comparison were employed.

RESULTS AND DISCUSSION

Results were determined as mean ± SD of dry weight from three replicates in each test. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Atomic Absorption Spectrophotometer in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University. The results of Cadmium and Lead contents in 150 samples of raw, washing, boiling – drained and cooking rice from 10 main agricultural areas in Gillan province; the north of Iran are shown in table 1 and 2 respectively. Results show that the mean content of Cadmium and Lead in all samples from Gillan province is over. These results indicated that the mean value of Lead and Cadmium concentrations in raw rice in 2012 are 1.73 and 0.3802 mg/kg on dry wt respectively.

Table 1. Mean content of Lead in rice from various agricultural areas from North of Iran in 2012

Agriculture Area	Mean Lead Content (mg/kg dw)±SD Raw Rice	Mean Lead Content (mg/kg dw)±SD Rinsing Rice	Mean Lead Content (mg/kg dw)±SD Draining Rice	Mean Lead Content (mg/kg dw)±SD Cooked Rice
Astara	1.8169±0.1417	1.4794±0.2199	1.7944±0.1523	1.7214±0.1315
Talesh	1.8014±0.0357	1.5142±0.0900	1.8380±0.1360	1.6492±0.0930
Rezvanshahr	1.5603±0.0565	1.4492±0.0338	1.7468±0.0234	1.5990±0.0759
Somesara	1.9075±0.2261	1.7246±0.1996	2.0450±0.2373	1.8556±0.1696
Bandar anzali	1.4072±0.4928	1.2991±0.4433	1.5661±0.4594	1.3870±0.4314
Rasht	1.7530±0.0585	1.5297±0.03305	1.8533±0.0210	1.8062±0.0184
Astane	1.8114±0.0306	1.6727±0.0259	1.8971±0.0592	1.8053±0.0359
lahijan	1.6509±0.0465	1.5497±0.0272	1.6848±0.1053	1.5607±0.0726
langrod	1.8717±0.1952	1.8058±0.2117	1.9426±0.2739	1.8620±0.2050
Total	1.7311±0.2386	1.5583±0.2294	1.8187±0.2339	1.6940±0.2235

SD= Standard Deviation

Table 2. Mean Level of Cadmium in rice from various agricultural areas from North of Iran in 2012

Agriculture Area	Mean Cadmium Content (mg/kg dw)±SD Raw Rice	Mean Cadmium Content (mg/kg dw)±SD Rinsing Rice	Mean Cadmium Content (mg/kg dw)±SD Cooked Rice	Mean Cadmium Content (mg/kg dw)±SD Draining Rice
Astara	0.4244±0.0056	0.4129±0.0101	0.4500±0.0117	0.4356±0.0095
Talesh	0.4034±0.0263	0.3866±0.0391	0.4497±0.04239	0.4068±0.0436
Rezvanshahr	0.3743±0.0130	0.3595±0.1704	0.4062±0.0118	0.3859±0.0137
Somesara	0.3739±0.0517	0.3679±0.0501	0.4318±0.0507	0.4193±0.5486
Bandar anzali	0.2985±0.0849	0.2812±0.0881	0.3387±0.1061	0.3063±0.0877
Rasht	0.3740±0.0086	0.3499±0.0116	0.4060±0.0354	0.3971±0.0194
Astane	0.4085±0.0059	0.3630±0.0273	0.4523±0.0192	0.4363±0.0142
lahijan	0.3394±0.0110	0.3347±0.0229	0.3823±0.0153	0.3534±0.0124
langrod	0.4256±0.0102	0.3950±0.0111	0.4699±0.0083	0.4500±0.0111
Total	0.3802±0.0508	0.3677±0.0678	0.4208±0.0565	0.3990±0.0560

SD= Standard Deviation

The results in figure 1 and figure 2 revealed that all samples (100%)had Cadmium and lead contents above maximum level 0.2 mg/kg and 0.3 mg/kg which is recommended by FAO/WHO Expert Committee on Food Additives (JECFA) .Average concentrations of lead and cadmium in cooking rice is lower than boiling and draining rice and the results show that washing and rinsing rice has the significant role ($p< 0.003$) in decreasing the cadmium and lead contents. Repetitive washing of the rice can greatly reduce level of heavy metal contents.

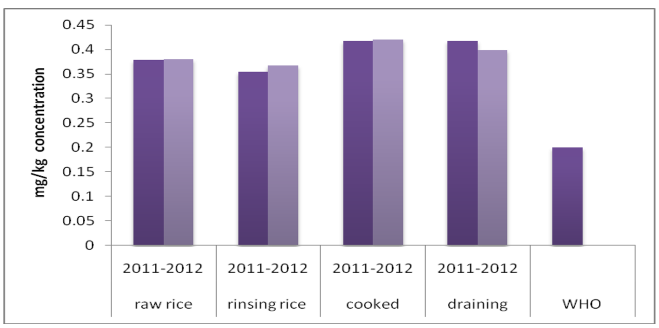

Figure 1. Cadmium content (mg/kg dw) in different forms of (*oryza sativa*) rice samplesin 2011 and 2012

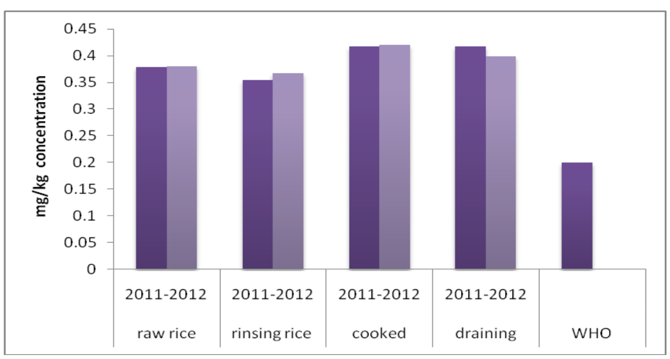

Figure 2. Lead content (mg/kg dw) in different forms of (*oryza sativa*) rice samples in 2011- 2012

Estimation of Cadmium and Lead Intake

The joint FAO/WHO Expert Committee on Food Additives (JECFA) has proposed a maximum level of 0.2 mg/kg Cadmium in rice but the community warned that " people who eat a lot of rice from regions containing the higher levels of cadmium could be significantly exposed".

JECFA has set PTWI for the Cadmium at 7 μg/kg of body weight (WHO, 2004). The total dietary exposure levels of Lead and Cadmium determined in this study were compared with the provisional tolerable weekly intakes (PTWLS) by the JECFA and showed higher than it. According to the published papers, daily consumption of rice in Asia countries ranges between 158-178 g/person-day with an average is 165 g/person-day, and the average body weight is 60 kg/person (Rivai et al., 1990; Nogawa and Ishizaki, 1979). The Weekly cd intake(µg/kg body weight/week)in this study was 8/06 μg/kg for cooked rice and 7/61 μg/kg for boiling and draining rice. The Weekly Lead intake(µg/kg body weight/week)in this study was31/54 μg/kg for cooked rice and 33.71 μg/kg for boiling and draining rice in comparison of 25 μg/kg which is recommended by JECFA, our results reveal that weekly Cadmium and lead intake from rice were higher than the maximum weekly intake recommended by WHO/FAO.

One of the major environmental pollution in the developing countries like Iran is the heavy metal pollution and the pollution from the use of excessive insecticides, pesticides and fertilizers in the agriculture fields. A questionnaire – based survey on dietary consumption showed that rice was grown in local region is the main source of the foodstuffs consumed by the people in Gillan province. Therefore the problem is rather more serious in Iran and the other developing countries in Asia as most people eat rice as the main foodstuff in their daily diet. By a comparison between acceptable global standards and the level of Cd and Pb on investigated in rice, our results showed that the majority of rice samples had higher level of these heavy metals .

To avoid entrance of metals into the food Chain, municipal or industrial waste should not be drained into rivers and farm lands without prior treatment. A port from treating the discharge that enters into the farms, it is also imperative to utilize alternative measures of cleaning up the already contaminated substrates. Probably the most important conclusion that could be drawn up from the findings of this study, is that science cereal and rice crops tend to absorb and accumulate Cd in them have been shown to accumulate relatively high concentration of heavy metals in compare to other crops , it is recommended that these type of planets should not be cultivated in the fields which irrigated by urban and industrial waste water or water contaminated by heavy metals. As heavy metal toxicity through contamination of preparation continues to be recognized risk, voluntary programs to provide community education regarding the potential risk of herbal preparations should be supported by the availability free heavy metal testing services.

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