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Assessing mixed trace elements in groundwater and their health risk of residents living in the Mekong River basin of Cambodia

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ABSTRACT

We investigated the potential contamination of trace elements in shallow Cambodian groundwater. Groundwater and hair samples were collected from three provinces in the Mekong River basin of Cambodia and analyzed by ICP-MS. Groundwater from Kandal ($n = 46$) and Kraite ($n = 12$) were enriched in As, Mn, Ba and Fe whereas none of tube wells in Kampong Cham ($n = 18$) had trace elements higher than Cambodian permissible limits. Risk computations indicated that 98.7% and 12.4% of residents in the study areas of Kandal ($n = 297$) and Kratie ($n = 89$) were at risk of non-carcinogenic effects from exposure to multiple elements, yet none were at risk in Kampong Cham ($n = 184$). Arsenic contributed 99.5%, 60.3% and 84.2% of the aggregate risk in Kandal, Kratie and Kampong Cham, respectively. Sustainable and appropriate treatment technologies must therefore be implemented in order for Cambodian groundwater to be used as potable water.

1. Introduction

Groundwater contamination by trace elements has created substantial concern among environmental health scientists. Although some trace elements are essential for normal growth of humans and animals, high intake and low intake of essential trace elements could lead to toxicity and nutritional deficiency, respectively (Goldhaber, 2003). For instance, trace elements in the drinking water (Muhammad et al., 2011; Kavcar et al., 2009) and food grains grown in contaminated soils (Huang et al., 2008) could pose significant non-carcinogenic effects to their consumers. In general, trace elements in groundwater could be derived from either natural or anthropogenic sources (Ramesh et al., 1995; Chen et al., 2007; Mondal et al., 2010; Banerjee et al., 2012). Ecological communities and living organisms in receiving water are also affected by direct discharge of effluents from various industries into aquatic systems (Krishna et al., 2009). Some of these trace elements in groundwater have been well documented in Vietnam (Agusa et al., 2006), Lao PDR (Chanpiwat et al., 2011), India (Ramesh et al., 1995) and China (Chen et al., 2007). The outcome of acute arsenic toxicity might include gastrointestinal discomfort, abdominal pain, vomiting, diarrhea, bloody urine, shock, coma and death (Hughes, 2002). Chronic exposure to arsenic through groundwater consumption may cause skin lesions (pigmentation, melanosis and keratosis) and development of hard patches of skin on palm of the hand and sole of the feet. Skin cancer, cancer of the bladder, kidney and lung as well as diabetes, high blood pressure and reproductive disorders have also been associated with chronic arsenic exposure (WHO, 2008; ATSDR, 2007a). Humans and animals can acquire Mn, an essential element for metabolism, through many food sources (WHO, 2008). However, an excess or deficiency of Mn might cause adverse health effects. Mn toxicity has been known to occur in particular occupational settings through

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inhalation of Mn-bearing dust and/or fumes (Laohaudomchok et al., 2011). Among all target tissues, the brain was most susceptible to excess Mn; high accumulation of Mn may cause neurodegenerative disorders and neurotoxicity (Wright et al., 2006; Laohaudomchok et al., 2011; ATSDR, 2008). Clinical symptoms of Mn toxicity include movement disorders, psychiatric disturbance, cognitive deficits such as memory loss/impairment, reduced learning capacity, decreased mental flexibility and cognitive slowing and behavioral and/or mood changes (Wright et al., 2006; Laohaudomchok et al., 2011). Neurological disorders resulting from drinking Mn-rich water have been reported in epidemiology studies (WHO, 2008). However, the World Health Organization has recently discontinued its drinking water guideline value of 400 g L^{-1} for Mn because this health-based value was well above Mn concentration normally found in drinking water (WHO, 2011). Although drinking Ba-contaminated water might lead to hypertension, Ba has not shown any evidence of carcinogenic or mutagenic effects (WHO, 2008; ATSDR, 2007b).

A general population census in 2008 revealed that 80% percent of Cambodians live in rural areas (NISC, 2012) and in 2010, 28.5% live under the national poverty—an estimate derived by the Cambodian Government (WFP, 2012). About 16.1% of Cambodian women suffered from malnutrition in 2008, and 29% of Cambodian children were underweight in 2010–2011 (WHO, 2012). It was estimated that 81% and 56% of populations in urban and rural areas had access to improved drinking water sources, which was equivalent to about 61% of the total populations (UNICEF, 2012). Although many populations lived alongside surface waters in Cambodia, shallow groundwater was the main source for drinking water (Phan et al., 2010). Unfortunately, shallow Cambodian groundwater is naturally enriched by arsenic and other trace elements (Polya et al., 2005; Berg et al., 2007; Benner et al., 2008; Kocar et al., 2008; Sthiannopkao et al., 2008; Robinson et al., 2009; Luu et al., 2009; Kim et al., 2011). Arsenic was released from the near-surface, river derived sediments within the Mekong River delta and transported to the underlying aquifer by groundwater flow (Polizzotto et al., 2008). Microbially mediated reductive dissolution coupled with redox cycling in near-surface sediments might play an important role in releasing trace elements into pore water in the Mekong River basin of Cambodia (Phan et al., 2010). Health risk assessment of inorganic arsenic intake through ingestion of contaminated has been investigated (Phan et al., 2010). Furthermore, adverse health impacts from chronic arsenic exposure have also been observed (Sampson et al., 2008; Phan et al., 2011). To date, however, studies on aggregate risk through consumption of mixed trace element intake and the resulting human health impacts have not been conducted. Therefore, the objectives of this study were to (1) investigate the distribution of trace elements in shallow groundwater in the Mekong River basin of Cambodia; (2) assess non-carcinogenic effects from both single and mixed trace elements through groundwater ingestion; (3) determine and compare the concentrations of trace elements in hair samples from local populations; and (4) correlate As, Ba and Mn in hair and groundwater samples as well as their respective average daily dose.

2. Materials and methods

2.1. Study area

The Mekong River originates in Tibet and flows 4600 km across six countries including China, Myanmar, Thailand, Lao PDR, Cambodia and Vietnam before discharging into the South China Sea. The area of the Mekong River basin is about 800,000 km^2 with 470 km^3 of annual water and 160 million tons of sediment deposition (Tamura et al., 2007; MRC, 2010). The furthest upstream reaches of the Mekong delta is defined close to the capital city of Phnom Penh; the delta expands downstream of Phnom Penh and covers an area of 62,520 km^2 with two major distributary channels, including the Mekong and Bassac Rivers (MRC, 2010). The present study was conducted in three provinces in the Mekong River basin of Cambodia. Kratie (Preak Samrong I and II villages, Khsarch Andaet commune, Chhloung district) and Kampong Cham (Andoung Chros and Veal Sbov villages, Ampil commune, Kampong Siem district) were located along the Mekong River upstream of Phnom Penh, whereas Kandal (Preak Russey village, Kampong Kong commune, Koh Thom district) was located between the Mekong and the Bassac Rivers, downstream of Phnom Penh.

2.2. Field work

Our field work was conducted after our research proposal was approved by the National Ethics Committee for Health Research (Reference No. 131NECHR, 12/12/2008) under the Cambodian Ministry of Health and informed consent was obtained. Groundwater samples were randomly collected from the study areas of Kandal ($n = 46$) and Kampong Cham ($n = 18$) in February 2009 and Kraite ($n = 12$) in August 2009. 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 an extended 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 sample was filtered (0.45 μm) into a polyethylene bottle and acidified with concentrated nitric acid in order to prevent the precipitation of Fe, Mn and As and adsorption of other trace elements to the bottle surface during field storage. Simultaneously, on-site measurements of pH and redox potential were taken using HORIBA pH/Cond meter D-54. The collected samples were kept in an ice box and then transferred to a fridge where they were stored at 4 °C until delivery to GIST, Republic of Korea for analyses. Concurrently, hair samples were collected using stainless steel scissors from the nape of the head as near as possible to the scalp of several members of the volunteered families in the Kandal ($n = 270$), Kratie ($n = 84$) and Kampong Cham ($n = 173$) province study areas, who claimed to routinely use a tube well. Individual demographic information was also collected to calculate the average daily dose of each trace element to individuals in the study populations. Age, gender, ingestion rates and exposure duration were collected using a structured questionnaire. Body weight was measured using a bathroom scale which was calibrated to zero prior to each measurement. The collected hair samples were separately kept in a labeled plastic ziplock bags and stored in darkness until analyses.

2.3. Sample preparation and analyses

Groundwater samples from Kandal and Kraite were diluted (1:25) with 2% nitric acid (prepared by 18.2 MΩ *MilliQ* deionized water and 70% nitric acid) to measure the concentrations of As, Mn, Ba and Fe. Samples from Kampong Cham did not require dilution, because the concentrations of As, Mn, Ba and Fe fell into the standard calibration curves. Concurrently, Ag, Al, Cd, Co, Cr, Cu, Ga, Ni, Pb, Se, U and Zn were determined using the original samples. Hair was cut into small pieces (3 mm) and alternately washed with acetone and deionized water as described in our previous report (Phan et al., 2010). Washed hair was dried at 60 °C overnight before digestion. Acid-digestion was performed following our previously used method (Phan et al., 2010). Chemical measurements of all groundwater and digestate of hair samples were employed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce) using an external calibration method. 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), also with 2% nitric acid. Two percent nitric acid was also used as an analytical blank. Standard reference material (Trace Element in Water, SRM 1643e) was analyzed to check the ICP-MS accuracy. If the recovery rate become out of the recommended range (90–110%), samples were reanalyzed with a new calibration curve. Digestions of two replicated hair samples were conducted to verify a validity of acid digestion method. Three replicates of human hair standard reference materials (GBW07601) were also treated in the same manner as the sample to check the accuracy of the digestion methods. The recovery rates of Ag (108.74%), As (94.80%), B (82.90%), Ba (87.29%), Cd (108.55%), Cu (101.00%), Mn (93.91%), Mo (83.68%), Ni (77.98%), Pb (93.94%) were in good agreements with the certified values.

2.4. Health risk assessment

Health risk assessment procedures from the USEPA (1989) were applied to calculate non-carcinogenic effects of single and mixed trace elements. The average daily dose of single element is calculated from the following Equation (1).

$$\mathrm{ADD} = \frac{C_w \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \tag{1}$$

where ADD is the average daily dose from ingestion (mg kg^{-1} d^{-1}); C_w is the trace element concentration in groundwater (mg L^{-1}); IR is the ingestion rate of groundwater (L d^{-1}); EF is the exposure frequency (d y^{-1}); ED is the exposure duration (y); BW is the body weight (kg) and AT is the averaging time (d). Field surveys (Table 1) showed that the residents in the study areas of Kandal ($n = 297$) and Kratie ($n = 89$) have consumed groundwater 9 months per year (EF = 270 d y^{-1})

Table 1
Field survey of body weight (BW), ingestion rates (IR), exposure duration (ED) and age of residents in each study area.

	Kandal				Kratie				Kampong Cham			
	BW	IR	ED	Age	BW	IR	ED	Age	BW	IR	ED	Age
Children												
Gender (F/M)	25/28				5/7				18/22			
Mean	19.1	1.0	5.2	8.2	20.5	1.0	5.9	8.2	18.0	1.0	4.0	6.9
Median	20.0	1.0	5.0	8.0	19.0	1.0	5.0	7.5	17.0	1.0	3.5	6.0
SD	4.7	0.2	2.3	2.2	4.2	0.0	1.9	1.9	4.8	0.3	2.3	2.5
Min	7.0	0.5	1.0	3.0	14.0	1.0	4.0	6.0	11.0	0.5	1.0	2.0
Max	28.0	2.0	9.0	11.0	28.0	1.0	9.0	11.0	30.0	2.0	10.0	11.0
Adults												
Gender (F/M)	144/100				51/26				96/48			
Mean	48.1	1.7	8.5	36.7	50.9	1.5	10.7	42.8	51.9	2.1	5.2	37.2
Median	48.0	1.5	8.0	35.0	51.0	1.5	11.0	44.0	50.0	2.0	5.0	32.5
SD	9.7	0.6	4.3	17.8	9.9	0.4	2.1	20.6	10.6	0.6	3.1	18.5
Min	20.0	1.0	1.0	12.0	26.0	1.0	5.0	12.0	23.0	1.0	1.0	12.0
Max	89.0	4.0	19.0	84.0	80.0	2.5	13.0	83.0	80.0	4.0	12.0	85.0

BW (kg); IR (L d^{-1}); ED (y); Age (y); SD, standard deviation; Min, minimum; Max, maximum; children (age $<$ 12 years old); adults (age $\geq$ 12 years old); F, female; M, male.

with rainwater and surface river floodwaters for the remainder of the year. However, Kampong Cham residents have used groundwater for the whole year (EF = 365 d y^{-1}). Non-carcinogenic effects of single elements, expressed as hazard quotient (HQ), are computed from the Equation (2) whereas non-carcinogenic effects of mixed trace elements (hazard index) are calculated from Equation (3).

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

HQ is a hazard quotient. Non-carcinogenic effects were considered if HQ > 1; RfD is a reference dose of single element. The oral reference dose (mg kg^{-1} d^{-1}) of Ag (5×10^{-3}), As (3×10^{-4}), Ba (2×10^{-1}), Cd (5×10^{-4}), Cr (3×10^{-3}), Cu (4×10^{-2}), Mn (1.4×10^{-1}), Ni (2×10^{-2}), Se (5×10^{-3}), U (3×10^{-3}) and Zn (3×10^{-1}) was obtained from a database of the Integrated Risk Information System (USEPA, 2012).

$$HI = \sum HQ = ADD_1/RfD_1 + ADD_2/RfD_2 + \ldots + ADD_i/RfD_i \quad (3)$$

HI is a hazard index which indicated the aggregate risk/risk of mixed trace elements. Non-carcinogenic effects of mixed trace elements are considered to occur in circumstance where HI is greater than one.

2.5. Statistical data analyses

All statistical analyses were employed by SPSS for windows (Version 16.0). Since the data sets were not normally distributed, nonparametric tests were applied. Kruskal–Wallis test was used to verify the difference in trace element concentrations in groundwater, average daily dose of each single element, hazard quotient of a single trace element and hazard index of mixed trace elements and trace element concentrations in human hair samples among the three study areas. Mann–Whitney U's tests were performed to certify the difference in trace element concentrations among acid/base and oxidizing/reducing groundwater environments. Likewise, it was also carried out to assess the difference in average daily dose of trace element, hazard quotient (risk of single element) and hazard index (aggregate risk/risk of mixed elements). In addition, Mann–Whitney U's test was used to verify the difference in trace element concentrations in human hair samples between male and female groups and children (<12 years old) and adults (≥12 years old) groups. The strength of correlations between As, Ba and Mn in groundwater and human hair samples were measured by a Spearman's rho correlation coefficients (r_s). Significance was considered in circumstance where $p < 0.05$.

3. Results

3.1. Trace elements in Cambodian groundwater

Chemical measurements of groundwater are presented in Table 2. All 46 tube wells from Kandal yielded detectable Al, As, Ba, Fe, Ga and Mn. However, Ag (4.3%), Cd (56.5%), Cr (4.3%), Cu (28.3%), Ni (95.7%), Pb (95.7%), Se (80.4%), U (43.5%) and Zn (4.3%) were not detected in all wells. Groundwater from this study area is polluted by As, Ba, Mn and Fe. All observed wells had As (817.52 ± 290.27 μg L^{-1}) and Fe

Table 2
Summary of trace element concentrations (μg L^{-1}) in groundwater of Kandal, Kratie and Kampong Cham in the Mekong River basin of Cambodia.

Elements	Kandal ($n = 46$)					Kratie ($n = 12$)					Kampong Cham ($n = 18$)				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
pH	7.17	7.13	0.26	6.58	7.85	6.48	6.36	0.73	5.53	7.37	6.75	6.84	0.23	6.21	6.96
Eh	−151.67	−154.75	20.27	−189.00	−55.00	−116.70	−101.35	37.34	−169.85	−57.95	221.44	200.50	46.27	164.50	319.00
Ag	0.014	0.008	0.016	0.003	0.092	0.054	0.010	0.069	0.005	0.180	0.008	0.006	0.006	0.004	0.027
Al	7.16	6.52	2.76	3.94	22.33	25.73	8.12	61.14	0.36	219.60	10.78	5.64	14.78	4.15	59.54
As	817.52	786.75	290.27	237.35	1832.25	19.41	0.57	41.96	0.07	132.30	1.18	1.06	0.59	0.10	2.34
Ba	1028.26	872.38	477.74	446.25	2652.50	102.38	64.45	102.96	0.25	359.90	18.56	18.06	10.08	4.04	47.40
Cd	0.693[a]	0.290	1.684	0.221	7.832	0.122[a]	0.093	0.056	0.085	0.218	0.018[a]	0.015	0.008	0.013	0.032
Co	0.395	0.361	0.205	0.064	0.920	0.310	0.258	0.249	0.074	0.921	0.043	0.043	0.024	0.016	0.081
Cr	0.118	0.106	0.059	0.057	0.308	0.298	0.235	0.200	0.078	0.648	0.497	0.419	0.390	0.061	1.589
Cu	2.217	1.394	3.968	0.069	23.070	0.290	0.271	0.142	0.101	0.584	2.474	0.916	3.397	0.106	13.570
Fe	5901.93	5564.18	3017.64	1367.41	17,134.49	899.18	74.84	1895.69	0.70	5114.00	16.49	15.77	13.10	2.18	59.78
Ga	151.50	128.83	69.13	68.93	381.75	26.61	16.77	26.45	0.47	90.79	2.73	2.63	1.57	0.58	7.52
Mn	584.23	405.75	516.49	88.18	3045.00	588.71	274.45	750.98	0.33	2139.00	4.31	0.81	8.16	0.22	26.26
Ni	3.785[b]	3.785	4.735	0.437	7.133	1.186	1.022	1.011	0.216	2.932	1.150	0.859	0.880	0.203	3.176
Pb	3.834[b]	3.834	5.390	0.023	7.645	0.211	0.165	0.117	0.068	0.412	0.916	0.547	0.946	0.091	3.542
Se	0.117[a]	0.090	0.057	0.068	0.237	0.874	0.455	0.931	0.337	2.832	0.153	0.147	0.047	0.090	0.237
U	0.014	0.004	0.028	0.003	0.110	0.852	0.183	2.042	0.033	7.276	0.422	0.272	0.587	0.056	2.650
Zn	1.768	1.329	1.789	0.258	10.050	5.491	5.176	3.238	0.273	11.950	16.768	3.850	30.119	0.471	103.700

SD, standard deviation; Min, minimum; Max, maximum (Adapted from Phan et al., 2010).
[a] Number of sample detected was less than 50%.
[b] Number of sample detected was less than 25%.

Table 3
Mean, median, standard deviation, minimum and maximum of the average daily dose (mg kg^{-1} d^{-1}) of each trace element from groundwater intake in the Mekong River basin of Cambodia.

Elements	Kandal					Kratie					Kampong Cham				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Ag	4.18E-08	3.19E-08	4.81E-08	8.00E-10	5.12E-07	3.15E-07	3.56E-07	2.97E-07	1.17E-08	9.49E-07	3.71E-08	2.19E-08	3.43E-08	1.62E-09	1.51E-07
As	3.41E-03	2.88E-03	2.42E-03	1.88E-04	1.07E-02	8.08E-05	1.65E-05	1.55E-04	2.25E-07	5.89E-04	5.01E-06	3.53E-06	4.30E-06	3.09E-07	2.10E-05
Ba	4.01E-03	3.34E-03	2.78E-03	1.32E-04	1.62E-02	5.51E-04	2.90E-04	5.91E-04	5.62E-07	2.35E-03	6.17E-05	3.95E-05	5.30E-05	5.90E-06	2.68E-04
Cd	2.57E-06	9.35E-07	8.94E-06	4.30E-08	7.04E-05	5.06E-07	4.66E-07	2.18E-07	1.79E-07	9.71E-07	5.89E-08	2.72E-08	5.50E-08	7.38E-09	2.11E-07
Cr	4.02E-07	3.48E-07	2.98E-07	1.92E-08	2.59E-06	1.01E-06	7.61E-07	7.49E-07	2.40E-07	3.41E-06	1.32E-06	1.05E-06	1.14E-06	6.84E-08	5.39E-06
Cu	6.26E-06	4.23E-06	1.13E-05	1.09E-07	1.09E-04	9.27E-07	8.83E-07	4.22E-07	2.51E-07	1.79E-06	4.88E-06	1.73E-06	7.66E-06	1.63E-07	3.74E-05
Mn	2.76E-03	1.72E-03	2.57E-03	5.73E-05	1.36E-02	2.09E-03	1.01E-03	2.64E-03	7.47E-07	9.96E-03	2.57E-05	3.05E-06	4.06E-05	1.21E-07	2.05E-04
Ni	9.22E-06	1.51E-06	1.25E-05	7.37E-07	2.59E-05	2.36E-06	1.27E-06	2.13E-06	5.22E-07	1.00E-05	2.09E-06	1.68E-06	1.44E-06	6.00E-07	9.26E-06
Se	4.76E-07	3.94E-07	4.17E-07	8.85E-08	2.13E-06	2.10E-06	1.50E-06	1.75E-06	7.44E-07	9.68E-06	3.94E-07	3.47E-07	3.08E-07	3.36E-08	1.28E-06
U	3.65E-08	1.93E-08	6.56E-08	8.37E-10	5.69E-07	2.79E-06	9.75E-07	5.70E-06	6.11E-08	3.39E-05	2.20E-06	9.22E-07	3.60E-06	4.76E-08	1.94E-05
Zn	6.48E-06	4.33E-06	9.25E-06	1.64E-07	7.88E-05	2.09E-05	1.97E-05	1.36E-05	6.15E-07	5.61E-05	1.06E-04	6.66E-06	2.10E-04	3.62E-07	1.03E-03

SD, standard deviation; Min, minimum; Max, maximum.

(5901.93 ± 3017.64 μg L^{-1}) greater than Cambodian drinking water standard 50 μg L^{-1} and 300 μg L^{-1}, respectively. A mean concentration of Ba was 1028.26 ± 477.74 μg L^{-1}; 73.9% of the observed wells had Ba greater than Cambodian drinking water standard 700 μg L^{-1} while 52.2% had Mn (584.23 ± 516.49 μg L^{-1}) greater Cambodian drinking water standard 400 μg L^{-1}. The frequency of undetectable trace elements in groundwater from Kratie was Ag (25%), Cd (58.3%), Pb (33.3%), Se (41.7%) whereas that in Kampong Cham was Ag (16.7%), Cd (72.2%), Co (22.2%), Ni (5.6%) and Se (27.8%). Among 12 observed wells from Kraite, 25% had As (19.41 ± 41.96 μg L^{-1}) greater than WHO's guideline 10 μg L^{-1} and 25% Mn (588.71 ± 750.98 μg L^{-1}) > 400 μg L^{-1}; 16.7% have Fe > 300 μg L^{-1} and 8.3% Al > 200 μg L^{-1} (Cambodian drinking water standard). However, none of the observed wells from Kampong Cham (n = 18) had trace elements higher than the Cambodian drinking water permissible limits.

A comparison revealed that there were significant regional differences in As, Ba, Cd, Co, Cr, Fe, Ga, Mn, Se, U and Zn among the three study areas (Kruskal–Wallis, $p < 0.001$). Significant regional differences in Ag ($p = 0.039$), Al ($p = 0.012$) and Cu ($p = 0.001$) among all study areas were also observed. However, Kruskal–Wallis analysis showed that there were not significant regional differences in Ni ($p = 0.726$) and Pb ($p = 0.161$) among these three study areas. This might be due to a large number of groundwater samples from Kandal not containing detectable Ni and Pb. Further comparison also indicated that there was not a significant difference in Ni concentration between Kratie and Kampong Cham (Mann–Whitney U's test, $p = 0.757 > 0.05$); however, there was a significant difference in Pb concentration between Kratie and Kampong Cham (Mann–Whitney U's test, $p = 0.04$).

In addition, there were significant differences in As, Ba, Co, Cr, Ga and Mn between acidic and basic groundwater (Mann–Whitney U's test, $p < 0.001$). Significant differences in Cd ($p = 0.019$), Fe ($p = 0.002$), Ni ($p = 0.016$), U ($p = 0.003$) and Zn ($p = 0.001$) between acidic and basic groundwater were also found. However, there were not significant differences in Ag, Al, Cu, Pb and Se (Mann–Whitney U's test, $p > 0.05$). Concurrently, there was significant difference in As, Ba, Cd, Co, Cr, Fe, Ga, Mn and U between oxidizing and reducing groundwater (Mann–Whitney U's test, $p < 0.001$). Significant difference in Al ($p = 0.046$) and Zn ($p = 0.029$) between oxidizing and reducing groundwater was also observed. However, there was not significant difference in Ag, Cu, Ni, Pb and Se between oxidizing and reducing groundwater (Mann–Whitney U's test, $p > 0.05$). Our field observations revealed that the study areas of Kandal and Kratie have been annually flooded during the rising river stage in the rainy season while the study areas of Kampong Cham have not. Flooding has been known to reduce the available oxygen levels in soils which leads to a reducing environment. In general, reducing condition favored a release of trace elements from solid phases into pore water. Since groundwater in the Kandal and Kratie province study areas were reducing, high concentration of As, Ba, Fe and Mn were found (Table 2). These findings suggest that the concentrations of trace elements in groundwaters of the Mekong River basin of Cambodia varied depending on its geological origins, acid–base and redox conditions.

Table 4
Mean, median, standard deviation, minimum and maximum values of hazard quotient of each trace element and hazard index of mixed trace elements from groundwater intake in the Mekong River basin of Cambodia.

HQ	Kandal					Kratie					Kampong Cham				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Ag	8.35E-06	6.38E-06	9.63E-06	1.60E-07	1.02E-04	6.29E-05	7.12E-05	5.94E-05	2.34E-06	1.90E-04	7.42E-06	4.38E-06	6.86E-06	3.25E-07	3.02E-05
As	1.14E+01	9.60E+00	8.08E+00	6.27E-01	3.56E+01	2.69E-01	5.51E-02	5.16E-01	7.52E-04	1.96E+00	1.67E-02	1.18E-02	1.43E-02	1.03E-03	6.99E-02
Ba	2.00E-02	1.67E-02	1.39E-02	6.59E-04	8.08E-02	2.76E-03	1.45E-03	2.96E-03	2.81E-06	1.17E-02	3.09E-04	1.97E-04	2.65E-04	2.95E-05	1.34E-03
Cd	5.15E-03	1.87E-03	1.79E-02	8.61E-05	1.41E-01	1.01E-03	9.33E-04	4.35E-04	3.57E-04	1.94E-03	1.18E-04	5.43E-05	1.10E-04	1.48E-05	4.23E-04
Cr	1.34E-04	1.16E-04	9.95E-05	6.39E-06	8.65E-04	3.36E-04	2.54E-04	2.50E-04	7.99E-05	1.14E-03	4.42E-04	3.51E-04	3.79E-04	2.28E-05	1.80E-03
Cu	1.56E-04	1.06E-04	2.83E-04	2.74E-06	2.73E-03	2.32E-05	2.21E-05	1.05E-05	6.28E-06	4.47E-05	1.22E-04	4.32E-05	1.92E-04	4.07E-06	9.35E-04
Mn	1.97E-02	1.23E-02	1.83E-02	4.09E-04	9.74E-02	1.49E-02	7.21E-03	1.89E-02	5.34E-06	7.11E-02	1.84E-04	2.18E-05	2.90E-04	8.67E-07	1.46E-03
Ni	4.61E-04	7.55E-05	6.25E-04	3.69E-05	1.29E-03	1.18E-04	6.37E-05	1.06E-04	2.61E-05	5.01E-04	1.05E-04	8.39E-05	7.20E-05	3.00E-05	4.63E-04
Se	9.52E-05	7.89E-05	8.33E-05	1.77E-05	4.26E-04	4.20E-04	3.00E-04	3.51E-04	1.49E-04	1.94E-03	7.89E-05	6.95E-05	6.16E-05	6.72E-06	2.57E-04
U	1.22E-05	6.44E-06	2.19E-05	2.79E-07	1.90E-04	9.30E-04	3.25E-04	1.90E-03	2.04E-05	1.13E-02	7.33E-04	3.07E-04	1.20E-03	1.59E-05	6.47E-03
Zn	2.16E-05	1.44E-05	3.08E-05	5.46E-07	2.63E-04	6.96E-05	6.56E-05	4.52E-05	2.05E-06	1.87E-04	3.53E-04	2.22E-05	7.00E-04	1.21E-06	3.42E-03
HI	1.14E+01	9.62E+00	8.10E+00	6.29E-01	3.57E+01	2.89E-01	6.42E-02	5.17E-01	2.97E-03	1.97E+00	1.90E-02	1.63E-02	1.56E-02	1.16E-03	7.75E-02

HQ, hazard quotient; HI, hazard index; SD, standard deviation; Min, minimum; Max, maximum; non-carcinogenic effect of single element and mixed elements are considered to occur in circumstance where HQ or HI is greater than 1.

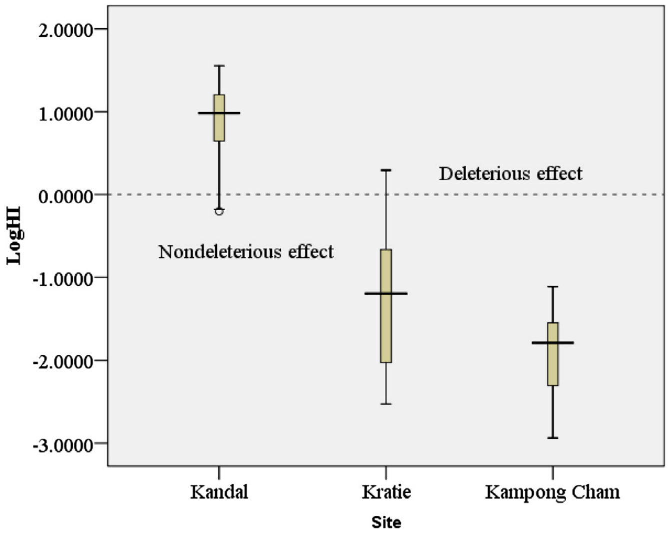

Fig. 1. Non-carcinogenic effect of mixed trace elements of the residents in the three study areas.

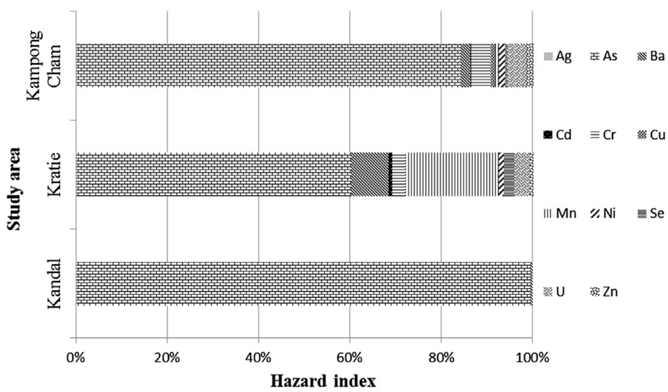

Fig. 2. Contribution (%) of each single element in hazard index of mixed trace elements in the Mekong River basin of Cambodia.

3.2. Health risks from drinking groundwater

The average daily dose of each single element was presented in Table 3. A comparison revealed that there were significant differences in the average daily dose of Ag, As, Ba, Cd, Cr, Cu, Mn, Se, U and Zn among the three study areas (Kruskal–Wallis test, $p < 0.001$); however, there was no significant regional difference in the average daily dose of Ni (Kruskal–Wallis test, $p = 0.103 > 0.05$). A comparison also indicated there were significant differences in the average daily dose of Ba, Cd, Cr, Cu, Ni, Se and Zn between male and female residents (Mann–Whitney U's test, $p < 0.05$); however, there was no significant difference in average daily dose of Ag, As, Mn and U (Mann–Whitney U's test, $p > 0.05$). Similarly, there was no significant difference in average daily dose of all observed elements between children (<12 years old) and adults (≥12 years old) (Mann–Whitney U's test, $p > 0.05$). These findings suggested that the intake of most trace elements likely depended on the source of the element and gender of the individual; however, it did not depend on the individual's age.

The hazard quotient (risk of single element) and hazard index (aggregate risk/risk of mixed trace elements) is presented in Table 4. None of residents in the Mekong River basin of Cambodia were at risk of non-carcinogenic effects from Ag, Ba, Cd, Co, Cr, Cu, Mn, Ni, Se, U and Zn alone. However, 98.7% and 12.4% of residents in the study areas of Kandal ($n = 297$) and Kraite ($n = 89$), respectively, were at risk of non-carcinogenic effect of As, yet none of residents in the study area of Kampong Cham ($n = 184$) were at risk. A comparison indicated that there were significant differences in risk of single Ag, As, Ba, Cd, Co, Cr, Cu, Mn, Se, U and Zn among residents in the three study areas (Kruskal–Wallis test, $p < 0.001$). However, there was no significant difference in risk for Ni exposure among residents in the three study areas (Kruskal–Wallis test, $p = 0.103 > 0.05$). There were significant differences in risk of Cu, Ni ($p < 0.001$), Ba ($p = 0.045$), Cd ($p = 0.036$), Cr ($p = 0.001$), Se ($p = 0.039$) and Zn ($p = 0.020$) between male and female residents (Mann–Whitney U's test, $p < 0.05$); however, there was no significant difference in risk of Ag ($p = 0.116$), As ($p = 0.079$), Mn ($p = 0.152$) and U ($p = 0.801$) between male and female residents (Mann–Whitney U's test, $p > 0.05$). There was no significant difference in risk of all single trace elements between children and adults (Mann–Whitney U's test, $p > 0.05$). Approximately 98.7% and 12.4% of residents in the study areas of Kandal ($n = 297$) and

Table 5
Mean, median, standard deviation, minimum and maximum concentration of trace elements (μg g^{-1}) in hair of residents in the Mekong River basin of Cambodia.

Elements	Kandal					Kratie					Kampong Cham				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Ag	0.23	0.05	1.18	0.00	18.14	0.34	0.03	1.44	0.00	9.50	0.62	0.11	2.12	0.01	22.27
Al	26.45	19.85	20.38	2.60	134.55	12.08	10.30	7.56	2.45	50.09	12.78	10.45	10.71	2.22	105.76
As	6.40	4.03	8.01	0.27	57.21	0.29	0.24	0.21	0.05	1.42	0.12	0.09	0.10	0.01	1.01
B	0.99	0.80	0.93	0.05	8.10	0.90	0.59	0.88	0.10	4.94	1.37	0.93	1.60	0.15	16.01
Ba	22.79	13.88	30.71	0.33	328.28	8.71	7.68	6.22	0.59	30.08	5.26	4.92	3.14	0.51	14.03
Cd	0.09	0.07	0.10	0.01	1.27	0.11	0.08	0.11	0.01	0.82	0.12	0.09	0.11	0.01	0.62
Co	0.18	0.10	0.22	0.01	2.17	0.16	0.10	0.21	0.004	1.34	0.24	0.16	0.39	0.02	3.53
Cr	0.21	0.18	0.16	0.02	1.60	0.13	0.08	0.29	0.02	2.63	0.18	0.14	0.12	0.06	1.00
Cu	15.01	11.83	10.25	3.74	76.07	15.08	11.20	12.90	4.88	86.83	24.70	12.38	60.85	6.27	705.62
Ga	7.18	4.03	10.56	0.07	126.65	3.38	2.93	2.39	0.23	11.21	1.80	1.62	1.12	0.14	5.34
Mn	35.00	26.39	27.93	2.04	181.37	41.01	24.80	53.72	1.04	236.62	11.44	8.25	9.25	1.03	58.01
Mo	0.06	0.06	0.03	0.00	0.20	0.05	0.04	0.02	0.02	0.12	0.06	0.05	0.03	0.02	0.20
Ni	0.93	0.44	1.65	0.03	14.74	1.28	0.53	2.34	0.04	14.70	1.17	0.52	1.82	0.06	13.11
Pb	14.75	7.04	26.47	0.34	255.80	10.89	3.87	22.30	0.90	97.30	7.76	5.18	7.06	0.48	31.81
Rb	0.24	0.15	0.34	0.02	3.65	0.32	0.14	0.44	0.02	2.25	0.27	0.10	0.45	0.01	3.55
Se	0.85	0.82	0.20	0.36	1.43	0.82	0.81	0.17	0.39	1.36	0.85	0.83	0.25	0.33	1.66
Tl	0.003	0.002	0.002	<DL	0.015	0.003	0.002	0.002	0.001	0.01	0.002	0.002	0.001	<DL	0.006
U	0.03	0.02	0.03	0.001	0.21	0.05	0.03	0.06	0.001	0.43	0.03	0.02	0.04	0.002	0.24

SD, standard deviation; Min, minimum; Max, maximum; DL, detection limit.

Kraite ($n = 89$), respectively, were at risk of non-carcinogenic effect of mixed trace elements while none of residents in the study area of Kampong Cham ($n = 184$) were at risk (Fig. 1). Although there was significant difference in aggregate risk among the three study areas (Kruskal–Wallis test, $p < 0.001$), there was no significant difference in aggregate risk between male and female (Mann–Whitney U's test, $p = 0.062 > 0.05$) and between children and adults (Mann–Whitney U's test, $p = 0.613 > 0.05$). A contribution of each single element in aggregate risk in Kandal was in the following order As > Mn > Ba > Cd > Cr > Cu > Zn > Se > Ni > Ag > U. The order of each single element contributed in aggregate risk in Kraite was As > Mn > Ba > U > Cr > Se > Ni > Cd > Zn > Cu > Ag whereas the order in Kampong Cham was As > Cr > U > Ba > Ni > Zn > Cu > Mn > Se > Cd > Ag (Fig. 2). Contribution of As in aggregate risk/risk

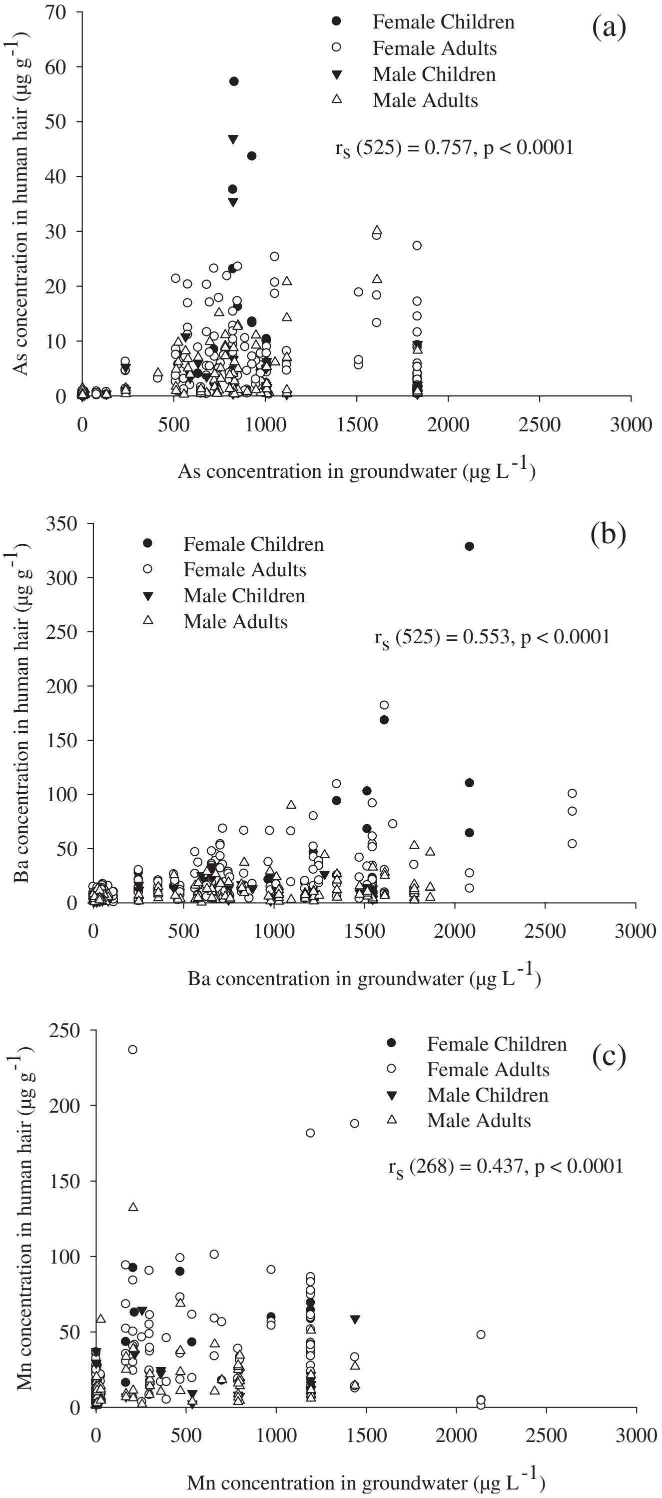

Fig. 3. Correlation between trace elements in groundwater and human hair (a) As (b) Ba and (c) Mn.

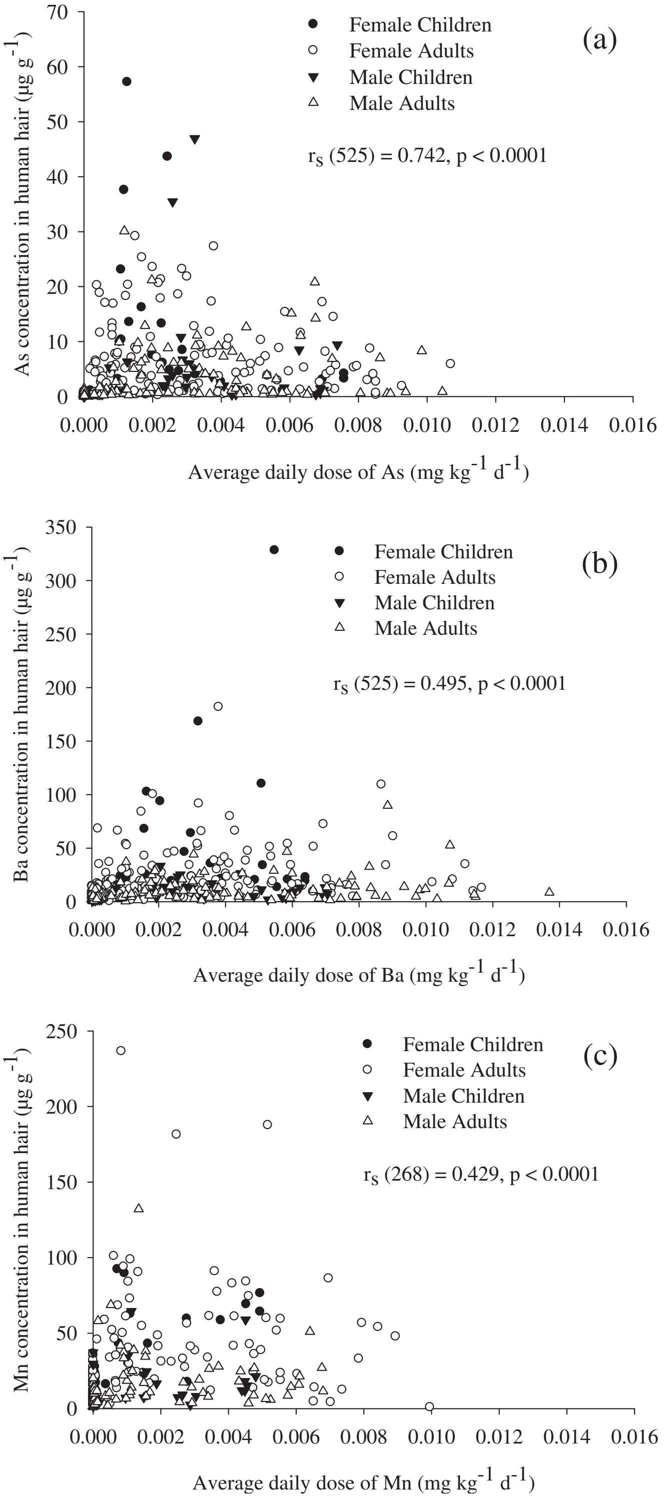

Fig. 4. Correlation between average daily dose of trace elements and human hair (a) As (b) Ba and (c) Mn.

of mixed trace elements in Kandal, Kratie and Kampong Cham were 99.5%, 60.3% and 84.2%, respectively. Although single Ba and Mn did not pose any non-carcinogenic effect, their contributions in aggregate risks in Kandal and Kratie province study areas were substantially considered.

3.3. Trace elements in human hair samples

Trace element concentrations in human hair samples are presented in Table 5. A Kruskal–Wallis comparison revealed that there are significant differences in Ag, Al, As, B, Ba, Cr, Ga and Mn ($p < 0.001$), Cd ($p = 0.003$), Co ($p = 0.001$), Cu ($p = 0.049$), Mo ($p = 0.001$), Pb ($p = 0.012$), Rb ($p = 0.009$), Tl ($p = 0.003$) and U ($p = 0.008$) concentrations in hair samples among residents in the three study areas. However, significant regional differences in Ni and Se in hair samples are not observed (Kruskal–Wallis test, $p > 0.05$). Mann–Whitney U's test indicated that there are significant difference in Ag, Ba, Cd, Co, Cu, Ga, Mn, Ni, Pb and U ($p < 0.001$), B ($p = 0.033$), Mo ($p = 0.004$) and Se ($p = 0.003$) in hair samples from male and female residents. However, there are no significant differences in gender between Al ($p = 0.133$), As ($p = 0.448$), Cr ($p = 0.974$), Rb ($p = 0.084$) and Tl ($p = 0.187$) in hair samples. Likewise, there are significant differences in Ag, Al, B, Cd, Co, Cr and Mo ($p < 0.001$), Pb ($p = 0.010$), Rb ($p = 0.033$), Se ($p = 0.037$), Tl (0.044) and U ($p = 0.013$) in hair samples from children and adults; however, there are no significant differences in As ($p = 0.945$), Ba ($p = 0.817$), Cu ($p = 0.204$), Ga ($p = 0.894$), Mn ($p = 0.600$) and Ni ($p = 0.468$) in hair samples from children and adults (Mann–Whitney U's test, $p > 0.05$). Spearman's rho correlation analysis indicated that there were positive significant associations between As, Ba and Mn in groundwater and human hair samples (Fig. 3). Likewise, positive significant correlations between As, Ba and Mn in hair samples with respective average daily dose of residents in the Mekong River basin of Cambodia were observed (Fig. 4). These findings suggested that a high accumulation of trace elements among residents in the Mekong River basin of Cambodia was due to intake of high trace elements in groundwater.

4. Discussion

A comparison of daily intake and concentration of trace elements of different countries was presented in Table 6. The present study suggests that the residents in the study areas of Kandal province ingested much higher amounts of As than those in Vietnam, Thailand and Turkey, and ingested as much As as those in West Bengal of India (Uchino et al., 2006). Risk computations indicated that Kandal residents were at higher risk of arsenic toxicity than those in Vietnam (Ha Nam residents who consumed treated water with HQ of 1–10 Nguyen et al., 2009), Thailand (via duplicate diet study with HQ of 6.98 Saipan and Ruangwises, 2009), Turkey (via drinking water with a maximum HQ of 5.77 Kavcar et al., 2009) and China (via drinking water with a maximum HQ of 4.76 Liu et al., 2009). However, Kandal residents were at risk of As as high as those in Brezno, Partizanske and Prievidza districts of Slovakia (HQ > 10 Rapant and Krcmova, 2007) and Vietnam (Ha Nam residents who consumed untreated water with HQ > 10 Nguyen et al., 2009). In the present study, As concentrations in hair samples of Kandal residents were much higher than those in the other areas of Kandal province (Gault et al., 2008; Sampson et al., 2008; Sthiannopkao et al., 2010). However, it was consistent to the findings of Mazumder et al. (2009) which observed in the same study area of Preak Russey, Cambodia. It was apparent that ranges of As concentrations in hair samples of residents in Bangladesh and India were comparable to that observed in the Kandal province study area where the range of As concentration in hair samples of Vietnam resident was close to those in Kratie and Kampong Cham provinces. Although there is significant difference in As concentrations in hair samples among the three study areas, no significant difference in As concentrations in hair between male and female residents and between children and adults are observed. A positive significant correlation between As concentration in hair and groundwater and its average daily dose are also found. The positive significant correlation between As concentration in hair samples and groundwater has also been reported elsewhere (Gault et al., 2008; Nguyen et al., 2009). These findings suggest that high accumulation of As in residents in the Mekong River basin of Cambodia are due to intake of As-rich groundwater. However, it was not associated with gender or age of an individual person.

Table 6 indicated that Mn concentration in hair samples from Kandal and Kratie residents are close to that of Vietnam while Mn concentration in hair samples from Kampong Cham residents are comparable to that of India. Although the hazard quotient of single Mn is less than one in the present study (Table 4), its contribution to aggregate risk (Fig. 2) is substantially of interest. It appears that children who received low IQ test scores are associated with concentrations of both As and Mn as high as 1.4–55.4 × 10^{-3} μg g^{-1}

Table 6
Comparison of daily intake (μg d^{-1}) and concentration of some trace elements in hair (μg g^{-1}) of different countries.

Elements	Countries	Exposure route	Daily intake	Concentration in hair
As	Bangladesh	Groundwater; cooked rice	19–232 (Smith et al., 2006)	1.10–19.84 (Karim, 2000)
	Cambodia	Groundwater, foodstuffs	0.043–8.386[a] (Phan et al., 2012); 3 × 10^{-4}–10.75[a] (Present study)	0.10–7.95 (Gault et al., 2008); 2.10–13.94 (Sampson et al., 2008); 0.06–30.00 (Sthiannopkao et al., 2010); 0.92–25.60 (Mazumder et al., 2009); 0.011–57.214 (Present study)
	India	Drinking water, food	37.1–1098 (Uchino et al., 2006); 141–179 (Roychowdhury, 2008)	0.70–16.20 (Mandal et al., 2003); 0.17–14.40 (Samanta et al., 2004)
	Thailand	Diet	15.8–146 (Ruangwises and Saipan, 2010)	–
	Turkey	Drinking water	4.46 × 10^{-6}–1.729[a] (Kavcar et al., 2009)	–
	Vietnam	Groundwater	1.1–4.3[a] (Nguyen et al., 2009)	0.088–2.77 (Agusa et al., 2006); 0.12–1.09 (Nguyen et al., 2009)
Mn	Cambodia	Groundwater	1.21 × 10^{-4}–13.6[a] (Present study)	1.03–236.62 (Present study)
	India	Groundwater	–	1.85–43.56 (Samanta et al., 2004)
	Pakistan	Drinking water	0.06–1.82 (Muhammad et al., 2011)	–
	Turkey	Drinking water	3.52 × 10^{-4}–0.83 (Kavcar et al., 2009)	–
	Vietnam	Groundwater	–	0.84–263 (Agusa et al., 2006)
Ba	Cambodia	Groundwater	5.62 × 10^{-4}–16.2[a] (Present study)	0.33–328.28 (Present study)
	Vietnam	Groundwater	–	0.32–34 (Agusa et al., 2006)

[a] Daily dose (μg kg^{-1} d^{-1}).

and 0.089–2.145 μg g^{-1}, respectively, in their hair (Wright et al., 2006). Although there is significant difference in Mn concentrations in male and female hair samples, no significant difference in Mn concentrations between children and adults' hair samples are observed. Mn concentration in hair is significantly positive correlated to that in groundwater and its average daily dose. These data suggest that high Mn accumulation in the body of residents in the Mekong River basin of Cambodia could be due to consumption of Mn-rich groundwater; also, it was more likely to be related to individual gender, but not an individual age.

A comparison revealed that Ba concentrations in groundwater from Kandal province study areas are comparable to that in Lao PDR while Ba concentrations in groundwater from Kratie province study areas are close to that in Vietnam Table 1. Ba concentration in hair samples of Hanoi residents ranged from 0.32 to 34 μg g^{-1}, which is much lower than that in Kandal province study areas, but comparable to that in Kratie province study areas of the present study (Table 6). Although there is significant difference in Ba concentrations in male and female hair, no significant difference in Ba concentrations in children and adults' hair was observed. Positive significant correlation between Ba concentration in hair samples and groundwater as well as its average daily dose were also found. These data suggest that high accumulation of Ba in residents in the Mekong River basin of Cambodia is due to intake of Ba-rich groundwater; it is also more likely to be associated to individual gender, but not individual age.

5. Conclusions

Analytical results revealed that groundwater in Kandal and Kraite are enriched in As, Ba, Fe and Mn. However, other trace elements are below the Cambodian drinking water permissible limits of both Cambodian and WHO. Risk assessment revealed that 98.7% and 12.4% of residents in the study areas of Kandal ($n = 297$) and Kratie ($n = 89$) are at risk of non-carcinogenic effects of mixed trace elements; however, none of those in Kampong Cham ($n = 184$) are at risk. Arsenic contributed 99.5%, 60.3% and 84.2% in aggregate risk/risk of the mixed trace elements in Kandal, Kratie and Kampong Cham, respectively. Although hazard quotient of single Ba and Mn are less than one, they are nevertheless interesting, due to their potential impact on residents of the Kandal and Kratie province study areas. The present study suggests that As, Ba, Fe and Mn were the most concerned contaminants in groundwater in the Mekong River basin of Cambodia. Therefore, sustainable and effective treatment technologies were needed in order for groundwater to be used as potable water in the Mekong River basin of Cambodia. Moreover, further studies were required to investigate health effects of mixed trace elements, in particular As in groundwater in the Mekong River basin of Cambodia and its subregions.

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