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Rice Cd Levels in Cambodia Ranged 3 Orders of Magnitude due to Season and Soil Cd Levels

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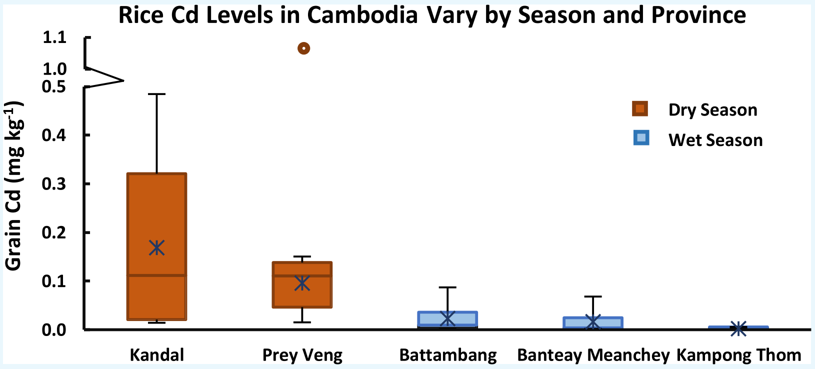

ABSTRACT: Cadmium (Cd) is a toxic trace element that can be transported from soil into rice grain, posing health threats to rice consumers. Among the global studies on rice grain Cd, only one market survey reported grain Cd levels from Cambodia, an important rice-growing country in Southeast Asia. Here, we collected paired rice and soil samples in the wet and dry seasons from major rice-growing regions across five provinces in Cambodia and report the relationships between plant Cd and soil Cd parameters. Both DTPA-extractable and nitric acid digestible soil Cd are significant predictors for Cd levels in rice straw and grain. Rice grain Cd concentrations ranged 3 orders of magnitude from 0.002 to 1.066 mg kg^{-1} with the median and mean concentrations of 0.024 and 0.091 mg kg^{-1}, respectively; these values have an upper range that is higher than previously reported. The highest grain Cd levels were found in rice grown in the dry season from two provinces located southeast of Phnom Penh along the Lower Mekong River, and their corresponding soil Cd levels were relatively higher than those collected during the wet season and around the Tonle Sap. While the source of higher Cd may be geogenic or due to anthropogenic activities, our data demonstrate that geographical and perhaps seasonal differences in grain Cd exist even within a small country that might not be reflected in market surveys.

■ INTRODUCTION

Cd is a toxic heavy metal and group I carcinogen that has a long biological half-life in humans and can cause kidney damage, cancer, osteoporosis, and cardiovascular disease.[1−5] Cd intake through food consumption is the major route for non-smokers, and rice in particular represents a significant portion because of its role as a staple food for billions of people.[6] Micronutrient (e.g., Fe, Zn, and Ca) deficiency can lead to more Cd absorption by rats or humans,[7−9] and white rice is typically low in these micronutrients. Historical rice Cd toxicity cases include *itai−itai* disease in Japan, which was caused by consumption of rice with high Cd content due to industrial Cd contamination in a rice-growing region.[10] The prevalence of Cd accumulation in rice is less-well studied compared to other contaminants (e.g., arsenic).

Cd is naturally present in soil at low background concentrations ranging from <0.1 to 2.0 mg kg^{-1},[9] but levels can be higher due to industrial release and deposition[11,12] and soil application of P fertilizer that has Cd impurity.[13] Regardless of the source, Cd can be solubilized depending on soil conditions. Cd mostly exists in soil solution in its divalent cationic form, and higher soil cation exchange capacity (CEC) enhances soil Cd retention and thus decreases Cd plant availability.[14] Cd is more plant available under highly weathered, acidic soil because Cd tends to precipitate as $Cd(OH)_2$ or $CdCO_3$ at higher pH.[15,16] However, in paddy soils where soil flooding buffers pH to near neutral, redox has an indirect control on Cd plant availability. Under flooded paddy conditions, Cd tends to precipitate as sparingly soluble CdS, but it can be mobilized under more oxic conditions when S^{2-} oxidizes to SO_4^{2-}.[17,18] If this occurs in highly weathered and acidic paddy

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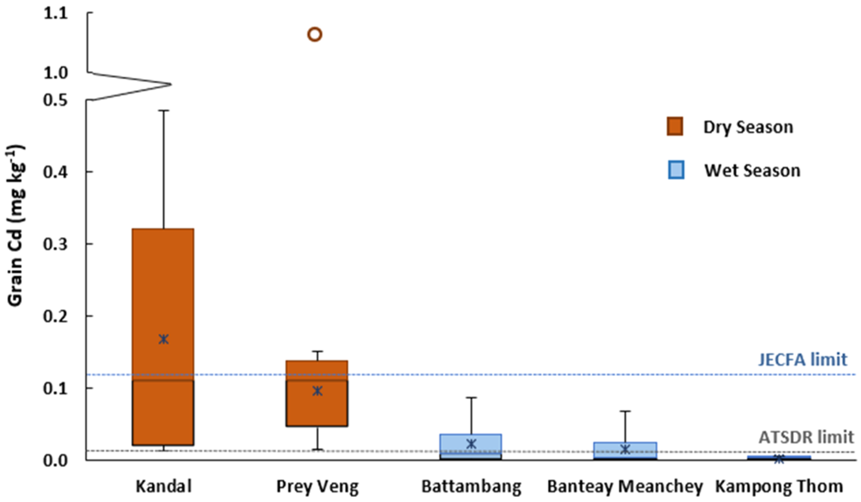

Figure 1. Rice grain Cd concentrations by province and season. Blue and gray broken lines represent the rice Cd limit for a 65 kg adult who consumes 450 g rice per day by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (5.8 μg kg^{-1} body weight per week) or US Agency for Toxic Substances and Diseases Registry (ATSDR) (0.7 μg kg^{-1} body weight per week), respectively.

soil, Cd^{2+} is available for plant uptake. It is therefore important to understand how water availability, soil Cd levels, and soil chemical factors affect grain Cd concentrations.

Some rice-producing regions may be prone to high grain Cd levels either due to proximity to industrial activities or because of rice production with less soil flooding, but little is known about Cambodia. For example, elevated rice Cd are found in metal-mining areas in parts of Hunan[19] and Guangdong[20,21] China, but no studies have reported geographical variation in Cambodia where rice is a major domestic crop and comprises over 70% of daily caloric intake. In addition, rice grown with limited water (i.e., higher soil Eh values) leads to higher rice grain Cd concentrations, which has been reported in multiple studies in China and Japan[17,22,23] but not in Cambodia. The tropical monsoonal climate brings Cambodia distinct wet and dry seasons, with a wet season typically from May to mid-November and the dry season from mid-November to April. Due to the limited irrigation infrastructure, Cambodian rice is typically grown in the floodplain of the Tonle Sap that swells during the wet season, but some rice is grown in the dry season along the Lower Mekong River. Therefore, rice grain Cd may be elevated in dry season Cambodian rice due to limited water availability and/or local droughts. However, to our knowledge, there is only one study that reports grain Cd levels from Cambodia; this market survey showed low grain Cd levels that ranged from 0.001 to 0.03 mg kg^{-1} based on 14 samples.[24] While market surveys are informative, there is often missing information about the timing (i.e., wet or dry season) and location (i.e., soil conditions) and of plant growth, which limits our understanding of the edaphic factors that influence the plant Cd levels. The soils of the rice-growing region in Cambodia are mostly highly weathered soil developed from old and recent alluvium or underlying parent material and are generally acidic with low soil organic matter (SOM) and CEC.[25] These soil conditions are likely to increase the plant availability of Cd particularly in the dry season, but there have been no reports of paired soil and rice samples across seasons from Cambodia.

Here, we examined the Cd content in rice grain, straw, and paddy soil, which were sampled in the wet or dry season from five major rice-growing provinces in Cambodia. We hypothesized that dry season rice would contain higher grain Cd than wet season rice in Cambodia due to the less water available for rice, which would result in more oxidizing soil conditions and higher plant-available Cd in the acidic soils. We further hypothesized that plant Cd concentrations would be positively correlated with soil Cd concentrations and negatively correlated with soil pH and CEC.

■ RESULTS

Rice Grain, Rice Straw, and Soil Cd Concentrations.

The rice Cd levels from Cambodia varied by province and season (Figure 1). Unpolished rice grain Cd concentrations ranged from 0.002 to 1.066 mg kg^{-1}, with a median concentration of 0.024 mg kg^{-1}. The mean of grain Cd of samples collected in wet and dry seasons was 0.018 ± 0.011 mg kg^{-1} ($n = 18$) and 0.196 ± 0.15 mg kg^{-1} ($n = 12$), respectively. Kampong Thom had the lowest grain Cd concentration of 0.002 mg kg^{-1}, but only one sample was collected from this province. Banteay Meanchey (wet season) had relatively low grain Cd concentration with a mean of 0.016 ± 0.014 mg kg^{-1} ($n = 8$), and Prey Veng (dry season) had the highest mean of 0.258 ± 0.291 mg kg^{-1} ($n = 6$). Rice straw Cd ranged from below detection to 1.95 mg kg^{-1}, and the median concentration was 0.098 mg kg^{-1}. The mean straw Cd was 0.056 ± 0.033 mg kg^{-1} ($n = 11$) for samples collected in the wet season and was 0.414 ± 0.500 mg kg^{-1} ($n = 16$) for those collected in the dry season (Table 1).

Soil physicochemical factors varied by province and season (Table 1). Soil DTPA-extractable Cd concentrations ranged from 0.002 to 0.109 mg kg^{-1} with a median of 0.006 and a mean of 0.024 ± 0.015 mg kg^{-1} ($n = 22$). The mean DTPA-extractable Cd of dry season soils was 0.088 ± 0.016 mg kg^{-1} ($n = 5$), and the mean of wet season soils was 0.006 ± 0.001 mg kg^{-1} ($n = 17$). Soil HNO_3-digestible Cd concentrations ranged from 0.007 to 0.214 mg kg^{-1} with a median of 0.025 mg kg^{-1} and a mean of 0.075 mg kg^{-1}. The mean of dry season soils was 0.180 ± 0.017 mg kg^{-1} ($n = 10$), and the mean of wet season soils was 0.020 ± 0.005 mg kg^{-1} ($n = 18$). Soil CEC ranged from 4.8 to 37.5 m eq 100 g^{-1} with a median of 12.5 and a mean of 14.8, with a dry season mean of 9.7 ± 1.6 m eq 100 g^{-1} ($n = 7$) and a wet season mean of 17.4 ± 3.8 m eq 100 g^{-1} ($n = 16$). Neither soil pH nor SOM had distinct seasonal patterns. Soil pH ranged from 5.0 to

Table 1. Grain, Straw, and Soil Cd Concentrations and Soil pH, CEC, and Organic Matter Content[a]

		grain	straw	soil-HNO_3 digestible	soil-DTPA extractable			
sampling time	sample ID	---------------------------------mg kg^{-1} Cd---------------------------------				soil pH	soil CEC meq $100g^{-1}$	SOM % by LOI
March	KD-1	0.274	0.568	0.125	0.066	6.4	12.3	2.7
	KD-2	0.191	0.556	0.175	ns	ns	Ns	4.8
	KD-3	0.266	0.698	Ns	ns	ns	Ns	ns
	KD-4	0.014	0.029	0.185	ns	ns	11.4	3.8
	KD-5	0.034	0.109	0.198	ns	ns	Ns	5.1
	KD-6	0.024	0.051	0.185	ns	ns	Ns	6.1
	PV-1	ns	1.95	0.174	0.089	5.7	9.2	5.0
	PV-2	ns	0.5	0.214	0.107	5.4	7.2	4.1
	PV-3	ns	0.075	0.196	ns	ns	11.1	4.3
	PV-4	1.066	ns	0.14	0.068	6.3	6.1	2.7
	PV-5	0.111	0.281	Ns	ns	ns	Ns	ns
	PV-6	0.078	ns	ns	ns	ns	Ns	ns
	PV-7	0.15	0.348	0.209	0.108	7.6	10.7	3.2
	PV-8	0.126	0.107	ns	ns	ns	Ns	ns
	PV-9	0.015	0.108	ns	ns	ns	Ns	ns
	Mean	**0.196**	**0.414**	**0.180**	**0.088**	**6.3**	**9.7**	**4.2**
	Median	**0.119**	**0.281**	**0.185**	**0.089**	**6.3**	**10.7**	**4.2**
August	K Thom-1	0.002	bdl	0.007	0.005	6.9	8.9	3.5
	Ban M-1	0.068	0.174	0.05	0.014	6.7	14.1	4.3
	Ban M-2	0.023	0.034	0.022	0.009	6.8	22.9	4.6
	Ban M-3	0.004	0.009	0.016	0.005	6.1	18.5	3.7
	Ban M-4	0.003	0.008	0.017	0.006	6.1	17.7	4.3
	Ban M-5	0.004	0.013	0.015	0.005	5.9	Ns	5.1
	Ban M-6	0.002	bdl	0.019	0.006	5.9	21.7	4.4
	Ban M-7	ns	0.029	0.015	ns	ns	25.8	5.1
	Ban M-8	0.025	0.054	0.007	0.002	6	4.8	2.1
	Ban M-9	0.002	bdl	0.01	0.004	6.4	6.3	3.3
	Bat-1	0.032	0.017	0.022	0.01	5	12.5	4.0
	Bat-2	0.005	ns	ns	ns	ns	Ns	ns
	Bat-3	0.002	bdl	0.014	0.002	6.3	16.7	3.0
	Bat-4	0.002	bdl	0.01	0.004	7	15.4	2.7
	Bat-5	0.019	0.055	0.017	0.007	5	17.7	3.9
	Bat-6	0.009	ns	ns	ns	ns	Ns	ns
	Bat-7	ns	bdl	ns	ns	ns	Ns	ns
	Bat-8	ns	bdl	0.029	0.006	8.2	37.5	3.1
	Bat-9	ns	bdl	0.029	0.008	5.8	Ns	4.9
	Bat-10	0.087	0.139	ns	ns	ns	Ns	ns
	Bat-11	0.003	bdl	0.025	0.005	5	Ns	4.6
	Bat-12	0.04	0.088	0.033	0.011	5.7	21	3.0
	Mean	**0.018**	**0.056**	**0.020**	**0.006**	**6.2**	**17.4**	**3.9**
	Median	**0.005**	**0.034**	**0.017**	**0.006**	**6.1**	**17.7**	**3.9**
All	**Mean**	**0.091**	**0.25**	**0.075**	**0.024**	**6.2**	**14.8**	**4.0**
	Median	**0.024**	**0.098**	**0.025**	**0.006**	**6.1**	**12.5**	**4.0**

[a] ns = no sample; bdl = below detection limit.

8.2 with a median of 6.1 and a mean of 6.2, and SOM ranged from 2.1 to 6.1% with both median and mean of 4.0% (Table 1).

Relation between Plant Cd and Soil Parameters. Regression analyses were performed between grain and/or straw Cd and measured soil parameters. The logarithms of grain ($R^2 = 0.71$, $p < 0.0001$) and straw ($R^2 = 0.44$, $p = 0.04$) Cd were positively correlated with the logarithm of DTPA-extractable soil Cd, and the logarithms of grain ($R^2 = 0.42$, $p = 0.001$) and straw ($R^2 = 0.44$, $p = 0.02$) Cd were positively correlated with the logarithm of acid-digestible soil Cd concentrations (Figure 2). No significant relationships were found between grain or straw Cd and soil pH or CEC (Figure 3). Significant positive linear relationships were also observed between the grain Cd and straw Cd ($R^2 = 0.93$, $P < 0.0001$) and soil DTPA-extractable Cd and soil nitric acid digestible Cd ($R^2 = 0.99$, $P < 0.0001$). We performed multiple linear regression and principal component analysis with our data, and these analyses confirmed that total Cd and DTPA-extractable Cd were the significant predictors of plant Cd levels (data not shown).

■ DISCUSSION

Despite the heavy reliance on rice both in production and food supply in Cambodia, to our knowledge, only one study has reported Cd levels in market-surveyed rice[24] and none has reported paired rice and soil samples from across Cambodia. Because Cd tends to be more plant available in acidic soil under less flooded conditions,[16−18] we hypothesized that rice grain Cd would be higher in rice grown during the dry season than the wet

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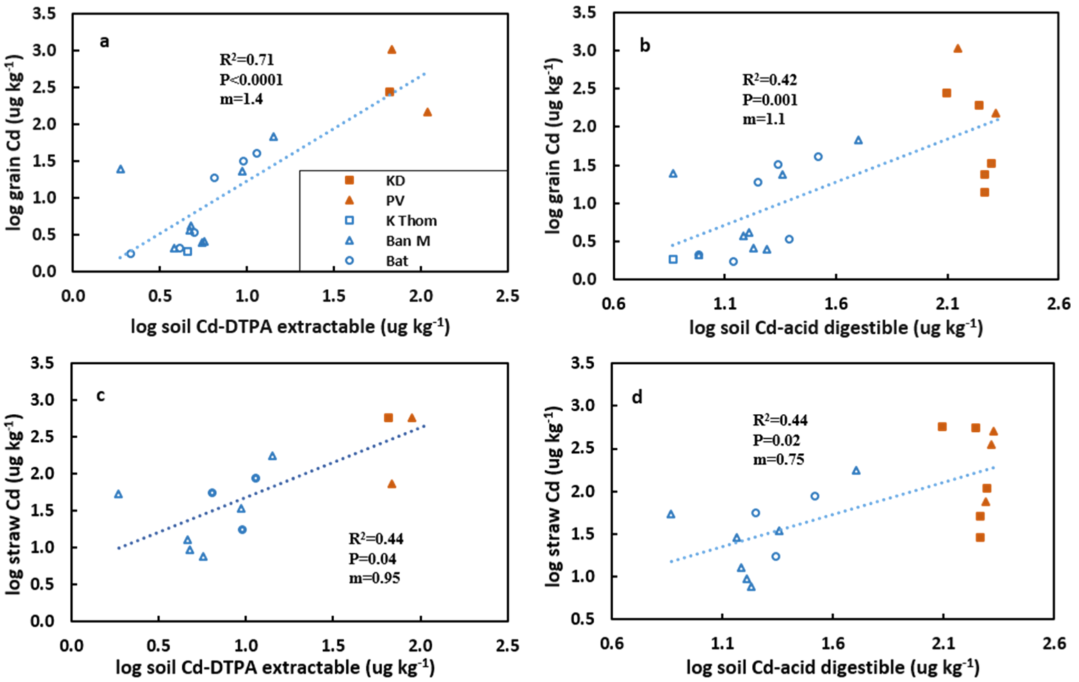

Figure 2. Relationships between the logarithms of grain Cd and soil DTPA-extractable Cd (a) or soil acid digestible Cd (b), and straw Cd and soil DTPA-extractable Cd (c) or soil acid digestible Cd (d). Note that the samples collected during the wet season are shown in blue and those collected during the dry season are shown in orange.

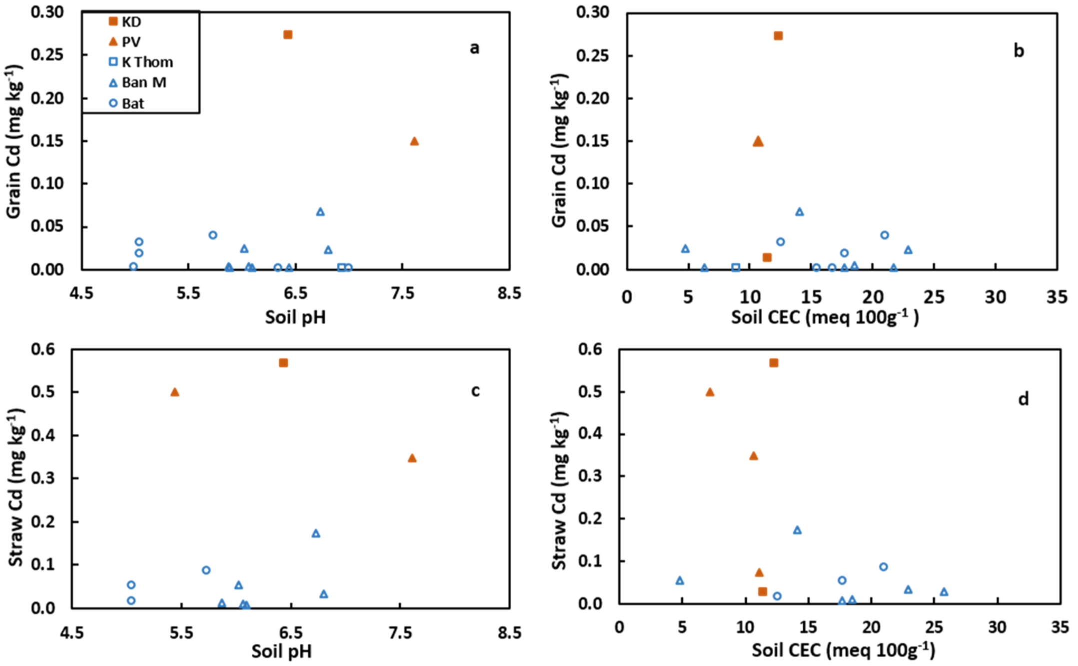

Figure 3. Relationships between grain Cd and soil pH (a) or soil CEC (b), straw Cd and soil pH (c), or soil CEC (d). None of these correlations were statistically significant. Note that the samples collected during the wet season are shown in blue and those collected during the dry season are shown in orange.

season. We further hypothesized that plant Cd would be inversely related to soil pH and CEC, as has been shown previously.[14−16,34,35] We examined paired rice and soil samples from across five provinces in Cambodia and found that plants collected in the wet season had lower Cd than those collected in the dry season, which supports our hypothesis. However, our hypothesis that grain or straw Cd would be inversely related to soil pH and CEC was not supported by our data. Instead, plant Cd was driven by soil Cd levels and potentially also by soil redox status caused by wet or dry season growth. Moreover, our data show that the health risk from Cambodian rice might be higher than previously reported.

Human Health Implications. The unpolished grain Cd levels reported here ranged from 0.002 to 1.066 mg kg^{-1}, which was higher than the range of 0.001 to 0.03 mg kg^{-1} previously reported in a market survey by Meharg et al.[24] Because our grain was unpolished, it may have slightly higher levels compared to white rice. Previous work suggests that polishing may decrease rice grain Cd by 20−40%.[24] Even after accounting for a 40% difference between brown and white rice, the grain Cd we measured from Prey Veng and Kandal provinces grown in the dry season was still higher than that reported in the Meharg et al.[24] study, which suggests that perhaps the previous market survey did not have samples from these provinces. Our data indicates that the risk of Cd from rice in Cambodia may be higher than previously thought. According to the weekly human Cd intake thresholds of 5.8 μg kg^{-1} body weight suggested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA),[36] grain Cd levels of 0.12 mg kg^{-1} would be the upper limit of grain Cd levels based on a 65 kg individual who consumes 450 g rice per day, 7 days per week (i.e., a typical Cambodian diet), and who does not ingest Cd from any other source. Our data show that 33% of rice samples grown in the dry season contain grain Cd above this safe limit, whereas all other samples including those collected in the wet season are below this threshold. If instead of the Cd weekly intake thresholds 0.7 μg kg^{-1} body weight suggested by US Agency for Toxic Substances and Diseases Registry (ATSDR)[5] is used, then grain Cd of 0.014 mg kg^{-1} would be the upper limit grain Cd levels; 89% of dry season rice and 39% of wet season rice samples exceed this limit (Figure 1).

Geographical Location. The highest soil, grain, and straw levels were obtained from Kandal and Prey Veng provinces, which were located adjacent to the Lower Mekong River southeast of Phnom Penh. This is in contrast to lower levels found in samples around the Tonle Sap. There was one rice grain sample from Prey Veng province with the Cd concentration as high as 1.066 mg kg^{-1} and one straw sample also from Prey Veng with the Cd concentration as high as 1.95 mg kg^{-1}, while soil Cd in these sites were not excessively high. The high plant Cd in these two samples may have arisen due to atmospheric deposition of Cd-rich dust or cultivars that are efficient in plant transfer.[6,24] Ignoring the outlier, grain Cd from Kandal and Prey Veng provinces was 3 orders of magnitude higher than previously reported in a market survey.[24] The higher soil Cd from Kandal and Prey Veng provinces may be due to geogenic differences between the sampling locations that result in different soil mineral compositions;[9,37] in other areas, high soil Cd is associated with alluvial deposits.[38,39] However, higher soil Cd in these provinces could also arise from the proximity of Kandal and Prey Veng sample locations to industrial activities near Phnom Penh.[40] While identifying the source of high soil Cd is beyond the scope of the present study, additional work on source tracking could elucidate the mechanism(s) for high soil Cd from these provinces. Nevertheless, our data show that the high soil Cd led to highest grain Cd levels, which may also be due to seasonal impacts.

Seasonality. Higher grain Cd concentrations were found in samples from the dry season than those from the wet season (Figure 1, 0.118 and 0.005 mg kg^{-1}, respectively), and this supports our hypothesis. This finding suggests at least partial (but indirect) redox control on plant-available Cd due to likely drier soil conditions during the dry season than the wet season and limited irrigation infrastructure in the region. Drier soil conditions are well known to increase the rice Cd levels in acidic soils,[17] especially during rice heading,[41] and it is likely that dry season rice experienced drier soil conditions than wet season rice in Cambodia;[42] however, more detailed field study of soil redox potentials throughout the season would be needed to confirm this. In this work, different rice cultivars were grown across the country, which limits our ability to directly compare soil conditions in one genotype. Indeed, Cd accumulation has been shown to vary across genotypes.[43] While our data support that wet season rice has lower Cd than dry season rice, we cannot disentangle the impact of seasonality on soil Cd levels in this work.

Soil Chemical Factors. Despite the dependence of Cd plant availability on soil pH[44] and soil CEC,[14] we did not observe significant relationships between rice grain or straw Cd and soil pH or CEC (Figure 3); instead, DTPA-extractable or acid-extractable Cd in soil were stronger predictors of plant Cd levels. This finding may be because many of the samples were collected from wet season rice, and the indirect effect of redox on plant-available Cd may have been stronger than pH or CEC effects.[15] The average soil pH of 6.2 indicates slightly acidic soil conditions, and the average soil organic content (3.95%) was slightly higher than what had been previously reported.[25] Despite this, no obvious trends were observed between plant Cd and pH or CEC. Instead, we observed strong and significant positive correlations between grain (Figure 2a) and straw (Figure 2c) Cd and DTPA-extractable soil Cd, which were expected because DTPA-extractable Cd is considered representative of the plant-available soil pool and correlates with the Cd accumulated by plants.[29] We also observed strong and significant positive correlations between grain (Figure 2b) and straw (Figure 2d) Cd and acid-digestible Cd, which represents soil total Cd,[29] and this is likely because the plant-available Cd pool correlated with the total Cd pool. These data suggest that both DTPA-extractable and total soil Cd are predictors of Cd levels in grain and straw. One of the limitations of this study was that the soil redox potentials of the rice paddies during rice-growing stages were not measured and can only be inferred due to seasonality. Nevertheless, the seasonal influence on rice grain Cd content may have reflected how soil redox status affected rice Cd uptake with wetter conditions resulting in lower soil redox potential and lower plant Cd and drier conditions resulting in higher soil redox potential and higher plant Cd.[35] While we could not perform correlations separately by season due to small sample size, the data suggest that the correlations between soil Cd and plant Cd were stronger for wet season than for dry season rice, the latter of which also has higher soil Cd levels. Future work should attempt to disentangle the impacts of soil redox potential driven by wet or dry season growth and soil Cd levels in predicating Cambodian rice concentrations.

■ EXPERIMENTAL SECTION

Sample Collection and Preparation. Paired rice (*Oryza sativa* L.) plant tissue and paddy soil samples were obtained from major rice-growing regions in Cambodia in March or August 2011 and previously reported for arsenic content.[26] The sampling sites were family owned small-scale rice paddies (ca. 1000 m^2) located in five provinces including Kandal (KD), Prey Veng (PV), Battambang (Bat), Banteay Meanchey (Ban M), and Kampong Thom (K Thom). Samples from KD and PV were located near the Lower Mekong River and obtained at the end of the dry season in March, while the others were obtained in the wet season in August around the Tonle Sap. Note that in Cambodia at the time of sampling, limited irrigation infrastructure resulted in rice being planted when water naturally reached the fields due to the monsoon-driven swelling and shrinking of the Tonle Sap and Mekong River. Therefore, no rice crop was present in the dry season around the Tonle Sap.

Sampling and processing details were reported in Seyfferth et al., 2014.[26] Briefly, rice and soil were obtained from at least three locations in each field and composited into one sample per field. Unpolished grain was separated from husk and straw and each was ground using stainless steel grinders. Soil was air-dried and sieved prior to analysis. A total of 30 rice grains, 33 straws, and 28 soil samples were analyzed here.

Rice Cd Analysis. Unpolished rice grain and straw samples were microwave-digested in concentrated trace metal grade (TMG) HNO_3 in Teflon digestion vessels (CEM Corp., Matthews, NC, USA) in a closed-vessel digestion system (MARS 6, CEM Corp.) following the established protocols.[27,28] During the digestion, the vessels were ramped to 200 °C in 20 min and held for 15 min and then allowed to cool. After digestion, the acid fraction was separated from non-dissolved Si-gel and diluted to 4% nitric acid matrix for total Cd analysis using inductively coupled plasma mass spectrometry (ICP−MS). The grain samples were analyzed on a Thermo iCap-TQ in the KED mode, whereas straw samples were analyzed with an Agilent 7500 equipped with a He collision cell. NIST1568a-certified rice, standard checks, and method blanks were included for assuring data quality. The Cd recovery of NIST1568a-certified rice was 95.3% on the Thermo iCAP-TQ and 90.7% on the Agilent 7500.

Soil Sample Analysis. The <2 mm sieved soil fractions were used for diethylenetriaminepentaacetic acid (DTPA) extraction to assess plant-available Cd.[29] For this, 10 mL of DTPA extraction solution (0.005 M DTPA, 0.1 M triethylamine, and 0.01 M $CaCl_2$) was added to 5 g of soil and shaken for 2 h. After centrifugation, the supernatant was passed through a 0.45 μm filter, acidified, and analyzed for Cd concentration via ICP−MS (Agilent 7500). Method blanks, duplicates, and check standards were used to ensure the data quality. The duplicates had 1.2 ± 0.3% error.

The same soil samples were also tested for acid-digestible Cd, soil pH, SOM, and CEC. For total Cd, soils were digested with concentrated TMG HNO_3 in a microwave digestion system using the EPA method 3051A,[30] where samples were ramped up to 175 °C in 5.5 min and held for 4.5 min. After centrifugation, the digest was diluted and analyzed for Cd with ICP−MS (Agilent 7500). Method blank, check standards, and NIST 2711-certified soil were used for data quality assurance. The NIST 2711-certified soil had 82% Cd recovery, likely because some of the Cd was associated with soil components that did not dissolve with HNO_3. Soil pH was measured in 1:10 (v/v) soil/water ratio,[31] SOM content was approximated by the percentage of loss of ignition (LOI),[32] and soil CEC at pH 7.0 was determined following the method by Ross (1995).[33]

Statistical Analysis. Regression analysis, multiple linear regression, and principal components analysis were performed to investigate the relations between rice Cd and Cd and soil parameters with JMP pro 15.

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Notes

The authors declare no competing financial interest.

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