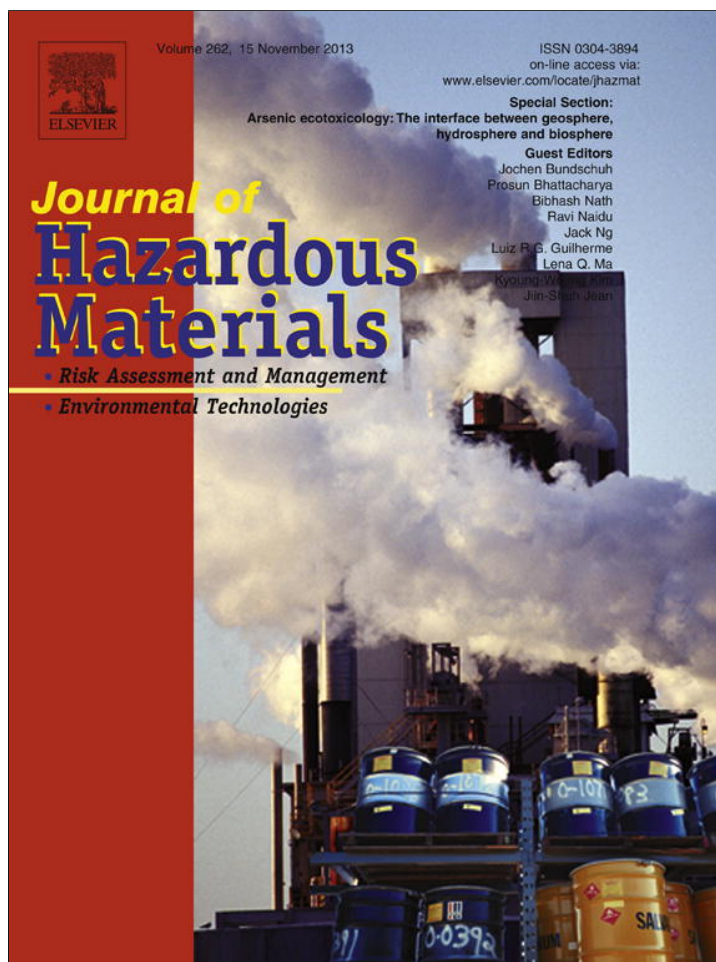


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Arsenic contamination in the food chain and its risk assessment of populations residing in the Mekong River basin of Cambodia

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HIGHLIGHTS

- ▶ We examined the arsenic exposure from daily food consumption in Cambodia.
- ▶ Paddy soil, rice, vegetable and fish were analyzed for total arsenic ([As]_{tot}).
- ▶ A positive correlation of the [As]_{tot} in paddy soil and paddy rice was found.
- ▶ Daily dose of inorganic arsenic ([As]_i) from daily food consumption was estimated.
- ▶ Kandal residents are at risk of [As]_i intake from their daily food consumption.

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ABSTRACT

In the present study, we investigated the potential arsenic exposure of Cambodian residents from their daily food consumption. Environmental and ecological samples such as paddy soils, paddy rice (unhusked), staple rice (uncooked and cooked), fish and vegetables were collected from Kandal, Kratie and Kampong Cham provinces in the Mekong River basin of Cambodia. After acid-digestion, digestates were chemically analyzed by inductively coupled plasma mass spectrometry. Results revealed that the means of total arsenic concentration ([As]_{tot}) in paddy soils and paddy rice from Kandal were significantly higher than those from Kampong Cham province (*t*-test, *p* < 0.05). Moreover, a significant positive correlation between the [As]_{tot} in paddy soils and paddy rice was found (*r*(14) = 0.826, *p* < 0.01). Calculations of arsenic intake from food consumption indicated that the upper end of the range of the daily dose of inorganic arsenic for Kandal residents (0.089–8.386 μg d⁻¹ kg⁻¹ body wt.) was greater than the lower limits on the benchmark dose for a 0.5% increased incidence of lung cancer (BMDL_{0.5} is equal to 3.0 μg d⁻¹ kg⁻¹ body wt.). The present study suggests that the residents in Kandal are at risk of arsenic intake from their daily food consumption. However, the residents in Kratie and Kampong Cham provinces are less likely to be exposed to arsenic through their daily dietary intake. To the best of our knowledge, this is the first report estimating the daily intake and daily dose of inorganic arsenic from food consumption in the Mekong River basin of Cambodia.

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1. Introduction

Chronic exposure to naturally occurring arsenic-rich groundwater has generally threatened and impaired the well-being of millions of people in South and Southeast Asia. For instance, it is

estimated that about 40 million people suffer from arsenic through the groundwater drinking pathway in India and Bangladesh [1]. Similarly, high concentrations of arsenic are also reported in Vietnam [2–4], Lao PDR [5,6] and Cambodia [7–12]. Toxicological studies have shown that chronic exposure to high arsenic concentrations in drinking water has led to dermatological manifestations (raindrop pigmentation, melanosis and hyperkeratosis) and skin cancer [13]. The outcomes of acute arsenic toxicity might include gastrointestinal discomfort, abdominal pain, vomiting, diarrhea, bloody urine, shock, coma and death [14,15]. A dose–response criteria such as no observable adverse effect level (NOAEL), lowest

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observable adverse effect level (LOAEL), and benchmark dose (lowest effect dose for 5% or 1% effect level, based on daily quality, expressed as LED₀₅ or LED₀₁) have also been observed [15]. One-hundred-twenty milligrams of arsenic trioxide is reported to be the most common minimum lethal dose of humans; however, it can vary from 70 to 180 mg (1–3 mg As kg⁻¹) [15]. Likewise, epidemiological studies indicate that ingested and inhaled arsenic can cause skin cancer and lung cancer, respectively. In addition, a number of studies have also shown that arsenic ingestion leads to internal cancer and cancer of the urinary bladder [15]. As a consequence, many approaches have been proposed to assess arsenic risks from drinking water because it has been identified and characterized as a toxin and carcinogen to humans and animals.

Our cross-sectional health risk assessment of inorganic arsenic intake of people residing in the Mekong River basin of Cambodia revealed that 98.65% of residents in the Kandal province study area were confronted with arsenic toxicity. Moreover, cancer risk index was found to average 5 in 1000 exposure [16]. Concurrently, individual variations in arsenic accumulation in human body were observed among the exposed populations while plotting arsenic content in scalp hair, fingernail and toenail with average daily dose (ADD) of arsenic, which was calculated through the groundwater drinking pathway using USEPA health risk assessment models [17]. These variations were explained by several factors. An assumption of high intake of arsenic was that people might ingest an additional amount of arsenic through their daily food consumptions while they were consuming arsenic-rich groundwater [16]. In fact, rice is a dietary staple of Cambodian people, and it is normally eaten three times per day. It was believed that rice grown in the arsenic-contaminated soils had greater arsenic concentration in the grain than that grown the arsenic-free soils. Pot experiments indicate that about 81% of the recovered arsenic in rice is found as inorganic arsenic species [18]. However, a gastrointestinal digestion simulation study revealed that arsenate bioavailability in cooked rice ranged from 63 to 99% [19]. Moreover, arsenic bioavailability in rice is highly dependent on its species in rice, irrigation water and cooking water [20].

Cambodia is an agriculture-based country, and most Cambodians live in rural areas alongside watersheds. They totally relied on their extensive farming methods, rain-fed flooding of paddies, for their rice productions in the past. However, some households in the Mekong River basin have recently gained access to shallow groundwater through the inexpensive and easily drilled boreholes. In addition to using this secured source of water for daily drinking water, some households have changed their traditional farming to an intensive one using shallow groundwater irrigation systems. However, a number of studies have reported that shallow groundwater in the Mekong River basin of Cambodia is highly contaminated with arsenic and other toxic trace elements [7,16,21,22]. The irrigation of paddy fields with arsenic-rich shallow groundwater may lead to accumulation of arsenic in paddy soils and potentially affect the production yield and rice quality. Although the mechanisms of arsenic transfer from arsenic-contaminated irrigation water to paddy soils and the transfer from arsenic-contaminated paddy soils to rice remained unclear [23], the study of arsenic contamination in the food chain may provide additional evidence and explanations for the variations in individual arsenic accumulations. Therefore, the objectives of the present study were to (1) investigate arsenic distributions and correlations between paddy soils and paddy rice in the Mekong River basin of Cambodia, (2) determine and compare a distribution of the total arsenic concentration ([As]_{tot}) among the three staple foodstuffs, namely rice, vegetable and fish, and (3) estimate the daily intake and daily dose of inorganic arsenic from daily food consumption of arsenic exposed populations.

2. Materials and methods

2.1. Study area

The design of the present project was a cross-sectional study. Sampling was carried out in three provinces in the Mekong River basin of Cambodia. Kratie (Sambok village, Sambok commune, Kracheh district) and Kampong Cham (Veal Sbov village, Ampil commune, Kampong Siem district) are located along the Mekong River upstream of Phnom Penh whereas Kandal (Preak Russey village, Kampong Kong commune, Koh Thom district) is located between the Mekong River and Bassac River, downstream of Phnom Penh (Fig. 1).

2.2. Field sampling and sample preparation

Sampling was conducted twice for the present study. The first batch of sampling was carried out in February 2009, when paddy soils and paddy rice were collected from households where groundwater was used to irrigate their paddy fields in Kandal (paddy soils $n=8$; paddy rice $n=8$) and Kampong Cham (paddy soils $n=8$; paddy rice $n=8$). Paddy soils and paddy rice were separately packed in paper bags in the field and transported to a laboratory in the Department of Chemistry, Royal University of Phnom Penh, Cambodia. Paddy soil samples were dried in the open air under diffused sunlight at room temperature for 48 h, and sieved (10 mesh) to separate debris. Paddy rice was manually ground with mortar and winnowed to separate the grain from its husk. The grain was then ground to fine powder. The second batch of sampling was carried out in March 2011, when staple rice (uncooked rice $n=10$; cooked rice $n=10$) and five types of popular fruit vegetables (cucumber $n=3$; gourd $n=3$; papaya $n=3$; pumpkin $n=3$; tomato $n=3$) were collected from the visited households and family gardens, respectively. Concurrently, two kinds of popular fish (snakehead fish $n=5$; catfish $n=5$) were collected from the natural habitats where people usually fish in the study areas of Kampong Cham and Kandal provinces. In addition, sampling was extended to Kratie (uncooked rice $n=10$; cooked rice $n=10$; snakehead fish $n=5$; catfish $n=5$; gourd $n=3$; papaya $n=3$; pumpkin $n=3$), which is known as a moderately arsenic-contaminated area [16]. However, cucumber and tomato were not available over there at the time of sampling. All rice, fruit vegetable and fish samples were kept separately in labeled plastic ziplock bags, placed in a cold box in the field, and transferred into a refrigerator where they were stored at 4 °C until further treatment. After thawing at room temperature for several hours, fruit vegetables were peeled and cut into small pieces with a quartz knife. Fish was dissected; viscera were removed and only the edible parts were taken. All rice, vegetable and fish samples were washed with deionized water and dried in the open air under diffused sunlight for 24 h; next, samples were oven-dried at 50 °C for several days to achieve complete dryness, and they were subsequently manually ground to a fine powder with a mortar and passed through 35-mesh sieve. Each sample was repacked in a labeled plastic ziplock bag and shipped to GIST, Korea for chemical analyses.

2.3. Sample analyses

Paddy soil samples were digested using a modified Aqua Regia method. Briefly, 0.50 g of the dried paddy soil was accurately weighed into a 15 mL polyethylene tube. Exactly 3.75 mL of HCl (36%) and 1.25 mL of HNO₃ (65%) were added and the mixture was allowed to stand overnight. In the next morning, the mixture was heated at 96 ± 3 °C for an hour using a heating block. After cooling down at room temperature, 5.00 mL of deionized water was added, after which it was centrifuged at 1824 × *g*-force (3000 rpm, rotor

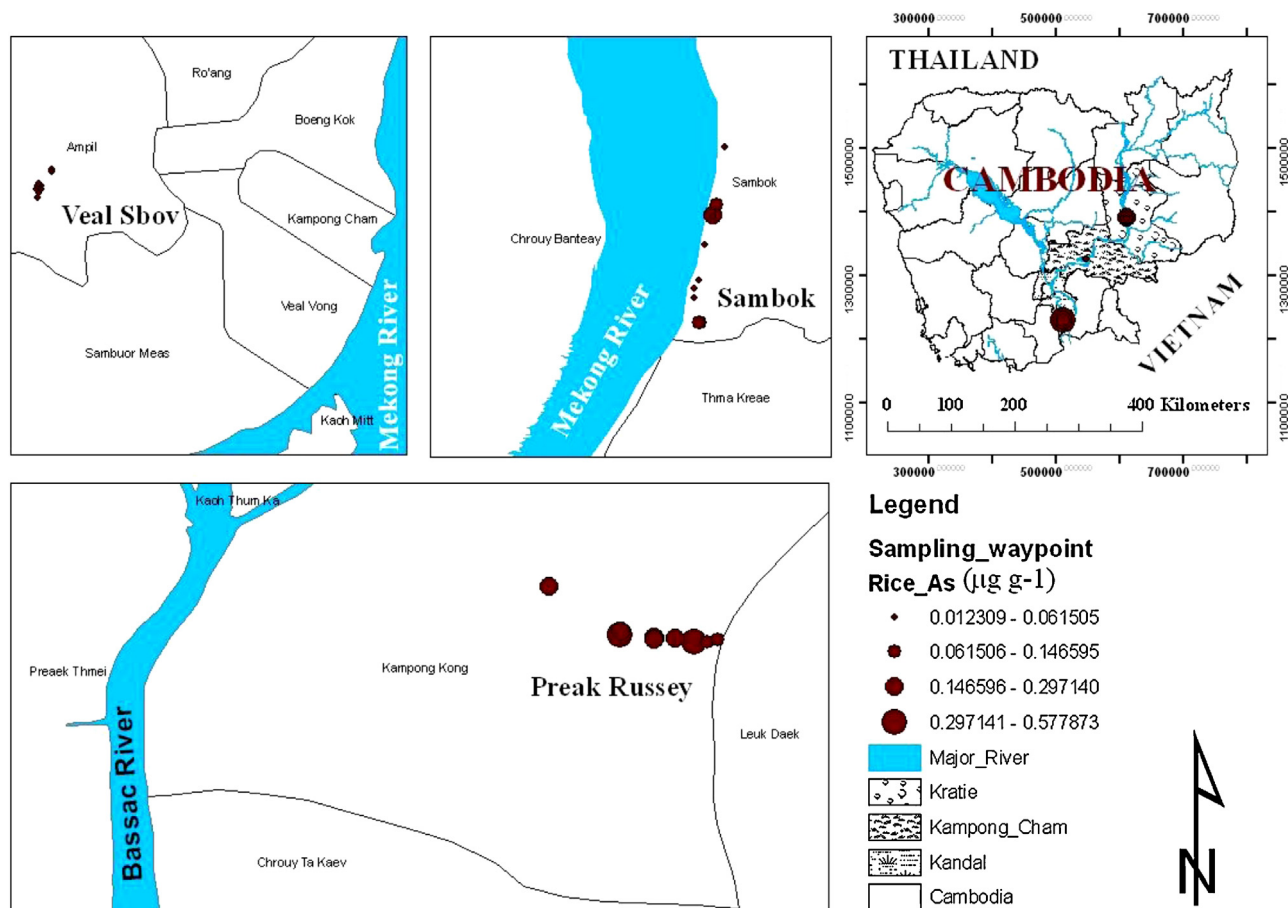


Fig. 1. Map of the study areas.

radius 181.3 mm) for 10 min (HANIL HA-1000-3, Republic of Korea). The supernatant was transferred to a fresh tube and stored at 4 °C. Since the supernatant was highly acidic and contained high concentrations of elements, dilution with 18.2 MΩ MilliQ deionized water (1:30) was made before analysis. Similarly, acid-digestion was performed for all foodstuff samples. Briefly, 0.10 g of rice or fish was weighed into a 15 mL polyethylene tube. One mL of the concentrated HNO₃ (65%) was added into each tube. The tube was then capped and left in a hood at room temperature. After 48 h, 9.00 mL of deionized water was added and filtered (0.45 μm) into a fresh tube. Similarly, 0.10 g of vegetable was weighed into a 15 mL polyethylene tube, 2.00 mL of HNO₃ (65%) was added into each tube, and the mixture was allowed to stand for 24 h in a hood at room temperature; subsequently, 2.00 mL of H₂O₂ (30%) was added, and the final mixture was heated (TAITEC Dry Thermo Unit DTU-2C, Japan) at 100 °C for 30 min. Heating was continued until the digestate became clear and made up to 10 mL volume. Finally, digestate was filtered (0.45 μm) into a fresh tube. All filtrates were stored in a refrigerator at 4 °C until analysis. All chemical

measurements of the [As]_{tot} were made by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 ce).

2.4. Data quality control and Statistical analyses

Digestions of two replicated samples were conducted. A number of Standard Reference Materials (NIST, 1568a: Rice flour; 1570a: Trace elements in Spinach leaves; 2976: Mussel tissue; 2710a: Montana I soil) were also treated in the same manner as the samples to verify the accuracy of the digestion methods. The recovery rates from the digestions were in the good agreement with the certified values (Table 1). Statistical data analyses were employed by SPSS for windows (Version 13.0). An Independent *t*-test was applied to assess the regional difference in [As]_{tot} in paddy soils and paddy rice in Kandal and Kampong Cham province study areas. Likewise, it was also used to verify the difference in [As]_{tot} in snakehead fish and catfish species in each study area. A strength of correlation between the [As]_{tot} in paddy soils and paddy rice was measured by a Pearson correlation coefficient (*r*). In addition, a Paired Samples

Table 1
Certified values (mg kg⁻¹) and recovery rates (%) of arsenic from acid digestion of various Standard Reference Materials (SRM).

SRM	Certified values	Recovery rate (%)			Average
		Trial 1	Trial 2	Trial 3	
1568a	0.290	106.460	100.810	104.000	103.757
1570a	0.068	96.340	77.900	101.280	91.840
2976	13.300	90.470	90.080	87.590	89.380
2710a	0.154	117.780	109.900	105.710	111.130

1568a: Rice flour; 1570a: Trace elements in Spinach leaves; 2976: Mussel tissue; 2710a: Montana I Soil.

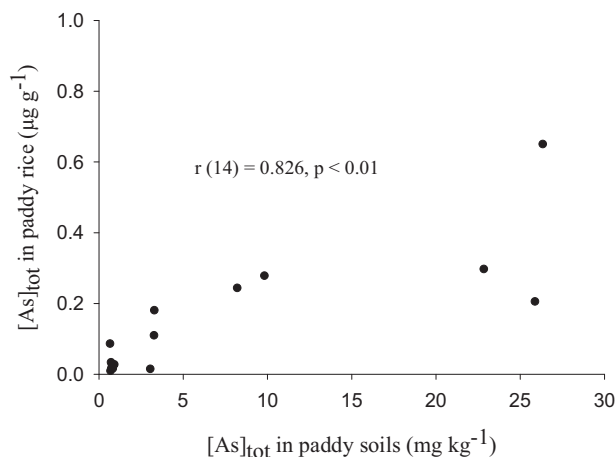


Fig. 2. Correlation between total arsenic concentrations ($[As]_{tot}$) in paddy soils and rice grains in the Mekong River basin of Cambodia.

t-test was performed to verify the difference in $[As]_{tot}$ in uncooked and cooked rice in each study area. One-Way ANOVA tests and post hoc tests (Tukey HSD and Games-Howell) were conducted to certify the regional differences in $[As]_{tot}$ in all foodstuffs among the three province study areas. Significance was considered in circumstances where $p < 0.05$.

2.5. An estimation of the daily intake of inorganic arsenic

In order to estimate the daily intake of inorganic arsenic from daily food consumption, inorganic arsenic concentration ($[As]_i$) was assumed to be 10% [24,25], 70% [25] and 80% [18,25–27] of the $[As]_{tot}$ in fish, vegetable and rice, respectively. The daily intake of inorganic arsenic ($\mu\text{g d}^{-1}$) from fish, vegetable and rice were calculated by multiplying the $[As]_i$ in fish ($\mu\text{g g}^{-1}$) by the fish consumption rate (g d^{-1}), the $[As]_i$ in vegetable ($\mu\text{g g}^{-1}$) by the vegetable consumption rate (g d^{-1}) and the $[As]_i$ in rice ($\mu\text{g g}^{-1}$) by the rice consumption rate (g d^{-1}). The $[As]_{tot}$ in fish, vegetable and rice were determined in this study, however, the consumption rates of fish, vegetable and rice were obtained from field surveys. Surveys showed that rural Cambodian people normally eat rice three times a day (approximately 450 g d^{-1}), 300 g of fish per week (42.86 g d^{-1}) and 500 g of fruit vegetable per week (71.43 g d^{-1}). The average body weight (52 kg) of residents in the Mekong River basin of Cambodia was obtained from our previous report [16]. The daily dose of inorganic arsenic ($\mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$) from daily food consumption was determined by dividing the daily intake of inorganic arsenic ($\mu\text{g d}^{-1}$) by the average body weight (kg) of residents in the study areas.

3. Results

3.1. Arsenic concentrations in paddy soils and paddy rice

Chemical measurements of the $[As]_{tot}$ in paddy soils and paddy rice are presented in Table 2. The mean of $[As]_{tot}$ in paddy soils in Kandal was $12.858 \pm 10.430 \text{ mg kg}^{-1}$ whereas it was $0.794 \pm 0.088 \text{ mg kg}^{-1}$ in Kampong Cham. The mean of $[As]_{tot}$ in paddy rice in Kandal was $0.247 \pm 0.187 \mu\text{g g}^{-1}$ whereas it was $0.029 \pm 0.024 \mu\text{g g}^{-1}$ in Kampong Cham. Further analysis showed that the means of $[As]_{tot}$ in paddy soil and paddy rice in Kandal province study area were significantly higher than those in Kampong Cham (*t*-test, $p < 0.05$) (Table 2). Moreover, a significantly positive correlation ($r(14) = 0.826$, $p < 0.01$) between $[As]_{tot}$ in paddy soils and paddy rice was observed (Fig. 2).

3.2. Arsenic concentrations in daily foodstuffs

Analytical results of the $[As]_{tot}$ in all foodstuffs are presented in Table 3. The mean of $[As]_{tot}$ in uncooked rice in Kandal was $0.256 \pm 0.141 \mu\text{g g}^{-1}$ whereas the mean of $[As]_{tot}$ in cooked rice was $0.255 \pm 0.343 \mu\text{g g}^{-1}$. Similarly, the mean of $[As]_{tot}$ in uncooked rice in Kratie was $0.075 \pm 0.049 \mu\text{g g}^{-1}$ whereas the mean of $[As]_{tot}$ in cooked rice was $0.079 \pm 0.057 \mu\text{g g}^{-1}$. The means of $[As]_{tot}$ in uncooked rice ($0.024 \pm 0.012 \mu\text{g g}^{-1}$) and cooked rice ($0.012 \pm 0.011 \mu\text{g g}^{-1}$) were relatively low in the Kampong Cham province study area. A comparison revealed that there were significant regional differences in $[As]_{tot}$ in both uncooked and cooked rice among the three province study areas (One-Way ANOVA test, Table 4). In addition, a pairwise comparison revealed that the $[As]_{tot}$ in the uncooked rice was significantly higher than that in the cooked one in Kampong Cham (Paired Samples *t*-test, $t(9) = 9.503$, $p < 0.01$). However, there were no significant differences in $[As]_{tot}$ in uncooked and cooked rice in Kandal (Paired Samples *t*-test, $t(9) = 0.021$, $p = 0.984 > 0.05$) and Kratie (Paired Samples *t*-test, $t(9) = -0.270$, $p = 0.793 > 0.05$). There were significant regional differences in the means of $[As]_{tot}$ in fish among Kandal ($0.178 \pm 0.034 \mu\text{g g}^{-1}$), Kratie ($1.502 \pm 1.837 \mu\text{g g}^{-1}$) and Kampong Cham ($0.080 \pm 0.004 \mu\text{g g}^{-1}$) (One-Way ANOVA test, Table 4). Games-Howell post hoc test analyses revealed that there were significant regional differences in $[As]_{tot}$ in snakehead fish between Kandal and Kratie ($p = 0.042$), Kandal and Kampong Cham ($p < 0.01$) and Kratie and Kampong Cham ($p = 0.036$). Post hoc Tukey HSD tests also indicated that there were significant regional differences in $[As]_{tot}$ in catfish between Kandal and Kratie ($p < 0.01$), Kandal and Kampong Cham ($p < 0.01$) and Kratie and Kampong Cham ($p = 0.011$). In addition, *t*-test verified statistically significant differences between the $[As]_{tot}$ in snakehead fish and catfish species in Kandal ($t = 10.167$, $df = 4.889$, $p < 0.01$) and Kratie ($t = 3.944$, $df = 4.000$, $p = 0.017$); however, there was no significant difference between the $[As]_{tot}$ in snakehead fish and catfish species in Kampong Cham ($t = 2.463$, $df = 4.623$, $p = 0.061 > 0.05$). These results suggest that arsenic accumulations in fish vary among fish species and their natural habitats, which are consistent to other studies [28,29]. Likewise, significant regional differences in the means of $[As]_{tot}$ in vegetables among Kandal ($0.062 \pm 0.048 \mu\text{g g}^{-1}$), Kratie ($0.020 \pm 0.012 \mu\text{g g}^{-1}$) and Kampong Cham ($0.043 \pm 0.033 \mu\text{g g}^{-1}$) were observed (One-Way ANOVA test, Table 4).

3.3. Inorganic arsenic intake from daily food consumption

A summary of the daily intake and daily dose of inorganic arsenic are presented in Table 5. The average daily intake of inorganic arsenic from rice of the Kandal residents ($91.784 \pm 123.436 \mu\text{g d}^{-1}$) was much greater than those in the Kratie ($28.590 \pm 20.691 \mu\text{g d}^{-1}$) and Kampong Cham ($4.484 \pm 3.840 \mu\text{g d}^{-1}$) province study areas. Likewise, the average daily intake of inorganic arsenic from fruit vegetables of the Kandal residents ($3.080 \pm 2.391 \mu\text{g d}^{-1}$) was higher than those in Kratie ($0.995 \pm 0.580 \mu\text{g d}^{-1}$) and Kampong Cham ($1.688 \pm 1.638 \mu\text{g d}^{-1}$). However, the average daily intake of inorganic arsenic from fish was highest in Kratie ($6.435 \pm 7.872 \mu\text{g d}^{-1}$) relative to Kandal ($0.765 \pm 0.144 \mu\text{g d}^{-1}$) and Kampong Cham ($0.341 \pm 0.018 \mu\text{g d}^{-1}$). The average daily dose of inorganic arsenic from total daily food consumption for the residents in the Kandal province study area ($1.839 \pm 2.423 \mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$) was higher than those in the Kratie ($0.693 \pm 0.560 \mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$) and Kampong Cham ($0.125 \pm 0.106 \mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$) province study areas. In addition, the upper end of the arsenic intake range (0.089 – $8.386 \mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$) was greater than the lower limits on the benchmark dose for a 0.5% increased incidence

Table 2
A comparison of the total arsenic concentrations in paddy soil (mg kg^{-1}) and paddy rice ($\mu\text{g g}^{-1}$) in Kandal ($n=8$) and Kampong Cham ($n=8$).

Variables	Mean \pm SD	Median	Range	<i>t</i>	<i>df</i>	<i>p</i>
Paddy soil				3.271	7.001	0.014
Kandal	12.858 \pm 10.430	9.040	3.070–26.360			
Kampong Cham	0.794 \pm 0.088	0.780	0.680–0.930			
Paddy rice				3.261	7.229	0.013
Kandal	0.247 \pm 0.187	0.224	0.014–0.649			
Kampong Cham	0.029 \pm 0.024	0.025	0.008–0.085			

The *t* and *df* were adjusted because variances were not equal; SD: standard deviation.

Table 3
Mean, median and range of the total arsenic concentrations ($\mu\text{g g}^{-1}$) in all foodstuffs in each of the study areas.

Foodstuffs	Kandal			Kratie ^a			Kampong Cham		
	Mean \pm SD	Median	Range	Mean \pm SD	Median	Range	Mean \pm SD	Median	Range
Rice									
Uncooked ($n=10$)	0.256 \pm 0.141	0.231	0.088–0.578	0.075 \pm 0.049	0.061	0.012–0.171	0.024 \pm 0.012	0.020	0.014–0.048
Cooked ($n=10$)	0.255 \pm 0.343	0.135	0.010–1.189	0.079 \pm 0.057	0.073	0.005–0.190	0.012 \pm 0.011	0.007	0.004–0.031
Fish									
Catfish ($n=5$)	0.148 \pm 0.004	0.147	0.144–0.155	0.086 \pm 0.002	0.085	0.084–0.088	0.077 \pm 0.005	0.076	0.071–0.083
Snakehead ($n=5$)	0.209 \pm 0.013	0.216	0.193–0.222	2.918 \pm 1.605	3.424	0.094–3.993	0.082 \pm 0.001	0.083	0.081–0.083
All ($n=10$)	0.178 \pm 0.034	0.174	0.144–0.222	1.502 \pm 1.837	0.091	0.084–3.993	0.080 \pm 0.004	0.081	0.071–0.083
Vegetable									
Cucumber ($n=3$)	0.131 \pm 0.009	0.128	0.123–0.141				0.085 \pm 0.046	0.066	0.053–0.137
Gourd ($n=3$)	0.043 \pm 0.017	0.033	0.032–0.062	0.026 \pm 0.004	0.023	0.023–0.031	0.017 \pm 0.006	0.018	0.010–0.021
Papaya ($n=3$)	0.089 \pm 0.042	0.093	0.045–0.128	0.026 \pm 0.014	0.019	0.016–0.042	0.020 \pm 0.003	0.021	0.017–0.022
Pumpkin ($n=3$)	0.011 \pm 0.002	0.010	0.010–0.013	0.009 \pm 0.005	0.007	0.004–0.014	0.022 \pm 0.015	0.018	0.009–0.038
Tomato ($n=3$)	0.035 \pm 0.013	0.043	0.020–0.043				0.025 \pm 0.012	0.023	0.014–0.038
All ($n=15$)	0.062 \pm 0.048	0.043	0.010–0.141	0.020 \pm 0.012	0.019	0.004–0.042	0.034 \pm 0.033	0.021	0.009–0.137

^a Cucumber and tomato were not available at the time of sampling; SD: standard deviation.

of lung cancer (BMDL_{0.5} of $2\text{--}7 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$ is equal to $3.0 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$) [25].

4. Discussion

Analytical results indicated that $[\text{As}]_{\text{tot}}$ in paddy soils were significantly higher in Kandal than Kampong Cham (*t*-test, $p < 0.05$). It is likely that higher $[\text{As}]_{\text{tot}}$ in paddy soils are consistent with higher As concentrations in groundwater. In fact, our previous study showed that groundwater arsenic concentrations in the Kandal province study area ranged from 247.08 to 1841.50 $\mu\text{g L}^{-1}$ ($n=46$, mean = $846.14 \pm 298.11 \mu\text{g L}^{-1}$) whereas it ranged from 0.12 to 2.37 $\mu\text{g L}^{-1}$ ($n=18$, mean = $1.28 \pm 0.58 \mu\text{g L}^{-1}$) in Kampong Cham

[16]. Because the residents in the Mekong River basin of Cambodia have had access to shallow groundwater, they have grown rice twice per year—rain-fed rice in the rainy season and groundwater-fed rice in the dry season. However, paddy fields in the Kandal province study area are annually flooded by the overflow of the Basac River water during the rising river stage in the rainy season. The residents in this area can grow rice whenever the river water moves out their paddy fields. Flooding has been known to reduce available oxygen levels, leading to reducing conditions in the surface and subsurface soils. These conditions enable rapid mobilization of arsenic, in the form of arsenite, into the soil solution [30]. The positive significant correlation between $[\text{As}]_{\text{tot}}$ in paddy soil and paddy rice ($r(14)=0.826$, $p < 0.01$) suggests that arsenic in paddy soils can be adsorbed, transported and accumulated in rice grains

Table 4
One-Way Analysis of Variance comparing regional groups on the total arsenic concentrations in uncooked rice, cooked rice, fish and vegetable.

Sources	<i>df</i>	SS	MS	<i>F</i>	<i>p</i>
Uncooked rice					
Between groups	2	0.298	0.149	19.907	0.000
Within groups	27	0.202	0.007		
Total	29	0.501			
Cooked rice					
Between groups	2	0.314	0.157	3.889	0.033
Within groups	27	1.089	0.040		
Total	29	1.403			
Fish					
Between groups	2	12.607	6.303	5.604	0.009
Within groups	27	30.372	1.125		
Total	29	42.978			
Vegetable					
Between groups	2	0.011	0.006	4.173	0.023
Within groups	36	0.048	0.001		
Total	38	0.059			

df: degree of freedom; SS: sum of squares; MS: mean square.

Table 5Summary of $[As]_{tot}$ ($\mu\text{g g}^{-1}$), $[As]_i$ ($\mu\text{g g}^{-1}$), daily intake ($\mu\text{g d}^{-1}$) and daily dose ($\mu\text{g kg}^{-1} \text{d}^{-1}$) of inorganic arsenic concentration in each of the study areas.

Foodstuffs	Kandal			Kratie			Kampong Cham		
	Mean \pm SD	Median	Range	Mean \pm SD	Median	Range	Mean \pm SD	Median	Range
Rice									
$[As]_{tot}$	0.255 \pm 0.343	0.135	0.010–1.189	0.079 \pm 0.057	0.073	0.005–0.190	0.012 \pm 0.011	0.007	0.004–0.031
$[As]_i$	0.204 \pm 0.274	0.108	0.008–0.951	0.064 \pm 0.046	0.059	0.004–0.152	0.010 \pm 0.009	0.005	0.003–0.025
Daily intake	91.784 \pm 123.436	48.510	3.546–428.108	28.590 \pm 20.691	26.398	1.917–68.360	4.484 \pm 3.840	2.413	1.478–11.124
Daily dose	1.765 \pm 2.374	0.933	0.068–8.233	0.550 \pm 0.398	0.508	0.037–1.315	0.086 \pm 0.074	0.046	0.028–0.214
Fish									
$[As]_{tot}$	0.178 \pm 0.034	0.174	0.144–0.222	1.502 \pm 1.837	0.091	0.084–3.993	0.080 \pm 0.004	0.081	0.071–0.083
$[As]_i$	0.018 \pm 0.003	0.017	0.014–0.022	0.150 \pm 0.184	0.009	0.008–0.399	0.008 \pm 0.000	0.008	0.007–0.008
Daily intake	0.765 \pm 0.144	0.746	0.618–0.951	6.435 \pm 7.872	0.390	0.358–17.115	0.341 \pm 0.018	0.347	0.305–0.358
Daily dose	0.015 \pm 0.003	0.014	0.012–0.018	0.124 \pm 0.151	0.008	0.007–0.329	0.007 \pm 0.000	0.007	0.006–0.007
Vegetable									
$[As]_{tot}$	0.062 \pm 0.048	0.043	0.010–0.141	0.020 \pm 0.012	0.019	0.004–0.042	0.034 \pm 0.033	0.021	0.009–0.137
$[As]_i$	0.043 \pm 0.033	0.030	0.007–0.098	0.014 \pm 0.008	0.013	0.003–0.029	0.024 \pm 0.023	0.015	0.006–0.096
Daily intake	3.080 \pm 2.391	2.151	0.480–7.030	0.995 \pm 0.580	0.960	0.190–2.085	1.688 \pm 1.638	1.071	0.450–6.865
Daily dose	0.059 \pm 0.046	0.041	0.009–0.135	0.019 \pm 0.011	0.018	0.004–0.040	0.032 \pm 0.031	0.021	0.009–0.132
All (foods)									
Daily intake	95.629 \pm 125.971	51.407	4.644–436.089	36.021 \pm 29.143	27.748	2.465–87.560	6.513 \pm 5.496	3.831	2.233–18.347
Daily dose	1.839 \pm 2.423	0.989	0.089–8.386	0.693 \pm 0.560	0.534	0.047–1.684	0.125 \pm 0.106	0.074	0.043–0.353

Inorganic arsenic was assumed to be 10% in fish, 80% in rice and 70% in vegetable. The daily consumption rates of fish, rice and fruit vegetable were 42.86 (g d^{-1}), 450 (g d^{-1}) and 71.43 (g d^{-1}), respectively. The average body weight of Cambodia residents was 52 (kg).

during plantation. The elevated $[As]_{tot}$ in paddy soils and paddy rice in Kandal province study area are consistent to a number of previous studies in arsenic-affected areas elsewhere [31–34].

The present study also investigated the distribution of arsenic concentrations in the staple foodstuffs of the residents in the Mekong River basin of Cambodia. The mean $[As]_{tot}$ in uncooked rice from the Kandal province study area ($n=10$, mean = $0.256 \pm 0.141 \mu\text{g g}^{-1}$, median = $0.231 \mu\text{g g}^{-1}$) is comparable to that in the arsenic-affected areas of Northern [35] ($n=10$, mean = $0.225 \mu\text{g g}^{-1}$) and Southern Vietnam [36] ($n=39$, mean = $0.225 \mu\text{g g}^{-1}$, median = $0.201 \mu\text{g g}^{-1}$), Bangladesh [37] ($n=46$, mean = $0.358 \mu\text{g g}^{-1}$) [38], ($n=18$, mean = $0.34 \pm 0.15 \mu\text{g g}^{-1}$) and India [39] ($n=34$, mean = $0.239 \mu\text{g g}^{-1}$). It is also quite close to the $[As]_{tot}$ in rice collected from different regions of Bangladesh [32] ($n=330$, 0.08 – $0.51 \mu\text{g g}^{-1}$) and Brazil [40] ($n=44$, mean = $0.223 \mu\text{g g}^{-1}$), as well as the $[As]_{tot}$ in rice in the markets of France ($n=33$, mean = $0.28 \mu\text{g g}^{-1}$, median = $0.23 \mu\text{g g}^{-1}$) and the United States ($n=163$, mean = $0.25 \mu\text{g g}^{-1}$, median = $0.25 \mu\text{g g}^{-1}$) [41]. However, the means of the $[As]_{tot}$ in uncooked rice in Kratie ($n=10$, mean = $0.075 \pm 0.049 \mu\text{g g}^{-1}$, median = $0.061 \mu\text{g g}^{-1}$) and Kampong Cham ($n=10$, mean = $0.024 \pm 0.012 \mu\text{g g}^{-1}$ and median = $0.020 \mu\text{g g}^{-1}$) are lower than those from markets (not specifically collected from arsenic-contaminated areas) of China, Egypt, Italy, Japan, Spain and Thailand [41].

Analytical results also showed that there was a significant difference in $[As]_{tot}$ in uncooked and cooked rice in Kampong Cham province (Paired Samples t -test, $p < 0.01$). This finding suggests that rice washing before cooking with arsenic-safe water might reduce arsenic from arsenic-laden rice. In fact, rural Cambodian people normally wash their rice three times before cooking with an aluminum cooking pot and charcoals and/or firewood. This finding is consistent to that observed by Sengupta et al. [42]. However, the present study revealed that there was no significant difference in uncooked and cooked rice in the Kandal and Kratie province study areas (Paired Samples t -test, $p > 0.05$). This might be due to some households in Kandal and Kratie using arsenic-rich groundwater for cooking. In rural parts of Cambodia, people normally cook their rice with adequate water, and no excess water is discarded during the cooking process. It is likely that $[As]_{tot}$ would be increase in cooked rice, if the rice was cooked with arsenic-rich groundwater. Field

observation indicated that some households in Kandal do in fact use arsenic-rich groundwater to cook their food when they cannot access safe alternative water sources. In fact, piped water, which is directly pumped from the Bassac River, had been distributed in the Kandal province study area (Preak Russey Village) since 2007, but not all households access it since local water vendors cannot supply enough water for the whole village. In addition, some families cannot afford a payment of the connection fee to get the piped line into their houses.

The daily intake and daily dose of inorganic arsenic were estimated in the present study by assuming 10%, 70% and 80% of the measured $[As]_{tot}$ to be the $[As]_i$ in fish, vegetable and rice, respectively. The calculation indicated that the average daily dose of inorganic arsenic from total daily food consumption of the residents in Kandal province study area ($1.839 \pm 2.423 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$) was higher than those in Kratie ($0.693 \pm 0.560 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$) and Kampong Cham ($0.125 \pm 0.106 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$). This finding is consistent to our previous study, which found that there was a significant regional difference in arsenic intake from the groundwater drinking pathway among the residents of Kandal, Kratie and Kampong Cham provinces [16]. The daily dose of inorganic arsenic from the groundwater drinking pathway in Kandal, which ranged from 0.19 to $10.75 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$ ($3.50 \pm 2.46 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$) [16,17], is higher than the daily dose of inorganic arsenic from food consumption. However, the daily dose of inorganic arsenic from food consumption is greater than the daily dose of inorganic arsenic from the groundwater drinking pathway in Kratie (0.0004 – $0.6261 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$, average = $0.0996 \pm 0.1637 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$) and Kampong Cham (0.0003 – $0.0221 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$, average = $0.0053 \pm 0.0044 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$) [16,17]. In addition, the upper end of the range of the daily dose of inorganic arsenic for the residents in Kandal is greater than the lower limits on the benchmark dose for a 0.5% increased incidence of lung cancer ($\text{BMDL}_{0.5}$ of 2 – $7 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$ is equal to $3.0 \mu\text{g d}^{-1} \text{kg}^{-1} \text{body wt.}$) [25]. It is clear that the residents in Kandal ingest an additional amount of inorganic arsenic from their daily food consumption, which may have influences the rapid development of various arsenicosis symptoms described by Sampson et al. [11]. However, the potential intake of inorganic arsenic of the residents in Kratie

and Kampong Cham provinces is more likely from food consumption rather than from a groundwater drinking pathway. Recently, Hanh et al. [36] reports that male and female residents of Southern Vietnam ingest inorganic arsenic from 28 to 102 $\mu\text{g d}^{-1}$, equivalent to the daily dose of 0.6–1.9 $\mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$ after they stopped using groundwater in 2008. The present study clearly indicates that residents in the Kandal province of Cambodia ingest higher amount of inorganic arsenic than those in the Southern Vietnam. Likewise, the residents in Kandal ingest higher amounts of inorganic arsenic than those in Ronphibun of Thailand [43] (15.8–146 $\mu\text{g d}^{-1}$, average = $82.4 \pm 27.8 \mu\text{g d}^{-1}$, equivalent to the daily dose of 0.31–2.01 $\mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$, average = $1.43 \pm 0.40 \mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$ from a duplicate diet study), Bangladesh [37] (19–232 $\mu\text{g d}^{-1}$ from cooked rice only) and India [39] (141–179 $\mu\text{g d}^{-1}$, average of the daily intake of arsenic from only rice and vegetable in Bengal delta).

5. Conclusions

Analytical results showed that the means of $[\text{As}]_{\text{tot}}$ in paddy soils and paddy rice in Kandal were significantly higher than those in Kampong Cham (t -test, $p < 0.05$). Moreover, a significant positive correlation ($r(14) = 0.826$, $p < 0.01$) between $[\text{As}]_{\text{tot}}$ in paddy soil and paddy rice was found, suggesting that arsenic in paddy soils could be adsorbed, transported and accumulated in rice grains during plantation. A comparison demonstrated that there were significant regional differences in $[\text{As}]_{\text{tot}}$ in both uncooked and cooked rice among the three study areas (One-Way ANOVA tests, $p < 0.05$). In addition, $[\text{As}]_{\text{tot}}$ in uncooked rice was significantly higher than that in cooked rice in Kampong Cham (Paired Samples t -test, $p < 0.01$), suggesting that rice washing with arsenic-safe water before cooking might reduce arsenic from arsenic-laden rice. An estimation of the daily intake and daily dose of inorganic arsenic from food consumption indicated that the upper end of the range of the daily dose of inorganic arsenic of the residents in Kandal (0.089–8.386 $\mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$) was greater than the lower limits on the benchmark dose for a 0.5% increased incidence of lung cancer (BMDL_{0.5} is equal to 3.0 $\mu\text{g d}^{-1} \text{ kg}^{-1} \text{ body wt.}$). The present study undoubtedly suggests that residents in Kandal are at risk of inorganic arsenic intake from their daily food consumption. However, the residents in Kratie and Kampong Cham are less likely to be exposed to arsenic through their daily dietary intake. The rapid development of arsenicosis in Kandal province might be due to the ingestion of excessive amounts of arsenic from either groundwater drinking pathway or daily food consumption, in particular rice irrigated with arsenic-rich shallow groundwater. Although some mitigating actions of arsenic risks from drinking arsenic-rich groundwater have been taken into account, arsenic in rice is substantially/equally dangerous. The development of information, education and communication (IEC) materials may play a vital role to educate people about their health risks from consumption of both arsenic-rich groundwater and arsenic-laden rice. In addition, the change of agricultural practices may reduce the arsenic burden in rice. Therefore, more attention should be paid to the development of irrigation systems in the arsenic-affected areas.

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