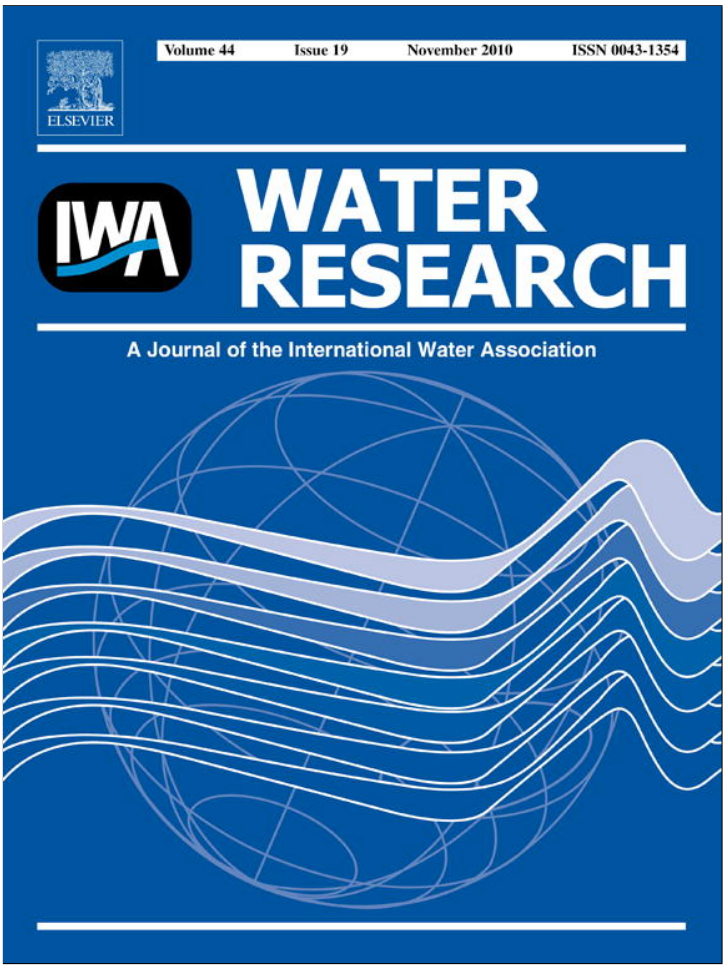

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Health risk assessment of inorganic arsenic intake of Cambodia residents through groundwater drinking pathway

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ABSTRACT

In order to compare the magnitudes and health impacts of arsenic and other toxic trace elements in well water, groundwater and hair samples were collected from three areas with different arsenic exposure scenarios in the Mekong River basin of Cambodia. Ampil commune in Kampong Cham province was selected as an uncontaminated area, Khsarch Andaet commune in Kratie province was selected as a moderately contaminated area, and Kampong Kong commune in Kandal Province was selected as an extremely contaminated area. Results of ICP-MS analyses of the groundwater samples revealed that As, Mn, Fe and Ba concentrations were significantly different among the three study areas (Kruskal–Wallis test, $p < 0.0001$). Out of 46 observed wells in the Kandal province study area, 100% detected As $> 50\ \mu g\ L^{-1}$ and Fe $> 300\ \mu g\ L^{-1}$; 52.17% had Mn $> 400\ \mu g\ L^{-1}$ and 73.91% found Ba $> 700\ \mu g\ L^{-1}$. In the Kratie province study area ($n = 12$), 25% of wells showed elevated arsenic levels above $10\ \mu g\ L^{-1}$ and 25% had Mn $> 400\ \mu g\ L^{-1}$, whereas samples from Kampong Cham province study area ($n = 18$) were relatively clean, with As $< 10\ \mu g\ L^{-1}$. A health risk assessment model derived from the USEPA was applied to calculate individual risks resulting from drinking groundwater. Computational results indicated that residents from Kandal Province study area ($n = 297$) confronted significantly higher non-carcinogenic and carcinogenic risks than those in Kratie ($n = 89$) and Kampong Cham ($n = 184$) province study areas (Kruskal–Wallis test, $p < 0.0001$). 98.65% of respondents from the Kandal province study area were at risk for the potential non-cancer effect and an average cancer risk index was found to be 5 in 1000 exposure. The calculations also indicated that, in the Kratie province study area, 13.48% of respondents were affected by non-cancer health risks and 33.71% were threatened by cancer, whereas none of respondents in the Kampong Cham province study area appeared to have non-carcinogenic effect. Positively significant correlations of the arsenic content in scalp hair (As_h) with both arsenic levels in

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groundwater (As_w) (r_s (304) = 0.757, $p < 0.0001$) and individual average daily doses (ADD) of arsenic (r_s (304) = 0.763, $p < 0.0001$) undoubtedly indicated that arsenic accumulation in the bodies of Cambodia residents in the Mekong River basin was mainly through a groundwater drinking pathway. To the best of our knowledge, this is the first comprehensive report comparing individual health risk assessments of arsenic exposure through a groundwater drinking pathway to enriched arsenic levels from groundwater in the Mekong River basin, Cambodia. This study indicates that elevated arsenic concentrations in groundwater may lead to thousands of cases of arsenicosis in the near future if mitigating actions are not taken.

1. Introduction

The widespread switch from microbiologically-unsafe surface water to microbiologically-unsafe groundwater has led to the unanticipated poisoning of large numbers of people in the developing world who have consumed various toxic trace elements. In particular, elevated concentrations of arsenic in groundwater have been reported in Taiwan (Tseng, 1977; Tseng et al., 1968), West Bengal (India) and Bangladesh (Das et al., 1994; Mandal et al., 1998; Nickson et al., 1998), resulting in a major public health issue. In Bangladesh and West Bengal (India), it is estimated that approximately 40 million people are suffering from drinking naturally occurring arsenic-rich shallow groundwater (Gault et al., 2008). Despite these concerns, groundwater is still a major source of drinking water in the developing world, especially in Southeast Asia. Recently, unsafe levels of arsenic have also been revealed in Vietnam (Berg et al., 2007, 2001; Buschmann et al., 2007; Nguyen et al., 2009) and Cambodia (JICA, 1999; Polya et al., 2003, 2005).

Individuals can be exposed to arsenic through several pathways, but the most critical one is daily diet and drinking water ingestion. Toxicological studies show that both trivalent and pentavalent soluble arsenic compounds are rapidly absorbed from the gastrointestinal tract and can be further metabolized. Reduction of As (V) to As (III) followed by oxidative methylation of As (III) takes place to form mono-, di- or trimethylated products (Hughes, 2002). Oral pathway exposure of organic arsenic compounds is less toxic since organic arsenicals are less extensively metabolized and more rapidly eliminated in urine than inorganic arsenicals (WHO, 2004; ATSDR, 2007). There is no evidence that arsenic is essential in human bodies. In contrast, chronic oral consumption of arsenic is considered to cause an adverse impact on human beings, known as “arsenicosis” or “arsenic poisoning disease”. Arsenicosis can cause skin lesions, pigmentation of the skin, and the development of hard patches of skin on the palm of the hands and soles of the feet. Arsenic poisoning finally leads to skin, bladder, kidney, and lung cancers, as well as diseases of the blood vessels of the legs and feet. Diabetes, high blood pressure and reproductive disorders may also be the side effects of chronic arsenic exposure (Tseng, 1977; WHO, 2004; ATSDR, 2007).

In Cambodia, unsafe levels of arsenic in shallow groundwater were first reported by JICA (1999) in its first unpublished draft report, named “The study on groundwater development in Southern Cambodia”, to the Cambodia Ministry of Rural Development. Consequently, numerous studies have been conducted and documented. Polya et al. (2003, 2005); Stanger et al. (2005); Berg et al. (2007); Buschmann et al. (2007); Quicksall et al. (2008); Sthiannopkao et al. (2008) & Luu et al. (2009) have described the distribution of arsenic in shallow Cambodian groundwater. The chemical, biological and physical processes that control the heterogeneous arsenic distribution in groundwater have also been widely studied (Polya et al., 2003, 2005; Rowland et al., 2008; Lear et al., 2007; Berg et al., 2007; Buschmann et al., 2007; Benner et al., 2008; Kocar et al., 2008; Polizzotto et al., 2008, Robinson et al., 2009). Arsenic treatment systems, modified from traditional sand filters, have also been developed to enhance arsenic removal from groundwater following seasonal and spatial variations in groundwater composition (Chiew et al., 2009).

In addition, studies of baseline concentrations of As in human hairs, nails and urine have been used to assess potential biomarkers of As exposure (Kubota et al., 2006; Berg et al., 2007; Gault et al., 2008; Sampson et al., 2008). The development of visual arsenicosis symptoms have been generally assumed to follow 8–10 years of consumption of water with unsafe level of arsenic; however, new cases discovered in Cambodia have followed exposure times as short as three years, due to extremely elevated arsenic levels (3500 $\mu g\ L^{-1}$), socioeconomic status, and malnutrition (Sampson et al., 2008). In Kandal alone, by using groundwater quality and population data, Sampson et al. (2008) have estimated that 100,000 people are at high risk of chronic arsenic exposure.

The objectives of the present study were (i) to determine the distribution of toxic trace elements in groundwater of the Mekong River basin of Cambodia, (ii) to determine the heterogeneous distribution of arsenic species in groundwater, (iii) to assess non-carcinogenic and carcinogenic risks among the populations exposed to arsenic through groundwater drinking pathways, (iv) to correlate the arsenic content in scalp hair (As_h) with arsenic levels in groundwater (As_w) and individual average daily doses (ADD) of arsenic, and (v) to compare the extent of health impacts with respect to different levels of arsenic-rich groundwater.

2. Materials and methods

2.1. Study area

The design of the present study was a cross-sectional study. Sampling was carried out within three purposely selected

areas with different arsenic exposure scenarios in the Mekong River basin of Cambodia. Kampong Kong commune (Preak Russey & Lvea Toung villages) in Kandal province was selected as an extremely contaminated area. Khsarch Andaet commune (Preak Samrong I & II villages) in Kratie province was selected as a moderately contaminated area, and Ampil commune (Andoung Chros & Veal Sbov villages) in Kampong Cham province was selected as an uncontaminated area. Kratie and Kampong Cham provinces are located along the Mekong River upstream of Phnom Penh, whereas Kandal province is located between the Mekong and the Bassac Rivers, downstream of Phnom Penh (Fig. 1).

2.2. Sample collection

After our research proposal was approved by the National Ethics Committee for Health Research (NECHR) under the Ministry of Health of the Kingdom of Cambodia and informed consent was obtained, groundwater samples were collected from the study areas of Kandal ($n = 46$) and Kampong Cham ($n = 18$) in February 2009 and Kratie province ($n = 12$) in August 2009. Concurrently, in the first batch of sampling, some scalp hair specimens were also sampled from the study areas of Kandal and Kampong Cham provinces, while the remainder were collected in the second batch. Sampling was conducted based upon the accessibilities to tube wells, the willingness of respondents to provide hair samples, and respondent claims of tube well use for a certain period of time. Each groundwater sample was collected from a tube well after 5–10 min of flushing in order to remove any standing water from the tube. Groundwater was filled in three separate polyethylene bottles for different purposes of analyses. Raw samples (no pretreatment) were analyzed for total arsenic. Filtered water samples (0.45 μm pore sized membrane filter) were analyzed for soluble arsenic and other trace elements. Inorganic As (III) was determined from the 0.45 μm filtered water samples which passed through disposable arsenic speciation cartridges. Simultaneously, on-site measurements for additional parameters were taken by using a *HORIBA pH/Cond meter D-54* (pH and Eh) and a *Thermo ORION 3 star* (conductivity and temperature). During field sampling, all of the collected water samples were kept in an ice box and then transferred to a refrigerator where they were stored at 4 °C until delivery to GIST, South Korea for analysis. Hair samples were randomly collected from several members of each household where people claimed to routinely use a tube well. Hair was cut from the nap of the head, as near as possible to the scalp, using stainless steel scissors. The collected hair samples were kept in labeled ziplock bags and stored in darkness until analyses.

2.3. Sample preparation and analyses

Groundwater samples from the Kandal province study area were diluted in order to analyze the concentrations of total As, As species, Mn and Ba. Dilution (1:25) was made for the final concentration of aliquots to meet the standard calibration curve as recommended by ICP-MS analytical technique. Dilution was done with 2% HNO_3 (prepared by 18.2 Ω *Milli*Q deionized water with 70% HNO_3); however, other trace elements were analyzed with original samples. Similarly, some samples

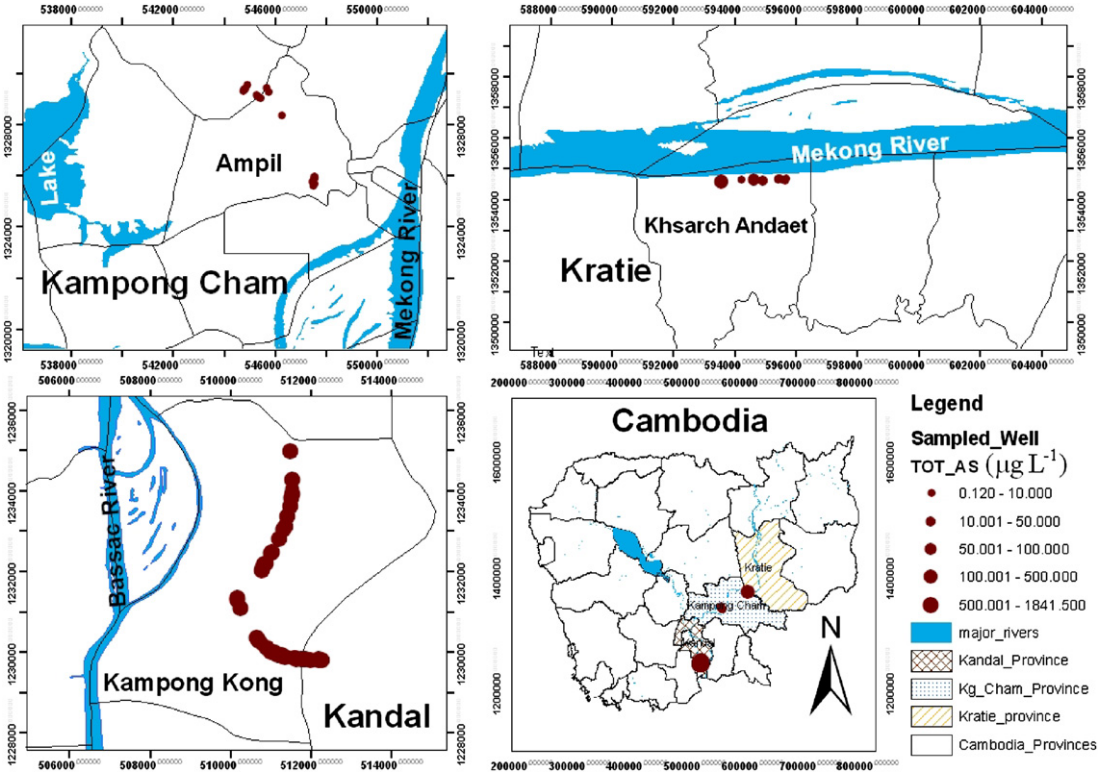

Fig. 1 – Map of groundwater sampling sites.

from the Kratie province study area were treated in the same manner, while all of groundwater samples from the Kampong Cham study area were analyzed without any treatment, aside from centrifuging for total arsenic analysis. Hair specimens were cut into small pieces (0.3 cm) and washed with the recommended method (Ryabukhin, 1978). Briefly, washing was conducted sequentially in five steps as follows: (1) 25 mL acetone, 10 min shaking, (2) 25 mL deionized water, 10 min shaking, (3) 25 mL deionized water, 10 min shaking, (4) 25 mL deionized water, 10 min shaking and (5) 25 mL acetone, 10 min shaking. Washed hair specimens were dried at 60 °C overnight prior to digestion. Acid digestion was performed using a slightly modified version of the method described by Gault et al. (2008). Approximately 100 ± 5 mg of two replicated subsamples of each dry washed hair sample were weighed into acid cleaned polyethylene tubes. 1.00 mL of concentrated HNO_3 (70% HNO_3) was added to each sample, and tubes were capped and left at room temperature. After 48 h, the digestate was diluted with 9 mL of deionized water and centrifuged at 4500 rpm for 10 min, after which the supernatant was transferred into a fresh acid cleaned polyethylene tube. A human hair standard reference material (GBW07601) was treated in the same manner as the samples to check the precision and accuracy of digestion method (Table 1). Calibration standard solutions (0.1 μg L^{-1}, 1 μg L^{-1}, 5 μg L^{-1}, 10 μg L^{-1}, 20 μg L^{-1}, 50 μg L^{-1} and 100 μg L^{-1}) were prepared from a stock solution (Multi element 2A) with the above 2% HNO_3. Concentrations of total arsenic, arsenic species and other trace elements were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce). Iron concentrations were determined by flame atomic absorption spectrometry (Flame-AAS, Perkin Elmer 5100).

2.4. Data quality control

In order to prevent the precipitation of As, Fe and Mn and the adsorption of other trace elements to container surfaces during field storage, samples were instantly acidified with concentrated HNO_3 to reach ~1% of acidic aliquot. Standard reference material (trace element in water, SRM 1643e) was analyzed every 20 samples during analysis to check the ICP-MS accuracy. If the recovery rate became out of the recommended range (80–120%), the samples were reanalyzed with a new calibration curve. Non-detectable parameters in some samples were reanalyzed with a spiking method, and results showed good agreement (data not shown). The portable pH meter used in the field was calibrated with buffer solutions of pH 4 and pH 7 (Thermo Orion, USA) prior to each trip. The conductivity probe was calibrated with a conductivity standard solution (Thermo Orion, USA). The Eh probe was checked by testing a standard solution (Thermo Orion, USA). All reported values showed good agreement with certified values.

Table 1 – Recovery rate from acid digestion (human hair CRMs GBW07601).

Elements	Certified value (μg g^{-1})	70% HNO_3 recovery rate (%)
Ag	0.029	108.74
As	0.28	94.80
Ba	17	87.29
Cd	0.11	108.55
Cu	10.6	101.00
Mn	6.3	93.91

2.5. Health risk assessment model

A health risk assessment model derived from the USEPA (Integrated Risk Information System (IRIS): arsenic, inorganic, CASRN 7440-38-2, 1998) was applied to compute the non-carcinogenic and carcinogenic effects to individuals who consume groundwater as their drinking water source.

$$\mathrm{ADD} = \frac{As_w \times IR \times EF \times ED}{AT \times BW} \quad (1)$$

where ADD: Average daily dose from ingestion (mg kg^{-1} d^{-1}); As_w: Arsenic concentration in ground water (mg L^{-1}); IR: Water ingestion rate (L d^{-1}); EF: Exposure frequency (d y^{-1}); ED: Exposure duration (y); AT: Average time/life expectancy (d); BW: Body weight (Kg)

$$\mathrm{HQ} = \frac{ADD}{RfD} \quad (2)$$

where HQ: Hazard Quotient (Toxic risk is considered occurring if HQ > 1.00); RfD: Oral reference dose (RfD = 3×10^{-4} mg Kg^{-1} d^{-1});

$$\mathrm{R} = 1 - \exp(-\mathrm{SF} * \mathrm{ADD}) \quad (3)$$

where SF is the slope factor, equal to 1.5 mg Kg^{-1} d^{-1}

2.6. Statistical analyses

Statistical data analyses were performed using SPSS for Windows (Version 13.0). Because the concentrations of arsenic, concentrations of other trace elements, risk factors, and risk indices were not normally distributed, non-parametric statistical tests were conducted. For the three study areas in Kandal, Kampong Cham and Kratie provinces, a Kruskal–Wallis test was applied to assess the regional differences in arsenic and other trace element concentrations in groundwater, arsenic content in scalp (As_h), individual average daily dose (ADD) of arsenic, and risk indices in terms of hazard quotient (HQ) and cancer risk probability (R). In addition, Mann–Whitney's *U* test was performed to verify the differences in gender and maturity between male and female and childhood and adulthood, respectively. The strength of inter-correlation between pH, Eh, Ba, Fe Mn and total As was measured by the Spearman's rho correlation coefficient. Spearman's rho was also used for correlating arsenic levels in groundwater (As_w), arsenic content in scalp hair (As_h), individual average daily doses (ADD) of arsenic, and risk factors such as age, body weight (BD), exposure duration (ED) and ingestion rate (IR). The values below the limit of detection were substituted by one-half of the respective limit of detection and used in the computations of mean, median and other statistical analyses. Significance was considered in circumstances where $p < 0.05$.

Table 2 – Mean, median, standard deviation, minimum and maximum values of groundwater analyzed in the Kampong Cham, Kandal and Kratie Province study areas (Location of each province, see Section 2.1 study area).

Parameters	Kampong Cham						Kandal					Kratie				
	N = 18						N = 46					n = 12				
	Units	Mean	Median	S.D	Min	Max	Mean	Median	S.D	Min	Max	Mean	Median	S.D	Min	Max
pH		6.75	6.84	0.23	6.21	6.96	7.17	7.13	0.26	6.58	7.85	6.48	6.36	0.73	5.53	7.37
Cond	μscm^{-1}	463	453	56	393	585	615	591	107	378	876	1203	1158	294	808	1634
Eh	mV	221.44	200.50	46.27	164.50	319.00	−151.67	−154.75	20.27	−189.00	−55.00	−116.70	−101.35	37.34	−169.85	−57.95
Tep	°C	30.28	30.20	0.55	28.90	31.30	29.04	29.13	0.68	25.05	29.90	29.01	29.03	0.48	28.36	30.12
Depth	m	31	31	6	19	47	24	24	5	16	37	30	30	12	14	47
Cons	yr	2003	2004	4	1997	2008	2000	2001	5	1987	2008	1999	1999	3	1996	2004
Ag	$\mu g\ L^{-1}$	0.007	0.005	0.006	<0.001	0.027	0.013	0.008	0.016	<0.001	0.092	0.041	0.008	0.064	0.002	0.180
Al	μgL^{-1}	10.78	5.64	14.78	4.15	59.54	7.16	6.52	2.76	3.94	22.33	25.73	8.12	61.14	0.36	219.60
Ba	μgL^{-1}	18.56	18.06	10.08	4.04	47.40	*1028.26*	*872.38*	*477.74*	*446.25*	*2652.50*	102.38	64.45	102.96	0.25	359.90
Co	μgL^{-1}	0.035	0.029	0.026	<0.007	0.081	0.395	0.361	0.205	0.064	0.920	0.310	0.259	0.249	0.074	0.921
Cr	μgL^{-1}	0.497	0.419	0.390	0.061	1.589	0.114	0.104	0.061	<0.022	0.308	0.298	0.235	0.199	0.078	0.648
Cu	μgL^{-1}	2.202	0.833	3.287	<0.027	13.570	1.598	0.711	3.491	<0.027	23.070	0.290	0.271	0.143	0.101	0.584
Fe	μgL^{-1}	16.49	15.77	13.10	2.18	59.78	*5901.93*	*5564.18*	*3017.64*	*1367.41*	*17134.49*	*899.18*	*74.84*	*1895.69*	*0.70*	*5114.00*
Ga	μgL^{-1}	2.729	2.633	1.573	0.575	7.515	151.499	128.825	69.126	68.925	381.750	26.607	16.770	26.455	0.470	90.790
Mn	μgL^{-1}	4.31	0.81	8.16	0.22	26.26	*584.23*	*405.75*	*516.49*	*88.18*	*3045.00*	*588.71*	*274.45*	*750.98*	*0.33*	*2139.00*
U	μgL^{-1}	0.422	0.272	0.587	0.056	2.650	0.008	0.003	0.022	<0.001	0.110	0.853	0.183	2.042	0.033	7.276
Zn	μgL^{-1}	16.768	3.850	30.119	0.471	103.700	1.697	1.287	1.781	<0.127	10.050	5.491	5.176	3.238	0.273	11.950
Tot As	μgL^{-1}	1.28	1.22	0.58	0.12	2.37	*846.14*	*822.63*	*298.11*	*247.08*	*1841.50*	*22.22*	*1.30*	*43.89*	*0.12*	*140.60*
Sol As	μgL^{-1}	1.18	1.06	0.59	0.10	2.34	817.52	786.75	290.27	237.35	1832.25	19.41	0.57	41.96	0.07	132.30
As(III)	μgL^{-1}	0.16	0.15	0.05	0.07	0.23	788.17	778.63	279.43	234.10	1747.00	17.59	0.18	40.83	0.03	127.80
Part As	μgL^{-1}	0.105	0.110	0.078	<0.03	0.240	28.614	11.750	56.620	0.750	363.750	2.804	0.815	5.514	<0.0	18.810
As(V)	μgL^{-1}	1.025	0.850	0.581	<0.03	2.220	29.358	22.325	26.728	0.450	138.800	1.820	0.415	2.602	0.040	8.180

<half of the limit of detection; S.D: Standard deviation; Min: Minimum; Max: Maximum; Cond: Conductivity; Cons: Construction year; Tot As: Total Arsenic; Sol As: Soluble Arsenic; Part As: Particulate Arsenic.

3. Results and discussion

3.1. Chemistry of groundwater in the Mekong River basin of Cambodia

The results from chemical measurements of Cambodian groundwater are presented in Table 2. In the Kampong Cham province study area, groundwater was slightly acidic with a pH range of 6.21–6.96. High Eh values (164.5–319 mV) indicate that the groundwater was under oxidizing conditions. In contrast, groundwater from the Kandal and Kratie study areas was quite similar; it had circumneutral pH and was under reducing conditions (low Eh values). Reducing condition might favor the dissolution of toxic trace elements from sediments to pore water. A significant regional difference was observed in As, Mn, Fe and Ba concentrations among the three study areas (Kruskal–Wallis test, $p < 0.0001$). In the Kandal province study area, analytical results indicate that groundwater was extremely polluted. Arsenic concentrations ranged from 247.08 to 1841.50 μg L^{-1} ($n = 46$, average 846.14 μg L^{-1}), with 100% exceeding the Cambodian standard of 50 μg L^{-1}; Mn concentrations were 584.23 ± 516.49 μg L^{-1} (mean ± σ) with 52.17% > 400 μg L^{-1}; Fe concentrations were 5901.93 ± 3017.64 μg L^{-1} (mean ± σ) with 100% exceeding the 300 μg L^{-1} regulation; and Ba concentrations were 1028.26 ± 477.75 μg L^{-1} (mean ± σ) with 73.91% > 700 μg L^{-1}. Out of the 12 observed wells in the Kratie province study area, 25% were found with elevated arsenic levels and 25% had Mn > 400 μg L^{-1}, whereas groundwater samples from the Kampong Cham study area were relatively clean, with arsenic concentrations less than WHO's guideline of 10 μg L^{-1} and no toxic trace elements found with elevated levels. The strongly positive significant correlation between total arsenic concentrations and pH (r_s (74) = 0.745, $p < 0.01$) suggests that arsenic might be released to pore water by desorption processes which are enhanced by alkaline pH (Fig. 2a; Table 3) and reducing conditions (Fig. 2b; Table 3) (Buschmann et al., 2007). The observed positive significant correlation between total As and soluble iron (r_s (74) = 0.559, $p < 0.01$) (Fig. 3; Table 3) suggests that reductive dissolution of arsenic-rich Fe (oxy) hydroxides is a possible mechanism, driving arsenic release to groundwater (Nickson et al., 1998, 2000; Rowland et al., 2008; Kocar et al., 2008). Using hydrologic and (bio) geochemical measurements, Polizzotto et al. (2008) showed that arsenic was released from the near-surface, river derived sediments within the Mekong River delta and transported to the underlying aquifer by groundwater flow, a mechanism also consistent with our geochemical data.

Common manganese minerals are secondary deposits of oxides MnO_2 (pyrolusite) and Mn_3O_4 (hausmanite) and carbonates $MnCO_3$ (rhodochrosite) (Greenwood and Earnshaw, 1997). However, the mechanisms involved in manganese release to the aqueous phase remain unclear. Recently, it has been reported that, under anaerobic conditions, some microorganisms can utilize Mn (IV) as an electron acceptor to oxidize elemental sulfur to sulfate (Prescott et al., 2002). This microbial activity might link the sulfur cycle to the Mn cycle. In the present study, a reversed significant correlation between soluble Mn and Eh ($r_s(74) = -0.591$, $p < 0.01$) was observed. This finding supports the notion that MnO_2 is reduced; Mn (II) is consequently released to pore water under the reducing conditions, which could be harmful to human beings, as in the cases of the Kandal and Kratie province study areas. Moreover, human and other animals can acquire Mn, an essential element for metabolism pathways, through many food sources (WHO, 2004). However, an excess or deficiency of Mn can cause adverse effects. Neurological disorders resulting from drinking very high levels of Mn have been reported in epidemiological studies (WHO, 2004).

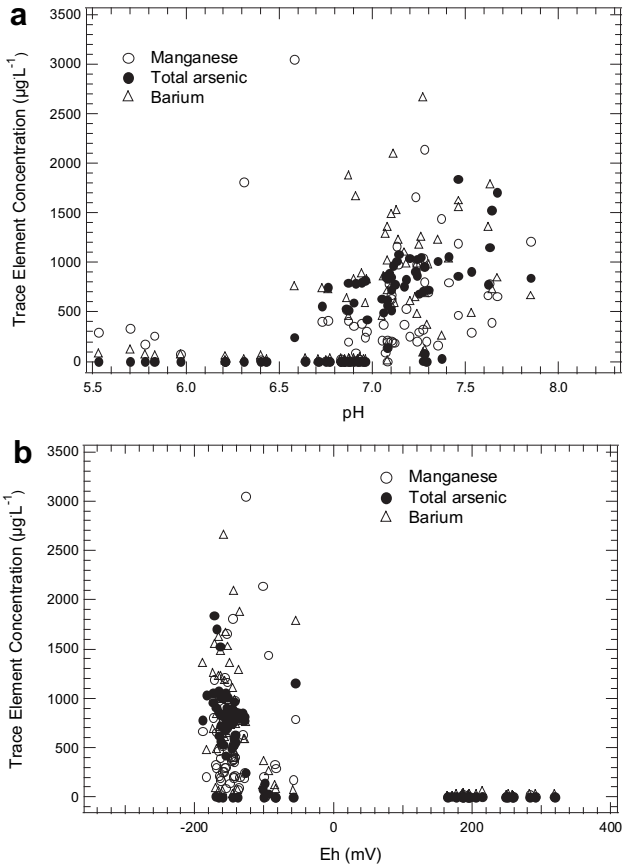

Fig. 2 – Association between Total As, Mn and Ba concentrations with (a) pH and (b) Eh.

Barium is a trace element present in igneous and sedimentary rocks. The most common barium mineral is $BaSO_4$ (Barite) (Greenwood and Earnshaw, 1997). To date, barium has not been proven to be carcinogenic or mutagenic, although drinking barium-contaminated water might lead to hypertension (WHO, 2004). In short, positively significant intercorrelation between As, Fe, Mn and Ba (Table 3) indicates that microbially mediated reductive dissolution coupled with redox cycling in near-surface sediments might play an important role in releasing toxic trace elements into pore water of the Mekong River basin in Cambodia.

3.2. Distribution of arsenic species

Analytical results of Cambodian groundwater samples show that the concentration of As ranges from <0.03 to 1841.50 μg L^{-1}(Table 2). Arsenic was predominantly found in

Table 3 – Inter-correlations, means and standard deviations (S.D) for pH, Eh, Ba, Fe, Mn and total As ($N = 76$).

Variables	pH	Eh	Ba	Fe	Mn	Total As	Mean	S.D
pH	–	−0.586**	0.571**	0.299**	0.526**	0.745**	6.96	0.45
Eh	–	–	−0.635**	−0.514**	−0.591**	−0.658**	−57.78	160.03
Ba	–	–	–	0.716**	0.608**	0.780**	642.93	608.20
Fe	–	–	–	–	0.532**	0.559**	3718.10	3670.93
Mn	–	–	–	–	–	0.614**	447.59	551.89
Total As	–	–	–	–	–	–	515.95	472.28

** $p < 0.01$.

the reduced form, As (III) in the more reducing groundwaters of the Kratie and Kandal province study areas. In contrast, As (V) was mainly present in the oxidizing groundwaters of the Kampong Cham province study area. This heterogeneous distribution in arsenic concentrations could be explained by Lear et al. (2007) who showed that annual loading of organic matter into the Mekong flood plain could increase the prevalence and activities of organisms which reduced As (V) to the potentially more mobile and toxic As (III). Within the Kandal and Kratie province study areas, over 80% of the arsenic species were trivalent arsenicals and less than 20% were pentavalent; this trend was reversed in the Kampong Cham study area (Fig. 4). This result corresponds to the findings of Rowland et al. (2008) who stated that "…arsenic was mainly in the reduced As (III), typically constituting 90% of the total present, with As (V) making up some 10% of the total". In the present study, As (V) and particulate arsenic concentrations are reported as subtractions between soluble arsenic and As (III) and between total arsenic and soluble form, respectively.

3.3. *Arsenic risk assessment*

3.3.1. *Hazardous identification*

Although many populations live alongside watersheds in Cambodia, shallow groundwater is still the main source for drinking water. This is due to the lack of safe water supplies and water treatment systems in rural areas, as well as the cutting edge campaign of "water and sanitation" that took place in the early 1990s when groundwater was promoted as microbiologically-safe water that could be consumed without any treatment. Many families were able to secure supposedly safe water through the inexpensive and easily drilled boreholes tapping into the shallow aquifer groundwater. While some people in certain areas are unwilling to use their existing water sources owing to the aesthetic properties and others have access to enough rainwater to last through the dry season, in rural Cambodia the majority of people are actively consuming shallow groundwater since they have no other alternative water sources. In the present study, individual survey questionnaires were conducted for demographic information as well as other parameters used in risk computation (See 2.5 Health risk assessment model). The survey results show that the residents in the Kratie ($n = 89$) and Kandal ($n = 297$) province study areas consume groundwater for approximately 9 months (270 days) per year and with rain water and surface river floodwaters for the remainder of the year. In contrast, residents in the Kampong Cham study area ($n = 184$) use the groundwater for the whole year (365 days). The surveys also indicate that groundwater in the Kandal, Kratie and Kampong Cham province study areas was used from the date of well installation until arsenic test results were released in August 2006, mid-2007, and present, respectively. Life expectancy of male and female Cambodian people is 59 (21535 days) and 65 (23725 days) years, respectively (WHO, 2007). Various symptoms of arsenicosis such as skin lesions, skin hyperpigmentation, hyperkeratosis and ulcers were found during a field survey in the Kandal province study area, resulting from the consumption of shallow groundwater over a decade.

3.3.2. *Exposure assessment*

The survey results, which were used in risk computation, are shown in Table 4 and calculated results of risk assessments are presented in Table 5. Although there was a significant regional difference in arsenic uptake of residents in each of

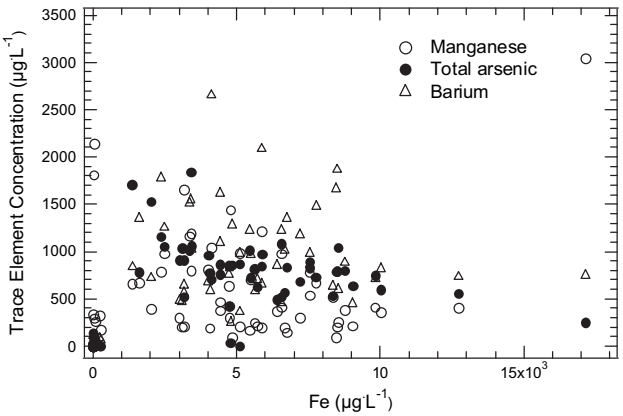

Fig. 3 – Bivariate plots between As, Mn and Ba with Fe.

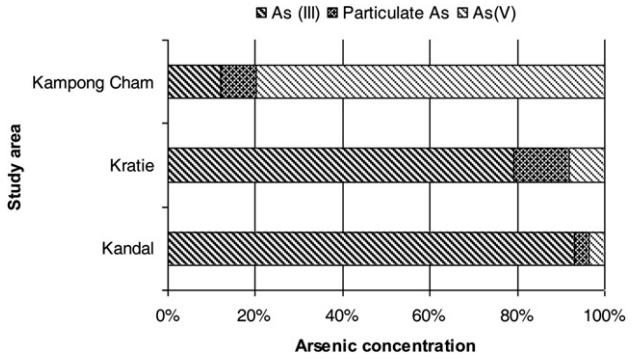

Fig. 4 – Distribution of arsenic species in Cambodian groundwater.

Table 4 – Summary of body weight (BW), ingestion rate (IR) and exposure duration (ED) of respondents in each of the study areas.

		Kandal				Kratie				Kampong Cham			
		BW	Age	IR	ED	BW	Age	IR	ED	BW	Age	IR	ED
Male children	N	32				8				23			
	Mean	20.1	8.5	1.1	5.8	22.0	8.3	1.1	6.0	19.0	7.4	1.1	4.0
	Median	20.0	8.0	1.0	6.0	18.5	7.0	1.0	5.0	18.0	7.0	1.0	3.0
	S.D (σ)	4.8	2.3	0.3	2.4	6.3	2.4	0.2	2.6	4.9	2.5	0.3	2.3
	Min	12.0	5.0	0.5	2.0	16.0	6.0	1.0	4.0	11.0	3.0	1.0	1.0
	Max	28.0	12.0	2.0	12.0	34.0	12.0	1.5	11.0	30.0	12.0	2.0	10.0
Male adults	N	96				25				47			
	Mean	51.1	37.4	1.9	8.5	57.4	44.0	1.8	10.1	52.2	34.7	2.3	5.2
	Median	52.0	33.0	2.0	8.0	56.0	47.0	2.0	11.0	52.0	31.0	2.0	5.0
	S.D (σ)	9.3	18.6	0.6	4.3	11.0	21.5	0.4	2.5	12.6	18.7	0.7	2.9
	Min	28.0	14.0	1.0	2.0	31.0	13.0	1.0	5.0	25.0	13.0	1.5	1.0
	Max	79.0	76.0	4.0	19.0	80.0	83.0	2.5	13.0	80.0	76.0	4.0	12.0
Female children	N	30				6				19			
	Mean	20.1	9.1	1.0	5.1	21.7	9.3	1.0	7.0	18.4	6.8	0.9	3.9
	Median	20.0	10.0	1.0	4.5	23.0	9.5	1.0	7.5	15.0	6.0	1.0	4.0
	S.D (σ)	5.5	2.5	0.3	2.9	4.5	2.2	0.0	1.8	7.3	2.9	0.3	2.2
	Min	7.0	3.0	0.5	1.0	14.0	6.0	1.0	4.0	12.0	2.0	0.5	1.0
	Max	32.0	12.0	2.0	10.0	26.0	12.0	1.0	9.0	42.0	12.0	1.5	10.0
Female adults	N	139				50				95			
	Mean	47.4	37.7	1.6	8.5	48.5	43.4	1.4	11.0	52.1	39.0	2.0	5.2
	Median	47.0	37.0	1.5	8.0	48.0	43.5	1.5	12.0	50.0	34.0	2.0	5.0
	S.D (σ)	8.2	16.8	0.5	4.4	6.9	19.7	0.3	1.8	9.0	18.2	0.6	3.2
	Min	29.0	13.0	1.0	1.0	35.0	14.0	1.0	5.0	35.0	14.0	1.0	1.0
	Max	89.0	84.0	3.5	19.0	65.0	80.0	2.0	13.0	78.0	85.0	4.0	12.0

Body Weight (BW) is in Kg, Age in year(s), Ingestion rate (IR) in L d^{-1} and Exposure Duration (ED) in year(s).

the study areas (Kruskal–Wallis test, $p < 0.0001$), no significant difference in gender (Mann–Whitney's U test, $p = 0.065 > 0.05$) and maturity (Mann–Whitney's U test, $p = 0.845 > 0.05$) were observed, suggesting that individual average daily dose (ADD) was not affected by the different genders and ages of Cambodia residents; this is likely due to the regional differences in groundwater arsenic levels. A strongly positive significant correlation between average daily dose (ADD) and arsenic levels in groundwater (As_w) (r_s (304) = 0.931, $p < 0.0001$) (Table 7) was observed. Moreover, a positively significant correlation of ADD with exposure duration (ED) (r_s (304) = 0.356, $p < 0.0001$) and a positively non-significant correlation with ingestion rate (IR) (rs (304) = 0.077, $p = 0.181 > 0.05$) (Table 7) were obtained, revealing that ADD was correlated with As_w, ED and IR. In addition, a negative, but non-significant correlation between ADD and body weight was also observed (r_s (304) = −0.099, $p = 0.084$) (Table 7), a finding that corresponds well to the conceptual health risk assessment model, expression (1) (See 2.5 Health risk assessment model). Recently, Nguyen et al. (2009) reported that Vietnam residents who consumed untreated groundwater (As > 100 μg L^{-1}) ingested 1.1×10^{-3}–4.3×10^{-3} mg As kg^{-1} d^{-1} Saipan and Ruangwises (2009) used a duplicate diet study to show that Ronphibun residents of Thailand ingested an average of 2.1×10^{-3} mg Askg^{-1} d^{-1}. The present study clearly indicates that residents in the Kandal province study area of Cambodia, ingest higher amount of arsenic than those in Vietnam and Thailand. The higher ingestion rates of arsenic might result in more significantly adverse health effects.

Toxic and cancer risk indices were calculated by expressions (2) and (3), respectively (See 2.5 Health risk assessment model). Computational results showed that the residents in the Kandal, Kratie and Kampong Cham province study areas had toxic risk indices (HQ) ranging from 0.63 to 35.82 ($n = 297$), 1.3×10^{-3} to 2.087 ($n = 89$) and 1.04×10^{-3} to 0.07 ($n = 184$), respectively (Table 5). The upper end of the ranges for Kandal and Kratie residents exceed the typical toxic risk index 1.00, suggesting that the residents in the Kandal and Kratie province study areas might confront more significant adverse toxic health impacts than those in the Kampong Cham province study area. In addition, cancer risk indices found in the Kandal, Kratie and Kampong Cham province study areas ranged from 3 in 10,000 to 2 in 100 ($n = 297$), <1 in 1,000,000 to 9 in 10,000 ($n = 89$) and <1 in 1,000,000 to 3 in 100,000 ($n = 184$), respectively (Table 5). Because the safe standards for cancer risks are in the range of 1 in 10,000 to 1 in 1,000,000, some residents from the study areas in all three provinces may be exposed to drinking water with arsenic concentrations that could lead to cancer. Although there were significant regional differences in toxic risk and carcinogenic risk (Kruskal–Wallis test, $p < 0.0001$), no significant differences in gender (Mann–Whitney's U test, $p = 0.065 > 0.05$) and maturity (Mann–Whitney's U test, $p = 0.845 > 0.05$) were observed. Health risk of arsenic poisoning was more dependent on the As level of groundwater (As_w), average daily dose (ADD) and other related factors of groundwater consumption such as exposure duration (ED) and ingestion rate (IR), rather than individual statuses such as gender and age.

Table 5 – Summary of Average Daily Dose (ADD), Hazard Quotient (HQ) and Cancer Risk (R) of respondents in each of the study areas.

		Kandal			Kratie			Kampong Cham		
		ADD	HQ	R	ADD	HQ	R	ADD	HQ	R
Male children	N	32			8			23		
	Mean	0.003656	12.18534	0.005463	0.000101	0.337805	0.000152	5.2E-06	0.017344	7.8E-06
	Median	0.003197	10.65623	0.004784	2.49E-05	0.083121	3.74E-05	2.7E-06	0.009004	4.05E-06
	S.D (σ)	0.002143	7.144362	0.003196	0.000192	0.639249	0.000288	5.87E-06	0.01956	8.8E-06
	Min	0.000664	2.21273	0.000995	1.32E-06	0.004399	1.98E-06	6.78E-07	0.00226	1.02E-06
	Max	0.007421	24.73739	0.01107	0.000567	1.888719	0.00085	2.21E-05	0.073823	3.32E-05
Male adults	N	96			25			47		
	Mean	0.003701	12.33591	0.005529	9.38E-05	0.312789	0.000141	6.43E-06	0.021424	9.64E-06
	Median	0.00332	11.06585	0.004967	5.61E-06	0.018712	8.42E-06	7.25E-06	0.02416	1.09E-05
	S.D (σ)	0.002492	8.306759	0.003712	0.000169	0.561997	0.000253	4.83E-06	0.016085	7.24E-06
	Min	0.000467	1.555275	0.0007	4.78E-07	0.001592	7.16E-07	6.11E-07	0.002037	9.17E-07
	Max	0.010495	34.98216	0.015619	0.00061	2.034005	0.000915	1.99E-05	0.066441	2.99E-05
Female children	N	30			6			19		
	Mean	0.003009	10.02889	0.004497	5.14E-05	0.17123	7.7E-05	4.71E-06	0.015692	7.06E-06
	Median	0.002333	7.7783	0.003494	2.45E-05	0.081688	3.68E-05	4.22E-06	0.014066	6.33E-06
	S.D (σ)	0.002324	7.745067	0.003464	5.88E-05	0.195911	8.82E-05	3.67E-06	0.012249	5.51E-06
	Min	0.000201	0.669493	0.000301	2.59E-06	0.008637	3.89E-06	5.77E-07	0.001923	8.65E-07
	Max	0.008383	27.94268	0.012495	0.000128	0.427994	0.000193	1.23E-05	0.041026	1.85E-05
Female adults	N	139			50			95		
	Mean	0.003436	11.45189	0.005133	0.000108	0.359845	0.000162	4.95E-06	0.016489	7.42E-06
	Median	0.002833	9.441875	0.00424	2.3E-05	0.076786	3.46E-05	4.78E-06	0.015923	7.17E-06
	S.D (σ)	0.002549	8.495685	0.003798	0.000168	0.560153	0.000252	3.91E-06	0.013021	5.86E-06
	Min	0.000189	0.629505	0.000283	3.87E-07	0.001288	5.8E-07	3.13E-07	0.001042	4.69E-07
	Max	0.010747	35.82394	0.015992	0.000626	2.087066	0.000939	1.51E-05	0.050301	2.26E-05

ADD: mg Kg^{-1} day^{-1}, if HQ >1.00 toxic risk is considered occurring; R (Cancer risk probability): 1 in 10,000 is the highest safe standard for cancer; 1 in 1,000,000 is the lowest safe standard for cancer risk.

3.3.3. *Arsenic content in scalp hair*

Hair is a keratin-rich tissue that can be used as a biomarker for chronic exposure to toxic trace elements in the environment. Analytical results of hair specimens are presented in Table 6. In the Kampong Cham province study area ($n = 68$), the arsenic content in residents' scalp hair ranged from 0.017 to 0.217 μg g^{-1} with an average of 0.092 ± 0.047 μg g^{-1} (mean ± σ). The upper end of this range is lower than the typical arsenic content in human scalp hair, 1.00 μg g^{-1}. The arsenic concentrations in Kratie residents' scalp hair ranged from 0.05 to 1.488 μg g^{-1} ($n = 84$, average 0.286 ± 0.217 μg g^{-1} (mean ± σ)) with 1.19% > 1.00 μg g^{-1}. The arsenic content in Kandal residents' scalp hair ranged from 0.271 to 30.09 μg g^{-1} ($n = 154$, average 5.474 ± 3.339 μg g^{-1} (mean ± σ)) with 72.08% exceeding the level of arsenic toxicity, 1.00 μg g^{-1}. Kubota et al. (2006) reported that the mean value of arsenic content in Kratie residents' scalp hair was 1.77 ± 2.94 μg g^{-1} (mean ± σ), with 42.6% of hair samples exceeding the level of arsenic toxicity, 1.00 μg g^{-1}, whereas Gault et al. (2008) found that arsenic concentrations in scalp hair of Kandal residents ranged from 0.10 to 7.95 μg g^{-1} ($n = 40$, median = 0.54 μg g^{-1}, mean 1.41 μg g^{-1}). These might be due to the differences in arsenic levels in groundwater (As_w) and average daily doses (ADD), which would lead to significantly different arsenic accumulation levels. Indeed, groundwater arsenic levels in the Kratie province study area in the present study ranged from 0.12 to 140.60 μg L^{-1} while the range of arsenic in previous study was from <1 to 886 μg L^{-1} (Kubota et al., 2006). Similarly, arsenic levels of groundwater in Kandal province in the present study ranged from 247.08–1841.5 μg L^{-1} while Gault et al. (2008) found levels from 0.21 to 943 μg L^{-1}.

Table 6 – Summary of arsenic in scalp hair of each of the study areas (μg g^{-1}).

	Kandal	Kratie	Kampong Cham
N	154	84	68
Mean	5.474	0.286	0.092
Median	3.339	0.236	0.085
S.D (σ)	6.478	0.217	0.047
Min	0.271	0.050	0.017
Max	30.09	1.488	0.217

Positively significant correlations between the arsenic content in scalp hair and arsenic levels in groundwater (r_s (304) = 0.757, $p < 0.0001$) and average daily doses (ADD) (r_s (304) = 0.763, $p < 0.0001$) were observed (Fig. 5; Table 7). No significant differences in gender (Mann–Whitney's U test, $p = 0.568 > 0.05$) and maturity (Mann–Whitney's U test, $p = 0.92 > 0.05$) were found, but significant regional differences (Kruskal–Wallis test, $p < 0.0001$) in the arsenic content of scalp hair were observed, undoubtedly suggesting that the arsenic accumulation in Cambodian residents' bodies, which was mainly through a groundwater drinking pathway, appeared uninfluenced by individual gender and age. The Positively significant correlation between arsenic content in

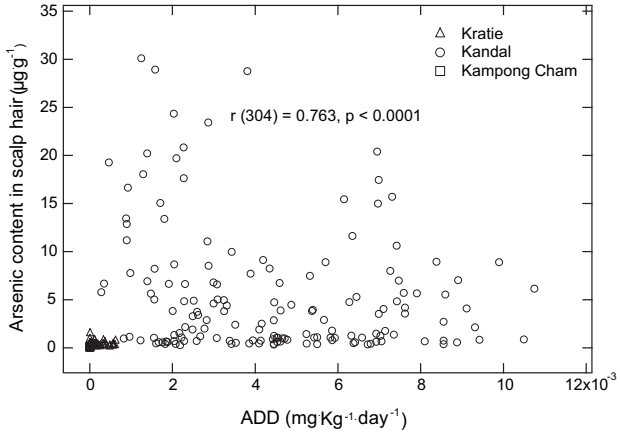

Fig. 5 – Variation of arsenic content in scalp hair as a function of average daily dose (ADD).

scalp hair (As_h) and exposure duration (ED) (r_s (304) = 0.198, $p < 0.01$) (Table 7) and the positive, non-significant correlation with ingestion rate (IR) (r_s (304) = 0.015, $p = 0.8 > 0.05$) and age (r_s (304) = 0.022, $p = 0.697 > 0.05$) demonstrate that arsenic accumulation might be induced by ED, IR and age.

Although there was a statistically significant positive correlation between As_h and ADD, the scatter plot of Fig. 5 indicates that the arsenic accumulation rate varies among individuals. Such variations – e.g. "low ADD, but high hair arsenic" or "high ADD, but low hair arsenic" – might be due to family socioeconomic status, malnutrition level, individual health status, hygiene and/or habits in drinking water maintenance and storage before consumption. Sampson et al. (2008) reported that higher family socioeconomic status and better nutrition could reduce physical susceptibility and delay the manifestation of arsenicosis symptoms. Moreover, healthier residents with sufficient macro and micronutrients might more efficiently remove arsenic from their bodies (Sampson et al., 2008). However, some residents might ingest an excessive amount of arsenic, not only through groundwater drinking pathway but also through their daily diet. In fact, some residents in the study areas used arsenic-rich groundwater to irrigate their farms as well as their rice fields. Therefore, the actual ingestion of arsenic content of this group would be much higher than that of the groundwater drinking pathway alone. Concurrently, in practice, some residents did not consume instantly pumped groundwater. Pumped groundwater may be stored for a period of time in traditional water storage containers, such as open rainwater jars, which may lead to natural oxidation and precipitation processes that lower arsenic levels; moreover, those who had more jars might store rainwater for use through the dry season. In short, a number of factors may play a role in arsenic accumulation in the bodies of residents living in the Mekong River basin of Cambodia.

Table 7 – Inter-correlations for BW, Age, IR, ED, As_w, ADD and As_h ($N = 306$).

Parameters	BW	Age	IR	ED	As_w	ADD	As_h
BW	–	0.595**	0.566**	0.233**	−0.06	−0.099	−0.072
Age	–	–	0.429**	0.292**	−0.002	−0.023	0.022
IR	–	–	–	0.130*	−0.014	0.077	0.015
ED	–	–	–	–	0.140*	0.356**	0.198**
As_w	–	–	–	–	–	0.931**	0.757**
ADD	–	–	–	–	–	–	0.763**
As_h	–	–	–	–	–	–	–

*$p < 0.05$; **$p < 0.01$.

Table 8 – Percentage of residents exposed to toxic and carcinogenic effects in each of the study area (%).

Study area	HQ > 1.00	Cancer Risk Probability (R)			
		>1 in 10^2	>1 in 10^3	>1 in 10^4	>1 in 10^6
Kandal ($n = 297$)	98.65	13.80	92.59	100.00	100.00
Kratie ($n = 89$)	13.48	0.00	0.00	33.71	97.75
Kampong Cham ($n = 184$)	0.00	0.00	0.00	0.00	93.48

HQ: Hazard Quotient; R: Carcinogenic risk probability.
1 in 10,000 is the highest safe standard for carcinogenic risk.
1 in 1,000,000 is the safe standard for carcinogenic risk.

3.3.4. *Risk characteristic*

Computational results revealed that 98.65% of respondents in the Kandal study area were at potential risk to non-cancer health effects. In addition, the cancer risk index was found to be 13.8% for >1 in 100 exposure and 92.59% for >1 in 1000 exposure, exceeding the highest safe standard for cancer risk (1 in 10,000). Similarly, there is a 13.48% chance that respondents from Kratie had non-cancer health risks and a 33.71% chance of increased cancer risk when compared to the highest safe standards. Additionally, there is a 93.48% chance Kampong Cham residents had elevated cancer risks when using the lowest safe standard for cancer risk (1 in 1,000,000) (Table 8). The present results are in agreement with the rapid manifestation of arsenicosis symptoms discovered by Sampson et al. (2008).

Nguyen et al. (2009) found that approximately 42% of Vietnam residents in the contaminated areas could be toxically affected by arsenic while carcinogenic effects were found to have the highest risk index, 5 in 1000. It is apparent that the residents in the Kandal province study area are suffering at a much higher risk of non-cancer and cancer effects. The highest risk index was found to be 2 in 100, 9 in 10,000 and 3 in 100,000 for the Kandal, Kratie and Kampong Cham provinces, respectively. Saipan and Ruangwises (2009) reported that residents of Ronphibun, Thailand, might confront significant health impacts because the risk indices for cancer and non-cancer were found to be 1.26×10^{-3} and 6.98, respectively, exceeding the safe standard for cancer (1 in 10,000) and typical toxic risk (1.00). However, cases discovered in the Kandal province, Cambodia, show residents confront higher risks, with carcinogenic effects that average 5 in 1000 and toxic risk indices of 11.67.

4. Conclusions

Analytical results demonstrated that groundwater in the Kandal province study area of Cambodia was more significantly enriched with As, Mn, Fe and Ba than that in Kratie and

Kampong Cham province study areas. Consequently, the computation of risk through arsenic consumption in drinking water indicated that residents in the Kandal province study area might be exposed to more elevated toxic and carcinogenic risks than those of the Kratie and Kampong Cham province study areas. Positive significant correlations between arsenic content in scalp hair (As_h), arsenic levels in groundwater (As_w), and individual average daily doses (ADD) of arsenic, undoubtedly suggested that arsenic accumulation in Cambodia residents' bodies was mainly through a groundwater drinking pathway. Rapid development of arsenicosis symptoms found during field sampling was closely correlated with risk factors such as extremely high arsenic level in groundwater (As_w), average daily dose (ADD), exposure duration (ED) and ingestion rate (IR). However, arsenic accumulation rate might vary among individuals and/or households owing to family socioeconomic status, malnutrition, individual health status, hygiene, and drinking water maintenance and storage habits. Dangerous As concentrations found in groundwater sources in the Mekong River basin of Cambodia might lead to thousands of cases of arsenicosis in the near future if mitigating actions are not taken.

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