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Research

Validating a portable device for groundwater iron measurement in Bangladesh

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Abstract
The mineral iron is essential for health at an optimum level. In Bangladesh iron is obtained from the drinking source groundwater, attributing largely to the good iron status in population. This groundwater iron poses two scenarios—predominantly high in many areas and predominantly low in other areas. Despite good iron status, burden of anemia in population is high. To control anemia, iron supplementation programs are operational against the backdrop of high iron status. This often leads to iron-associated side effects, plausibly due to excess iron, especially among the individuals exposed to high amount of iron from the drinking water. This geological phenomenon warrants the supplementation program to deliver different doses depending on the groundwater iron level. To enable that, the country needs an area-representative "mapping" of groundwater iron. However, such a mapping using the gold standard laboratory methods is expensive and logistically intensive. The aim of the study is to validate Handheld Colorimeter (HI721 Checker® HC), a portable device ("spot kit" device) to measure iron concentration in the drinking groundwater. The study was carried out in the rural Belkuchi upazila of the Sirajganj district in the Rajshahi division. In the study 25 tubewells were selected and categorized into five groups. Among them, four were categorized based on their iron concentration, low iron conc. (0-<1 mg/L), medium iron conc. (1-2 mg/L), high iron conc. (2-10 mg/L), very high iron conc. (>10 mg/L). One additional group was included for tubewell water that was used after filtering. The study compared the iron estimates measured in groundwater samples of the selected tube wells by the spot kit device and the same samples measured by atomic absorption spectrophotometry (AAS). There was a high agreement between the estimates by the spot kit and AAS (Kappa coefficient= 0.72, $p < 0.001$; Lin's concordance = 0.829, $p < 0.001$). Pitman's test of difference in variance exhibited that the two methods produced equally precise results (r=0.227, p=0.275). The study concluded that the Handheld Colorimeter (HI721 Checker® HC) was valid to measure the iron concentration of groundwater under field conditions. This device can facilitate testing processes, particularly in remote or resource-constrained areas where sophisticated testing methods (i.e: AAS) are unavailable and thus may help the re-design of the anemia control program in settings like Bangladesh with high and variable level of iron in the drinking groundwater.

INTRODUCTION
Iron has an invaluable role in numerous biological processes of the body, including hemoglobin synthesis, oxygen transport, energy metabolism, and immune function (Ravingerová et al., 2020). Iron deficiency is associated with decreased productivity, impaired cognitive development, increased susceptibility to infection, disrupted reproductive health, and finally results in anemia (Scrimshaw, 1991, Cappellini et al., 2019). It is assumed that the risks of iron deficiency and anemia are high, in resource poor settings, like Bangladesh, where minimally bioavailable cereal-based diet contributes the major share of iron (Bhatnagar & Padilla-Zakour, 2021, Bhargava et al., 2001).

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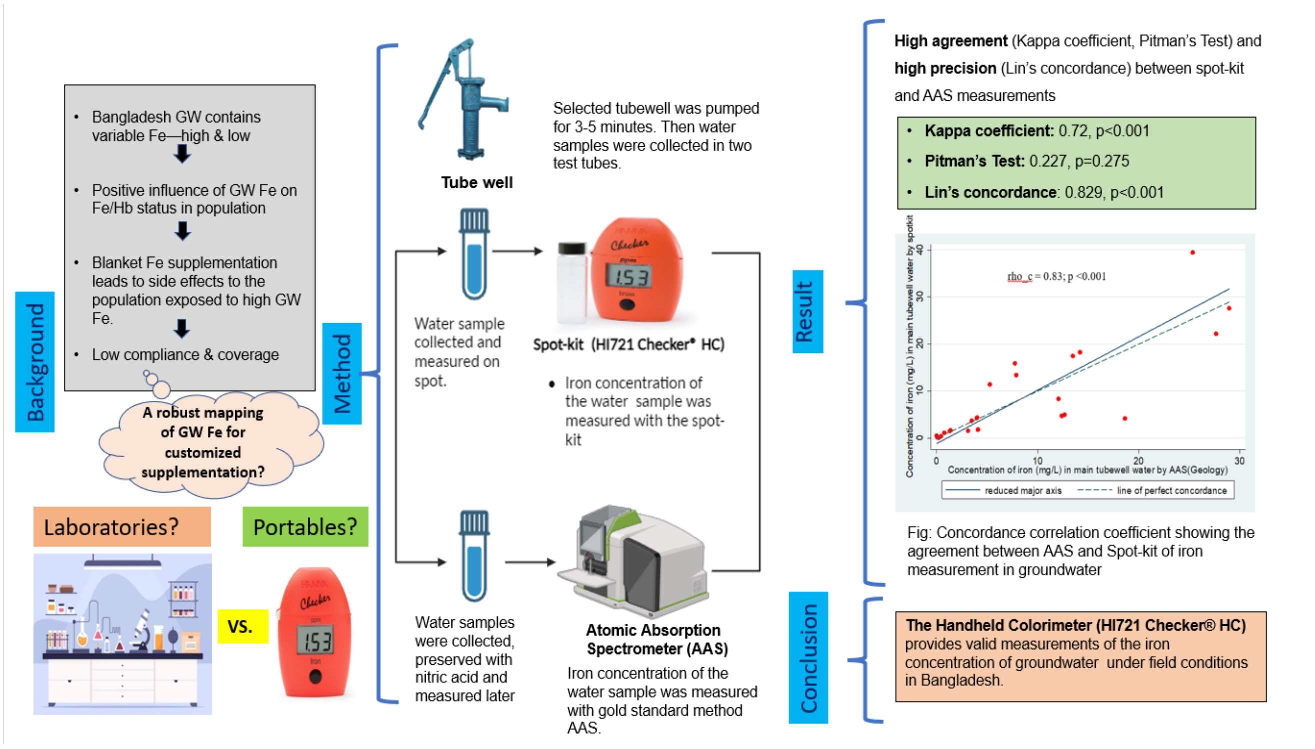

The high prevalence of anemia among children (33.1%) and women (26%) is a pressing public health concern in Bangladesh (Rahman et al., 2016). To control anemia, over the decades, the government of Bangladesh operates and promotes nationwide iron supplementation programs for pregnant women, adolescent girls and young children (Institute of Public Health Nutrition and United Nation Children's Fund 2016).

Recently, it has been revealed in Bangladesh that groundwater which is the principal source of drinking water in population, contains a variable but fair concentration of iron. (Merrill et al., 2011, Rahman et al 2016). Bangladesh is one of those countries where iron in groundwater derived from geological formation, an enabling level of the pH and water temperature, and oxygen reduction potential (Merrill et al., 2009). About 90% of the Bangladeshi population is relying on groundwater to fulfill their daily water requirement (Ghosh et al., 2020). Recent studies, in native settings, suggest, this ground water iron is readily absorbable and favorably contributes to elevated serum ferritin and hemoglobin status in Bangladeshi populations (Merrill et al., 2011; Rahman et al., 2016, Ahmed et al, 2018), despite the fact that the traditional Bangladeshi diet is predominantly cereal based and low in animal source foods with a poor potential of bioavailable iron. Henceforth, groundwater iron is considered as a novel and potent way to maintain iron status in the Bangladeshi population (National Anemia Consultation, 2016).

However, iron concentration in groundwater varies geographically throughout the country. The British Geological Survey 2001 (BGS) identified variable degree of iron in tube well water, ranging from <0.004 mg/L to 61 mg/L (BGS & DPHE, 2001). The findings of National Drinking Water Quality Survey (NDWQS) conducted in 2009 measured the median iron concentration of 0.95 mg/L and the highest concentration of iron was reported to be 43 mg/L (UNICEF Bangladesh, 2009); and thus comparable to the BGS data. Regionally, the northeast Sylhet area, South to the Ganges and the either side of the Brahmaputra basins have higher than average concentrations of iron in the groundwater. On the other hand, low iron concentrations are found in the Barind and Madhupur tracts, deep groundwater in the Barishal/Patuakhali areas, and the western section of the Tista-fan region (BGS & DPHE 2001, Rahman et al., 2020). The wide variation of the level of groundwater iron implies, there are the smaller pocket of areas within predominantly high groundwater iron regions, where some wells contain low level of iron in groundwater. Conversely, in a predominantly low groundwater iron setting, smaller areas exist where the wells contain higher amount of iron. Consequently, determining whether a particular area should be defined as high or low groundwater iron region is difficult without the assessment of water iron content from a representative sample.

In the high groundwater iron areas, where people are by and large replete in iron status from the drinking water, supplemental iron from anemia control programs might be counterproductive to many of them, as excess of iron may lead to the side effects such as loose stools, nausea, diarrhea, vomiting (Rahman et al., 2019), which are mostly attributed to the alteration of the gut microbiome (Zimmermann 2010, Jaeggi 2014). Using the screen and treat principle for iron supplementation is infeasible to implement, because of the resources and logistical constraints for the very large population of the country. Hence, adjustment of doses of iron supplement, sorted by the groundwater iron content across the country might be a plausible solution.

Therefore, assessment of groundwater iron levels or iron mapping for guidance to anemia prevention/iron supplementation program is of utmost importance. The surveys undertaken previously to map the level of iron in the drinking groundwater had limited sample size; and was non-representative at the level of the smaller administrative area units (Rahman et al., 2020).

Measuring iron content of groundwater representative at the smaller area units (i.e. subdistrict or upazila level) across the country warrants a robust survey. Such a survey across the country (~500 upazilas) using the gold standard laboratory-based method (e.g. Atomic Absorption Spectrophotometry, AAS) will be highly expensive and logistically challenging. Hence, it is important to have validated low-cost and easy-to-use, portable field instruments for assessing iron levels in groundwater samples. Previously a study validated a portable iron testing device which used color-matching principle (Merrill et al., 2009). Nonetheless, the color-matching devices are prone to have subjective-errors. In the present study, we aim to validate a portable instrument, Handheld Colorimeter (HI721 Checker® HC), Hanna Inc. Woonsocket, RI, USA (denoted in the text as "spot kit" device), for measuring water iron, which provides results on the digital display. The measurement done by the device will be compared against the gold standard, AAS.

METHODS

STUDY SITE

The study was carried out in the rural Belkuchi upazila of the Sirajganj district in the Rajshahi division (24.2917ºN, 89.7000ºE). It is located in northwest part of Bangladesh. The Belkuchi upazila was selected purposively according to the report of the DPHE/BGS 2001, which identified Belkuchi among predominantly high groundwater iron areas.

SAMPLE SIZE AND SAMPLING STRATEGY

As a pilot study, a small number of tube wells were purposively selected. The inclusion criteria of tube wells were: a) must be a source of drinking and cooking water for households, b) belonging to a subgroup as per study design with particular range of the iron concentrations. An iron level classification was developed depending on the iron concentration of groundwater (BGS & DPHE 2001). A community engagement phase involved for facilitating iron content estimation in tube well water. Selection of the tube wells was guided by:

A. Qualitative experience of the villagers of what they perceive about the iron content in the water of the wells they use for cooking and drinking.
B. At next, the actual measurement of iron content of the tube well water by a spot kit device was done.

A total of forty eligible tube wells were identified, eight belonging to each group (Table 1). Some of the households were found to consume filtered water, using traditional sand filtration technique, therefore one group of the user of filtered water was considered. Finally, five tube wells were randomly selected form each of the groups; thus, 25 tube wells were considered for final data collection. Before data collection, the participants (the household head or his wife) were verbally informed about the objectives of the study and only after taking their written consent, the samples were collected.

Table 1. Selection of the tube wells sorted by iron concentration in groundwater

Groundwater iron concentration	No. of tube wells identified	No. of tube wells selected for data collection
Group 1: low iron conc. (0-<1 mg/L)	8	5
Group 2: medium iron conc. (1-2 mg/L)	8	5
Group 3: high iron conc. (2-10 mg/L)	8	5
Group 4: very high iron conc. (>10 mg/L)	8	5
Group 5: using filtered water	8	5
Total	40	25

ESTIMATION OF IRON IN GROUNDWATER SAMPLES

The tube wells were firstly pumped for at least 3 minutes to access the deeper aquifer and to remove any lingering minerals that may have accumulated in the inner surface of the pipes. The samples were collected in a pair. One water sample from the pair was used for the assessment of iron by spot kit on the study site and another sample was stored in acidified condition by adding 2 mL of nitric acid in polyethylene falcon tubes for the iron assessment by AAS.

MEASURING IRON CONCENTRATION IN GROUNDWATER BY SPOT KIT

To measure the iron concentration in groundwater, a spot kit device, Handheld Colorimeter (HI721 Checker® HC), Hanna Inc. Woonsocket, RI, USA was used. First, the Checker®HC device is "zeroed" with the vial inside. Next, in the manufacturer-provided test beaker, 10 mL water sample was drawn and combined with the supplied phenanthroline reagent, ensuring thorough mixing. The test beaker with the reagent mixed sample water is placed in device. On pressing the button, the result is displayed in numerical digits, with a maximum estimation of 5 mg/L. If necessary, the test was repeated using two-, five-, or ten-fold dilutions of the original sample, and the final reading was adjusted based on the dilution factor.

GROUNDWATER SAMPLE COLLECTION AND ANALYSIS FOR IRON CONCENTRATION BY ATOMIC ABSORPTION SPECTROMETRY (AAS)

A 50 ml of water sample from each selected tube well was collected in polyethylene falcon tubes. The falcon tubes were previously rinsed with deionized water. About 1 mL of concentrated nitric acid was added to the collected samples and mixed immediately to avoid the iron precipitation and preserved for further analysis. All the collected samples were stored in a safe place of study site below 20ºC in a dark room. Within the three days of sample collection, they were transferred to the laboratory of Institute of Nutrition and Food Science (INFS) for safer storage at room temperature. Next the collected samples were sent to the Geochemistry Laboratory of Geology Department, University of Dhaka for the estimation of iron concentration by AAS standard method.

In atomic absorption spectrometry method, iron ions get vaporized into a flame and the iron vapor absorbs radiation from the specific hollow cathode lamp in proportion to the number of iron atoms present. Beer's Law is followed in the part-per-million (ppm or mg/L) range. Atomic absorption Spectrophotometer (Savant AA, GBC, Australia) with built-in software was used for this purpose. As per anticipated concentration of iron in the groundwater, a series of standard solution (blank, 0.5ppm, 1.0 ppm, 2.0 ppm, 4.0 ppm and 8.0 ppm) were used for preparing standard curve. A wavelength of 248.30 nm with slit width of 0.2 nm and 7.0 mA lamp current was used for iron measurement. The acceptable value of coefficient of determination (R^2) of the standard curve was set 0.995 to continue for running the samples. Standard reference sample and duplicate sample were run after every ten samples to check the accuracy and reproducibility of the analyses. Dilution method was applied, where necessary, to determine the higher concentration of iron in the sample.

STATISTICAL ANALYSIS

Descriptive analysis was carried out to determine the mean iron concentration in the collected samples measured by spot kit and AAS. The Mann-Whitney test was used to report the statistical differences between the mean estimates measured by the instruments. The Spearman Rank correlation coefficient assessed the relationship of the iron concentration measures by spot kit vs. AAS in the groundwater samples. The agreement between the methods was assessed using Kappa statistic. The bias and limit of agreement between the methods were examined by Bland - Altman plot, (Bland-Altman, 1986). Lin's Concordance Correlation Coefficient measured the precision and accuracy between the two iron concentration estimation methods (Lin, 2000). Precision and accuracy are the combined in the form of Concordance Correlation Coefficient, or rho_c (Rahman et al., 2020). Data analysis was done with the statistical software, STATA version 16 and SPSS version 26.0.

ETHICAL CONSIDERATIONS

The study was nested in the project assessing the availability of the groundwater iron from cooked rice. The main project received approval (reference no: 179/biol.sci) from Institutional Review Board (IRB) of Faculty of Biological Science, University of Dhaka.

RESULTS

In the collected groundwater samples, iron concentration was measured using atomic absorption spectrometry (AAS) and the spot kit device (Table 2). The mean (SE) concentrations of iron in the water samples tested by AAS and spot kit were 8.38 (1.79) mg/L and 8.36 (2.03) mg/L respectively. The Mann Whitney test reported no significant (p=0.97) mean difference between the iron concentrations obtained by two methods (Table 2).

Spearman Rank correlation coefficient revealed strong association between ground water iron concentration measured by the spot kit and the AAS (r=0.91, p<0.001) (Table 3). Further, the Table 3 describes that the observed agreement between the AAS and spot kit methods of iron estimation was higher than the expected agreement (observed agreement: 90.61% vs. expected agreement: 65.89%). The Kappa coefficient was 0.72 (SE = 0.13), which was statistically significant (z= 5.79, p < 0.001).

Table 2. Groundwater iron concentration using atomic absorption spectrometry (AAS) and spot kit

Total iron concentration (mg/L), n=25	Mean (SE)	95% CI
AAS	8.38(1.79)	4.69,12.06
Spot kit	8.36(2.03)	4.17, 12.54
p value	0.977[a]	

[a]Mann-Whitney test

Table 3. Association and agreement of iron concentrations in groundwater sample measured by the spot kit method and atomic AAS method.

Variable			Spot kit vs. AAS		
Association					
Correlation coefficient (rho) [a]				0.91[b]	
Agreement					
Observed agreement	Expected agreement	Kappa	SE	z	p
90.61%	65.89%	0.72	0.123	5.79	0.000

[a] Spearman Rank correlation coefficient.
[b]p value < 0.001

According to Table 4, on average, the mean difference (SD) between the AAS and spot-kit methods of groundwater iron estimation was -0.021(5.584). The correlation coefficient between the mean difference and individual mean of the methods was 0.227. The analysis showed the relationship between the mean difference and individual mean measurements is not significantly different (Bradley Blackwood F = 0.625, p = 0.54398).

Table 4. Bradley Blackwood value of linearity, showing the difference in estimation and deviation from mean between two methods (AAS and spot kit) of iron measurement in groundwater

Difference between estimated values		
Bland and Altman, 1986	Average (SD)	95% limits of agreement
	-0.021 (5.584)	-10.966, 10.924
Correlation between difference and mean		0.227
Bradley Blackwood F		0.625 (p=0.54398)

Figure 1 illustrates Bland-Altman plots using the average of two concentrations (iron concentration using spot kit and AAS) on the x-axis and the difference between them on the y-axis. It also depicts that the two methods have a mean difference of -0.021 indicating the spot kit tends to exhibit slightly lower results than the AAS method. The 95% limits of agreement are -11.189 to 11.147. The figure further suggests that 8% of the observations (2/25) fell outside the upper and lower limits of agreement i.e. 95% confidence interval for the difference between the methods—hence, the results (measures by the two methods) are consistent with a high degree of agreement. The Pitman's Test of difference in variance exhibited a non-significant weak positive correlation between the two methods (r=0.227, p=0.275), which means that the two methods are able to produce equally precise results.

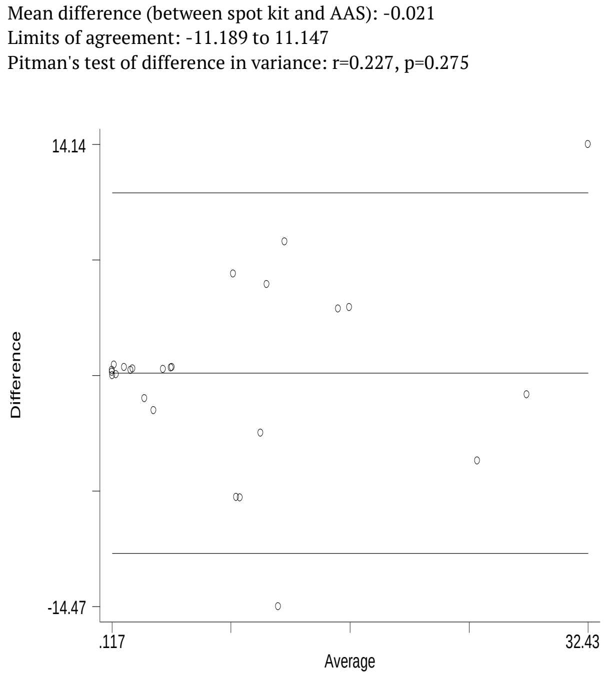

Figure 1. Bland-Altman plot: Groundwater iron concentration spot kit vs AAS

Figure 2 revealed the extent of agreement between two methods (AAS and spot kit) of groundwater iron concentration measurement. The Lin's correlation coefficient (rho_c) indicated a significant ($p<0.001$) strong positive (rho_c=0.83) linear relationship between the iron measurements estimated by AAS and spot kit.

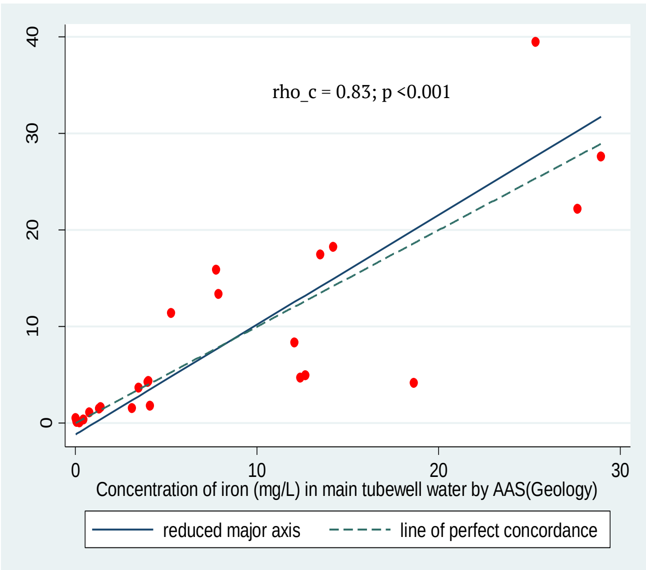

Figure 2. Concordance Correlation coefficient showing the agreement between two methods (AAS and spot kit) of iron measurement in groundwater

DISCUSSION

The aim of the study was to validate a portable, easy-to-operate and budget friendly groundwater iron measurement spot kit: the Handheld Colorimeter (HI721 Checker® HC), (Hanna Inc. Woonsocket, RI, USA), against the gold standard method AAS. The water samples collected from 25 tube-wells of rural north-western (Belkuchi, Sirajganj) Bangladesh were measured for the iron concentration by the spot kit device and the AAS. It further estimated the extent of the association and agreement between the values to determine whether the device has the potential to be a reliable tool in the field tests for iron measurement. Our findings suggested a positive association and strong agreement between the methods.

According to the kappa statistics, the results showed that the values obtained by AAS and test kit were in a good degree of agreement. The Bland-Altman plot analysis showed that the spot kit device reported slightly lower results than the AAS method. But the results were consistent as only 8% (2/25) of the observations fell outside the 95% CI of the limits of the agreement (LoA). An earlier study, that validated the HACH DR/890 portable colorimeter (colorimeter) and HACH Iron test-kit, Model IR-18B (test-kit) against AAS, also found only 8% (2/25) and 4% (1/25) of observations outside of their LoAs. Therefore, the finding of our study is consistent to earlier findings (Merill et al., 2009). The statistical non-significant result of the Pitman's test of difference indicated that the measurement produced by the device is likely to be in considerable agreement with the AAS measurement. A high value of the Lin's concordance correlation coefficient against the AAS measurement indicated that the measurement by the spot-check device are precise and accurate. All these analyses suggest that the device is a valid tool to measure groundwater iron concentration.

There are a few limitations of the study. A small number of samples resulted in relatively large amount of SE to measure to groundwater iron concentration; hence suggesting a limited internal validity of the findings. Nonetheless, the measurement by the AAS also suffered from a large SE. Thus, the error in the instrumentation is not inherent to the device, but largely accounted for the low sample size. A larger study is likely to reduce the statistical error margin and thus might have improved the internal validity to measure the water iron status. The analyzer and the reagents need to be exported from the foreign countries, which is a limitation for wide scale usage.

The device is a portable, easy-to-administer machine which can evaluate the groundwater iron profile in Bangladesh. The AAS is a gold standard method for iron estimation, however it is not field friendly. The method maintains a sophisticated protocol and requires the laboratory grade expertise. Moreover, AAS method is logistically challenging. Therefore, the spot-check method can be a promising alternative.

The device can be used for updated groundwater "iron-mapping" of Bangladesh. The data of water iron concentration mapping can guide to identify and classify the upazilas (lowest administrative area in Bangladesh) with predominantly "high" and "low" groundwater iron loads. The updated groundwater iron map may help to administer the customized iron supplement dosage (traditional dose in the low iron areas, and the low iron dose in the high iron areas) throughout the country, which has the potential to increase coverage and compliance of the supplement with probable limitation of the side-effects of excess iron intake. Thus, potentially, it might contribute to the success of the national anemia control programs for the targeted populations in Bangladesh.

CONCLUSION

The Hanna portable colorimeter (HI721 Checker® HC) is a valid, easy-to-administer, and logistics friendly tool for measuring the iron concentration in groundwater. The device may be used to update the groundwater iron mapping of Bangladesh. The updated groundwater iron mapping may inform the re-design of the national anemia control programs by adjustment of iron doses, thus potentially contributing to the better control of anemia in Bangladeshi population.

AUTHOR CONTRIBUTIONS

SR, AAS, and NS (corresponding author) contributed to conceptualization, and method establishment. SS and NS conducted field investigation, and sample collection. MAH contributed in laboratory analysis of the samples. SR, SS and NS contributed in statistical data analysis and visualization. SS wrote the first draft. SR, AAS and NS (corresponding author) provided critical inputs to finalize. NS (corresponding author) acquired the funding. All the authors read the final version and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY

Data can't be made publicly available; readers are requested to contact the corresponding author for details.

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