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Arsenic and Manganese Contamination of Rice and Wheat Grains in District Chiniot, Punjab, Pakistan

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***Abstract*—As and Mn contaminated groundwater is widely supplied as agricultural and drinking water in District Chiniot, Punjab, Pakistan. In order to assess the impact of contaminated groundwater to agricultural products, trace elements were analyzed for samples of rice and wheat grains harvested from the field in Chiniot. Total concentrations of As and Mn were measured using an inductively coupled plasma mass spectrometer (ICP-MS) after a nitric acid extraction. Chemical speciation of As, including organic/inorganic As (III) and As (V), was also examined applying a combined method of a high performance liquid chromatography (HPLC) and ICP-MS. The analytical results showed that the concentration of total As in rice and wheat grains ranges from 0.05 to 0.10 mg/kg, while that of total Mn from 3.1 to 38.9 mg/kg. The concentration of manganese is much higher in wheat than in rice. The chemical speciation of arsenic contained in rice and wheat grains shows that inorganic As (III) ranges from 0.03 to 0.06, inorganic As (V) from 0.01 to 0.02, and dimethylarsinic acid (DMA) from 0.02 to 0.05 mg/kg. Monomethylarsonic acid (MMA) was not detected in either rice or wheat grain. The contamination level of total As in rice grains is less than 0.2 mg/kg that is the recommended maximum limit by Codex, which means no immediate health risk.**

Keywords— ***Groundwater contamination, Arsenic, Manganese, Rice and wheat grains, Chemical speciation***

I. Introduction

Groundwater quality is constantly deteriorating due to overexploitation in Punjab plain, Pakistan. In Chiniot District, a typical agricultural area of Punjab plain, groundwater quality showed high electric conductivity (averaged EC = 4,584 µS/cm), high total dissolved solids (averaged TDS = 2,505 mg/L), high salinity (averaged Cl = 1,241 mg/L), high sulfate ion (averaged SO_4^{2-} = 884 mg/L), and high hardness (averaged Ca = 209 mg/L) [1]. These data indicate distinct groundwater contamination; however, it is widely used for both drinking and agricultural purposes in the area.

In addition to above-mentioned general water quality problems, the contamination of trace elements in groundwater was recently measured in the area (Table 1), where total arsenic (As) concentration reaches 0.0251 mg/L and total manganese (Mn) concentration marked 0.130 mg/L [2]. The concentration of bromine (Br) is relatively high, showing a maximum of 0. 540 mg/L.

Arsenic is potentially toxic to humans, known to be a human carcinogen [3], and has been able to induce epigenetic changes and genetic mutations (a leading cause of cancer) in the body [4]. According to these health risks, the WHO provisional guideline value for drinking water is defined as 0.01 mg/L (total As) for maximum permissible limit for drinking water quality [5]. On the other hand, manganese is an essential element for human, and it is naturally occurring in many surface water and groundwater sources, particularly in anaerobic or low oxidation conditions where it may stay in solution above 0.1 mg/L. The health-based value is 0.4 mg/L (Mn) while levels exceeding 0.1 mg/L can cause some metallic tastes and concentrations at 0.2 mg/L may cause the formation of a coating on pipes, which may slough off into drinking-water as a black precipitate [6]. Thus, WHO defined that Mn concentrations below 0.05 mg/L were usually acceptable to consumers, although this may vary with local circumstances [7].

Therefore, in the area, a migration of these elements to rice and wheat grains are highly concerned; because the uptake of As and Mn in human bodies mainly occurs via a food chain. Rice plants generally accumulate As at higher levels than other terrestrial plants [8].

Table 1: Basic statistics of trace element concentration of groundwater in Chiniot area, analyzed by an ICP-MS (data source: [2])

Element	Detection Limit	Unit	Average	Standard Deviation	Maximum	Guideline values
As	0.5	µg/L	9.77	8.45	25.1	10*
B	5	µg/L	111.5	46.8	192	2,400*
Ba	0.05	µg/L	74.534	56.289	186.79	700*
Br	5	µg/L	244.0	169.4	540	c.500***
Cd	0.05	µg/L	0.019	0.049	0.13	3*
Cr	0.5	µg/L	11.27	3.03	17.3	50*
Cu	0.1	µg/L	4.58	2.21	7.8	2,000*
Hg	0.1	µg/L	-	-	BDL	1*
Mn	0.05	µg/L	45.444	50.989	130.39	50**
Ni	0.2	µg/L	-	-	BDL	70*
Pb	0.2	µg/L	0.02	-	0.2	10*
Sb	0.05	µg/L	0.033	0.046	0.12	20*
Se	0.5	µg/L	1.55	1.12	3.0	40*
U	0.02	µg/L	5.349	3.166	9.78	30*
Zn	0.5	µg/L	22.62	34.40	86.8	5,000**

* WHO Drinking water guideline (version 4) [5], ** Secondary maximum contamination level (SMCL) by US EPA [27], *** Maximum level of bromide concentration in natural freshwater [5].

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Another problem is chemical species of arsenic in rice and wheat grains. Different chemical species of arsenic exhibit different toxicity. Inorganic arsenic appears in different oxidation state, and methylation in an anaerobic condition generates organo-arsenic species. These chemical transformations are closely related to agricultural field conditions, and both total As and As speciation within grains are affected by the irrigation management, after all [9].

In this paper, the concentration of various chemical species of arsenic and manganese for rice and wheat grains harvested from the Chiniot area is examined for assessing the risk to public health.

II. Materials and Method

Two samples, 180216-GI (rice grains harvested in 2017) and 180216-G2 (wheat grains harvested in 2017), were obtained directly from local farmers of which locations are shown in Figure 1. The other sample 180216-G3 (rice grains) was procured from a marketplace in Chiniot, which was harvested from unknown location(s) in District Chiniot. The chemical analysis method applied was based on Nagaoka et al. and Nishimura et al. ([10],[11]) as follows:

A. *Measurement of total As and Mn*

A 0.5g split of each sample was extracted by a mixture of 5 mL of nitric acid and 1 mL of hydrogen peroxide with a microwave digestion system, where the first step was 2 min, 70°C at 1,000 W; the second step was 5 min, 50°C at 0 W; the third step was 20 min, 200°C at 1,000 W; the fourth step was 30 min, and 200°C at 1,000 W.

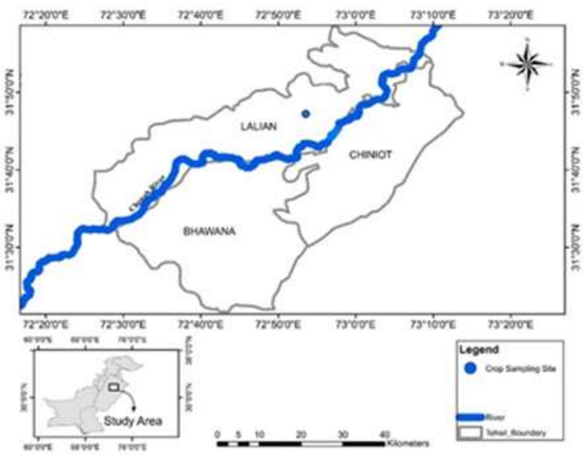

Figure 1: Location map for sampling sites.

After cooling of the digested solution, 1 mL of acetic acid and 1 μL of Ge solution, as an internal standard, were added, then the solution was made up to 50 mL with water. It was measured by an ICP-MS Agilent 7500ce in the analytical laboratory of Japan Grain Inspection Association (JGIA), Tokyo, Japan.

B. *Chemical Speciation of As in Grain Samples*

Most of organo-arsenic compounds are non-toxic arsenobetaine (AB; $C_5H_{11}AsO_2$), trimethylarsine oxide (TMAO) or arsenosugars (Figure 1; [12]) which are generally found in fish, shellfish, and seaweed. In contrast to these seafoods, rice and wheat grains biologically accumulate more toxic inorganic As species, arsenate ($As(V)O^{3-}$) and arsenite ($As(III)O_3^{3-}$), and also relatively toxic organic As compounds such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Figure 2).

The contribution of rice and wheat to the total intake of inorganic As may be relatively high for humans but cannot be negligible the factors of intake of MMA and DMA. Therefore, in present study total arsenic, arsenate, arsenite, MMA, DMA, and total manganese were analyzed.

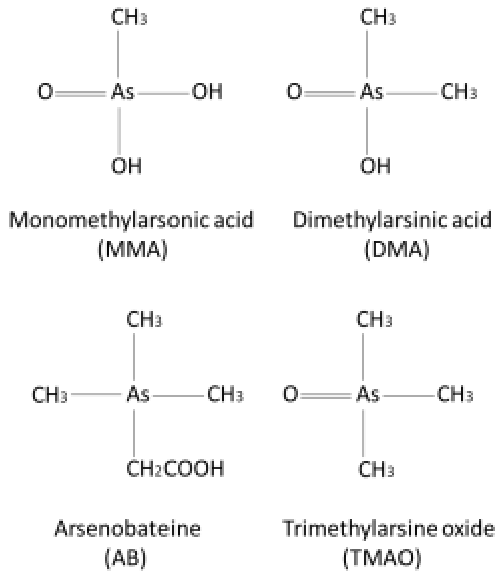

Figure 2: Major organo-arsenic compounds

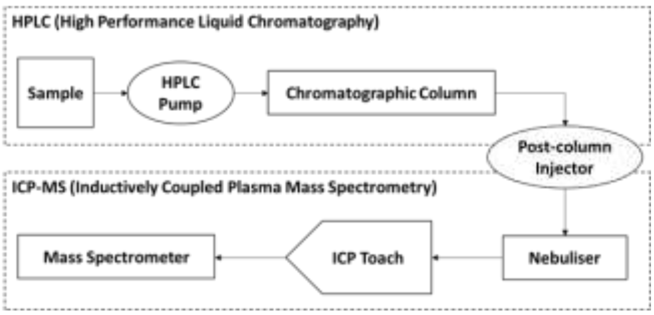

Figure 3: Schematic diagram of HPLC-ICP-MS coupling

Chemical speciation analysis was carried out with an HPLC-ICP-MS combined system (Figure 3), where a high performance liquid chromatography (Agilent HPLC 1200) equipped with an HPLC column (CAPCELL PAK C18 MG, 4.6 mm i.d. x 250 mm, Shiseido Ltd.) was coupled with the ICP-MS, in the analytical laboratory of Japan Grain Inspection Association, Tokyo, Japan.

Applied analytical conditions were based on Nagaoka et al. (2008) [10] as follows: The HPLC solvent, a solution (pH 3.0) composed of 10 mmol/L sodium 1-butanesulfonate, 4 mmol/L tetramethylammonium hydroxide (TMAH), 4 mmol/L malonic acid, and 0.05% methanol, was used for isocratic elution at a flow rate of 0.75 mL/min. The sample

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solution (20 mL) was loaded onto the column and eluted at room temperature. In this study, the retention time did not change at this temperature.

The analytical conditions of ICP-MS were the same as the total As determination described above.

III. Analytical Results

The HPLC-ICP-MS system could analyze four chemical species of arsenic: arsenate (As (V)), arsenite (As (III)), monomethylarsonic acid (MMA, organic As (V)), and dimethylarsinic acid (DMA, organic As (III)). Concentrations of total As and Mn were also determined. The results of chemical analysis are summarized as the following table (Table 2):

Table 2: Results of chemical analysis (unit: mg/kg)

	Detection Limit	180216-G1 (rice)	180216-G2 (wheat)	180216-G3 (market)
As (III)	0.01	0.06	0.03	0.06
As (V)	0.01	BDL	0.02	0.01
MMA	0.02	BDL	BDL	BDL
DMA	0.02	0.04	0.02	0.05
Total As	0.01	0.08	0.05	0.10
Total Mn	0.1	6.7	38.9	3.1

BDL = below the detection limit.

Arsenic concentration in rice grains is higher than that of wheat. On the other hand, the manganese concentration in wheat grains is much higher than that in rice. MMA was not detected in any samples, but other arsenic species, As (III), As (V), and DMA were identified in all samples, which indicates some uptakes of inorganic arsenic and organo-arsenic by rice and wheat. Relatively high concentration of total manganese was observed to wheat grains (38.9 mg/kg).

IV. Discussions

A. *Risks of Manganese Contamination*

Manganese is an essential nutrient element in plants, and deficiency of this element impairs healthy growth of plants and causes deficiency symptoms [13]. According to agricultural study in Japan, healthy plants have a Mn concentration ranging from 20 to 500 mg/kg, and healthy rice grains contain 50 to 70 mg/kg while healthy wheat grains 9.3 to 57.4 mg/kg [14]. The concentration of manganese in rice and wheat grains greatly depends on the variety, but it has been known that deficiency disorders sometimes occur for rice at 16 mg/kg or less, for wheat at 22.2 mg/kg or less [14].

Mn is required in small quantities obtained through dietary intake. The average intake for many Western diets is between 2.3 and 8.8 mg Mn/day, but infants can consume about 3 µg Mn/day for 0–6 months [15]. Adverse health effects such as neurological effects can be caused by inadequate intake or overexposure of manganese. The health effects are collectively characterized by a “Parkinson-like syndrome”, including weakness, anorexia, muscle pain, apathy, slow speech, monotonous tone of voice, emotionless mask-like facial expression and slow, clumsy movement of the limbs [16]. There is no clear definition of maximum concentration of Mn in rice and wheat grains.

Present analytical results indicate that manganese concentration level in rice (6.7 and 3.1 mg/kg) is rather smaller than the data in Japan, but that in wheat (38.9 mg/kg) is at normal level in comparing with the data in Japan.

No immediate health risk can be considered due to consumption of these grains.

B. *Risks of Arsenic Contamination*

The concentration of total arsenic in rice varies depending on the agricultural field conditions. Figure 4 shows the mean and standard deviation of the total arsenic concentration for each country, Japan, Australia, Brazil, China, Thailand, and the United States, based on the data submitted to the Codex Committee on Contaminants in Foods (CCCF) until 2014 [17].

The total arsenic concentration of unpolished brown rice (0.10 to 0.29 mg/kg) tends to be higher than that of polished rice (0.05 to 0.12 mg/kg). It is also noteworthy that unpolished brown rice has a higher inorganic arsenic content than polished rice (Figure 5). These facts indicate that arsenic is more concentrated in the outer shell of rice grains that can be removed by polishing, and the outer shell of the rice grains contains relatively higher level of inorganic arsenic.

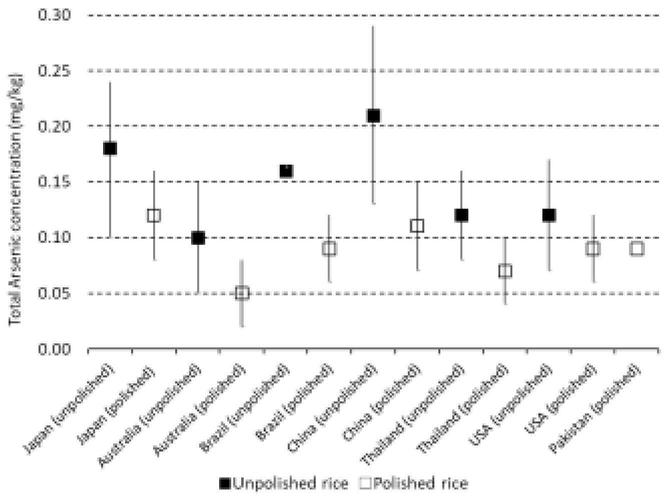

Figure 4: Total arsenic concentration of unpolished and polished rice grains in various countries. Symbols indicates averaged values and bars indicate the range of standard deviation (Data source: Ministry of Agriculture, Forestry and Fisheries, Japan and present study [17])

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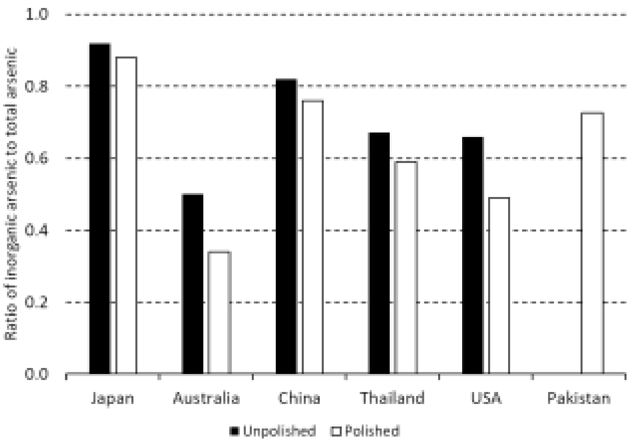

Figure 5: Concentration ratio of inorganic arsenic to total arsenic in unpolished and polished rice grains in various countries (Data source: Ministry of Agriculture, Forestry and Fisheries, Japan and present study [17])

The total arsenic concentration of unpolished brown rice (0.10 to 0.29 mg/kg) tends to be higher than that of polished rice (0.05 to 0.12 mg/kg). It is also noteworthy that unpolished brown rice has a higher inorganic arsenic content than polished rice (Figure 5). These facts indicate that arsenic is more concentrated in the outer shell of rice grains that can be removed by polishing, and the outer shell of the rice grains contains relatively higher level of inorganic arsenic.

The analytical data of Pakistan in this study are plotted in both graphs for comparison (Figures 4 and 5). It can be recognized that the total arsenic concentration of polished rice samples in present study is not high level in comparing with other countries data, but the ratio of inorganic arsenic to total arsenic is relatively high. Moreover, it has been reported that rice grains harvested from Nagarparkar area, southern part of Pakistan, the total arsenic concentration reaches 4.47 mg/kg in maximum [18], which means that there is a possibility of highly contaminated rice grains by total arsenic.

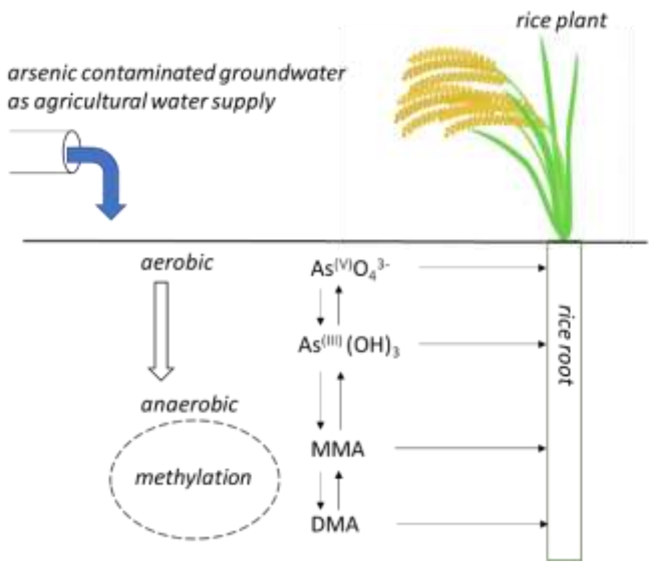

Figure 6: Schematic diagram of arsenic migration to rice plant. Chemical transformation is adopted from [21].

Picture 1: Groundwater well for agricultural water supply

Picture 2: Groundwater irrigation in Chiniot, Punjab

It is widely known that the toxicity is highest for arsenite, followed by arsenate and then organo-arsenic ([19], [20]). In present study, special interest is on arsenite (As (III)) which shows the highest concentration in total arsenic. Next higher concentration is shown by DMA, and arsenate (As(V)) shows the smallest concentration which is more toxic than DMA.

The estimated chemical transformation of arsenic species within rhizosphere of rice field is shown in Figure 6. In general, near surface of the agricultural soil section arsenate (As(V)) is dominant under an aerobic condition but arsenate gradually transforms to arsenite (As(III)) under an anaerobic condition. In these zones, iron plaque plays an important role in the absorption of inorganic arsenic into rice root [21]. In deeper part of the rhizosphere, a methylation occurs in an anaerobic environment to form MMA and further methylated DMA, which are directly adsorbed by rice root.

The characteristics of the composition of arsenic contained in rice and wheat as described above suggest that an anaerobic reducing environment is predominant in the agricultural field of study area.

Continuous exposure of inorganic As is associated with an increased health risk for various carcinomas [3]. According to the present study, the content of arsenic in

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grains is higher in rice than in wheat, and most toxic arsenite is the majority of chemical type of arsenic contained. The toxicity of As mainly depends on its oxidation state and chemical speciation. WHO previously defined the provisional tolerable weekly intake (PTWI) as 0.015 mg/kg bw (body weight) per week [22], but the Seventy-second Meeting of the Joint FAO/WHO Expert Committee on Food Additives (72JECFA) [23] withdrew the previous PTWI. Because the lower limit on the benchmark dose for a 0.5% increased incidence of lung cancer (BMDL0.5) from epidemiological data to be 2 to 7 μg/kg bw per day (= 0.014 to 0.049 mg/kg bw per week), which partly overlaps with the previous PTWI level (= 0.015 mg/kg bw per week). However, no new tolerable intake level could be established by the 72JECFA due to insufficiency of available data [24], and the provisional guideline value (in view of scientific uncertainties surrounding the risk assessment for arsenic carcinogenicity) was determined for drinking-water quality as 10 μg/L [5]. Based on those discussions, the Codex Alimentarius Commission (CAC) tentatively set a maximum level (ML) for inorganic arsenic in polished rice at 0.2 mg/kg based on ALARA (As Low As Reasonably Achievable) principle [25] [26]. According to the Codex General Standard [24], if the total arsenic concentration is below the value of ML (<0.2 mg/kg), no further testing is required, and the sample is determined to be compliant with the ML. If the total arsenic concentration is above the value of ML (≥0.2 mg/kg), follow-up testing shall be conducted to determine if the inorganic arsenic in concentration is above the ML.

As a result of this study, the total arsenic concentration was below the maximum limit (0.2 mg/kg) in all samples. The rice samples measured here meet Codex standard for arsenic content, indicating that eating them has no health consequences.

However, high level of arsenite (As(III)) concentration suggests that long-term continuous consumption of rice harvested in the area requires special caution, especially for children. It should be noted that arsenic can pass through the placenta. Pregnant women chronically exposed to arsenic-contaminated foods are at increased risk for spontaneous abortion, stillbirth and preterm birth. In utero and/or early-life exposures to arsenic have been linked to increases in deaths due to multiple cancers, lung disease, heart attacks and kidney failure as well as effects on cognitive development, intelligence and memory later in life [24].

V. Conclusions

(1) We examined the content of arsenic and manganese contained in the rice and wheat grains harvested from District Chiniot, Punjab, Pakistan.

(2) Arsenic is relatively concentrated in rice grains, while manganese is rich in wheat grains.

(3) The manganese concentration in wheat grains in the area is not cautious level for public health.

(4) According to the chemical speciation analysis using HPLC-ICP-MS, arsenite (AS(III)) is the most abundant and arsenate (AS(V)) and DMA are relatively few among total arsenic. MMA was not detected from both rice and wheat grains.

(5) The concentration of As is below the maximum limit of the Codex food safety standards and has no immediate health effect. However, since arsenite is the most abundant within arsenic species, careful attention should be paid for the constant consumption of rice by children.

(6) Arsenic and manganese contamination of rice and wheat grains harvested in the area is likely caused by groundwater which is used as agricultural water.

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