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Arsenic Speciation and Contamination in Cereals from Chhattisgarh, India

Madhuri Khute[1], Saroj Sharma[1], Khageshwar Singh Patel[2*], Piyush Kant Pandey[2], Jasmina Allen[3], Warren Corns[3], Nelina Georgieva[4], Elena Bozhanina[4], Borislav Blazhev[4], Simge Varol[5], Pablo Martín-Ramos[6], Yanbei Zhu[7]

[1]Department of Chemistry, Government Nagarjuna Post Graduate College of Science, India
[2]Department of Applied Sciences, Amity University, India
[3]PS Analytical Ltd, United Kingdom
[4]Central Laboratory for Chemical Testing and Control, Bulgaria
[5]Department of Geology, Suleyman Demirel University, Turkey
[6]Agricultural and Forestry Engineering Department, ETSIIAA, Universidad de Valladolid, Spain
[7]National Institute of Advanced Industrial Science and Technology (AIST), Japan

ABSTRACT

Cereals serve as a major food source for humans and animals. This investigation explored the presence of arsenic species in cereal crops grown in the contaminated area of Ambagarh Chouki (Chhattisgarh, India). Rice, wheat and maize, along with husk, straw and soil samples, were analyzed using hydride generation–atomic fluorescence spectrometry (HG–AFS), and inductively coupled plasma-mass spectrometry (ICP-MS). Significant inorganic arsenic (iAs) contamination was found in rice, wheat, and maize plants, with the highest levels in roots, followed by husk, straw, and grain. Inorganic arsenic content in rice grain ranged from 229.9 mg kg^{-1} to 684.7 mg kg^{-1}, while in wheat and maize it ranged from 84.6 mg kg^{-1} to 218.5 mg kg^{-1} and from 20.0 mg kg^{-1} to 26.2 mg kg^{-1}, respectively. All cases exhibited a hazard quotient exceeding 1. Organic arsenic, specifically monomethyl arsenic (MMAs) and dimethyl arsenic (DMAs), were detected in rice plants. The findings address speciation, enrichment, sources, transfer factors, and health risk assessment. Overall, this study emphasizes the detrimental health effects of consuming cereals grown in this region, necessitating intervention by the Indian Government.

Keywords: Arsenic speciation; Grain; Health risk assessment; Pollution; Toxicity

INTRODUCTION

Exposure to arsenic and heavy metals from contaminated drinking water and food sources is a major concern, prompting extensive research in this field [1-3]. Among these contaminants, the presence of arsenic in grains has garnered global attention due to its varying concentrations both within and across countries [4-7]. The toxicity of arsenic is intricately linked to their speciation patterns, which exhibit differences among different species [8]. Rice, in particular, is highly vulnerable to arsenic contamination, primarily due to its aquatic nature [9]. In the Ambagarh Chouki area of Chhattisgarh, India, the issue of arsenic contamination has become particularly prominent [10,11]. This study aims to investigate the contamination levels and sources of arsenic species in various organs (grain, husk, straw, and root) of cereal crops, including rice, wheat, and maize, cultivated in the contaminated soil of this region. By examining these aspects, we can gain valuable insights into the

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Corresponding author Khageshwar Singh Patel, Department of Applied Sciences, Amity University, India, E-mail: kspatel@rpr.amity.edu

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extent and distribution of contamination, as well as understand the potential risks associated with consuming crops from this area.

MATERIALS AND METHODS

Study Area

The study was carried out in different agricultural areas in Ambagarh Chouki (20.78209°N 80.74117°E) region of India. The climate is hot tropical with maximum temperature (≈47°C) in month of May. The average annual precipitation in the region is about <1200 mm, and most of the precipitation occurs between June and September. The major predominant rocks that occur are Dongargarh granite and rhyolite, which contain quartz, feldspar, ferromagnesium and iron oxide [12]. The most common soil groups occurred in the study area are the Bhata (Entisol), Matasi (Inceptisol), Dorsa (Alfisol) and Kanhar (Vertisol) [13].

Samples

During March-April 2021, a total of 24 locations in Ambagarh Chouki were chosen to collect grain samples (18 rice, 3 wheats and 3 maize), as shown in **Figure 1**. Additionally, 6 soil, 6 straw, 3 husk and 6 root samples were obtained. The husk was manually separated from the grain. The husk, straw, and root samples were washed three times using deionized water. All samples were sundried for one week, heated overnight at 50°C in a hot air oven, and finally crushed into powdered form, removing particles sized ≤ 0.1 mm. The processed samples were subsequently refrigerated at −4°C until analysis.

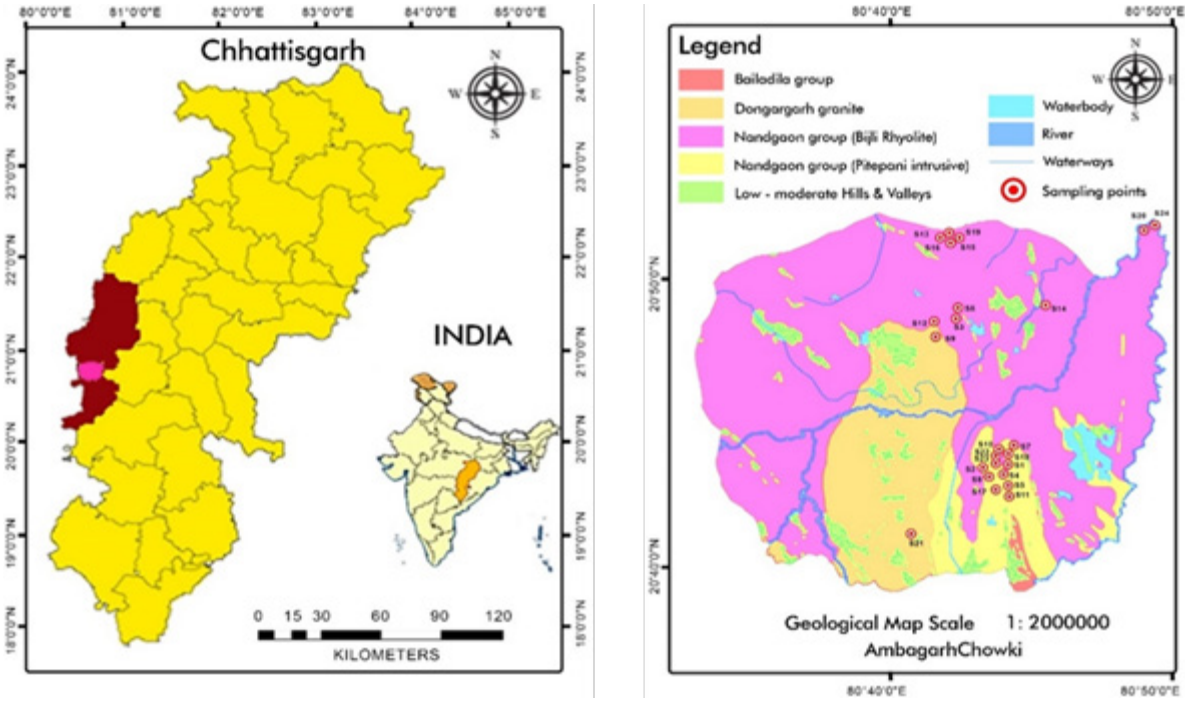

Figure 1: Left: Area of study. Right: Sampling points in Ambagarh Chouki, CG, India

Analysis

Two analytical techniques, namely hydride generation–atomic fluorescence spectrometry (HG–AFS), and inductively coupled plasma–mass spectrometry (ICP–MS), were used for the analysis of arsenic in soil, husk, straw and grain samples.

Quantification of Total As and As Speciation Analysis in Soil

Soil samples from 6 locations were selected to analyze total As and and As speciation, using the HG-AFS and LC-HG-AFS, respectively (**Table 1**). Analyses of total arsenic were conducted with a Millennium Excalibur (PS Analytical, Orpington, Kent, UK). An aliquot of soil sample (0.2 g) was digested with 10 mL of aqua regia (3:1 ratio of HCl to HNO_3) for 2 hours at 120°C in a 50 mL polypropylene tube. After cooling down, the solution was diluted with deionized water to 50 mL, it was filtered using a Whatman 541 filter paper and further diluted to fit the calibration range. The samples were then analyzed with the HG–AFS system using 0.7% $NaBH_4$ (m/v) in 0.1M NaOH as the reductant against the reagent blank, namely 25% HCl (v/v)+2% KI (m/v) + 10% ascorbic acid (m/v). Calibration standards of 0.10 ng mL^{-1} were prepared in the reagent blank, as shown in **Figure 2**. The mean value of three replicate measurements is reported. For the arsenic speciation analysis, an aliquot of soil sample (0.2 g) was weighed into a 50 mL clean polypropylene vial, followed by the addition of 5 mL of 0.5 M phosphoric acid. Samples were then capped and heated at 60°C overnight on a hot block and then shaken for 3 hours at room temperature, followed by centrifuging for 20 minutes. The supernatants were filtered by a 0.2 µm polytetrafluoroethylene (PTFE) syringe filter and transferred into a clean vial. These solutions were then further diluted in 20 mM phosphate buffer mobile phase and analyzed using HPLC-HG-AFS, where HPLC stands for high-performance liquid chromatography. A mixed standard with 10 µg L^{-1} of As(III) and As(V) was also prepared and extracted using the same method as the samples to ensure no species conversion occurred. A single analysis was done for speciation.

Table 1: Arsenic content in soil samples from Ambagarh Chouki (Chhattisgarh, India)

Sample	Location	pH	Total As (mg.kg^{-1})	As(V) (mg.kg^{-1})	Extraction efficiency (%)
LGC 6138	-	-	24.121 ± 0.060	23.33	97.2
S1	Koudikasa	7.5	51.425 ± 0.223	40.26	78.3
S2	Muletitola	7.4	21.405 ± 0.274	17.11	79.9
S3	Sonsai tola	7.5	36.732 ± 0.401	30.314	82.5
S4	Koudikasa	7.5	42.762 ± 0.412	35.086	82.1
S5	Koudikasa	7.3	61.949 ± 1.400	47.504	76.7
S6	Joratarai	7.5	37.079 ± 0.370	28.351	76.5

S=Soil sample, LGC 6138=Soil reference sample

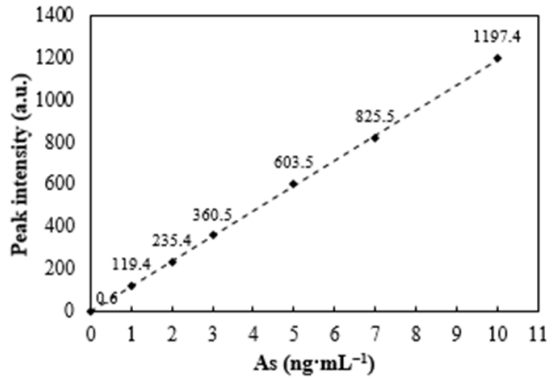

Figure 2: Calibration curve for the determination of total As

Quantification of Inorganic Arsenic and Arsenic Species in Grain

An aliquot of powdered plant sample (0.2 g) was taken into a 50 mL polypropylene tube and mixed with 10 mL solution of 1% 0.1 N HNO_3 and 3% H_2O_2 (v/v), heated by placing in hot water (90°C) for 2 hours. The cold solution was centrifugated, and the supernatant liquid (2 mL) was filtered through a 0.45 µm syringe into a polypropylene vial for injection into an Agilent 8900 × ICP-MS/MS equipped with Thermo Surveyor. The carrier gas, plasma gas, and auxiliary gas in the ICP-MS flowed at rates of 1.05 L min^{-1}, 15 L min^{-1} and 0.90 L min^{-1}, respectively. Mass resolution, integration time, and isotope conditions were as follows: 0.6 amu-0.8 amu, 0.6 seconds-0.8 seconds, and Q1-75/Q2-91 m/z, respectively. The Agilent speciation column was operated at the flow rate, temperature, and pressure conditions of 0.9 mL min^{-1}, 35°C, and 75 bar by injecting 10 µL of the sample solution at room temperature over a measuring time of 12.5 minutes. **Figure 3** showed a typical chromatographic separation. Blank tests were performed, and the results showed that the signals were negligibly low. The mean value of three replicate measurements is reported in the following text.

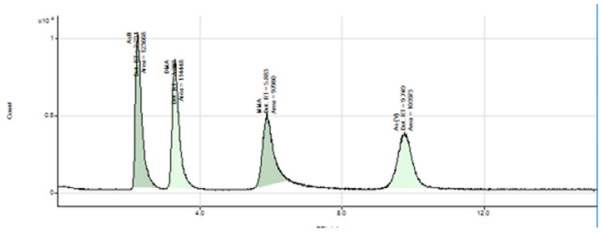

Figure 3: Chromatographic separation of arsenic standards at 10 ng.g^{-1}

Total As and As(V) Analysis in Soil

Soil samples from 6 locations were selected to analyze total As and As(V) contents using the HG–AFS technique. Analyses of total arsenic were conducted with a Millennium Merlin and Excalibur AFS (P S Analytical, Orpington, Kent, UK). An aliquot of soil sample (0.2 g) was digested with 10 mL of aqua regia (3:1 ratio of HCl to HNO3) for 2 hours at 120°C in a 50 mL polypropylene tube. After cooling down, the solution was diluted with deionized water to 50 mL, it was filtered with Whatman 541 filter paper and further diluted to fit the calibration range. The samples were then analyzed with the HG–AFS system using 0.7% NaBH4 (m/v) in 0.1M NaOH as the reductant against the reagent blank, namely 25% HCl (v/v)+2% KI (m/v) + 10% ascorbic acid (m/v). Calibration standards of 0.10 ng mL^{-1} were prepared in the reagent blank, as shown in **Figure 2**. The mean value of three replicate measurements is reported. For the arsenic speciation analysis, an aliquot of soil sample (0.2 g) was weighed into a 50 mL clean polypropylene vial, followed by the addition of 5 mL of 0.5 M phosphoric acid. Samples were then capped and heated at 60°C overnight on a hot block and then shaken for 3 hours at room temperature, followed by centrifuging for 20 minutes. The supernatants were filtered by a 0.2 µm polytetrafluoroethylene (PTFE) syringe filter and transferred into a clean vial. These solutions were then further diluted in 20 mM phosphate buffer mobile phase and analyzed using HPLC-HG-AFS, where HPLC stands for high-performance liquid chromatography. A mixed standard with 10 µg L^{-1} of As(III) and As(V) was also prepared and extracted using the same method as the samples to ensure no species conversion occurred. A single analysis was done for speciation.

Quantification of As Species in Grain

An aliquot of powdered plant sample (0.2 g) was taken into a 50 mL polypropylene tube and mixed with 10 mL solution of 1% 0.1 N HNO_3 and 3% H_2O_2 (v/v), heated by placing in hot water (90°C) for 2 hours. The cold solution was centrifugated, and the supernatant liquid (2 mL) was filtered through a 0.45 µm syringe into a polypropylene vial for injection into an Agilent 8900 × ICP-MS/MS equipped with Thermo Surveyor. The carrier gas, plasma gas, and auxiliary gas in the ICP-MS flowed at rates of 1.05 L min^{-1}, 15 L min^{-1} and 0.90 L min^{-1}, respectively. Mass resolution, integration time, and isotope conditions were as follows: 0.6 amu-0.8 amu, 0.6 seconds-0.8 seconds, and Q1-75/Q2-91 m/z, respectively. The Agilent speciation column was operated at the flow rate, temperature, and pressure conditions

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Transfer Factor

The transfer factor (Tf) of As and other elements was calculated using equation 1, which indicates the ratio of the metal content in the plant to that in the soil [14].

$$T_f = C_{plant} / C_{soil} \quad \text{Eq 1}$$

Health Risk Assessment

Different parameters, like Average Total Dose (ATD), Chronic Daily Intake (CDI), Cancer Risk (CR), and Hazard Quotient (HQ), were used to assess the health risk faced by the population. These evaluations were calculated based on the equations given by [15].

ATD=Asg*IR Eq 2

CDI=Cm*DI/BW Eq 3

HQ=CDI/RfD Eq 4

HI=ΣHQi Eq 5

CRlim=RfD*BW/Cm Eq 6

Cancer risk=CDI*SF Eq 7

The variables ATD, Asg, IR, Cm, DI, BW, RfD, CDI, HI, HQi, CRlim and SF represent the average total dose, arsenic contamination of grain (in mg kg^{-1}), grain ingestion rate (kg day^{-1}), mean concentration of As in food, amount of food consumed per day (0.5k g day^{-1}), the mean body weight of the individual (60 kg), reference dose, chronic daily intake, hazard index, summation of HQ of non-carcinogens, the maximum allowable food consumption rate (in kg d^{-1}), and the slope factor (in mg kg^{-1} day^{-1}), respectively.

QA/QC Analysis

Samples were digested using analytical grade acids from Sigma-Aldrich. Quality control and method validation were ensured through the use of LGC 6138 (coal carbonization, site soil, certified for total arsenic, 24 ± 1 µg g^{-1}), and rice flour ERM BC211 reference materials. The LOD for all arsenic species are 0.5 µg L^{-1}. The LOQ for iAs (As3+As5), MMA and DMAs in the rice was 30 µg kg^{-1}. The recovery values for inorganic arsenic (iAs), monomethylarsenic (MMAs), and dimethylarsenic (DMAs) were within the range of 84%-112%, with a relative standard deviation (RSD) of 8%.

Statistical Analysis

IBM SPSS v.20 was used to analyze the data. The mean value of three measurements was recorded. The arsenic concentration in the samples was recorded in µg kg^{-1} dry weight (DW).

RESULTS AND DISCUSSION

Soil Characteristics

The soil pH in the field samples was neutral, averaging 7.5 ± 0.1. Total and As(V) concentrations ranged from 21.4 to 62.0 mg kg^{-1} and from 17.1 to 47.5 mg kg^{-1} respectively (**Table 1**). Neither As(III) nor organic arsenic was found in the soils. Notably, arsenic concentration in the studied area's soils greatly surpassed the background level of 5 mg kg^{-1}. The level of arsenic exceeded values found in other regions of the country [16-19].

Cereal Grain Contamination

The arsenic speciation (iAs, MMAs, and DMAs) results for the 24 cereal grain samples (18 rice, 3 wheat, and 3 maize) are summarized in **Table 2**. All grain samples exhibited a significant concentration of iAs. Specifically, iAs concentrations in rice (n=18), wheat (n=3), and maize (n=3) grains were 229.9 to 684.7 µg kg^{-1}, 84.6 to 218.5 µg kg^{-1} and 19.9 to 26.2 µg kg^{-1}, respectively. The mean values for iAs content were 342.8 ± 82 µg kg^{-1}, 96.2 ± 106.2 µg kg^{-1}, and 44.1 ± 35.3 µg kg^{-1}, respectively (**Table 2**). Thus, rice and wheat grains displayed concentrations approximately 8 and 2-fold higher than those of maize grains. MMAs were found only in one rice grain sample, while almost all rice grain samples exhibited substantial DMAs content. Among rice varieties, DMAs concentration in rice grain (n=16) displayed notable variations, ranging from 17.5 to 267.5 µg kg^{-1}, with a mean value of 101.9 ± 69.8 µg kg^{-1}. A low DMAs concentration was also detected in one maize grain sample. The arsenic speciation pattern observed in these grains is in line with previous reports in the literature [20-22].

Table 2: Arsenic species contents in rice, wheat, and maize plant parts, expressed in µg.kg^{-1}

Sample Number	Sample	Location	DMAs	MMAs	iAs
Blank	-	-	-	-	0.046
BC 211	ERM BC 211	-	126.889	7.829	122.248
RG1	Rice (Sonam) grain	Kaudikasa	90.277	ND	282.819
RG2	Rice (Sonam) grain	Muletitola	63.239	ND	232.973
RG3	Rice (Sonam) grain	Sonsaytola	ND	ND	330.894
RG4	Rice (Sarna) grain	Kaudikasa	32.224	ND	250.614
RG5	Rice (Sarna) grain	Muletitola	ND	ND	229.912
RG6	Rice (Sarna) grain	Sonsaytola	125.949	ND	471.3
RG7	Rice (Naya 1010) grain	Kaudikasa	178.461	ND	293.303
RG8	Rice (Naya 1010) grain	Muletitola	134.094	ND	261.893

RG9	Rice (Naya 1010) grain	Sonsaytola	92.112	ND	346.771
RG10	Rice (RI) grain	Jadutola	93.722	ND	260.683
RG11	Rice (RI) grain	Sangali	97.043	ND	317.777
RG12	Rice (RI) grain	Majhitola	50.256	ND	398.151
RG13	Rice (PRN) grain	Jadutola	117.603	ND	346.043
RG14	Rice (PRN) grain	Muletitola	208.358	ND	684.738
RG15	Rice (PRN) grain	Kaudikasa	267.478	ND	384.992
RG16	Rice (Luchai) grain	Jadutola	37.467	7.726	386.256
RG17	Rice (Luchai) grain	Muletitola	24.229	ND	320.007
RG18	Rice (Luchai) grain	Kaudikasa	17.474	ND	371.268
MG1	Maize grain	Daihan	8.451	ND	84.637
MG2	Maize grain	Joratarai	ND	ND	27.592
MG3	Maize grain	Netamtola	ND	ND	19.954
WG1	Wheat grain	Kaudikasa	ND	ND	26.211
WG2	Wheat grain	Kaudikasa	ND	ND	218.471
WG3	Wheat grain	Joratarai	ND	ND	43.914
RS1	Rice (Naya 1010) straw	Kaudikasa	29.718	ND	8002.237
RS2	Rice (Naya 1010) straw	Muletitola	26.747	ND	6019.72
RS3	Rice (Naya 1010) straw	Sonsaytola	ND	ND	15460.911
WS1	Wheat straw	Koudikasa	ND	ND	862.216
WS2	Wheat straw	Koudikasa	ND	ND	388.408
WS3	Wheat straw	Joratarai	11.834	ND	4092.089
RH1	Rice (Naya 1010) husk	Kaudikasa	186.81	ND	1056.251
RH2	Rice (Naya 1010) husk	Muletitola	135.719	ND	756.116
RH3	Rice (Naya 1010) husk	Sonsaytola	ND	ND	951.901
RR1	Rice (Naya 1010) root	Kaudikasa	293.383	26.908	77404.395
RR2	Rice (Naya 1010) root	Muletitola	281.836	80.956	48697.176
RR3	Rice (Naya 1010) root	Sonsaytola	443.464	91.345	87051.697
WR1	Wheat root	Kaudikasa	ND	ND	1242.98
WR2	Wheat root	Kaudikasa	ND	ND	319.762
WR3	Wheat root	Joratarai	ND	ND	6682.693

Straw and Husk Contamination

In the rice husk and straw, only two types of arsenic, i.e., iAs and DMAs, were detected (**Table 2**). The iAs concentration in rice straw, wheat straw, and rice husk were 6046.5 to 15460.9 μg kg^{-1}, 388.4 to 4092.1 μg kg^{-1}, and 756.1 to 1056.3 μg kg^{-1}, respectively. The higher As content in the root straw samples (5.5-fold and 9.6-fold compared to wheat straw and rice husk) can be attributed to high arsenic translocation from the roots to the shoot [23].

Root Contamination

An extremely high uptake of iAs was noted in the root samples. The MMAs, DMAs, and iAs levels in the rice root samples varied from 26.9 to 91.3 μg kg^{-1}, from 281.8 to 443.5 μg kg^{-1} and from 48697.2 to 87051.7 μg kg^{-1}, respectively, with average values of 66.4 ± 34.6 μg kg^{-1}, 339.6 ± 90.2 μg kg^{-1}, and 71051.1 ± 19951.0 μg kg^{-1}. However, only iAs showed accumulation in wheat roots, with contents ranging from 319.7 to 6682.7 μg kg^{-1} and a mean value of 2748.5 ± 3438.3 μg kg^{-1}. The As concentrations in the different parts of the rice plant generally followed the same pattern previously reported [22]but were considerably higher than the values reported for that study in rice from Bangladesh.

Transfer factor

The mean transfer factor (Tf) values of the total As for rice grain (n=3), Triticum (wheat) grain (n=3), rice husk (n=3), rice straw (n=3), Triticum straw (n=3), rice root (n=3) and Triticum root (n=3) evaluated were 0.01 ± 0.003, 0.002 ± 0.001, 0.03 ± 0.006, 0.29 ± 0.11, 0.05 ± 0.05, 2.05 ± 0.39 and 0.07 ± 0.08, respectively. In terms of As, the rice root samples exhibited the highest Tf value (2.05), whereas other parts of the rice and wheat plants showed significantly lower values (0.03–0.29).

Toxicity

Rice serves as the primary food source for the residents of this region, yet grains from all rice varieties were discovered to be contaminated with iAs, surpassing the permissible limit of 200 μg kg^{-1} (as mentioned earlier, the mean iAs concentration in rice, wheat and maize grain was observed to be 433 ± 1 54.1 μg kg^{-1}, 96.2 ± 106.2 μg kg^{-1}, and 44.1 ± 35.4 μg kg^{-1}, respectively). The concentrations found in Ambagarh Chouki were higher than those detected in rice samples collected from 13 major rice-producing provinces in China, where concentrations ranged from 25 to 327 μg kg^{-1} [23]. As for the husk and straw samples, utilized as feed for domestic animals, they exhibited iAs contamination at levels several times higher than the grains, posing a clear risk. The ATD, CDI, CR, and HQ values for the

grains are presented in **Table 3**. The HQ values for rice, wheat, and maize were 14.28, 4.01 and 1.84, respectively, much higher than those reported for the Bengal Delta and the Bahama basin, likely due to the elevated As content in the soil and water of Ambagarh Chouki [24,25]. The HQ and CR values for these grains were observed to exceed above 1 and acceptable limit of 1×10^{-4} (US EPA 2005), respectively suggesting potential non-cancer and cancer health risk [26,27].

Table 3: Health risk assessment of grains samples

Grain	ATD	CDI	CR	HQ
Rice	0.17 ± 0.05	0.0029 ± 0.0009	0.0043 ± 0.0013	14.28 ± 4.46
Wheat	0.05 ± 0.05	0.0008 ± 0.0009	0.0012 ± 0.0013	4.01 ± 4.43
Maize	0.02 ± 0.02	0.0004 ± 0.003	0.0006 ± 0.004	1.8 4± 1.47

ATD, CDI, CR, and HQ stand for average total dose, chronic daily intake, cancer risk, and hazard quotient.

CONCLUSION

Elevated concentrations of arsenic in soil in the form of As(V) was found in all six locations. Neither As(III) nor organic arsenic species were found. Very high levels of arsenic were found in rice grain, husk, straw and root samples obtained from Ambagarh Chouki (Chhattisgarh, India). The order of increasing arsenic concentration was grain << husk << straw << root. Arsenic levels in rice plant parts were significantly higher than in wheat grain and plant parts, as well as maize grain. A significant portion of the total arsenic (tAs) came from toxic inorganic arsenic (iAs), which accounted for 80.9%, 97% and nearly 100% of the tAs in rice grain, husk, and straw/root samples, respectively. Only rice root samples contained MMAs, while DMAs were present in most rice plant parts. The average iAs content in rice grain (433 ± 154.1 µg kg^{-1}) largely exceeded the permissible limit of 200 µg kg^{-1}, whereas wheat and maize grain (96.2 ± 106.2 µg kg^{-1} and 44.1 ± 35.4 µg kg^{-1}, respectively) remained within the safe range, indicating them as safer food sources. Hazard quotient values for rice, wheat, and maize grain were 14.28, 4.01 and 1.84 respectively. The presence of high levels of arsenic in the grains can be attributed to both natural and human activities. Urgent action is required from the Indian Government to address the adverse health effects associated with consuming cereals grown in this region.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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