ORIGINAL RESEARCH



Recent status of cadmium-contaminated paddy field and its impact on food safety: a field study over two years in North 24 Parganas, West Bengal, India

Ankita Biswas¹ · Suparna Pal¹ · Subhabrata Paul²

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Abstract

Asian people depend on rice for its significant nutritional value. The depreciating effect of cadmium (Cd) on rice crops is notably impulsive, leading to high changes in field and negotiating consumer health. A regional two-year survey of 30 fields of the district North 24 Parganas was conducted to study soil parameters contributing Cd uptake in rice grains and subsequently to compare phytochemical properties of seeds among 3 common cultivars. Based on soil Cd concentration, rice cultivars grown in high Cd (HCd) and low Cd (LCd) contaminated fields were chosen. The efficacy of grain nutrition status of each cultivar with HCd and LCd was compared. Our results revealed that soil pH, organic matter, carbonate, and minerals pronouncedly affected Cd accumulation in grains. Cd-exposed Khitish HCd grains displayed significant reduction of phytochemicals like sugar, phenol, thiamine, ascorbic acid, and riboflavin contents imposing major nutritional degradation of grains compared to Khitish LCd. Contrarily, Pratiksha grains exhibited moderate levels of degradation while Maharaj HCd showed the least alteration in phytochemical status with minimum-grain-quality degradation compared to other HCd cultivars. The element analysis also confirmed that the highest Cd content in Khitish grains holds this cultivar as Cd accumulator while low Cd in Maharaj grain makes this "Cd safe" cultivar. This investigation may be recommended to avert consumption of such rice cultivars that accumulate high Cd in grains, and precautions should be drawn to limit Cd accumulation in rice grains by implementing Cd remediating agents for better food security.

Suparna Pal suparnap486@gmail.com

¹ Department of Botany, Lady Brabourne College, P-1/2, Suhrawardy Ave, Beniapukur, Kolkata, West Bengal 700017, India

² Institute of Health Sciences, Presidency University (2nd Campus), Newtown, Kolkata, West Bengal 700156, India

Graphical abstract



Keywords Bioavailability · Cadmium · Cancer risk · Grain nutrients · Rice · West Bengal

Introduction

Increasing occurrences of numerous abiotic stresses together with ever-increasing population are putting major constraints for achieving global food security. Rice (Oryza sativa L.), the most popular staple food, is very much prone to various abiotic stress, among which heavy metal stress is one of the major environmental issues in the world agricultural market (Maksymiec 2007). Cd is a potent toxic, non-biodegradable heavy metal, and hazardous soil contaminant with a long biological half-life (Ismael et al. 2019). Cd is released into the environment by mining, electroplating, sludge dumping, and smelting and gets accumulated in agricultural soil due to overuse of phosphate fertilizers and contaminated irrigated water (Kubier et al. 2019). Cd stress imposes detrimental effects on plants like poor growth, turgor loss, inhibiting respiration, leaf chlorosis, poor seed germination rate, necrosis, genotoxicity, crippled photosynthetic apparatus, dysfunction of protein, membrane rupture, and anti-oxidative disparity through the generation of reactive oxygen species (Arif et al. 2019). Cd accumulation in plants depends on several edaphic factors like soil pH, soil texture, availability of organic matter, presence of other minerals, nitrogen source, soil microorganisms, etc. (Sarwar et al. 2010; Hussain et al. 2020). Jeopardizing effect of Cd stress on rice crops is considerably more challenging and complicated, generating effective impairments in the cropland. According to the report on the National General Survey of Soil Contamination by Chinese officials, 16.1% of China's farmland had soil pollution levels over the threshold for heavy metals, and 7% of it was contaminated with Cd (Niu et al. 2022). Being highly mobile, Cd is easily translocated from soil to grains and consequently compromises consumer health menace via entering food chain (Grant et al. 1998; Paul et al. 2022) (According to WHO, PTMI of Cd is 25 µg/kg BW/month).

In India, 40 out of 424 water quality monitoring stations, Ganga, Kopili, Tungabhadra, Rapti, and Yamuna Rivers are highly contaminated with Cd, with levels exceeding $3 \mu g/L$ and going up to 7.8 $\mu g/L$. Vautha water quality monitoring station at Sabarmati River (Gujarat) revealed the highest levels of hazardous Cd content (70.51 μ g/L). Uttar Pradesh, Assam, Gujarat, Karnataka, Arunachal Pradesh, Madhya Pradesh, Andhra Pradesh, Rajasthan, and Haryana are the major states affected by Cd contamination of water. West Bengal is less affected (Source: Department of Water Resources, River Development and Ganga Rejuvenation, 2019). Based on most recent available data, Bichhri in Rajasthan has no fertile land left for agriculture due to large clusters of industries. However, recent information has revealed that enrichment factor of Cd is 45 in Indian agricultural land (Xiaofang and Dongmei 2019). Within West Bengal, Dhapa-Bantala (Garbage farming), a small quaint locality in EM Bypass, Kolkata, is one of the richest Cd-contaminated areas. Average soil Cd concentration in this site was about 0.721 (µg/g dry weight) recorded by Banerjee et al. (2010).

According to recent reports, overuse of phosphate fertilizers and application of organophosphate pesticides are prominent contributors to Cd contamination in arable soil (Yang et al. 2020; Alengebawy et al. 2021). Wastewatercontaminated irrigation promotes Cd deposition in soil (Lien et al. 2021). Allowable limits of uncontaminated soil Cd are less than 0.5 mg/kg, yet this can be tolerated up to 3.0 mg/ kg of soil subject to soil chemistry (Zhang et al. 2021). Cd bioavailability is regulated by soil physicochemical properties (pH, electrical conductivity, and soil organic matter) (Li et al. 2018a) and extractable soil Cd content (Barman et al. 2020) while Cd accumulation is rice genotype-dependent (Li et al. 2018b; Barman et al. 2020). Acidic pH promotes metal solubility, thus facilitating heavy metals (HMs) accumulation in rice grain (Yu et al. 2016) while soil organic matter sequesters HMs, making it less bio-available.

Based on soil Cd concentration and bio-concentration factors, three rice cultivars (Maharaj, Khitish, and Pratiksha) with high and low Cd accumulation abilities were collected from high Cd-contaminated (HCd), and low Cd (LCd) contaminated sites and their grain nutritional status were explored. Rice grains were collected from same fields during the harvesting season in May and November for two consecutive years-2021 and 2022. HCd cultivars are better capable of Cd uptake, translocation, and accumulation (Guo et al. 2018) than LCd cultivars. Rice, the grain of life, is the major source of starch, protein, and micro-nutrients. Rice has more tendency to absorb Cd in comparison to other crops (Lien et al. 2021). Oral intake of Cd-contaminated rice grain increases health risk as humans lack natural Cd disposal pathway other than urine, which is not enough as Cd is sequestered by metallothionein and reabsorbed in renal tubule (ATSDR 2006). Long-term exposure to Cd affects the nervous system, brain, kidney, gastrointestinal tract, and bone density (Jehan et al. 2018). Rice carcinogenicity is a well-known fact (Lien et al. 2021). Hence for health risk assessment study, the carcinogenic risk has been estimated in terms of cancer risk (CR) factor and weekly oral intake.

According to FMO, rice supplies 20% of the world's dietary energy. Asian people depend on rice mostly for its caloric value (45%), total protein (40%), nutrients (thiamine, riboflavin, and niacin), etc. (Wordu et al. 2021). In recent years, Chinese and Korean people are quite aware of the health benefits of wild rice grain because of its protein, minerals, and vitamins providing antihypertensive, antiallergic, anti-cholesterolemic, anti-inflammatory, and immunomodulatory properties (Yu et al. 2020). Furthermore, it was observed that nutritional compositions of rice cultivars vary from one another depending on climate, irrigation, and fertilizer utilization (Surendran et al. 2021). Since each cultivar has distinct nutritional distribution, it is imperative to compare varietal differences in grain nutritional values in response to field Cd contamination and to assess the degree of nutritional degradation, which will be helpful in selecting Cd safe cultivars for human consumption. In this context, this study evaluated nutrient and anti-nutrient (phytic acid) compositions in rice grains of 3 cultivars collected from low Cd-contaminated (average Cd concentrations in soil $< 1 \text{ mg kg}^{-1}$) and high Cd-contaminated (average Cd concentrations in soil > 1 mg kg⁻¹) fields.

Materials and methods

Study area

North 24 Parganas District in West Bengal was selected for this study. The area of the district is 4094 km². Rice, jute, oilseeds, wheat, pulses, and potato are mainly cultivated in this area. The average yield of rice within this district is 3540 kg/ha. Though water of River Ganga is primarily used for irrigation, wastewater irrigation is a common practice for 8–10% land of our study area, mainly mouzas of Barrackpore II block. The entire sampling location map is given in Fig. 1 and Table 1. Total Cd-contaminated study area is classified into two zones based on Cd concentration in soil (Table 2). Details of 3 locally available rice cultivars are specified in Table 3.

Collection of grain and soil samples

Rice grains and corresponding rice field soil samples were collected directly from the field during harvesting season (May and November of 2021 and 2022) via direct communication with the local farmers and the district administration office of N 24 Parganas, West Bengal. Several grains from individual plants were randomly collected from the sampling area and then mixed to give a composite grain Fig. 1 Location of the study area at North 24 Parganas, West Bengal, India



sample of each field. In order to eliminate possibility of incorrect sampling, soil samples were collected in zip lock packets, 5 sub-samples were taken from each field of each site at a depth of 10–20 cm using a spud. All sub-samples were mixed to prepare a composite sample of each field. Soil samples were air-dried for 5–7 days and ground to pass through a 1 mm nylon mesh. Metal analyses of the plant and soil samples were carried out by acid digestion (3:1 Conc. HNO₃ and Conc. HClO₄ v/v) of accurately 1 g of plant tissue and soil samples, respectively.

Riboflavin content

To assay riboflavin content, about 1 g powdered rice grain was homogenized with 50% ethanol, following filtration collected supernatant was mixed with 2.5 ml 5% KMnO₄ and 30% H_2O_2 solution. The whole extract is placed in water bath at 80 °C for 30 min. Then, 1 ml 40% Na_2SO_4 was added, and riboflavin contents were estimated by measuring absorbance

Table 1	Details of mouza	and respect	ve block, No	orth 24 Parganas,	West Bengal
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Field no	Location	Block	Area (in Hector)	Cultivars	Location coordinates
1	Angnara	Hasnabad	38	Sujala	22.5745°N, 88.9174°E
2	Angrail Shutia-Mahishakati	Gaighata	685.97	Pratiksha	23.0082°N, 88.8536°E
3	Ankhola village	Habra I	116.78	Khitish	22.8158°N, 88.728 ⁰ E
4	Arbelia village	Habra I	205.52	Satabdi, Khitish	22.5613°N, 88.5843°E
5	Babanpur Mouza	Barrackpore II	143	Khitish, Pratiksha	22.7855°N, 88.3877°E
6	Berachapa	Deganga	109.16	Prtaiksha	22.627°N, 88.716°E
7	Chandpara	Gaighata	106.7	Pratiksha	22.9831°N, 88.7843°E
8	Dogachhia	Barasat I	66.32	Pratiksha, Khitish	23.002°N, 88.7984°E
9	Fazilpur	Deganga	290.72	Prtaiksha	22.7485°N, 88.6128 ⁰ E
10	Gambhirgachhi	Deganga	169.11	Maharaj	22.923°N, 88.6294°E
11	Gazna Kancha Rasta	Gaighata	267.19	Pratiksha	22.9°N, 88.8056°E
12	Ghoragachha	Baduria	118.5	Maharaj, Pratiksha	22.8286°N, 88.8023°E
13	Gopalnagar	Bongaon	74.02	Khitish	23.0613°N, 88.7618°E
14	Haidarpur	Baduria	245.96	Kanak	22.7823°N, 88.7486°E
15	Jaleshwar	Gaighata	368.67	Pratiksha	22.9225°N, 88.7192°E
16	Jhaudanga Panchpota Road	Gaighata	247.55	Maharaj, Khitish	22.923°N, 88.8284°E
17	Khejurdanga	Deganga	270.99	Khitish	22.725°N, 88.6483°E
18	Kulpi road, Charchalki	Bongaon	318.63	Pratiksha	23.0613°N, 88.7843°E
19	Natgram	Gaighata	102.79	Khitish	22.8759°N, 88.6863°E
20	Nilganje	Barrackpore II	93.68	Khitish	22.7776°E, 88.4107°E
21	Noradaha More	Gaighata	133.21	Pratiksha	22.6914°N, 88.0052°E
22	Noradaha Rd	Gaighata	133.21	Pratiksha	22.760°N, 88.140 ⁰ E
23	Panchpota Rd	Amdanga	164.31	Pratiksha	22.872°N, 88.8534°E
24	Patulia gram panchayat	Barrackpore II	137.58	Maharaj, Pratiksha	22.7776°N, 88.4107°E
25	Rampur	Barrackpore I	46.93	Maharaj, Pratiksha	22.7218°N, 88.2141°E
26	Ruiya	Barrackpore II	198	Pratiksha	22.7451°N, 88.3997°E
27	Selampur, Sewli	Barrackpore II	39.02	Pratiksha, Maharaj	22.7474°N, 88.4074°E
28	Singerbar, Bandipur	Barrackpore II	144.64	Pratiksha	22.94260°N, 88.7985°E
29	Takipur	Hasnabad	238.76	Khitish	22.5451°N, 88.8997°E
30	Tegharia	Barasat II	105.72	Maharaj, Pratiksha	22.9046°N, 88.7969°E

Table 2	Location area of cadmium-	polluted site at North 24 Parganas	District (22°07' 48.00"N and	88°30'0.00"E) and availability of cultivars
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Location	Field	Maharaj LCd	Maharaj HCd	Pratiksha LCd	Pratiksha HCd	Khitish LCd	Khitish HCd
Barrackpore	Patulia Gram Panchayat		Yes		Yes		
	Babanpur Mouza				Yes		Yes
Bongaon	Kulpi Road, Charchalki			Yes			
Gaighata	Jhaudanga Panchpota Road		Yes				Yes
Deganga	Gambhirgachhi	Yes					
Habra	Arbelia village					Yes	
Hasnabad	Takipur					Yes	
Bongaon	Gopalnagar	Yes					
Barasat	Tegharia	Yes		Yes			

Table 3 Details of 3 locally available rice cultiva	ars
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Cultivars	Maturation time (days)	Ecosystem		
Maharaj	140-145	Rainfed		
Pratiksha	142	Irrigated/Rainfed		
Khitish	132	Rainfed, Irrigated, Uplands		

at 510 nm as described previously by Nandagoapalan et al. (2016). Riboflavin content was expressed as $\mu g/Kg$ DW.

Thiamine content

The thiamine contents in grains were estimated by the method proposed by Bhattacharyya and Roy (2018). Thiamine was extracted from 1 g of crude powdered rice grain by blending with 10 ml of 20% ethanolic NaOH. After that, assay mixture contained 1 ml filtrate and 1 ml of 2% $K_2Cr_2O_7$ solution, and absorbance was monitored at 360 nm. The result of thiamine content was expressed as $\mu g/Kg$ DW.

Assay of soluble sugar and starch

Total soluble sugar content of grain was measured by following the details of Dubois et al. (1956) protocol. About 1 g of powdered rice grain sample was homogenized with 80% ethanol followed by centrifugation for 20 min at 2000 rpm. Alcoholic extract was done by phenol–sulfuric acid reagent and expressed as mg/g DW. These end residues collected after centrifugation were suspended in 2.5 ml d.H₂O. 3.25 ml HClO₄ was added to suspension and centrifuged. Final volume of resultant supernatant was made upto 50 ml with d.H₂O, and starch content was measured from filtrate by phenol–sulfuric acid reagent at 490 nm according to method of McCready et al. (1950). The amount of total starch was expressed as g/g DW.

Determination of total protein content

The protein content from 1 g powdered sample has been estimated by the method previously described by Lowry et al. (1951). Total protein contents were expressed as mg/g DW.

Ascorbic acid (AsA) estimation

The method of Okolie et al. (2014) was followed to estimate AsA content. About 100 mg powdered sample was blended with 1.5 ml of 10% TCA and centrifuged. The final reaction

mixture was obtained by adding 0.5 ml supernatant with 0.5 ml DNPH reagent. After 3 h of incubation period at room temperature (RT), 2.5 ml of 85% H₂SO₄ was added slowly, and absorbance was taken at 530 nm after 30 min of incubation. AsA content was expressed as μ g/Kg DW.

Phenol estimation

Phenol extraction was carried out using the **Folin–Ciocâlteu** reagent using gallic acid as standard. About 1 g of powdered grain samples was homogenized with 10 ml of 80% ethanol and centrifuged. After that, 200 μ l of supernatant was added with 800 μ l diluted **Folin–Ciocâlteu** reagent and 2 ml of sodium carbonate (7.5%). The resulting reaction mixture was incubated for 30 min in dark. The absorbance was determined at 765 nm using UV/VIS spectrophotometer (Su et al. 2007). Total phenolics were expressed as mg/g DW.

Flavonoid estimation

Total flavonoid concentration was quantified according to the method of Lamaison and Carnet (1990). The methanolic extract of powdered grain was evaporated to dryness in hot air oven. The end residue was mixed with 10 ml of aluminum chloride solution and kept at RT for 30 min. The intensity of resultant yellow color sample was estimated at 420 nm. The result of flavonoid content was expressed as µg quercetin/g DW.

Phytic acid content

The phytic acid content was estimated following the method of Vaintraub and Laptera (1988). About 500 mg powdered rice grain samples were homogenized with 10 mL of 3.5% HCl. After stirring for 1 h, mixture was cold-centrifuged at 10,000 g for 10 min. Next 1 mL of supernatant was diluted with 2 mL of 3.5% HCl and 1 mL Wade reagent, and centrifuged again. The absorbance was taken at the wavelength of 500 nm using spectrophotometer and expressed as g/Kg.

Fatty acid analysis

Fatty acids in rice grains were estimated by the method of Garces and Mancha (1993). For methylation of fatty acid, 1 ml of methylation reaction mixer carrying methanol: n-heptane: benzene: DMP: H_2SO_4 (37:36:20:5:2) was mixed to 100 mg rice powder taken in 2 ml microfuge tube and incubated for 2 h at 80 °C in water bath. Next, the tube was cooled at RT, mixture was allowed to separate into two phases, and the upper layer containing fatty acid methyl ester was analyzed using Gas Chromatograph with high-resolution Mass Spectrometer (GC-HRMS) (AccuTOF GCV).

Quantification of macro- and micro-nutrient composition by ICP-AES

Rice grains were dried in hot air oven at 80 °C for 72 h prior to estimation of nutrient contents. The oven-dried samples were then ground into fine powder following digestion with a tri-acid mixture of HNO_3 : HCl: $HClO_4$ (4:2:1), and the solutions were adjusted to 25 ml with deionized water. The concentration of macronutrients (Ca, K, and Mg) and micronutrients (Zn, Fe, and Na) and Cd in digested solution was determined using Inductively Coupled Plasma-Optical Emission Spectroscopy instrument (ARCOS, Spectro, Germany).

Estimation of bio-available Cd in the soil samples

Bio-available Cd contents in soil samples were determined according to the procedure of Mohamadiun et al. (2018). First, collected soil samples from each field were oven-dried at 60 °C for 48 h. About 1 g of fully dried soil samples was dissolved in 15 ml d.H₂O and the soluble fraction was collected after incubation for 24 h using a shaker overnight. After every process, sample was quantified for Cd concentration by the UV/VIS spectrophotometric method. The soil precipitation was washed with d.H₂O repeatedly and again dried overnight at 60°C. Cd content in soil fractions was determined by referencing a standard curve of Cd. Soil parameters, such as pH and EC, were also measured.

Statistical data analysis

All lab experiments were conducted in triplicates and all data were represented as an average of triplicates. All experimental data were statistically analyzed using two-way analysis of variance (ANOVA) test. Heat map was generated to determine the degree of nutritional degradation among 3 cultivars. The results of statistical analysis were performed in SPSS 23 and Past3 software.

Results

Grain qualities

Vitamin B complexes like riboflavin and thiamine have already been recognized in a wide range of rice landraces throughout countries (Asaduzzaman et al. 2013). As shown in Fig. 2A, the percentage degradation of riboflavin content in Khitish HCd collected in 2021 (12.64%) and 2022 (14.52%) was significantly higher and Pratiksha HCd with no significant alteration in riboflavin content relative to that of Pratiksha LCd. In grains collected in 2021, the data recorded significant depletion of thiamine content (Fig. 2B) in Khitish (30.93%) and Pratiksha (17.59%) HCd than





Fig. 2 Effects of Cd on rice grain qualities—riboflavin (A), thiamine content (B), total soluble sugar (C) and starch content (D) in LCd and HCd soil for years 2021 and 2022. The error bars in this figure indicate standard deviation obtained from the mean value of three

individual replicates. Bars followed by (*), (**), (***) and (****) represent significant differences at $P \le 0.05$, $P \le 0.01$, $P \le 0.001$, $P \le 0.001$ level, respectively. (ns): P > 0.05

Maharaj HCd (6.86%). Similarly, in grains collected in 2022, thiamine content was reduced in the following order—Khitish (36.94%), Pratiksha (12.82%) and Maharaj (7.34%).

All the rice HCd sets had significantly declined soluble sugar contents (Fig. 2C) than their LCd counterparts. Reduction of sugar concentration in 'Khitish' was 18.93% and 26.57%, in Pratiksha was 5.07% and 4.6% in the years 2021 and 2022, respectively, while in Maharaj HCd, sugar concentration was elevated by 1.25% in 2021. The percentage of starch content was not uniform in both seasons. Prominent reduction of starch content (Fig. 2D) was observed in Khitish (7.06% and 6.02%) in both years, and enhancement was noted in Pratiksha (6.83%) and Maharaj (4.5%) in 2021 grains, while grains in 2022, starch content declined significantly in Maharaj (16.64%) and Pratiksha (17.56%). In 2021 and 2022, a significant decrease in total

protein content (Fig. 3A) of Khitish HCd (40.75%) and (21.14%) was documented, respectively.

In contrast, total protein (Fig. 3A) of Pratiksha and Maharaj HCd was statistically insignificant from their LCd set. In comparison to Maharaj HCd, AsA content (Fig. 3B) was found to be decreased significantly in Khitish (35.33%, 24.8%) and Pratiksha (23.86%, 13.94%) in both seasons, respectively. A decreasing trend of the phenol and flavonoid content of all HCd was observed (Fig. 4). The study revealed significant reduction of flavonoid (Fig. 4B) (28.73% and 24.27%), (6.11% and 6.47%) and (1.29% and 2.2%) and phenol content (Fig. 4A) of grains (29.1% and 18.56%), (28.07% and 14.14%) and (3.87% and 4.48%) of Khitish, Pratiksha and Maharaj in the year 2021 and 2022, respectively. Phytic acid content (Fig. 4C) was mentioned to be significantly increased in Khitish HCd (57.14%, 51.93%) and



Fig. 3 Effects of Cd on rice grain qualities—total protein (A), ascorbic acid content (B) in LCd and HCd soil for years 2021 and 2022. The error bars in this figure indicate standard deviation obtained from

the mean value of three individual replicates. Bars followed by (*), (**), (***) and (****) represent significant differences at $P \le 0.05$, $P \le 0.01$, $P \le 0.001$, $P \le 0.001$ level, respectively. (ns): P > 0.05



Fig. 4 Effects of Cd on rice grain qualities—phenol (**A**), flavonoid (**B**) and phytic acid content (**C**) in LCd and HCd cultivars for the years 2021 and 2022. Error bars in this figure indicate standard deviation obtained from the mean value of three individual replicates. Bars

followed by (*), (**), (***) and (****) represent significant differences at $P \le 0.05$, $P \le 0.01$, $P \le 0.001$, $P \le 0.001$ level, respectively. (ns): P > 0.05



Fig. 5 Fatty acid composition of Cd-contaminated rice grains collected in the years 2021 and 2022

Table 4 Effect of Cd on macro-nutrient and micro-nutrient content of rice grains collected in 2021 (a) and 2022 (b)

	Ca	К	Mg	Fe	Zn	Na
	(a) Macronutrient (gm	n kg ⁻¹ dry seed)		Micronutrient (µg k	g ⁻¹ dry seed)	
Khitish LCd	0.522 ± 0.66^{a}	0.475 ± 0.13^{a}	1.237 ± 0.03^{a}	0.3 ± 0.01^{a}	0.021 ± 0.002^{a}	11 ± 0.28^{a}
Khitish HCd	0.4775 ± 0.04^{ab}	0.436 ± 0.08^{ab}	1.726 ± 2.29^{ab}	$0.06 \pm 0.028^{\rm bc}$	0.01 ± 0.02^{a}	4.8 ± 1.05^{b}
Pratiksha LCd	0.461 ± 1.43^{ac}	0.53 ± 0.1^{a}	1.443 ± 0.12^{a}	0.4 ± 0.007^{b}	0.015 ± 0.97^{a}	10.12 ± 0.008^{ab}
Pratiksha HCd	0.452 ± 0.59^{a}	0.375 ± 0.07^{b}	1.466 ± 0.88^{a}	$0.09 \pm 3.35^{\circ}$	0.0117 ± 0.14^{a}	7.48 ± 0.26^{bc}
Maharaj LCd	0.45 ± 0.001^{a}	0.405 ± 0.004^{a}	1.037 ± 0.052^{a}	0.25 ± 0.9^{a}	0.02 ± 0.83^{a}	$10.3 \pm 0.06^{\rm ac}$
Maharaj HCd	0.435 ± 1.5^{ad}	$0.365 \pm 0.02^{\circ}$	$1.388 \pm 0.007^{\rm ac}$	0.07 ± 0.02^{ab}	0.025 ± 0.05^{a}	8.87 ± 0.8^{bd}
(b)						
Khitish LCd	0.5 ± 0.4^{a}	0.458 ± 0.6^{a}	0.322 ± 1.9^{a}	0.389 ± 1.1^{a}	0.025 ± 0.8^{a}	8.01 ± 0.2^{a}
Khitish HCd	0.423 ± 0.07^{b}	0.460 ± 0.9^{a}	0.382 ± 1.2^{b}	0.076 ± 0.3^{ab}	0.014 ± 0.004^{a}	4.7 ± 1.6^{be}
Pratiksha LCd	0.421 ± 0.13^{a}	0.544 ± 0.05^{a}	0.627 ± 0.04^{b}	0.4 ± 0.08^{a}	0.028 ± 0.001^{a}	9.62 ± 0.2^{ab}
Pratiksha HCd	0.38 ± 1.9^{ab}	0.312 ± 0.7^{ab}	0.675 ± 0.1^{ab}	0.09 ± 0.027^{ac}	0.0102 ± 0.062^{a}	$9.76 \pm 0.07^{\rm ac}$
Maharaj LCd	0.69 ± 0.52^{a}	0.402 ± 0.01^{a}	0.842 ± 1.02^{d}	0.33 ± 0.005^{a}	0.031 ± 0.06^{a}	$10.6 \pm 0.05^{\circ}$
Maharaj HCd	$0.66 \pm 0.003^{\rm ac}$	0.373 ± 0.023^{a}	$1.03 \pm 0.017^{\rm ac}$	0.097 ± 0.1^{bd}	0.032 ± 0.004^{a}	9.9 ± 0.003^{ad}

The data show means \pm SD of three independent replicas. Values carrying different letters (^{a-e}) are significantly different at *P* < 0.05 and means sharing identical letters in a column are not significantly different according to Tukey multiple range test (*P* < 0.05)

Pratiksha HCd (30.17%, 28.18%), whereas in Maharaj, the value remained the least altered in both seasons.

In both years, due to soil Cd contamination, fatty acid profile (Fig. 5) of Khitish and Pratiksha was affected significantly in comparison with Maharaj grain.

Cd accumulation has an impact on mineral distribution and uptake in grains. In both seasons, the nutritional degradation was approximately uniform, which was evident from significant depletion of some nutrients in grains like Fe, Na, Ca, and K, but very minimal increase in Mg concentration in all 3 cultivars was noted (Table 4). For health risk assessment, the rate of oral Cd intake through consumption of contaminated rice grains was calculated. Results of Cd content, bio-concentration factor (BCF), oral weekly intake and cancer risk factor in low Cd and high Cd-contaminated cultivars collected in the years 2021 and 2022 were encapsulated in Table 5. It was noticed that maximum level of Cd in grain was in Khitish, which was followed by higher levels of oral weekly intake of Cd. The increment in weekly intake of Cd positively increased CR factor. Moderate level of grain Cd was found in Pratiksha. On the contrary, the lowest Cd accumulation was found in

Table 5	Estimation of grain C	d content, Cd intake and	subsequent risk of cance	r development for y	ears 2021 (a) and 2022 (b)
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	Grain Cd (µg/g)	BCF	CR factor	Oral week intake (µg)	Oral week intake per kg body weight (µg/ kg)
(a)					
Khitish LCd	0.21 ± 0.022^{a}	0.214 ± 0.01^{a}	0.451 ± 0.007^{a}	478.8 ± 0.008^{a}	9.576 ± 0.05^{a}
Khitish HCd	0.68 ± 0.003^{b}	0.475 ± 0.07^{a}	1.46 ± 0.21^{a}	1550.4 ± 0.25^{b}	31 ± 0.84^{b}
Pratiksha LCd	$0.19 \pm 0.017^{\circ}$	0.2 ± 0.63^{a}	$0.408\pm0.09^{\rm b}$	$433.2 \pm 0.01^{\circ}$	8.66 ± 0.001^{ac}
Pratiksha HCd	0.48 ± 0.007^d	0.350 ± 0.052^{b}	0.817 ± 1.21^{a}	866.4 ± 0.036^{d}	17.3 ± 0.06^{d}
Maharaj LCd	0.16 ± 0.002^{a}	0.197 ± 0.05^{a}	0.316 ± 0.95^{b}	720.48 ± 1.95^{e}	14.4 ± 0.09^{e}
Maharaj HCd	0.27 ± 0.001^{e}	0.254 ± 0.023^{ac}	0.58 ± 0.02^d	$615.6 \pm 0.76^{\rm f}$	$12.31 \pm 0.004^{\rm f}$
(b)					
Khitish LCd	0.18 ± 0.08^{a}	0.184 ± 0.74^{a}	0.645 ± 0.005^{a}	684 ± 0.003^{a}	13.68 ± 0.17^{a}
Khitish HCd	0.72 ± 0.051^{b}	0.431 ± 0.006^{a}	1.54 ± 0.08^{a}	1641.6 ± 0.27^{b}	32.83 ± 0.005^{b}
Pratiksha LCd	0.15 ± 0.012^{a}	0.15 ± 0.98^{a}	0.365 ± 0.52^{a}	$382.5 \pm 0.005^{\circ}$	$7.65 \pm 0.62^{\circ}$
Pratiksha HCd	$0.446 \pm 0.227^{\circ}$	0.353 ± 0.04^{ab}	0.729 ± 0.99^{a}	772.92 ± 0.7^{d}	15.45 ± 0.013^{d}
Maharaj LCd	0.17 ± 0.139^{d}	0.155 ± 0.01^{a}	0.322 ± 0.074^{ab}	342 ± 0.66^{e}	6.84 ± 0.81^{ce}
Maharaj HCd	0.339 ± 0.035^{a}	$0.256 \pm 1.07^{\rm ac}$	0.529 ± 0.02^{a}	$560.88 \pm 0.021^{\rm f}$	$11.217 \pm 0.001^{\rm f}$

The data show means \pm SD of three independent replicas. Values carrying different letters (^{a-f}) are significantly different at P < 0.05, and means sharing identical letters in a column are not significantly different according to Tukey multiple range test (P < 0.05)

Maharaj, and also dietary Cd intake risk and CR factor were compressed.

The significant differences in pH and EC values of soil were estimated, which are given in Fig. 6. Here, more acidic pH was recorded in the field of Khitish (Fig. 6A) than in other 2 fields. High Cd-contained Khitish soil had relatively higher amounts of Cd in soluble, exchangeable and carbonate fractions and lower level of non-bio-available Cd content (residual, organic, and oxide-hydroxide) relative to Khitish LCd soil which is listed in Table 6. Also, Pratiksha HCd contained moderate amounts of Cd for soluble, exchangeable, carbonate fraction, oxide–hydroxide, residual, and organic matter, which is also mentioned in Table 6. But Maharaj soil contained higher residual Cd, organic matter and pretty negligible bio-available Cd.

Discussion

Heavy metal toxicity and nutritional value monitoring in rice grain have been recommended as oral consumption of contaminated grains may have significant long-term health risks. Maharaj, Pratiksha, and Khitish are Cd-accumulating rice cultivars. Thus, if cultivated in Cd-contaminated agricultural lands, grain Cd accumulation will be facilitated, and intake of such type of contaminated nutritionally compromised grains may cause serious health problems. To address this global issue, nutritional quality alteration of low Cdaccumulating grains and high Cd-accumulating grains of each cultivar have been investigated. Phytochemicals are significant bioactive non-nutrient chemical compounds with antioxidant capabilities, found in plant parts like vegetables, fruits, and whole grains, which reduce the risk of chronic diseases (hypertension, heart disease, diabetes, cancer, and other undesirable clinical disorders) (Liu 2013; Arendt and Zannini 2013; Guha et al. 2021).

Riboflavin content was found to be barely degraded in Maharaj maybe because of the cultivar restoring nutritional profile, mitigating free radicals via glutathione redox cycle and under Cd stress (Zeddies et al. 2014; Acosta-Estrada et al. 2019). Thus, Maharaj may likely be safer for consumption. Banerjee and Roychowdhury (2019) have reported that riboflavin content gradually dropped in fluoride-stressed rice seedlings. Interest in thiamine content has grown in the past decade as its deficiency leads to beriberi (Acosta-Estrada et al. 2019). Health-promoting properties of thiamine in controlling metabolism, nerve cell damage, maintaining a healthy cardiovascular function, boosting immunity, and alcoholism treatment are well known (Khalifa et al. 2016; DiNicolantonio et al. 2018). The thiamine content in Maharaj HCd is pretty less compromised than Pratiksha and Khitish HCd which indicates that Maharaj is likely nutritionally richer than Khitish and Pratiksha. Besides this, thiamine content in Maharaj breaks carbohydrate from rice grains to produce sufficient energy (Bhattacharya and Roy 2018). The sugar content was downgraded due to high Cd toxicity in grains. But greater loss of sugar content in Khitish HCd and Pratiksha HCd indicates poor grain quality with compromised nutritional value for consumption and disables to regulate blood glucose level and insulin metabolism, fatty acid



Fig.6 Effect of Cd on soil pH (A), soil EC (B) in LCd and HCd fields for the years 2021 and 2022. The error bars in this figure indicate standard deviation obtained from the mean value of three

metabolism, energy supply, bile acid dehydroxylation, protein glycosylation, etc. (Wylie-Rosett et al. 2013), whereas no remarkable reduction of sugar contents in Maharaj grain indicates its better contribution to release of brain chemicals like serotonin (Singh et al. 2018). The lesser decay of protein content in Maharaj HCd than other HCd grains may be possible as the cultivar is competent enough to withstand Cd genotoxicity and possesses long chain amino acids that catalyze important biochemical reactions (Singh et al. 2018). Being a primary nutrient in rice, protein accomplishes vital functions throughout human body system and validates health benefits of Maharaj for consumption (Singh et al. 2018). Ascorbic acid is another appreciated ingredient of our diet chart. The content of AsA was not very much affected in Maharaj HCd grain which could be linked to possessing effective antioxidant system, which scavenges free radicals

individual replicates. Bars followed by (*), (**), (***) and (****) represent significant differences at $P \le 0.05$, $P \le 0.01$, $P \le 0.001$, $P \le 0.001$ level, respectively. (ns): P > 0.05

generated by Cd toxicity. AsA is efficiently employed in collagen, skin, tendons, ligaments, blood vessels and certain neurotransmitters synthesis for better functioning in human body. So the effects of AsA on human health and nutrition are substantially considerable (Bhattacharya and Roy 2018; Acosta-Estrada et al. 2019). ROS generation in plants is directly correlated to stress. Flavonoids are polyphenolic compounds and potent antioxidants (Asaduzzaman et al. 2013). The minimal degradation of flavonoid content in Maharaj grain marks its better Cd tolerance and hence capable of scavenging free radicals, mitigating oxidative cell damage, controlling inflammation, allergic and carcinogenic activity (Wordu et al. 2021; Bhattacharyya and Roy 2018). Phenolic compounds are key phytochemicals in plants for having hydroxyl groups to confer scavenging ability and control chronic inflammatory diseases in human beings (Eleazu

Table 6 Estimation of different fractions of soil Cd collected in 2021 (a) and 2022 (b) from location sites

	Soil Cd (mg/kg)	Soluble (mg/kg)	Exchangeable (mg/kg)	Carbonate (mg/ kg)	Oxides and hydroxides (mg/kg)	Residual frac- tion (mg/kg)	Organic matter (mg/kg)
(a)							
Khitish LCd	0.98 ± 0.35^{a}	4.3 ± 0.005^{a}	3.08 ± 0.033^{a}	2.94 ± 0.031^{a}	5.5 ± 0.007^{a}	18.7 ± 0.56^{a}	18.13 ± 0.016^{a}
Khitish HCd	$1.43\pm0.150^{\rm b}$	7.01 ± 0.032^{b}	$4.25\pm0.14^{\rm b}$	3.47 ± 0.004^{a}	4.88 ± 0.043^a	18.11 ± 0.007^{ab}	15.75 ± 0.006^{b}
Pratiksha LCd	$0.95 \pm 0.166^{\circ}$	3.77 ± 0.05^{ab}	2.96 ± 0.053^{ab}	2.74 ± 0.36^{ab}	5.36 ± 0.07^{a}	19.6 ± 0.08^{ad}	$24.18 \pm 0.77^{\circ}$
Pratiksha HCd	1.37 ± 0.020^d	$4.21\pm0.011^{\rm ac}$	3.62 ± 0.006^{a}	$2.9\pm0.002^{\rm b}$	5.15 ± 0.23^{ab}	$19.52 \pm 0.66^{\mathrm{ac}}$	22.69 ± 0.13^{d}
Maharaj LCd	0.81 ± 0.007^{e}	2.59 ± 0.004^{bd}	1.96 ± 0.001^{bc}	$2.51 \pm 0.088^{\circ}$	4.93 ± 0.008^a	20.53 ± 0.31^{bd}	$23.95\pm0.98^{\rm ce}$
Maharaj HCd	$1.06\pm0.040^{\rm f}$	3 ± 0.002^{be}	2.47 ± 0.01^{ad}	2.68 ± 0.004^d	5.01 ± 0.4^{bc}	$21.89 \pm 0.21^{\rm ce}$	24.44 ± 0.001^{df}
(b)							
Khitish LCd	0.95 ± 0.31^{a}	4.78 ± 0.012^a	3.34 ± 0.03^{a}	3.55 ± 0.09^{a}	5.7 ± 0.58^{a}	17.4 ± 0.53^{a}	17.68 ± 0.09^{a}
Khitish HCd	1.67 ± 0.001^{b}	$6.51 \pm 0.84^{\rm b}$	5 ± 0.001^{bd}	3.91 ± 0.005^{a}	4.23 ± 0.33^{b}	17.1 ± 1.94^{ab}	15.25 ± 0.65^{b}
Pratiksha LCd	1 ± 0.040^{c}	$3.59 \pm 0.95^{\rm ac}$	3.76 ± 0.04^{a}	$3.07\pm0.02^{\rm b}$	5.63 ± 0.02^{ab}	18.6 ± 0.004^{bc}	$22.91 \pm 0.066^{\circ}$
Pratiksha HCd	1.26 ± 0.002^d	3.81 ± 0.74^{d}	$4.12 \pm 0.01^{\circ}$	3.36 ± 0.47^{a}	$5.49 \pm 0.49^{\circ}$	$18.71 \pm 0.17^{\circ}$	21.56 ± 0.72^{cd}
Maharaj LCd	$0.89 \pm 0.030^{\rm e}$	$2.64 \pm 0.007^{\rm bc}$	2.02 ± 0.28^{ab}	$2.82\pm0.86^{\rm c}$	5.02 ± 0.51^{a}	$21.66\pm0.81^{\rm be}$	$23.45\pm0.19^{\rm d}$
Maharaj HCd	$1.52\pm0.07^{\rm f}$	2.88 ± 0.02^{bd}	2.37 ± 0.53^{ac}	2.51 ± 0.003^{bc}	5.54 ± 0.03^d	22.08 ± 0.02^{bd}	24.73 ± 0.27^{e}

The data show means \pm SD of three independent replicas. Values carrying different letters (^{a-f}) are significantly different at P < 0.05, and means sharing identical letters in a column are not significantly different according to Tukey multiple range test (P < 0.05)

et al. 2020). Limited degradation of phenol in Maharaj grain suggested better management of Cd stress via retardation of lipid peroxidation and acts as immune enhancer in humans, whereas compromised phenol and flavonoid production in Khitish HCd could be attributed to inferior PAL activity, unable to withstand stress with exposure to pathogens resulting severe damage in grain nutritional value (Bhattacharyya and Roy 2018). As a result of fluoride stress, sensitive rice cultivar fails to accumulate the antioxidant, resulting in declined rate of flavonoids in rice grains (Banerjee and Roychowdhury 2019). Phytic acid, familiar as an anti-nutrient, is a major storage form of phosphorus in grains and chelates calcium, zinc, and iron and exerts inhibitory action in proteolytic digestion of monogastric animals, including humans (Wordu et al. 2021). Here pronounced phytic content in Khitish and Pratiksha impedes the bioavailability of these minerals, leading to impairment of Fe-catalyzed oxidative reactions, rendering iron in its inert form (Goufo and Trindade 2014). In our data, phytic acid declined in Maharaj grain, which is desirable and suitable for consumption. This result agreed with the previous finding, which mentioned the anti-nutrient effect of phytic acid in rice grains (Khan et al. 2019).

Fatty acids are the fundamental building units of fats and lipids. It has diverse functions including establishment of healthy cell membranes, proper working of the brain and nervous system, blood pressure regulation, and hormone secretion (Andersson et al. 2015; Ristić-Medić et al. 2013). Interestingly, our results revealed positive correlation between fatty acid composition of Khitish and Pratiksha, which is congruent with the finding that these cultivars can be considered as Cd accumulator (Majumder et al. 2022).

The exposure to high Cd caused alteration in grain macro- (K, Ca, and Mg) and micro- (Fe, Zn, and Na) nutrients, perhaps due to restricted translocation to the shoot and accumulation in grains. This can be supported by the fact that Cd and mineral nutrients not only share physical and chemical similarities but also use same transporters for uptake (Hussain et al. 2021). Cd substitutes Ca²⁺ in Ca-calmodulin signaling pathway by restricting enzymatic activity and gene expression that are involved in signal transduction pathway (de Araujo et al. 2017). The correlation of Fe content under Cd stress has implicated synergistic interactions between absorption and translocation of Cd and Fe, which is congruent to the previous findings in rice plants (Liu et al. 2003). Here, ample macro- and micro-mineral deficiencies in Khitish and Pratiksha cultivars pose severe health threats which are not preferable. This data is further supported by the report of Raghuvanshi et al. (2022) where they have revealed that Arsenic and Cd toxicity disrupted the balance of minerals in rice grains, reducing crop output.

Our results manifested disparity in Cd accumulation, which can be substantiated by the fact that cultivars may possess variability in Cd transporter, and grain accumulation acts as a prime source of Cd that enters our body through the food chain (Takahashi et al. 2011). According to CODEX, the recommended dietary Cd level in rice grain must be below 0.2 mg/kg. According to the report of WHO, provisional tolerable monthly intake (PTMI) of Cd is near about 7 μ g/kg body weights per week. Our evaluation suggests



Fig. 7 Heat map showing changes of nutritional status among rice grains of 3 cultivars collected from high Cd-contaminated fields with respect to low Cd-contaminated fields for the years 2021 and 2022

that oral weekly intake of Cd upon consumption of Khitish and Pratiksha grain (high Cd-accumulating cultivars) exceeded 15 μ g kg⁻¹ body weight. Likewise, these 2 cultivars highly correlated with the increased level of CR that can be approved by the results of Majumder et al. (2022). The value of CR of Maharaj grain (Cd safe) is out of concern.

The assessment of soil Cd content makes our data more obvious. Our results revealed an indirect linear relationship between soil pH and Cd content in grains due to low soil pH influencing metal solubility and Cd bioavailability by altering the chemical speciation, which is in accordance with previous reports (Guo et al. 2020; Daulta et al. 2021). Increased amount of soil pH in field area of Maharaj led to Cd immobilization through better adsorption (Zhao et al. 2014; Mu et al. 2020). This data was similar to the previous finding by Li et al. (2020), where Cd-polluted calcareous paddy soil improved nutrient profile of soil by lowering soil Cd content. Fahad et al (2015) reported that bio-availability of Cd in soil governs the degree of toxicity rendered by Cd on croplands, as bio-available Cd is only conveyed to whole plant parts. Cd forms complexes with different soil particles like soil geological elements, oxides, organic matter, inorganic components, and soil solid particles (Simmler et al. 2013). It is reported that increased organic matter levels in LCd soils are believed to be key factors in lowering soluble Cd phyto-availability (Saengwilai and Meeinkuirt 2021). From our experiments, the exchangeable Cd fraction has a lofty capability to emigrate and consequently make direct availability for plants, whereas residual Cd has high stability, so non-bio-available for plants (Memoli et al. 2018). Barman et al. (2020) also demonstrated an increase in bio-available Cd fraction (soluble, exchangeable, and carbonate fractions) in high Cd-containing soil. A higher rate of Cd binding with the organic matter was noted in all LCd cultivars which is associated with the least amount of available Cd content with respect to HCd cultivars. Moreover, Maharaj HCd shows better efficiency to diminish phyto-available Cd in soil than other Cd accumulator cultivars and sequentially reduces the translocation of Cd to the grain which is established in our results. To understand the variation in Cd accumulation in rice cropping zones due to seasonal rainfall, we collected rainfall data. Surprisingly, the average annual rainfall of LCd field sites was about 1800 mm with high precipitation triggering Cd ions to submerge into deeper soil, thereby reducing Cd ion transport from roots and shoots to grains by dropping transpiration rate. Rather, HCd fields that received low annual rainfall (average 1600 mm) might be accelerating transpiration rate of rice plants, assisting Cd transport from deep soil layers to vicinity of the root, from roots to shoots and grains (Gill et al. 2014; Liu et al. 2017). Hence, water levels in paddy fields are predominantly controlled by different rainfall amounts and they are a salient factor for generating variation in Cd availability and accumulation in plants.

A clustered heat map was created with contrasting color intensity to visualize all considered nutritional parameters, where it was obvious that grain nutritional quality degradation was higher in Khitish and Pratiksha than Maharaj in every aspect and indicates poor efficiency of these two cultivars to resist Cd toxicity (Fig. 7). To best of our knowledge, this is the first reported attempt where Cd-inflicted nutritional degradation of rice grains has been studied, justifying the potential beneficial role of Maharaj cultivar compared to Pratiksha and Khitish in nutrition of people within the study location.

Conclusion

Cd accumulation in rice grains affects nutritional quality and makes it unsafe for consumption. Therefore, it becomes crucial to have an idea of the nutritional quality degradation of rice cultivars due to Cd contamination in fields. Based on nutritional quality deterioration of rice grains, we concluded that the Maharaj cultivar was superior to Khitish cultivar. Maharaj grain was offered with minimal nutritional degradation while Khitish rice cultivar displayed significant reduction of phytochemicals along with high content of anti-nutritional phytic acid. Therefore, this study envisages urgent need to reduce consumption of such rice cultivars which accumulate high Cd, and precautions should be taken by farmers to build global food security.

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Data availability All data analyzed or generated during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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