

## Elevated levels of iron in groundwater in Prey Veng province in Cambodia: a possible factor contributing to high iron stores in women

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### ABSTRACT

Iron is a natural element found in food, water and soil and is essential for human health. Our aim was to determine the levels of iron and 25 other metals and trace elements in groundwater from 22 households in Prey Veng, Cambodia. Water analyses were conducted using inductively coupled plasma–mass spectrometry and optical emission spectrometry. Compared to the 2011 World Health Organization guidelines for drinking water quality, aluminum, iron and manganese exceeded maximum levels (in 4.5, 72.7 and 40.9% of samples, respectively). Compared to the 2004 Cambodian drinking water quality standards, iron and manganese exceeded maximum levels (in 59.1 and 36.4% of samples, respectively). We found no evidence of arsenic contamination. Guidelines for iron were established primarily for esthetic reasons (e.g. taste), whereas other metals and elements have adverse effects associated with toxicity. Iron in groundwater ranged from 134 to 5,200 µg/L (mean ~1,422 µg/L). Based on a daily consumption of 3 L groundwater, this equates to ~0.4–15.6 mg iron (mean ~4.3 mg/day), which may be contributing to high iron stores and the low prevalence of iron deficiency anemia in Prey Veng women. Elevated levels of manganese in groundwater are a concern and warrant further investigation.

**Key words** | anemia, arsenic, Cambodia, groundwater, iron, manganese

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### INTRODUCTION

Iron is an abundant element and is found naturally occurring in soil, water and food (Haynes 2012). It is an essential mineral in the human body and is required for oxidative energy metabolism, red blood cell production and oxygen transport, as well as other important functions (Gibson 2005; Ganz 2007). The majority of iron that is absorbed and utilized in the body is obtained from dietary food sources. However, in many parts of the developing world,

diets are low in iron and cannot provide adequate amounts of iron to meet daily requirements (De Benoist *et al.* 2008).

In Cambodia, the diet is iron-poor consisting mainly of rice and few iron-rich animal food sources (CARD 2011). Impaired absorption and loss of iron can also result from infection and disease (Gibson 2005; Weiss & Goodnough 2005) such as dengue, malaria, hookworm and parasites, which are prevalent in some areas of rural Cambodia.

Therefore, it is often assumed that iron deficiency is prevalent among women in Cambodia.

However, in a recent study in Prey Veng province (Karakochuk *et al.* 2015) we detected very low prevalence of iron deficiency in women ( $n = 420$ ). Iron stores, based on serum ferritin concentrations, were unexpectedly high (mean unadjusted ferritin  $\sim 103 \mu\text{g/L}$ ). Serum ferritin is an acute phase protein which means that in the presence of inflammation, levels are elevated. Therefore, it is recommended that serum ferritin concentrations be adjusted for levels of inflammation using inflammation biomarkers (Thurnham *et al.* 2010). However, even after adjustment for inflammation, serum ferritin concentrations remained high in the women (mean adjusted ferritin  $\sim 93 \mu\text{g/L}$ ). The prevalence of iron deficiency anemia among women was very low ( $\sim 1\%$ ) based on common biomarkers (hemoglobin  $< 120 \text{ g/L}$  and ferritin  $< 15 \mu\text{g/L}$ , for non-pregnant women of reproductive age). Based on self-reported data, the women in the study were not taking iron supplements, using iron cooking pots, nor consuming iron fortified food products. With the published evidence of elevated iron levels in groundwater in Cambodia (Feldman *et al.* 2007; Murphy *et al.* 2010), it is possible that groundwater could be a contributing factor to the high iron stores in the women in Prey Veng.

In addition to iron, elevated levels of arsenic and manganese have also previously been detected in groundwater in some areas in Cambodia (Buschmann *et al.* 2007; Feldman *et al.* 2007; Sthiannopkao *et al.* 2008; Luu *et al.* 2009). The Southeastern provinces of Kandal, Prey Veng and Kampong Cham in Cambodia are the most severely affected areas for arsenic contamination (levels in groundwater exceeding  $\geq 50 \mu\text{g/L}$ ), with the worst affected areas along the Mekong, Bassac and Tonle Sap riverbanks (RDIC 2012; Berg *et al.* 2007). This evidence of metal and trace element contamination in groundwater is a public health concern. Inorganic arsenic, the form most often found in drinking water, is classified as a human carcinogen (ATSDR 2007). Chronic exposure to inorganic arsenic through contaminated water or food has been associated with an increased risk of morbidity and mortality (Berg *et al.* 2001; Argos *et al.* 2010). Elevated levels of manganese ( $\geq 400 \mu\text{g/L}$ ) in drinking water have been associated with adverse neurological effects in children (Wasserman *et al.* 2006; Bouchard *et al.* 2011). Elevated levels of iron in drinking water

( $\geq 300 \mu\text{g/L}$ ), however, have not previously been associated with significant health risks. The maximum levels of iron in drinking water are set by the World Health Organization (WHO) because of undesirable esthetic properties that are associated with high iron content in water (i.e. poor taste, color and smell) (WHO 2011).

One successful approach to reduce the iron content and to improve microbial water quality at the household level has been the implementation of point-of-use water filtering systems. In Cambodia, the BioSand filter is a commonly used household filter. It is a slow sand filter that was originally designed to reduce microbial contamination in drinking water (WSP 2010). The BioSand filter, when installed correctly, can achieve reductions of up to  $\sim 99\%$  removal of both bacteria and iron (Murphy *et al.* 2010; Stauber *et al.* 2012). However, the availability and regular use of filtering systems in Prey Veng province is not well known.

The primary aim of this study was to determine the level of iron in groundwater in a subset of households including women participating in a larger study in Prey Veng (Karakochuk *et al.* 2015). We hypothesized that groundwater could be a contributing factor to the high iron stores of these women. The secondary aims were to determine the levels of 25 other metals and trace elements in groundwater and compare those levels to recommended minimum and maximum levels based on both chemical and nutritional quality; and to investigate the availability of and compliance with BioSand filtering systems in households.

## METHODS

Water samples were collected from groundwater wells in households participating in a larger trial in Prey Veng province (Karakochuk *et al.* 2015). Ethical approval for the study was granted by the Clinical Research Ethics Board at the University of British Columbia (Canada) and the National Ethics Committee for Health Research (Cambodia).

Water samples were collected from 22 households across 10 villages in four districts (Ba Phnum, Kamchay Mear, Me Sang and Svay Antor) of rural Prey Veng province in May 2014 at the end of the dry season. The 10 villages were randomly selected from a list of all villages in the four districts in Prey Veng province. From each of the 10 villages, two to

three water samples were collected from randomly selected households within the catchment area of the village. If a household did not have a groundwater well, or if no family members were home at the time of visit, the next nearest household was visited. A hand-held global position satellite device (Garmin® eTrex 10) was used to record coordinates of each groundwater well in order to confirm the geographical location of each well. Water consumption and treatment practices of the household were recorded at the time of the water sample collection, including availability of a water filtering system, compliance and regular use of the filtering system if it was available, and descriptions of the esthetic properties of the water (i.e. taste, color and smell).

A total of 22 water samples were taken directly from the well at each household. Before collection of the water samples, the wells were pumped for 90 seconds to initiate continuous water flow and avoid contamination of samples due to stagnant water in the pipe. An estimated quantity of 125 mL water was collected in an acid-washed polyethylene plastic container provided by the laboratory conducting the analyses (Agat Laboratories, Canada). In addition, filtered water samples were taken from a BioSand filter at three random households that reported use of a filter. The water samples from these filters were collected immediately and directly from the spout of the BioSand filter. For these households, untreated water samples were also collected directly from the well.

Temperature and pH were recorded for all 25 water samples. Temperature of the water sample was recorded to the nearest 1 °C using a standard glass thermometer (Thermo Scientific® ERTCO precision model). The pH of the water sample was recorded to the nearest 0.1 value using pH indicator test strips (Merck®). The pH strip was immersed in the pumped water for 15 seconds, removed and left to stand for 30 seconds. It was then compared to the pH color strip by two different field researchers (for confirmatory purposes) to estimate the pH level reading in the water sample.

After temperature and pH were recorded, concentrated nitric acid (2 mL) was immediately added to preserve the concentrations of metals (BCME 2009). Water samples were placed in an icebox and kept at 4 °C during transportation. They were then stored in a 4 °C refrigerator at the Helen Keller International office in Phnom Penh. Within 7 days of collection, the samples were shipped to Canada

and analyzed for metal and trace element content by Agat Laboratories (Burnaby, BC, Canada). Inductively coupled plasma–mass spectrometry and optical emission spectrometry were used to determine metal and trace element content of the water as per standardized methods (USEPA 1994). A total of 26 metals and trace elements were analyzed: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, molybdenum, nickel, selenium, silver, sodium, thallium, titanium, uranium, vanadium and zinc.

Detected levels of the 26 metals and trace elements were compared to the CMIME (2004) and WHO (2011) water quality guidelines to evaluate the chemical quality of the groundwater. The maximum standard levels are set based on expected risk of adverse health outcomes or undesirable esthetic properties (i.e. taste, color and smell) associated with high levels in drinking water.

Of the total 26 metals and trace elements analyzed, 13 also have dietary reference intakes (DRIs) established for women 19–30 years of age, including at least one of the following: adequate intakes (AI), estimated average requirements (EAR), recommended dietary allowances (RDA) or tolerable upper intake levels (UL). The DRIs are nutrient reference values developed by the Institute of Medicine of the National Academies (NRC 2006). They are established based on scientific evidence and provide reference values for minimum requirements and maximum limits for safety and are specified on the basis of age, gender and life stage. The EAR is the average daily nutrient intake level estimated to meet the requirements of ~50% of a healthy population. The RDA is an average daily dietary intake level that is sufficient to meet the nutrient requirements of ~97.5% or more of a healthy population and is calculated using the EAR value. The AI is an estimated value that suggests a recommended intake when there is insufficient scientific evidence to establish an EAR. The UL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to ~99% in the general population (NRC 2006).

For the 13 metals or trace elements with an established EAR, AI, RDA or UL, estimated levels of daily intakes (based on consumption of ~3 L groundwater daily) were compared to the available DRIs. We speculate that the

average Cambodian woman would consume ~3 L of groundwater daily. A recent unpublished report on the dietary intakes of women in Prey Veng (using 24-hour dietary recall methods and survey questionnaires) suggests that the mean water intake of women in Prey Veng is ~7.9 cups per day (~2 L water) solely as drinking water (V. Verbowski, personal communication, The University of British Columbia). However, this does not include water from tea, soups or other cooking sources using groundwater. In Cambodia, rice is a staple food and is consumed on a daily basis in most households. It requires water for cooking and rice is usually prepared with one part rice to four parts water. Most of the water will be evaporated during the cooking process; however, the iron in the water does not freely evaporate and remains in the rice. Therefore, the groundwater used to cook rice is also included in our estimation of daily water consumption of women. Therefore, we speculate the average Cambodian woman would consume ~3 L groundwater daily but recognize that this value is an estimation and would vary among individuals. Our results that refer to the estimated ~3 L groundwater should therefore be interpreted with these considerations in mind.

IBM SPSS™ software v.22 (Armonk, NY, USA) was used to conduct statistical analyses. Median values, mean values and standard deviations were used to describe the data.

## RESULTS

A total of 25 water samples were collected from 22 households in 10 villages in Prey Veng province. Data on the consumption, water treatment and filtering practices were obtained from women caregivers at the household level (self-report) at the time of water sample collection (Table 1). Data on whether or not a household had a filtering system were missing for four households (~18%) and data on whether or not a household reported red sediment in the water after 1 day were missing for six households (~27%). The study area of Prey Veng province is shown in Figure 1.

The groundwater samples (unfiltered) were analyzed for a total of 26 metals and trace elements. Table 2 presents the units of measure, reporting detection limit (RDL), minimum value observed, maximum value observed, mean value and

**Table 1** | Water consumption and treatment characteristics of 22 households in 10 villages in Prey Veng province<sup>a</sup>

	<i>n</i>	%
Water treatment for drinking source in household ( <i>n</i> = 22)		
No treatment (drinks directly from well)	17	77
Boils well water	2	9
Filters well water	3	14
Household has a filtering system available ( <i>n</i> = 18) <sup>b</sup>		
No	12	67
Yes	6	33
Household reports red sediment in water after 1 day ( <i>n</i> = 16) <sup>b</sup>		
No	9	56
Yes	7	44

<sup>a</sup>Values are *n* (%); *N*, number. Reported by women caregivers in households.

<sup>b</sup>Data were missing for four households (filtering system available) and six households (red sediment in water).

standard deviation, median value of each metal and trace element, and comparisons to the CMIME (2004) and WHO (2011) water quality guidelines. Quality assurance tests confirmed that control samples were within acceptable limits for all metals and trace elements analyzed. The values of the three filtered groundwater samples were not included in this summary table.

When compared to the WHO guidelines for drinking water quality (WHO 2011), a total of three metals and trace elements exceeded the recommended maximum levels in one or more samples of unfiltered well water: aluminum (one of 22 samples, or 4.5%), iron (16 of 22 samples, or 72.7%) and manganese (nine of 22 samples, or 40.9%). When compared to the Cambodia drinking water quality standards (CMIME 2004), a total of two metals and trace elements had eight or more samples that exceeded the recommended maximum levels: iron (13 of 22 samples, or 59.1%) and manganese (eight of 22 samples, or 36.4%).

Of the total 26 metals and trace elements analyzed, 13 are recognized as essential for human health and have DRIs established for women 19–30 years of age, including at least one of the following: AI, EAR, RDA or UL. For these 13 metals and trace elements, estimations of daily intake (based on consumption of 3 L groundwater daily) and comparisons to the DRIs are presented in Table 3. Of the 13 metals and trace elements, 10 have recommended



**Figure 1** | Study area of Prey Veng province in Cambodia.

intakes (either an RDA or an AI) for women of 19–30 years: calcium, chromium, copper, iron, magnesium, manganese, molybdenum, selenium, sodium and zinc. None of the groundwater samples had levels that met the recommended intakes (RDA or AI) for these elements, although an intake of 3 L would provide about a quarter of RDA for iron and two-thirds of the AI for manganese. For other minerals, 3 L of groundwater would provide <8% of the intake recommendations. Of the 13 elements, all but chromium have a UL established for women of 19–30 years (NRC 2006). On average, an intake of 3 L would provide ~10% of the UL for iron and manganese, however much less (<2%) for the remaining metals and trace elements. None of the individual groundwater samples came close to levels that exceeded the UL for these 12 elements.

In a subset of three households, water samples were taken directly from the groundwater well and also from the BioSand filter (after filtering). Levels of iron (Table 4) were compared before and after filtering with the BioSand filter. Water filtered with the BioSand filter had significantly

less iron content: ~98–99% of iron was removed through the filtering process.

## DISCUSSION

### Iron levels in groundwater

We detected elevated levels of iron in groundwater from wells in rural Prey Veng province in Cambodia. The groundwater iron levels ranged from 134 to 5,200 µg/L across households, with a mean value of 1,422 µg/L and a median value of 1,150 µg/L. Iron was elevated in 73 and 59% of samples based on recommended maximum levels of WHO (2011) and CMIME (2004) water quality guidelines, respectively. These maximum levels are recommended based on undesirable esthetic properties that are associated with high iron content in water (i.e. poor taste, color and smell). However, iron is an essential mineral in the body and a daily intake of 18 mg/day (RDA) is recommended

**Table 2** | Summary of metals and trace elements analyzed for chemical quality in 22 samples of groundwater from household wells in Prey Veng province and comparisons to the 2004 Cambodia Ministry of Industry, Mines and Energy and the 2011 World Health Organization water quality guidelines<sup>a</sup>

Metals and trace elements	Unit of measure	RDL	Min. value	Max. value	Mean ± SD	Median value	Global WHO guideline 2011	No. of samples exceeding WHO guideline	Cambodia CMIME guideline 2004	No. of samples exceeding CMIME guideline
Aluminum	µg/L	5	5	122	25.9 ± 35.0	5	100	1	–	–
Antimony	µg/L	0.5	0.5	0.5	0.5 ± 0.0	0.5	20	0	–	–
Arsenic	µg/L	0.1	0.1	7.7	2.1 ± 2.2	1.1	10	0	50	0
Barium	µg/L	0.5	24.9	349.0	111.0 ± 82.8	85.6	700	0	1,000	0
Beryllium	µg/L	0.05	0.05	0.58	0.13 ± 0.16	0.05	–	–	–	–
Boron	µg/L	5	5	18	10.2 ± 4.9	8	500	0	–	–
Cadmium	µg/L	0.01	0.01	0.09	0.03 ± 0.02	0.015	3	0	10	0
Calcium	mg/L	0.05	1.2	50.5	12.0 ± 11.9	9.4	–	–	200	0
Chromium	µg/L	0.5	0.5	1.5	0.6 ± 0.2	0.5	50	0	50	0
Cobalt	µg/L	0.05	0.05	7.38	2.22 ± 2.30	1.16	–	–	–	–
Copper	µg/L	0.5	0.5	4.4	0.85 ± 0.92	0.5	2,000	0	1,500	0
Iron	µg/L	10	134	5,200	1,422 ± 1,296	1,150	300	16	1,000	13
Lead	µg/L	0.05	0.05	6.06	0.51 ± 1.29	0.13	10	0	10	0
Lithium	µg/L	0.5	8.1	26.4	17.2 ± 4.0	16.6	–	–	–	–
Magnesium	mg/L	0.05	0.6	17.8	6.1 ± 4.8	5.5	–	–	150	0
Manganese	µg/L	1	47	827	393.9 ± 236.1	352.5	400	9	500	8
Molybdenum	µg/L	0.1	0.1	0.6	0.2 ± 0.1	0.1	70	0	–	–
Nickel	µg/L	0.5	0.1	9.5	2.5 ± 3.0	0.8	20	0	–	–
Selenium	µg/L	0.5	0.5	0.5	0.5 ± 0.0	0.5	10	0	10	0
Silver	µg/L	0.02	0.02	0.02	0.02 ± 0.0	0.02	–	–	–	–
Sodium	mg/L	0.1	2.46	67.0	28.4 ± 4.5	23.8	200	0	–	–
Thallium	µg/L	0.02	0.02	0.26	0.04 ± 0.06	0.02	–	–	–	–
Titanium	µg/L	1	2	7	3.6 ± 1.5	3	–	–	–	–
Uranium	µg/L	0.01	0.01	0.28	0.05 ± 0.06	0.015	–	–	–	–
Vanadium	µg/L	1	1	2	1.1 ± 0.2	1	–	–	–	–
Zinc	µg/L	5	5	26	10.7 ± 6.7	8	4,000	0	1,500	0
Hardness	µgCaCO <sub>3</sub> /L	0.1	5.8	166.0	55.4 ± 46.9	47.1	–	–	300	0
Temperature	°C	–	30	34	31.4 ± 1.0	31	–	–	–	–
pH	–	–	4	7	5.85 ± 1.0	6.0	–	–	–	–

<sup>a</sup>CMIME, Cambodia Ministry of Industry, Mines and Energy; RDL, reporting detection limit; SD, standard deviation; WHO, World Health Organization. Values of the three filtered water samples (filtered by BioSand filters) were not included in this summary table as the process of BioSand filtering removes the metals and trace elements.

for women aged 19–30 years (NRC 2001) as iron is required for required for oxidative energy metabolism, red blood cell production and oxygen transport, as well as other important functions (Gibson 2005; Ganz 2007). Therefore, it was the aim of our study to determine levels of iron in groundwater and assess if the water source could be contributing to the high iron stores observed in women in Prey Veng. We

speculated that the average Cambodian woman would consume ~3 L of groundwater daily, including drinking water, tea, soup or other cooking sources using groundwater. This would equate to ~0.4–15.6 mg of iron consumed daily, with a mean value of 4.3 mg iron and a median value of 3.5 mg iron. This is a large contribution of iron to daily dietary intakes (~24% of the RDA or ~53% of the

**Table 3** | Estimated daily intakes of 13 metals and trace elements (based on consumption of 3 L groundwater) and comparisons to established dietary reference intakes<sup>a</sup>

Metals and trace elements	Unit of measure	Estimated daily intake (3 L water) <sup>b</sup>	Daily DRIs			
			EAR	RDA	AI	UL
Boron	µg/day	30.6	–	–	–	20,000
Calcium	mg/day	36	800	1,000	–	2,500
Chromium	µg/day	1.8	–	–	25	ND
Copper	µg/day	2.55	700	900	–	10,000
Iron	µg/day	4,266	8,100	18,000	–	45,000
Magnesium	mg/day	18.3	225	310	–	350 <sup>c</sup>
Manganese	µg/day	1,181.7	–	–	1,800	11,000
Molybdenum	µg/day	0.6	34	45	–	2,000
Nickel	µg/day	7.5	–	–	–	1,000
Selenium	µg/day	1.5	45	55	–	400
Sodium	mg/day	85.2	–	–	1,500	2,300
Vanadium	µg/day	3.3	–	–	–	1,800
Zinc	µg/day	32.1	6,800	8,000	–	40,000

<sup>a</sup>AI, adequate intakes; DRI, dietary reference intakes; EAR, estimated average requirements; ND, not determined; RDA, recommended dietary allowances; UL, tolerable upper intake levels. DRI values based on females 19–30 years of age are reported for comparisons (NRC 2006).

<sup>b</sup>Estimated daily intakes of minerals and trace elements were calculated using mean values and based on consumption of 3 L groundwater daily (drinking water, tea, soups, or other cooking sources using groundwater, namely rice).

<sup>c</sup>The UL for magnesium applies only to magnesium consumed as a supplement or fortificant.

**Table 4** | Iron levels in groundwater before and after household BioSand filtering in three households<sup>a</sup>

Households	Iron level from groundwater (µg/L)	Iron level after filtering (µg/L)	Change in iron level
1	1,150	24	98% decrease
2	1,020	10	99% decrease
3	5,200	10	>99% decrease

<sup>a</sup>Iron levels from groundwater wells all exceed the recommended maximum levels; however, after BioSand filtering all iron levels are decreased to below the recommended maximum levels (CMIME 2004; WHO 2011).

EAR for women aged 19–30 years), assuming that the iron in the groundwater is bioavailable.

Bioavailability of iron is suspected to be high given the form of iron in groundwater. The groundwater aquifer environment is reducing, which means the majority of iron is in the ferrous form (Fe<sup>2+</sup>) which is more dissolvable and bioavailable than the ferric form (Fe<sup>3+</sup>) (Hurrell 1997; Merrill *et al.* 2010). In addition, low pH concentrations were detected in our water samples, indicating acidic environments. An acidic environment increases iron bioavailability and absorption in the gastrointestinal tract (Hallberg 1981). It is likely

that some of the iron is oxidized to Fe<sup>3+</sup> during the pumping of the well water, and during storage if the water is not consumed immediately (Murphy *et al.* 2010). This theory can be substantiated by the fact that some women would see red precipitate (Fe<sup>3+</sup>) in their water storage containers after a day of storage (Table 1). Although Fe<sup>3+</sup> is not as bioavailable as Fe<sup>2+</sup> (Hurrell 1997), once consumed, Fe<sup>3+</sup> can be reduced to Fe<sup>2+</sup> in the body with the help of stomach acid, the contents of the intestine and the cell membrane enzyme ferric-reductase (Aster 2007).

Worwood *et al.* (1996) demonstrated that iron bioavailability was high in naturally occurring mineral water (~300,000 µg iron/L) in England. Absorption studies in adults ( $n = 13$ ) using water labeled with ferrous sulphate indicated absorption rates of ~23% among individuals who consumed 10 mL of the mineral water on an empty stomach. Furthermore, Worwood *et al.* demonstrated higher absorption rates (~40%) among individuals with low iron stores (ferritin < 10 µg/L) compared to absorption rates of ~10% among individuals with high iron stores (ferritin > 200 µg/L). As expected, the rates of iron absorption increase when iron body stores are deficient. Although the

iron content of the mineral water in the England study (~300,000 µg iron/L) was substantially higher than the groundwater in our study (mean ~1,422 µg iron/L), the groundwater in our study was very acidic, which increases the solubility and bioavailability of the iron. We suspect the iron in our study would be comparatively bioavailable.

Researchers in other parts of Asia have observed that increased groundwater iron is positively and significantly associated with increased iron stores in women. In rural Bangladesh, the median daily iron intake from groundwater was estimated at ~41 mg/day (Merrill *et al.* 2010). Merrill *et al.* (2011) then found that increased groundwater iron content was significantly correlated with plasma ferritin ( $r = 0.36$ ) and total body iron ( $r = 0.35$ ) in women in Bangladesh. Although median levels of iron in groundwater were much lower in our study (~3.5 mg/day) as compared to the Bangladesh study (~41 mg/day), the iron could still be accumulating and contributing to iron stores in women in our study, but at a slower rate than observed in the Bangladesh study. We did not attempt to correlate groundwater iron content with iron status of individual women in our study as the number of unfiltered water samples collected was too small ( $n = 22$ ) to detect statistically significant associations.

As noted previously, the recommended maximum levels of iron in drinking water are based on esthetic concerns (WHO 2011). Iron content in groundwater has not previously caused concern in terms of potential health risks (WHO (World Health Organization) 2011). However, there is a potential risk of iron overload in populations with hemoglobinopathies, or genetic hemoglobin disorders (Bain 2006). In Cambodia, these disorders are common and affect ~50% of the population (Carnley *et al.* 2006). Some forms of hemoglobinopathies can result in accumulated iron stores in the body (high serum ferritin concentrations) (Thurlow *et al.* 2005; George *et al.* 2012; Karakochuk *et al.* 2015). Some forms of severe thalassemias can also result in decreased erythropoiesis which further enhances iron absorption in the gastrointestinal tract and may contribute to even higher iron stores (Rees *et al.* 1998). These severely affected individuals often require life-long blood transfusions and other serious medical treatment (Bain 2006). However, many of these individuals go undiagnosed and/or untreated in rural Cambodia. In these affected individuals, large quantities of iron from untreated and unfiltered groundwater

sources may potentially increase the risk of iron overload and associated adverse effects.

### Manganese levels in groundwater

The other metal that was elevated in the groundwater samples and is of potential concern was manganese. These findings are consistent with the findings of other researchers in Cambodia (Feldman *et al.* 2007; Murphy *et al.* 2010). Elevated manganese in drinking water is a concern as it has been associated with neurological impairment in children (Wasserman *et al.* 2006; Ljung & Vahter 2007; Bouchard *et al.* 2011). A recent systematic review and meta-analysis (including 17 studies on manganese toxicity) published evidence that elevated manganese (detected in hair) was associated with decreased IQ levels in children aged 6–13 years. The authors also reported that manganese exposure was associated with attention deficit disorder with hyperactivity in children (Rodriguez-Barranco *et al.* 2013).

However, manganese, similar to iron, is also an essential nutrient in the human body. A daily intake of 1.8 mg/day (AI) is recommended for women aged 19–30 years (NRC 2001) as manganese is required for optimal bone health, energy and protein metabolism, and regulation of cell metabolism (Keen *et al.* 2000). Although none of the groundwater samples had levels of manganese that met the AI, some samples were relatively close, suggesting that groundwater provides a substantial contribution to daily recommended intakes of manganese. Of particular interest, none of the samples were close to reaching the UL, not even the samples that exceeded the CMIME and WHO water quality standards.

Bouchard *et al.* (2011) speculated that manganese from groundwater was significantly more bioavailable than manganese from food sources based on correlations between manganese in hair samples and manganese in water. However, some researchers have highlighted several limitations of the Bouchard study (Chen & Lopes 2011), indicating that these findings would ideally be substantiated with studies that quantify the bioavailability of manganese from both water and food sources. Hence, more research is warranted in this area before we can draw conclusions that excess manganese presents a greater risk of toxicity when consumed from water rather than food sources. Further research is also



warranted to investigate levels of manganese in groundwater in other geographical regions and the consequences of elevated manganese levels in drinking water in populations of differing age and geographical location.

### Other metals and trace element levels in groundwater

Aluminum was elevated ( $\geq 100 \mu\text{g/L}$ ) in one sample. However, the majority of samples (18 of the 22) were well below  $50 \mu\text{g/L}$ , suggesting that aluminum in groundwater is not a major concern. Some researchers have speculated that elevated aluminum levels in drinking water is a risk factor for the development of Alzheimer's disease. However, there is insufficient evidence to prove an association. Therefore, it has been concluded that elevated aluminum in drinking water does not currently pose any significant health risk to humans (WHO 1997).

None of the water samples contained arsenic levels that exceeded WHO recommended maximum limits ( $> 10 \mu\text{g/L}$ ) despite previous research indicating arsenic in groundwater as a major public health problem in Cambodia (Feldman *et al.* 2007; Sampson *et al.* 2008). Sthiannopkao *et al.* (2008) reported arsenic contamination in well water in Prey Veng and neighboring Kandal province, where 15 of 28 samples (54%) exceeded the WHO maximum limits ( $> 10 \mu\text{g/L}$ ). It has been speculated that although high iron and manganese concentrations are often found in areas of high arsenic concentration (further to the reducing environment of aquifers), statistical correlations between arsenic, iron and manganese are of only moderate strength (Feldman *et al.* 2007). We did not detect high arsenic concentrations in our groundwater samples, despite the aforementioned studies in Cambodia that did. We conclude that the relationship between iron, arsenic and other chemical parameters is complicated. There are many factors which contribute to the determination of arsenic in groundwater samples that we neither investigated nor discussed in this manuscript. Further to arsenic, none of the other remaining 22 metals and trace elements tested in our samples were elevated beyond the maximum limits according to either WHO (2011) or CMIME (2004) guidelines: antimony, barium, beryllium, boron, cadmium, chromium, cobalt, copper, lead, lithium, magnesium, molybdenum, nickel, selenium, silver, sodium, thallium, titanium, uranium, vanadium and zinc.

Other than manganese and iron, none of the other 11 metals and trace elements that have established DRIs provided a substantial contribution to recommended dietary intakes or indicated a risk of excess intake in the groundwater samples.

For simpler interpretation (Table 3) the levels of the 13 metals and trace elements were compared to DRIs for women aged 19–30 years. However, women in our study were between the ages of 18 and 45 years. Therefore, it is important to note that for some metals and trace elements, the DRIs are slightly higher or lower for women outside of the 19–30 years age category (NRC 2006). In brief, women aged 14–18 years have a slightly higher EAR for calcium (1,100 mg/day), magnesium (300 mg/day) and zinc (7.3 mg/day), a slightly lower EAR for copper (685  $\mu\text{g/day}$ ), iron (7.9 mg/day) and molybdenum (34  $\mu\text{g/day}$ ), and a slightly lower AI for chromium (24  $\mu\text{g/day}$ ) and manganese (1.6 mg/day). Women aged 31–50 years have a slightly higher EAR for magnesium (265 mg/day).

### Biosand filters

As expected, the BioSand filter was successful in removing iron from the water (98–99% iron removal) in the three households in our study. Murphy *et al.* (2010) previously reported ~99% iron removal from Cambodian groundwater using BioSand filters. Other studies have found that these filters are effective at reducing levels of bacteria (Sobsey *et al.* 2008; Stauber *et al.* 2012). However, in one evaluation study, it was reported that the BioSand filters do not consistently remove bacteria to levels considered acceptable by the WHO under field conditions (Murphy *et al.* 2010). Although ~33% of the households reported having a water filtering system available, only ~14% of households ( $n = 3$ ) reported using any type of filter before consuming water from groundwater wells. This rate of compliance (~42%) is lower than observed in another cross sectional study of 336 households across five provinces in Cambodia evaluating compliance and use of BioSand filters (~88% compliance) (WSP 2010). The low compliance rate of BioSand filters reported in our study highlights a potential problem and warrants further investigation.

## Strengths and limitations

Strengths of this study include that it is the first study to our knowledge to evaluate both the chemical and nutritional quality of groundwater in Cambodia. Limitations of this study include that it was conducted in small sample of 22 households and findings cannot be extrapolated to other geographical regions. We did not measure the oxidation–reduction potential in the ground water samples collected, which could have provided more information about the form and bioavailability of the iron in the samples. We did not investigate microbiological contamination of the water which is another important factor related to water quality. Microbiological contamination of drinking water can cause an increased risk of diarrhea or other co-morbidities and thereby increase the risk of mortality in infants and children (Lanata *et al.* 2013). Whereas the primary aim of our study was to investigate the levels of iron in groundwater. We did not attempt to detect an association between groundwater iron content in the household and biochemical iron stores (serum ferritin concentration) in women in the study. Therefore, we can only speculate that higher levels of iron in groundwater could contribute to iron stores in women, based on similar research conducted in Bangladesh (Merrill *et al.* 2011).

## CONCLUSIONS

We conclude that groundwater from wells in our study in the province of Prey Veng, Cambodia contained elevated levels of iron and manganese, but not arsenic. Iron in the well water from some households was considerably elevated (up to 5.2 mg/L). It is theorized that iron found in the households' drinking water could be contributing up to 15.6 mg iron/day to some women's diets. Consequently, it is likely that the iron was contributing to the high serum ferritin concentrations (and therefore a lack of iron deficiency anemia) observed in a recent study (Karakochuk *et al.* 2015) including women from the same households where groundwater samples were collected.

A daily intake of 3 L groundwater would provide about a quarter of the RDA for iron (and about a half of the EAR for iron) and two-thirds of the AI for manganese. Other than

manganese and iron, none of the other 11 metals and trace elements that have established DRIs provided a substantial contribution to recommended dietary intakes or indicated a risk of excess intake in the groundwater samples.

As expected, the BioSand filter was successful in removing iron from the water (98–99% iron removal) in the three households in our study. However, it is important to note that only ~33% of the households ( $n = 7$ ) reported having a filtering system available, and only ~14% of households ( $n = 3$ ) reported using any type of filter before consuming water from ground wells (~42% compliance).

Further research is needed to characterize the groundwater quality in other geographical regions of Cambodia, particularly the occurrence of iron and manganese. Studies linking the presence of elevated iron in groundwater with elevated iron stores in women are needed to substantiate the potential of groundwater as a contribution to dietary iron intakes. Investigation of the potential risk of iron overload from groundwater in those individuals with severe forms of hemoglobinopathies cannot be ignored and warrants further research.

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