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Abu Mohd Naser Titu Date

Drinking Water Salinity: Mineral Intake and Cardiovascular Health
By
Abu Mohd Naser Titu
Doctor of Philosophy
Environmental Health Sciences

Thomas F. Clasen, PhD, MSc
Chair

Matthew O. Gribble, PhD
Co-Chair

Howard Chang, PhD
Committee Member

K.M. Venkat Narayan, MD, MSc
Committee Member

Dana Barr, PhD
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D. Dean of the James T. Laney School of Graduate Studies

Date

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By

Abu Mohd Naser Titu
MPH, James P Grant School of Public Health, BRAC University, 2010
MBBS, Sir Salimullah Medical College, University of Dhaka, 2006

Thomas F. Clasen, PhD, MSc, JD
Advisor

Matthew O. Gribble, PhD, DABT
Advisor

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of Doctor of Philosophy
in Environmental Health Sciences
2018

Abstract

Drinking Water Salinity: Mineral Intake and Cardiovascular Health

Many coastal communities drink saline groundwater, which is a source of high sodium and other minerals. Groundwater salinity will increase in many coastal areas in future due to increased groundwater extraction and global climate change. Managed aquifer recharge (MAR), an intervention to lower drinking water salinity by infiltrating rainwater and pond water in order to dilute brackish aquifers, is a candidate solution for lowering drinking water salinity. The objective of this dissertation was to evaluate the health effects of access to MAR water on communities' blood pressure, and to evaluate the association between drinking water salinity and blood pressure in southwest coastal Bangladesh using a stepped-wedge cluster randomized trial. This dissertation also assessed the association between drinking water chemical concentrations and blood pressure by analyzing the nationally representative surveys of Bangladesh.

The stepped-wedge trial enrolled 1,191 participants in 16 communities and conducted 5 visits between December 2016 and April 2017. Following baseline, 4 communities were randomly assigned to receive MAR water access in successive visits. We measured households' drinking water electrical conductivity and participant's blood pressure, weight and 24-hour urinary total protein, sodium, potassium, calcium, and magnesium. We used multilevel regression models to estimate effects of MAR water access and drinking water salinity on participants' blood pressure. We analyzed blood pressure data of ≥35 year adults from the 2011 Bangladesh Demographic and Health Survey, and groundwater chemical concentrations data from the British Geological Survey (BGS) and the Department of Public Health Engineering (DPHE) survey 1998-1999 using survey estimation regression models to determine the association between drinking water chemical concentration and blood pressure.

Participants from communities randomized to have access to MAR water had 1.89 [95% Confidence Interval (CI): 0.51, 3.27; p=0.007] mmHg higher mean systolic blood pressure, and 1.39 [95% CI: 0.23, 2.55; p=0.018] mmHg higher mean diastolic blood pressure than communities with no access to MAR. Participants drinking moderately saline water [electrical conductivity: 2-10 μS/cm] had 1.60 [95% CI: 0.15, 3.04] mmHg lower mean systolic blood pressure and 1.21 [95% CI: 0.43, 1.99] mmHg lower mean diastolic blood pressure compared to fresh water drinkers [electrical conductivity <0.7 μS/cm]. Participants consuming moderately saline water had 17.57 [95% CI: 12.40, 22.74] mmol/day higher mean daily sodium, 0.32 [95% CI: -1.65, 2.28] mmol/day higher mean daily potassium, 1.23 [95% CI: 1.09, 1.39] times higher median daily calcium, and 1.28 [95% CI: 1.10, 1.48] times higher median daily magnesium in 24-hour urine compared to fresh water drinkers. Survey data analyses suggest the geometric mean ratio of systolic blood pressure for 10 mg/L increase in groundwater magnesium concentration was 0.992 (95% CI: 0.986, 0.999) after controlling for all chemicals and confounders but other chemicals were not associated with blood pressure.

Our findings show no evidence that MAR systems reduced blood pressure. We identified drinking water salinity and magnesium concentration in drinking water have an inverse association with blood pressure. Saline water is a source of cardio-protective minerals such as calcium and magnesium that may have blood pressure lowering effect. Salinity lowering interventions need to critically assess the contribution of drinking water to the daily intake of these minerals. Without remineralization of calcium and magnesium, lowering drinking water salinity may put people at risk for cardiovascular disease.

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Acknowledgements

First and foremost I want to express my gratitude to everyone who made my time at Emory a success. I am thankful to my committee members, who provided immense mentorship and support along this path: Thomas Clasen, my committee chair, for his phenomenal mentorship throughout my time at Emory, who is flexible, a great listener and always keen to support my ideas; Matthew Gribble, for his all-time enthusiasm for teaching statistics and epidemiology and great review of manuscripts; Howard Chang, for teaching statistical modelling with fun; Venkat Narayan, for his thoughtful suggestions on my papers; and to Dana Barr, for her insightful suggestion on exposure and biomarker measurement.

My colleagues at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) played critical roles in both the design and implementation of the trial. I am grateful to Mahbubur Rahman, Solaiman Doza and Leanne Unicomb for the scientific, logistic and institutional support to make this trial possible. I am also grateful to the laboratory officers Mohammad Nasir Uddin, Rabiul Islam, Ruhel Gazi and Raisul Alam Robin, as well as all the field research assistants for their incredible effort for collection and processing of data. Thanks to Professor Kazi Matin Ahmed from University of Dhaka who gave me necessary hydro-geological knowledge essential for my dissertation project.

Thank you to Stephen Luby from Stanford University, my long-time mentor since 2009, who is a great support and inspiration for me, and gave me the initial idea to work in coastal Bangladesh that eventually converted into my dissertation project. I doubt my path to earn PhD would be so smooth if Steve did not agree to be my MPH thesis advisor in 2009.

My time at Emory University was made enjoyable in large part due to the many friends. I am grateful for time spent with all Environmental Health Sciences students. Thank you to the faculty and staff of the Rollins School of Public Health. I have gained so much from your mentorship and experience, in particular: Dr. Karen Levy, Dr Jeremy Sarnat and Dr. Paige Tolbert.

I gratefully acknowledge the funding sources that made my Ph.D work possible. I was funded by Laney Graduate School (LGS) at Emory University for my first two years, and Wellcome Trust, UK and Unilever Ltd for years 3 & 4. My research activities were supported by Wellcome Trust, UK through Our Planet, Our Health scheme. I also appreciate the British Geological Survey for allowing to analyze the Bangladesh National Hydrogeological Survey 1999-2000 dataset, and USAID for permitting to analyze the Bangladesh Demographic Health Survey 2011 dataset for my dissertation.

Finally, thank you to my wife Nafisa for her tremendous moral support and care at home, my daughters Hasnat and Yusrah for their playful nature that makes life more beautiful, my parents and family and friends from Bangladesh and Afghanistan for their prayers, love and encouragement.

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List of Abbreviations

MAR – Managed Aquifer Recharge
EC – Electrical Conductivity
μS/cm – Microsemiens per Centimeter
TDS – Total Dissolved Solids
BP – Blood Pressure
SBP – Systolic Blood Pressure
DBP– Diastolic blood Pressure
mmHg – Millimeter Mercury
DALY – Disability-Adjusted Life-Year
BDHS – Bangladesh Demographic and Health Surveys
EED – Environmental Enteric Dysfunction
BGS – British Geological Survey
DPHE – Department of Public Health Engineering
GPS – Geographic Positioning System
OR – Odds Ratio
RR – Risk Ratio
RCT – Randomized Controlled Trial
SD – Standard Deviation
CI – Confidence Interval
DE – Design Effect
IV – Instrumental Variable
BMI – Body Mass Index
WHO – World Health Organization
UNICEF – United Nations Children’s' Fund

Chapter 1. Introduction

Seawater Intrusion and Climate Change

More than one billion people globally rely on coastal aquifers as the source of water [1]. Of them, nearly 204 million currently reside in areas affected by seawater intrusion [2], a process of groundwater salinity due to movement of fresh-saline groundwater interface towards the inland along the shores [3]. In general, freshwater flows from areas with higher groundwater levels to areas with lower groundwater levels [4]. This natural movement of fresh water towards the sea prevents salt water from entering freshwater coastal aquifers [4]. Saltwater intrusion has increased the salinity of groundwater in aquifers of many coastal regions and small islands [2,5-8] including North Africa, the Middle East, the Mediterranean, China, South Asia, Australia, Mexico, the Atlantic and Gulf Coasts of the United States, and Southern California [9-14] (Appendix 1).

A number of climatological and anthropogenic factors are associated with saltwater intrusion. Among the climatological factors, sea-level rise due to global warming, increased cyclones and tidal surges in many coastal regions, and decreased recharge of groundwater and freshwater flow of rivers as a result of change in pattern of rainfall are important [15,16]. Increased groundwater withdrawal due to high population and economic demand, construction of dam and barrage are important anthropogenic factors contributing to saltwater intrusion [4,17]. If excessive freshwater is extracted from coastal aquifers that are hydraulically connected with the sea, induced gradients may cause the migration of saltwater from the sea toward the freshwater aquifers [18]. Projections suggest high extraction of groundwater to meet the water demand of the population will contribute more to seawater intrusion than the climatic variables such as sea-level rise [8].

Communities in many coastal regions rely on groundwater as their main source of freshwater for domestic, agricultural, and industrial purposes. Often, there is a total dependence on groundwater aquifers for a reliable source of drinking water in many coastal communities. For instance, communities have few options but to drink brackish groundwater in southwest coastal Bangladesh [19,20]. Nearly half of the world population and 8 out of 10 major cities in the worlds are located in coastal areas [6]. Ten percent of the world population live in low lying coastal area within 10 m elevation from sea level [21]. It is expected that saltwater intrusion in coastal areas will increase in the future due to increase withdrawal of water due to population and economic growth, increase sea-level rise and decrease recharge of coastal aquifers [6]. As the world's population and economic activities continues to grow, freshwater supplies are constantly being depleted, which raise the importance of monitoring, management and conservation of coastal freshwater aquifers [22].

Managed Aquifer Recharge

The saltwater intrusion in southwest coastal Bangladesh has contributed to a severe drinking water scarcity during the dry season when there is no rainfall. People in Bangladesh mainly consume groundwater for drinking purposes, however, aquifers in southwest coastal Bangladesh are too brackish to consume. Communities drink pond water during the dry season if available. Rainwater harvesting systems and rainwater-supplied pond sand filters are also currently being implemented in the region to promote household use of low-salinity water during the dry season [23,24]. Nevertheless, neither pond water nor rainwater harvesting is a sustainable water source and often may not ensure year-round availability of drinking water. Moreover, ponds are often inundated by tidal surges due to cyclones and pond water become unsuitable for drinking.

One of the potential solution to adapt the effect of saltwater intrusion is the enhancement of recharge in coastal aquifers [25]. The natural recharge of freshwater in coastal aquifers may be affected by a number of climatological factors in future such as change in pattern of rainfall [15]. Higher temperatures would increase evapotranspiration that may result in decrease recharge [16]. Future sea level rise [26-28] would likely cause more cyclones, tidal surges and flood in this region that will further affect the surface water and groundwater salinity [16].

Managed aquifer recharge (MAR) is a viable technology of artificial recharge of aquifers to enhance the quantity and/or quality of groundwater [29]. MAR is a technology to infiltrate water in a controlled way into the aquifers to increase the storage of groundwater or improve the water quality (Figure 1). Globally MAR systems are used for the agricultural, domestic, industrial and ecological purposes (Appendix 2). In southwest coastal Bangladesh, MAR systems were installed to infiltrate rainwater and pond water into the brackish aquifer to create a lens of freshwater on top of the brackish aquifer for drinking and cooking purposes (Figure 1). [30, 31]

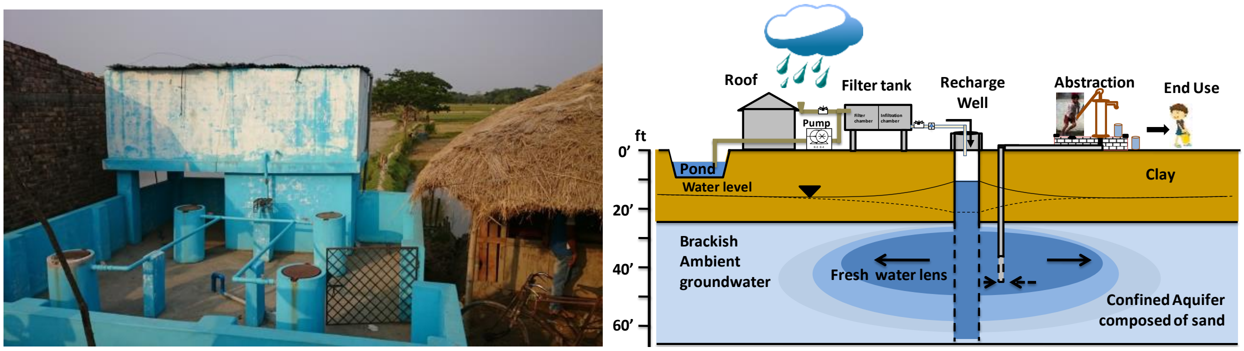

Figure 1a

Figure 1b

Figure 1: Schematic diagram of MAR systems for reducing aquifer salinity for the delivery of low-salinity drinking water in southwest coastal Bangladesh

MAR can be adapted to lower the aquifer salinity and to have a sustainable source of low-salinity drinking water in southwest coastal Bangladesh. It adapted MAR system for potable water supply in southwest coastal Bangladesh involves storage of freshwater in aquifers by infiltrating freshwater (e.g. rainwater and pond water) to aquifers for creating a lens of freshwater (Figure 1) on top of brackish water [31]. MAR represent a promising adaptive strategy for a year-round drinking water supply that is protected from evaporation, and is expected to be resilient to tidal storms, cyclones and salinity of surface water bodies since freshwater infiltration and storage is done at groundwater level [32]. The primary aim of this dissertation is to determine whether MAR systems can provide low-salinity water that confers health benefit thereby justifying scale-up in southwest coastal Bangladesh.

Drinking saline water and mineral intake and cardiovascular health

Saline water is a source of minerals and chemicals. Accurate exposure measurement of chemicals and minerals through drinking water is essential to evaluate the health impact of drinking saline water and salinity lowering interventions. Water salinity is typically expressed as Total Dissolved Solids (TDS) ― i.e, milligrams dissolved solids per liter of water, or Electrical Conductivity (EC), which means the ability of water to conduct an electrical current where the dissolved ions are the conductors. The major cations contributing to the electrical conductivity are sodium, calcium, potassium and magnesium [33]. The dissolved ions in saline water vary highly from one location to another, both in terms of specific ions and concentration level [2].

Drinking saline water has been associated with high sodium intake and blood pressure among communities in southwest coastal Bangladesh. People consume an estimated 5 — 16 gm sodium during the dry season through drinking water [34] whereas the daily target sodium intake from all sources is < 2gm/day [35]. Studies from southwest coastal Bangladesh have focused on only sodium intake through drinking saline water. These studies measured sodium in water as a measure of salinity exposure but the ignored other potential chemicals in saline water contributing to blood pressure regulation. Epidemiological studies suggest potassium, magnesium and calcium intake, which may be also derived from drinking saline water, have inverse association with blood pressure [36,37] as opposed to sodium. Currently, no data exists on how all minerals together in saline water contribute to blood pressure.

Potential effect of reducing salinity on other essential minerals intake and cardiovascular health

Any intervention that reduces drinking water salinity would reduce the sodium exposure through drinking water, which would likely provide health benefits. However, salinity reduction would also reduce the intake of beneficial minerals lime calcium, magnesium and potassium that may be associated with adverse health consequences. Therefore, a huge uncertainly exists on the overall health benefits of drinking water salinity lowering interventions. This uncertainty will depend on the intake of these cardio-protective minerals through diet of the population. If diet of the population have low or borderline content of these cardio-protective minerals, the contribution of drinking saline water for the daily intake of these cardio-protective minerals becomes important. Densely populated communities in the seawater intrusion affected coastal regions of Bengal, Mekong, and Red River deltas in South and Southeast Asia have an appreciable intake of these cardio-protective chemicals through drinking saline water [38].

Mineral concentrations in drinking water and blood pressure

Measuring drinking water “electrical conductivity” or “total dissolved solids” is convenient and less expensive. However, drinking water salinity or electrical conductivity do not provide any information about the concentrations of minerals in drinking water. Measuring the concentrations of all chemicals and minerals in drinking water is expensive and require specialized laboratory support, which is a constraint in large population based study, particularly in the low income settings. To overcome this limitation, we analyzed the available data of two nationally representative survey of Bangladesh. The blood pressure data were collected from the 2011 Bangladesh Demographic Health Survey (BDHS), and groundwater chemical concentration data from the British Geological Survey (BGS) - Department of Public Health Engineering (DPHE) 1998-1999 survey.

Dissertation Aims

Aim 1. To determine the cardiovascular and renal health effects of managed aquifer recharge initiatives designed to reduce drinking water salinity in southwest coastal Bangladesh

Aim 2. To determine the dose-response association of drinking water electrical conductivity (a measure of salinity) with blood pressure and urinary protein excretion in southwest coastal Bangladesh

Aim 3. To determine the association between groundwater chemicals concentrations and blood pressure in Bangladesh using the 2011 BDHS and 1998-99 BGS-DPHE data.

These aims will be addressed throughout this dissertation. Chapter 2 describes the detailed rationale and study design of the stepped-wedge trial to assess the cardiovascular health evaluation of MAR systems. Chapter 3 describes the findings of cardiovascular effect of MAR system from the stepped-wedge trial. Chapter 4 illustrate the dose response association between drinking water electrical conductivity (salinity) and blood pressure and urinary total protein. Chapter 5 summarizes the findings of secondary data analyses highlighting the association between groundwater chemicals and blood pressure. Chapter 6 provides a summary of the dissertation findings and overall implications, reflects on limitations of the research, and proposes future directions.

Chapter 2. Stepped-wedge cluster-randomized controlled trial to assess the cardiovascular health effects of a managed aquifer recharge initiative to reduce drinking water salinity in southwest coastal Bangladesh: study design and rationale[1]

Abu Mohd Naser[1*], Leanne Unicomb[2], Solaiman Doza[2], Kazi Matin Ahmed[3], Mahbubur Rahman[2], Mohammad Nasir Uddin[2], Shamshad B. Quraishi[4], Shahjada Selim[5], Mohammad Shamsudduha[6], William Burgess[7], Howard H. Chang[8], Matthew O. Gribble[1], Thomas F. Clasen[1], Stephen P. Luby[9]

[1] Department of Environmental Health Sciences, Rollins School of Public Health, Emory University, Atlanta, USA

[2]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), GPO Box 128, Dhaka 1000, Bangladesh

[3] Department of Geology, University of Dhaka, Dhaka, Bangladesh

[4]Analytical Chemistry Laboratory, Atomic Energy Centre, Dhaka, Bangladesh

[5]Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

[6] Institute for Risk and Disaster Reduction, University College London, UK

[7] Department of Earth Sciences, University College London, London, UK

[8] Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, USA

[9]Woods Institute for the Environment, Stanford University, Stanford, California, USA

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[1] This article was published in BMJ Open following peer review and can also be viewed on the journal's website at http://bmjopen.bmj.com. 39. Naser AM, Unicomb L, Doza S, et al. Stepped-wedge cluster-randomised controlled trial to assess the cardiovascular health effects of a managed aquifer recharge initiative to reduce drinking water salinity in southwest coastal Bangladesh: study design and rationale. *BMJ open* 2017; **7**(9): e015205.

Abstract

Introduction: Saltwater intrusion and salinization have contributed to drinking water scarcity in many coastal regions globally, leading to dependence on alternative sources for water supply. In southwest coastal Bangladesh, communities have few options but to drink brackish groundwater which has been associated with high blood pressure among the adult population, and preeclampsia and gestational hypertension among pregnant women. Managed aquifer recharge (MAR), the purposeful recharge of surface water or rainwater to aquifers to bring hydrologic equilibrium, is a potential solution for salinity problem in southwest coastal Bangladesh by creating a freshwater lens within the brackish aquifer. Our study aims to evaluate whether consumption of MAR water improves human health, particularly by reducing blood pressure among communities in coastal Bangladesh.

Methods and analysis: The study employs a stepped-wedge cluster-randomized controlled community trial design in 16 communities over five monthly visits. During each visit, we will collect data on participants' source of drinking and cooking water and measure the salinity level and electrical conductivity of household stored water. At each visit, we will also measure the blood pressure of participants' ≥ 20 years of age and pregnant women and collect urine samples for urinary sodium and protein measurements. We will use generalized linear mixed models to determine the association of access to MAR water on blood pressure of the participants.

Ethics and dissemination: The study protocol has been reviewed and approved by the Institutional Review Boards of the International Centre for Diarrheal Disease Research, Bangladesh (icddr.b). Informed written consent will be taken from all the participants. This study is funded by Wellcome Trust, UK. The study findings will be disseminated to the government partners, at research conferences, and in peer-reviewed journals.

Registration: (http://www.clinicaltrials.gov): NCT02746003

Strengths and limitation of the study

- This is the first study to evaluate the health impact of managed aquifer recharge in southwest coastal Bangladesh.
- The stepped-wedge trial ensures we will have counterfactual data as well as gradual access to MAR water in all communities.
- Objective measurement of exposure (drinking water salinity) and outcomes (urinary sodium and blood pressure).

- The magnitude of exposure will vary geographically and across time period. Therefore, MAR water salinity will differ across communities at a single point of time, and also for the same community at different points of time.
- Compliance of the intervention may be different across sites and for individuals of different socioeconomic status.

Introduction

Background and rationale:

Saltwater intrusion and salinization have increased groundwater salinity in many coastal aquifers and small islands across the world.[2,5-8] This is driven by a number of climatological and anthropogenic factors including global warming, increased cyclones and tidal surges, reduced river discharge, and increased groundwater abstraction in excess of recharge.[15-17] Communities in many coastal regions rely on groundwater as their main source of drinking water [40] as well as freshwater for domestic, agricultural and industrial purposes.[41] Nearly half of the world's population resides in coastal areas [6] and 10% of these live in low-lying coastal areas where surface elevation is generally $\leq$10 meters above mean sea level.[21] Salinization in coastal areas is expected to increase in the future because of increased groundwater withdrawal due to population and economic growth and sea-level rise.[6] As the world's population and economic activities continue to grow, groundwater supplies are progressively under threat of depletion, which increases the importance of monitoring, management and conservation of coastal freshwater aquifers.[42,43]

One approach to minimize the impact of groundwater salinization is to enhance groundwater recharge into coastal aquifers.[44] Managed aquifer recharge (MAR) is an approach to artificially promote freshwater recharge to increase storage. MAR involves infiltration of freshwater (e.g. rainwater and pond water) into aquifers to create a store of freshwater within the naturally brackish aquifer (**Figure 1**).[31,45,46] MAR represents a promising adaptive strategy for increasing freshwater availability and sustaining a year-round drinking water supply that is protected from evaporation, and could be resilient to tidal storms, cyclones and surface water salinity since freshwater infiltration and storage occur under confined conditions.[32]

The brackishness of groundwater in southwest coastal Bangladesh is caused by a combination of climatic and anthropogenic factors including sea level rise, frequent cyclones and tidal storms, and shrimp cultivation.[47-50] In the future, climate change and sea-level rise are expected to cause more cyclones, tidal surges and flood in this region that will further affect surface water and groundwater salinity.[16] In many areas in southwest coastal Bangladesh, both shallow and deep aquifers contain naturally brackish water causing acute scarcity of drinking water (**Figure 2A**).[51,52] Water salinity in southwest coastal Bangladesh follows a clear seasonal pattern ─ higher in the dry season than the wet season.[53] Salinity of water bodies builds up from October to May, peaking during February to early May. After May, salinity decreases sharply due to rainfall and increased upstream river flow. People collect and use rainwater for drinking and cooking purpose during the monsoon when precipitation is intense, but during the dry season, they generally rely on pond water or saline tube well water.[54]

The Geology Department of the University of Dhaka, in collaboration with UNICEF and the Department of Public Health Engineering (DPHE), Government of Bangladesh have piloted 20 small-scale MAR projects in three districts of southwest coastal Bangladesh to evaluate the feasibility of MAR for drinking water supply in rural communities.[55] The shallow brackish aquifer was the target storage zone that is overlain by Holocene clay aquitard of 3 to 15 meter thickness.[45] In the pilot phase, an average storage of approximately 900 m^3 of fresh water per year per site was established, sufficient to deliver 15 L of safe drinking water per day per household, which can fulfill the demand for drinking and cooking for approximately 300 people in 60-70 households during the dry season.[56,57] The second phase of the MAR project started in 2014 aiming to install 75 MAR systems in three districts of southwest coastal Bangladesh: Satkhira, Khulna and Bagerhat (**Figure 2B**). In March, 2016, 30 MAR sites were handed over to the community for water consumption. The remaining 45 sites are expected to be ready by November 2016 following infiltration of water during monsoon of 2016 (June – October, 2016).

Epidemiological studies have demonstrated that high sodium intake is associated with elevated blood pressure [26-28] and other cardiovascular diseases.[58] A study conducted among the adult population residing in southwest coastal Bangladesh suggest drinking saline water was associated with high blood pressure after adjusting for personal, lifestyle and environmental factors.[59] Among study participants, the mean systolic blood pressure for those consuming water with sodium from the lowest quintile was 119.4 (SD 13.7) and from the highest sodium quintile was 126.7 mm Hg (18·0).[59] Excess sodium intake from drinking brackish water has been also associated increased gestational hypertension and preeclampsia among pregnant women in southwest coastal Bangladesh.[10,11] Mean systolic and diastolic blood pressure were higher among the pregnant women who drank tube well or pond (saline sources) water compared to those who drank rain water.[10,60] The mean systolic blood pressure of pregnant women from these areas was 102.4 mm Hg among those who drank rainwater, 112.6 mm Hg among pond water users and 119.4 mm Hg among brackish groundwater users.[10] High blood pressure during pregnancy is associated with high maternal mortality, and adverse pregnancy and fetal outcomes.[61]

There are 37 million people living in the southwest coastal region and 20 million are currently affected by drinking water salinity.[62] The estimated mean global sodium consumption is 3.95 g per day (range 2.2 to 5.5 g per day),[63] but people in southwest coastal Bangladesh consume up to 16 g of sodium per day through drinking brackish groundwater.[64] While MAR water can potentially reduce exposure to saline water, the health effects of providing access to MAR water have not been investigated. Characterizing the health consequences of shifting from current drinking water supply to MAR water can assist decision-makers to assess the value of scaling up MAR. Many water interventions, for example, failed to achieve health impact, because people do not use them. There is also considerable controversy on whether reducing sodium intake improves health.[65] In addition the MAR system will also alter the intake of other cations such as calcium and magnesium that may have health impact.[66-69] Because the cost of these systems is high and their long-term feasibility an open question, clarifying the amount of health benefit, if any, is an important step that can help inform future efforts to provide water to communities faced with high levels of groundwater salinity. The human subjects committees who reviewed the study appreciated the uncertainty in bringing solutions to scale in this setting and considered it appropriate to include health measurements of participating residents to objectively evaluate the potential impact.

Objectives

Randomized controlled trials demonstrate that reduction in dietary sodium in adults decreases blood pressure among people both with and without hypertension. [70-72] Meta-analysis of randomized trials also suggest modest reduction in salt intake for four or more weeks causes significant reduction in blood pressure at the population-level. [73,74] The primary objective of the study is to assess whether access to low-salinity MAR water can reduce blood pressure of community members ≥20 years of age. Secondary objectives include whether access to MAR water can reduces urinary sodium and total protein excretion. We will evaluate water salinity, urinary sodium excretion and blood pressure so that we understand whether or not we achieved our immediate targets along the causal pathway.

Methods

Study design: We will implement a stepped-wedge cluster randomized controlled trial in 16 MAR communities. MAR system is a community based intervention designed to supply low-salinity water for 60-70 households in a village or community. MAR intervention cannot be implemented and randomized at individual- or household-levels. Once a community will have access to MAR water, it is difficult to withhold the access of MAR water for some households. Therefore, we will conduct a cluster randomized trial where each community will be considered as a cluster. The stepped wedge design allows communities to gradually have access to MAR water; however, the point at which their access commences will be randomly assigned.[75] In this way, each MAR site will contribute data for both the intervention and the control time periods. We will have five monthly steps in the stepped wedge trial (**Figure 3**). In the first step, none of the communities will have MAR water available for drinking and we will collect baseline information. During each subsequent month, four randomly selected communities will receive access to MAR water for drinking and cooking. In the last (fifth) monthly step, all the communities will have access to MAR drinking water.

Study settings: The study will be conducted at the community-level in three districts in southwest coastal Bangladesh ─ Khulna, Satkhira and Bagerhat ─ where 75 MAR systems have been installed. Of these, 30 systems are already in use. We will select 16 communities for study based on consultation with the implementers of MAR systems (Dhaka University and UNICEF, Bangladesh). Three criteria need to be met for inclusion of communities in the study: communities have not started drinking MAR water, acceptable level of arsenic (<50 µg/L) in drinking water set by the government of Bangladesh,[76,77] and electrical conductivity (a measure of salinity) of water below 2000 µS/cm, an indication that the MAR system has successfully reduced salinity in the aquifer.

Participant eligibility criteria: The inclusion criteria will be households whose ≥ 20 year old members and pregnant women willingly agree to exclusively use MAR water during the dry season for drinking and cooking purposes. The Dhaka University team have determined the catchment area for each MAR site based on geographic distance from MAR infrastructure and have developed a list of households who expressed willingness to drink MAR water. Any ≥ 20 year old hypertensive household members will be also eligible for participation but research staff will collect information about their medication if any. Research staff will enrol post-adolescent ≥ 20 year population as an individual's response to salt intake (salt-sensitivity) increases with age[78] and adolescence is associated adrenal and nervous system maturation that may contribute to salt sensitivity.[79,80] They will approach households living near the MAR water access point. They will enroll the 28 closest households surrounding each MAR systems that meet the inclusion criteria and consent (Supplemental Information 1) to study participation. During the informed written consent process, research staff will inform each household that they will be randomly selected when to start consuming MAR water as part of the scientific process of the study. All household members ≥ 20 year of age will be eligible and enrolled in the study from the selected 28 households. In addition, research staff will enroll households that include a pregnant woman in each selected MAR catchment community irrespective of household selection, from a list of pregnant women developed by a female promoter.

Randomization and intervention delivery: We will randomly select four MAR communities to drink MAR water in each step following the first (baseline) step (**Figure 3**) by computer-generated random numbers. Randomization will be conducted by an investigator who will not be directly involved in implementation of the stepped wedge study and this will be done before commencement of the study. The study could not be blinded therefore there was no concealment from the cluster participants. Although infiltration of rainwater and pond water into the brackish aquifer through the MAR systems will be ongoing for 1-2 years in each community, people will not have access to MAR water until a formal handover of MAR systems to community members. The implementers hire a caretaker or gatekeeper for each MAR community who is responsible for maintenance of the respective MAR system prior to the community hand over. The implementers form a community management team who are responsible for maintenance of each MAR system following handover. We have synchronized the community handover with the randomization schedule in the 16 communities after consulting with the gatekeepers. Community health promoters will inform the participants about when the MAR water will be available for consumption once the community management team is formed. Agreement of the implementers and gatekeepers was sought at the beginning of the study during site selection for inclusion of any site in trial and access of MAR water as per the randomization.

We will deploy a local trained promoter at each MAR site, who will visit these households, list members and identify pregnant women in these and other households in catchment areas of each MAR site. Promoters will encourage all household members to drink MAR water exclusively while they are at home, carry a bottle of MAR water while they go out for work and other activities, and to cook with MAR water. The community health promoters will visit households with promotional materials (e.g. flip charts) from the beginning of the study to inform household members about adverse health effects of drinking brackish water and potential benefits of drinking low-salinity MAR water. As per the randomization schedule, they will inform households when MAR water will be available for consumption.

Sample size: The sample size of the stepped wedge trial was calculated based on the effect of MAR systems to reduce systolic blood pressure of the ≥ 20 years old community members. Data from southwest coastal Bangladesh demonstrated a 7 mmHg lower mean systolic blood pressure among pregnant women who drank pond water compared to those who drank brackish water, and a 17 mmHg lower mean systolic blood pressure for pregnant women who drank rainwater compared to those who drank brackish water [10]. The mean reduction in systolic blood pressure following long term modest salt reduction is 4.2 mmHg.[73] For calculations we considered a mean systolic blood pressure reduction of at least 3 mmHg among the communities who will have access to MAR water for drinking and cooking purposes compared to those who will use brackish water. The entire population under the MAR community catchment area will be considered as one cluster. Each MAR community serves approximately 300 people, constituting 60-70 households in the catchment area.[56] The mean household size in southwest coastal Bangladesh is 4.2, approximately 52% of household members are ≥ 20 years.[81,82] Since we will collect blood pressure for all household members ≥ 20 years, we estimated an average of 2.2 participants in this age group per household. We will select 28 households per cluster (28 x 2.2 = 60 people per cluster). A previously published study in Bangladesh reported standard deviation (SD) for systolic blood pressure as 13.51,[83] but we considered standard deviation of systolic blood pressure 20 to capture a greater variation of blood pressure. We calculated the sample size for the stepped wedge trial adopting the formula used by Hussey and Hughes 2007.[84] Although their formula assumed no within participant correlation over time (cross sectional design), we applied their formula for a cohort design (same participant followed up over time). The salinity problem and the MAR water quality may be influenced by seasonality over the progression of dry season and participants' blood pressure may respond differently to different level of sodium exposure in MAR drinking water. Therefore, it is important that we investigate the effect of the MAR system on the same participants over the entire dry season. Since we will investigate the same participants in each steps, we will have less participant-level variability and sufficient power compared to a cross-sectional design to investigate the effect of MAR systems. We initially calculated the sample size for a simple unadjusted trial (N_U), and then multiplied by the design effect for a stepped wedge trial (DE_{SW}). The sample size for a simple unadjusted trial was calculated as 1,396 participants (effect size= 3, standard deviation of 20 for both groups, 80% power, and 5% Type I error). The DE_{SW} was 3.04 based on a formula provided by Hussy and Hughes therefore sample size was 1396 x 3.04. We also inflated the total sample size considering 10% loss to follow-up. We then followed the approaches of determining the number of clusters required for total sample size considering a fixed cluster size of 60.[85] We then calculated the number of steps that we need to randomize by dividing total cluster by steps. We calculated that 16 MAR communities will be required for the study with four communities randomized to access MAR water in each step (**Figure 3**).

Data collection methods: The outcomes of interest will be measured from selected participants of 16 communities irrespective of their access to MAR water during five monthly visits. The interval between two successive blood pressure measurements or two consecutive data collection visits will be at least four weeks for each participant. After taking informed written consent from household heads and each participant, research staff will administer a survey using a structured questionnaire to collect information on drinking water sources, household members' socio-demographic status, medical history, family history of cardiovascular diseases, medication intake and cardiovascular risk factors including smoking history and alcohol intake during the first monthly visit. Exposure and outcome data will be collected in all five visits. Participants' weight and hip circumference will be measured in all visits but height will be measure in one visit.

As part of the MAR uptake evaluation, the research staff will collect self-reported data on MAR water use for each households and whether households exclusively or intermittently use MAR water for drinking and cooking during follow-up visits. Research staff will also collect stored drinking and cooking water samples for measurement of electrical conductivity and will ask respondents the source of stored water.

Exposure assessment: During each visit, research staff will inquire about household members' reported primary water sources used for drinking and cooking purposes in last 24 hours, collect reported information on whether participants exclusively used the primary water sources and explore whether any alternative water sources were used. They will also ask about the frequency of collection and cost of primary water sources for drinking and cooking, time required to collect water, amount of collected water and when the last water was collected. Research staff will observe the presence of stored water in households, ask the sources of each container of stored water and will collect water samples that have been stored for drinking and cooking (if any) to measure the salinity level, electrical conductivity, resistivity, total dissolve solutes (TDS) and temperature of water samples using Hanna Salinity™ meter. They will also collect the MAR water from the source MAR outlet to measure the salinity level, electrical conductivity, resistivity, total dissolve solutes (TDS) and temperature of water. Research staff will collect 24 hour urine from each participant to measure the urinary sodium excretion as a proxy for sodium intake.[86,87]

Outcomes assessment: Systolic blood pressure of the ≥ 20 year old participants is the primary outcome of this study. Secondary outcomes will be diastolic blood pressure, mean arterial pressure, and pulse pressure (**Table 1**). The different components of blood pressure are independent cardiovascular risk factors associated with sodium intake,[88-92] and indicate future risk for different cardiovascular diseases.[93] High systolic blood pressure puts more stress on the vascular wall and is strongly associated with risk for future intracerebral haemorrhage, subarachnoid haemorrhage, angina, myocardial infarction and peripheral vascular diseases.[93] Raised diastolic blood pressure is associated with aortic and thoracic aneurysm.[93] Some effects of high sodium intake are independent of high systolic and diastolic blood pressure such as arterial stiffness and left ventricular mass ─ both of these are independent predictor of future cardiovascular diseases. High pulse pressure (difference between systolic and diastolic blood pressure) is associated with increased arterial stiffness,[89,91] and high mean arterial pressure (diastolic blood pressure plus one-third of systolic blood pressure) and high pulse pressure is independently associated with increased left ventricular mass.[89,91]

We will also measure the creatinine adjusted protein excretion of all participants as tertiary outcomes (**Table 1**). Proteinuria is a biomarker for future risk of cardiovascular diseases,[94-97] and is associated with the pathogenesis of cardiovascular diseases, including hypertension,[98,99] chronic kidney disease,[100] myocardial ischemia,[101] carotid artery thickness,[102,103] left ventricular hypertrophy,[104,105] hyperlipidemia,[106] atherosclerosis,[107] and coronary artery calcification.[95,103,108,109] Reduced salt intake for four weeks has been associated with decreased proteinuria in blinded randomized controlled trial.[110] Preeclampsia is associated with high maternal mortality and adverse pregnancy and fetal outcomes [61] and is characterized by high blood pressure and proteinuria after 20 weeks of pregnancy.[111]

Blood pressure measurement: Blood pressure will be measured for all $\geq$20 year old participants and pregnant women during each step. Research staff will use a Omron® HEM–907 blood pressure device, which is comparable to the gold standard mercury sphygmomanometers in terms of measurement and meets the Association for the Advancement of Medical Instrumentation's (AAMI) criteria.[112] Blood pressure will be measured following the guidelines described by Pickering et al. 2005 and Giorgini et al. 2014.[113,114] Caffeine (tea, coffee, carbonated beverages), eating, heavy physical activities and smoking will be proscribed for 30 minutes prior to measuring blood pressure. Participants will rest for 5 minutes sitting on a chair keeping their arm supported. An appropriate sized cuff and calibrated instrument will be used for different age groups and the blood pressure instrument will be positioned at heart level. Blood pressure will be measured three times; first left arm, then right arm, then again left arm. Both systolic and diastolic blood pressure will be recorded from all measurements. The arithmetic mean of three systolic blood pressure measurements will be used as the primary outcome. However, if a systolic blood pressure measurement differs by 10% from the other measurements, that measurement will be excluded when calculating the arithmetic mean systolic blood pressure.

Biomarker measurements: Field research staff will instruct the participants' to collect 24 hours urine sample during each household visit. The volume of the urine samples will be noted at household level, and a sample of 25 ml urine will be collected and transported to the field laboratory at 2-8° C within 6 hours of collection for processing, analysis and storage. Aliquots of each participant's urine sample will be made for biochemical and electrolyte measurements. Urinary creatinine concentration will be measured by a colorimetric method (Jaffe reaction) using a semi-auto biochemistry analyzer (Evolution 3000, BSI, Italy). Urinary total protein will be estimated using a light sensitive colored reagent (Randox.UK). We will use the direct Ion Selective Electrode (ISE) method for urinary sodium measurements using a semi-auto electrolyte analyzer (Biolyte2000, Bio-care Corporation, Taiwan). We will measure the uric acid concentration in blood by an enzymatic colorimetric method (Evolution 3000, BSI, Italy). We will perform routine Quality Control for all tests using standard quality control reagents (Bio-Rad Laboratories, USA) and 5% of samples will be cross checked at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) central laboratory, in Dhaka.

Data management and monitoring: The data collection instrument will be programmed and field research staff will use hand held computers to collect data. Appropriate range values and data values will be programmed to minimize data entry errors. The dataset downloaded from handheld devices will be cleaned and checked by the site investigators. All laboratory data will be double entered. Data will be stored in icddr,b's data repository system, in compliance with the system's requirements and will be publicly available after analyzing the primary result.

The research staff will be trained for identifying adverse events such as hypertension and hypertensive disorders in pregnancy. They will report to the investigators following identification of these patients and the investigators will assess whether these adverse events need to be reported to icddr,b's Ethical Review Committee.

Statistical methods: We will conduct an intention-to-treat analysis for the primary analysis. For the primary analysis we will assess whether access to MAR water reduce the systolic blood pressure (continuous outcome) of the ≥20 years old participants. Pregnant women will be included in the primary analysis because it is likely few pregnant women will be identified in the 16 communities and separate analysis of pregnant women will be underpowered. We will use generalized linear mixed models with appropriate links for the primary analysis considering random effects for community, households and participants, and a fixed effects of steps or visits. We will adjust the effect of MAR systems on blood pressure for age, sex, weight and height, personal, and socioeconomic factors (Table 2). We will inspect the missing data patterns and use multiple imputation with chained equations to jointly impute data on missing exposure and confounders to preserve an unbiased association estimate if the data are missing at random conditional on measured variables. We will also conduct subgroup analyses among the households that adhere with the MAR intervention and that exclusively use MAR water. If the trial demonstrates drinking MAR water has health effects, we will investigate the time required for health effects following receiving MAR intervention in secondary analysis.

Ethics: Informed written consent will be taken from all participants and household heads. Consent will be also taken for ancillary studies and future use of specimens collected from study participants. This study protocol has been reviewed and approved by the icddr,b Ethical Review Committee. Approval will be taken for any addition or modification of the protocol from icddr,b Ethical Review Committee. If research staff members identify hypertensive patients or cases of hypertension during pregnancy, they will refer patients to the local government health facilities for further management. In addition, research staff will train pregnant women and family members to recognize the danger signs during pregnancy, and will also instruct them where to seek medical care if such danger signs appear. During monthly household visits research staff will encourage pregnant women to attend prenatal visits. All dataset will be anonymous without the personal identifiers and participants' privacy will be maintained during data storage, analysis and dissemination.

Dissemination: Study findings will be shared with the Department of Public Health Engineering (DPHE), Department of Environment of the government of Bangladesh and other partner NGOs working in southwest coastal Bangladesh for access to safe drinking water. We will discuss the scope and limitations of the MAR system to address the demand of safe drinking water based on findings from this study. We will be submitting abstracts to international conferences for dissemination to the international audience working on safe water. We will develop manuscripts and submit to peer-reviewed journals to publish our research.

Discussion

This will be the first study to assess the health impact of an environmental intervention to reduce groundwater salinity in southwest coastal Bangladesh. Our study has several strengths. The stepped-wedge trial ensures that we will have counterfactual data as well as gradual access to MAR water in all communities. In a stepped-wedge design, treatment effect of an intervention can be estimated from between- and within-cluster comparisons as participants will act as their own control, compared to only between-cluster comparison in a parallel cluster randomized design.[84]

We explored other study designs such as non-randomized study. As per suggestion of icddr,b's Ethical Review Committee, we conducted a pilot study in four communities (two MAR communities and two non-MAR communities) during May-August 2016. Pilot study findings suggested that because of the heterogeneity in village level hydrogeological conditions and in participation by community residents with different characteristics, non-randomized quasi-experimental studies would expected to generate unequal distribution of confounders that would undermine scientific inference. The objective of the pilot study was to pre-test our data collection instruments laboratory protocols prior commencement of the main study We identified unequal distributions of several important observed confounders across the communities such as socio-economic status, and household characteristics such as supply of electricity and ownership refrigerator. We identified that some covariates such as supply of electricity was perfectly predictive of communities receiving MAR interventions. We were unable to derive satisfactory matching of MAR intervention and control households after principal component analysis. The findings highlighted the importance of a randomized trial to evaluate the health effects of MAR systems since there are many observed and unobserved household and community-level and hydro-geological confounders that may influence blood pressure.

We also explored the possibilities of parallel group randomized trial but several programmatic issues barred us to implement a parallel group cluster randomized trial. The implementers of MAR systems (UNICEF Bangladesh and University of Dhaka) installed the 75 new sites in different stages and planned to allow communities to access these over three dry seasons. The implementers informed us that 20-25 MAR sites may be suitable for community access during our study period and they wanted to allow all communities to drink MAR water in the same season after satisfactory reduction in salinity. Therefore, a parallel group cluster randomized trial was not suitable as we could not randomly select control sites and postpone the community to drink water for the dry season. We believe that the stepped-wedge trial design was appropriate to obtain counterfactual data as well as fulfilling the programmatic need.

We will use the same instruments for data collection and outcome measurements for all steps for exposed and unexposed communities, which will mitigate bias in data collection. Measurement of water source salinity and urinary sodium concentration will explain the biologically plausible effect of drinking MAR water on blood pressure. All outcomes planned to measure in the study are objective outcomes that will reduce the risk of reporting bias. Collection of detailed exposure and co-variate data will help in determining a valid association between drinking MAR water and health benefits. We will have several outcome variables and biomarkers that will ensure a comprehensive health benefit evaluation of access to MAR water.

The study has several limitations. We will be unable to control salinity level of drinking water across the 16 MAR communities due to different hydro-geological conditions and volume of infiltrated water across the aquifers. Although the initial electrical conductivity of MAR water will be < 2000 μS/cm, electrical conductivity is likely to vary across different MAR communities with the intensification of dry season. Therefore, MAR water salinity will differ across communities at a single point of time, and also for the same community at different points of time. This will result in different versions of exposures (i.e. access to MAR water with different salinity-levels) that may affect different responses for some participants depending on the version of exposure they will receive.[115,116] To account this, we will specify the versions of exposure by measuring the salinity level of MAR water available in participants' households and interpret the response as unit change of salinity at different MAR water salinity-levels. As compliance of drinking MAR water may be different across communities despite active encouragement by promoters, one problem of the intention to treat analysis is that if the proportions of participants who will always drink MAR water is low compared to those who will not, the potentially greater effect of MAR intervention on blood pressure of fully participating individuals may be washed out by the smaller effect of those who will not comply. To account for this, as a secondary analysis, we will conduct "instrumental variable" (IV) analyses by jointly running two regression models: a regression model for predicting urinary sodium excretion by drinking water salinity, and a regression model for blood pressure prediction given the urinary sodium excretion. The assumption of instrumental variable analyses is fulfilled in our context as the exogenous variable (instrumental variable) can only affect outcome variable (blood pressure) through influencing the endogenous variable (urinary sodium excretion).[117] If MAR water has lower-salinity than other water sources and if participants always drink MAR water, their urinary sodium excretion will be low compared to those who will not fully participate.

Acknowledgements: The study has been funded by Wellcome Trust, UK, Our Planet, Our Health Award. Wellcome Trust reviewed and approved the design as the condition of providing funding but will not have a role in data collection, data analysis, data interpretation, or writing of the report. The study was implemented by International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). icddr,b acknowledges with gratitude the commitment of Wellcome Trust, UK to its research efforts. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support. Dr. Gribble is supported by funding from the National Institute for Environmental Health Sciences to the Emory Health and Research Exposome Center (P30 ES019776). The authors would like to thank Dr. Karla Hemming, Institute of Applied Health Research, University of Birmingham for her input in design and sample size calculation. We are grateful to the Dhaka University/UNICEF field team for their assistance in providing preliminary data about the readiness of MAR sites.

*Contributors***:** SPL and AMN developed the study concept. AMN, SPL, LU, KMA, and SD have developed the study design. MR, MOG, TFC and HHC reviewed the epidemiological study design. KMA, MS, WB, SS and MNU provided input in environmental and biological sample collection and analysis. HHC and MOG provided input in statistical analysis. AMN drafted and all author reviewed the manuscript.

Competing interest: None

Funding: Wellcome Trust, UK (Grant # 106871/Z/15/Z).

Data sharing statement: The anonymous dataset without personal identifiers will be publicly available following publication of the primary trial results.

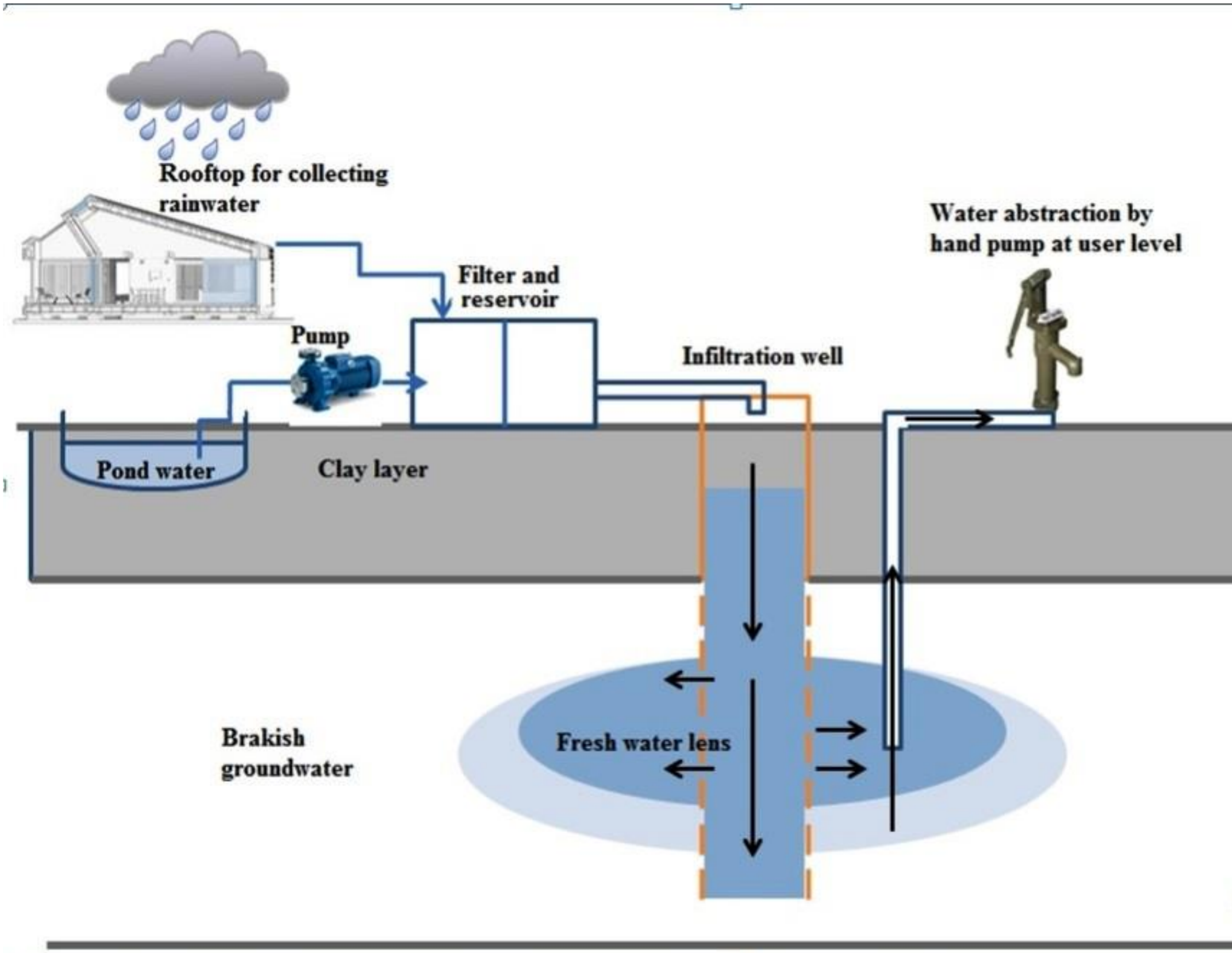

Figure 1: Schematic diagram of the southwest coastal Bangladesh MAR systems

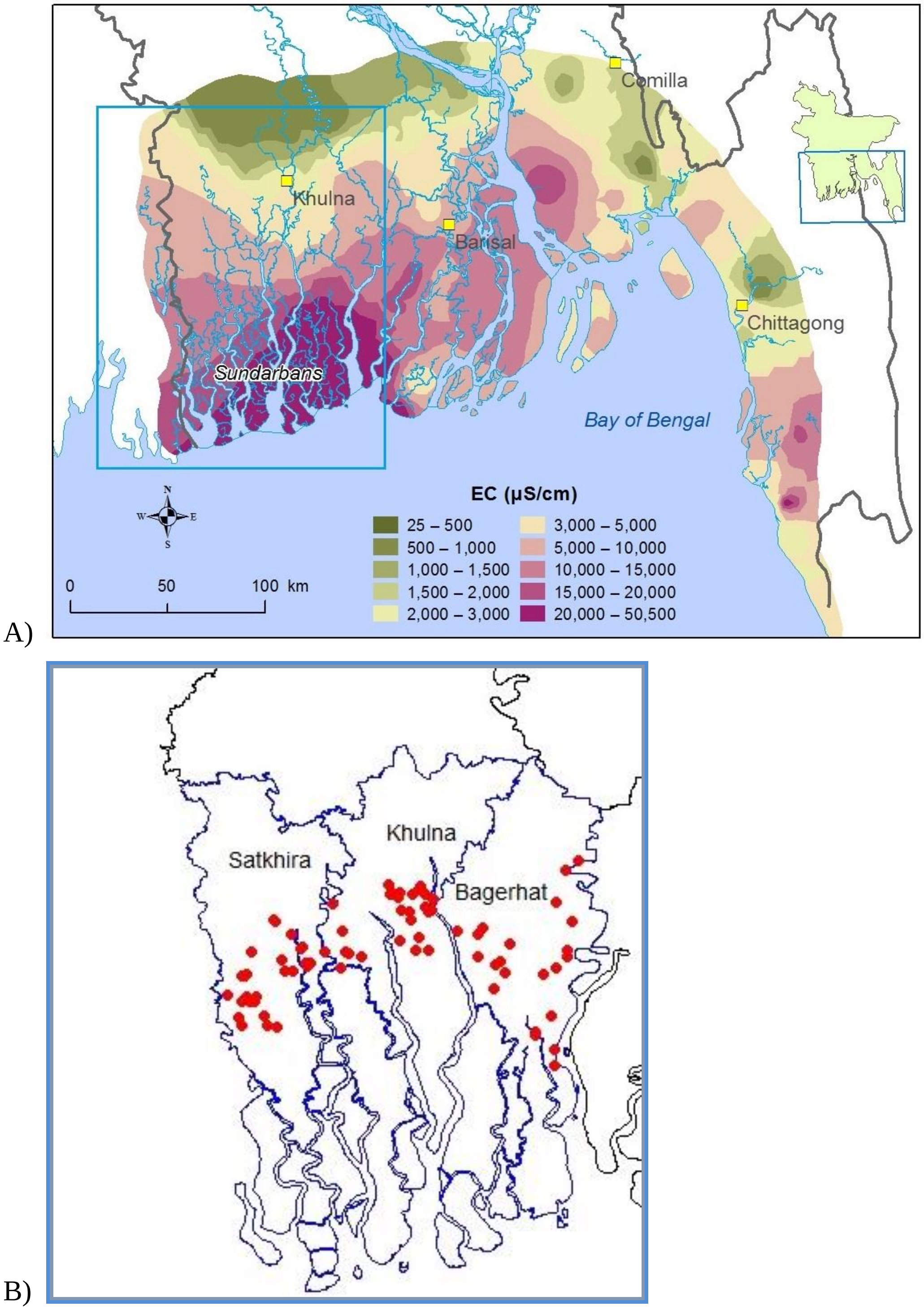

Figure 2: A) Groundwater salinity (measured as EC: electrical conductivity) distribution in coastal Bangladesh (data source: Bangladesh Water Development Board[52]) and B) location of the 75 MAR sites in southwest coastal region illustrated by red dots [data source: Professor Kazi Matin Uddin Ahmed].

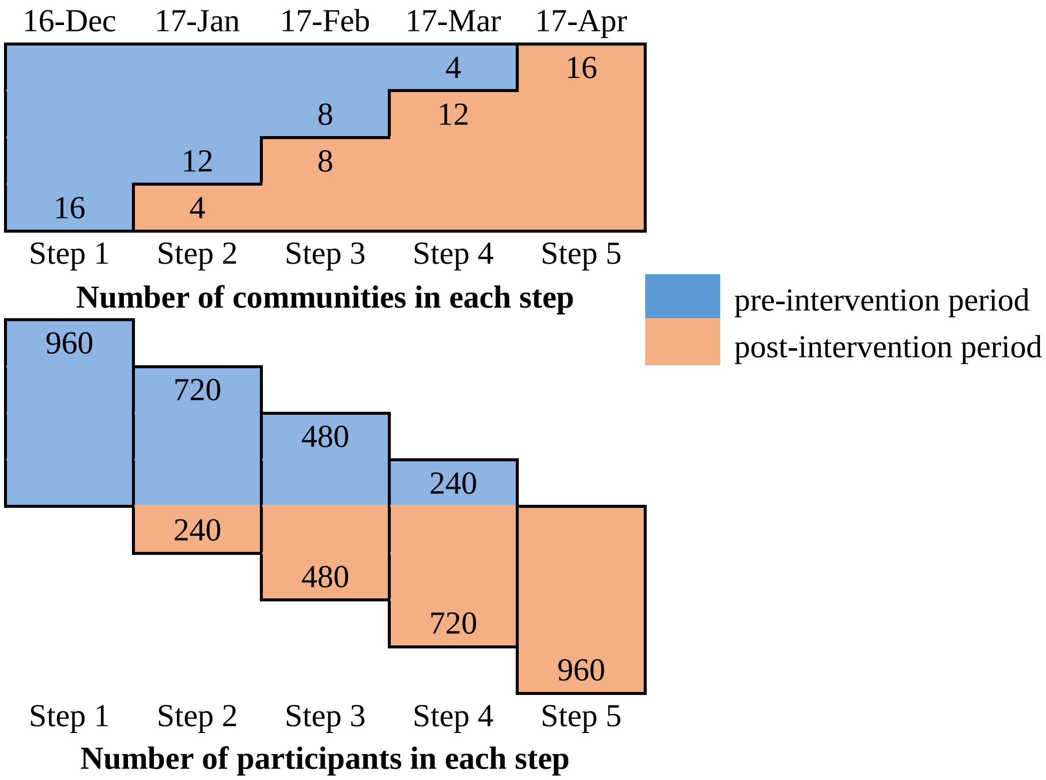

Figure 2: Timeline of blood pressure measurement with number of MAR communities and participants in pre-intervention (blue color) and post-intervention (grey color) time period in different steps

Table 1: Study outcomes including normal ranges of blood pressure and biomarkers

Outcomes	Study population		Measures	Normal range/calculation
	≥ 20 years old	Pregnant women		
Primary outcome: Systolic blood pressure	Yes	Yes	Systolic blood pressure	90 -140 mmHg
Secondary outcomes: Diastolic blood pressure	Yes	Yes	Diastolic blood pressure	60 -90 mmHg
Mean arterial pressure	Yes	Yes	Mean arterial pressure	Diastolic blood pressure + 1/3 systolic blood pressure
Pulse pressure	Yes	Yes	Pulse pressure	Systolic blood pressure – diastolic blood pressure
Tertiary outcomes: Urinary sodium excretion	Yes	Yes	Urinary sodium-creatinine ratio	40-220 mEq/L/24 hours
Proteinuria	Yes	Yes	Urinary protein-creatinine ratio	< 0.11 mg/mg

Table 2: Key variables to be used for assessing the health impact of access to MAR water

Outcomes	Exposures	Confounders	Co-variates	Moderators
Systolic blood pressure	Access to MAR water	Sex	Age	Drinking/co from anothe sources
Diastolic blood pressure	Drinking water salinity	Socio-economic status	Weight	
Pulse pressure	Cooking water salinity	Education	Height	
Mean arterial pressure			Waist circumference	
	Drinking water sources			
	Cooking water sources			
Urinary total protein			Mid upper arm circumference	
Urinary sodium excretion				
			Adding table salt	
			Adding salt for cooking	
			Exercise level	
			Smoking status	
			Use of betel nuts	
			Stress	
			Diet	
			Urinary creatinine	

Chapter 3. Cardiovascular and renal health effects of managed aquifer recharge to lower drinking water salinity in southwest coastal Bangladesh: a stepped-wedged cluster-randomized trial[2][3]

Abu Mohd Naser, Solaiman Doza, Mahbubur Rahman, Leanne Unicomb, Kazi M. Ahmed, William Burgess, Nargis Akther, Boluwaji Onabolu, Shuchi Anand, Shahjada Selim, Mohammad Shamsudduha, K. M. Venkat Narayan, Howard Chang, Thomas F. Clasen, Matthew O. Gribble, Stephen P. Luby

Target Journal: Lancet Global Health/ Lancet Planetary Health (4500 words for RCTs; 300 word abstract)

Correspondence to:

Abu Mohd Naser, MBBS, MPH
Department of Environmental Health Sciences
Rollins School of Public Health | Emory University
2nd floor, CNR building, Room # 2030E
Atlanta, GA 30322, USA
email: atitu@emory.edu
Department of Environmental Health Sciences, Rollins School of Public Health
Emory University
Atlanta, GA, USA
(AM Naser MPH, MO Gribble PhD, Prof TF Clasen, PhD)

International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh
(M Rahman MBBS, L Unicomb PhD, S Doza MPH)

Department of Biostatistics, Rollins School of Public Health
Emory University
Atlanta, GA, USA
(Prof H Chang PhD)

Water Sanitation & Hygiene Section, UNICEF, Bangladesh
Dhaka, Bangladesh
(B Onabolu, N Akhter)

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[2] This chapter is a manuscript prepared for submission to Lancet Planetary Health. The structure, length and format are in keeping with journal requirements.

[3] AMN, SPL, LU, KMA, and SD have developed the study design. MR, MOG, TFC and HHC reviewed the epidemiological study design. KMA, MS, WB, SS and MNU provided input in environmental and biological sample collection and analysis. HHC and MOG provided input in statistical analysis. AMN conducted the analyses and drafted the manuscript. Other authors reviewed the manuscript.

Emory Global Diabetes Research Center, Hubert Department of Global Health, Rollins School of Public Health
Emory University
Atlanta, GA, USA
(Prof KMV Narayan, MD)

Department of Geology, University of Dhaka,
Dhaka, Bangladesh
(Prof KM Ahmed, PhD)

Department of Earth Sciences, University College London
London, UK
(Prof W Burgess, PhD, M Shamsudduha PhD)

Department of Nephrology, Stanford University
Stanford, CA USA
(S Anand, MD)
Division of Infectious Diseases and Geographic Medicine
Stanford University
Stanford, CA USA
(Prof SP Luby, MD)

Abstract

Background: Coastal aquifer salinity has been associated with high sodium intake and blood pressure among communities in southwest coastal Bangladesh. The objective of this study was to evaluate the impacts of managed aquifer recharge (MAR), a hydrological intervention to lower drinking water salinity by pumping rainwater and surface water into wells to dilute brackish aquifers, on blood pressure and kidney function in southwest Bangladesh.

Methods: We conducted a stepped-wedge cluster randomized trial (NCT02746003) with 5 monthly visits among 1,191 participants from 542 households in 16 communities in 3 districts of southwest coastal Bangladesh. At each visit subsequent to baseline, 4 additional communities were randomized to receive access to MAR water. We measured salinity of the drinking and cooking water sources, measured sodium, potassium, calcium, magnesium and total protein in 24-hour urine samples, collected socio-demographic data, and measured blood pressure of the enrolled participants. Systolic blood pressure was the primary outcome. We used multilevel regression to estimate the effects of MAR water on participants' blood pressure and urine protein. We performed intention-to-treat, as-treated and per-protocol analyses.

Findings: Participants from communities randomized to have access to MAR had 1.89 (95% CI: 0.51, 3.27; p=0.007) mmHg higher systolic blood pressure, and 1.39 (95% CI: 0.23, 2.55; p=0.018) mmHg higher diastolic blood pressure than communities with no access to MAR. The adjusted ratio of median 24-hour urine protein was 1.10 (95% CI: 0.96, 1.25; p= 0.159) among participants randomized to have MAR water access compared to those without access. When access to MAR water was provided, 40% of households in 2nd, 21% in 3rd, 20% in 4th and 40% in last visit reported that they did not consume MAR water for drinking and cooking. Per-protocol analyses estimated that those reporting actually consuming MAR water for both drinking and cooking purposes had 1.08 (95% CI: 0.11, 2.06) mm Hg higher systolic blood pressure.

Conclusions: Our findings show no evidence that MAR systems reduced blood pressure and urine protein of the population. Although the intention-to-treat analyses suggest MAR interventions increase population blood pressure, poor adherence to the MAR intervention due to availability of pond water limits the intention-to-treat findings.

Funding: Wellcome Trust, UK

Panel: Research into context

Evidence before the study

Salinity of coastal aquifers is a growing concern particularly for populations relying on groundwater for drinking purposes. Several observational studies from southwest coastal Bangladesh suggest drinking brackish water has contributed to high sodium intake and has been associated with high blood pressure among the population. Communities in southwest coastal Bangladesh consume an estimated 5 - 16 gm sodium[34] during the dry season through drinking water whereas the daily target sodium intake is < 2gm/day.[35] High salinity groundwater drinkers were 2.4 times more likely to consume high sodium (≥ 2gm/day) than the low salinity pond water drinkers (Odds Ratio: 2.43, 95% confidence interval [CI] 1.38–4.30). [118] A cross sectional study found that 19-25 year old adults consuming high salinity water (> 600 mg/L) had 3.5 (95% CI: 0.75 — 6.17) mmHg higher systolic blood pressure and 2.8 (95% CI: 0.31 — 5.24) mmHg higher diastolic blood pressure compared to those who consumed low salinity water (< 600 mg/L).[119] A case control study found that pregnant women with pre-eclampsia or gestational hypertension had a 5.5 (95% CI: 3.30 — 9.11) times greater odds of drinking water with >900 mg/L Na concentration compared to those drinking water with <300 mg/L after adjustment for age, parity, socio-economic and nutritional status. [120] A further study identified that normotensive pregnant women consuming high salinity groundwater had 4.85 (95% CI: 2.55 — 7.15) mmHg higher systolic blood pressure and 2.30 (95% CI: 0.33 — 4.23) mmHg higher diastolic blood pressure compared to normotensive women who consumed low salinity rainwater after adjusting for age, parity, socioeconomic status and nutritional status, physical activity, outdoor temperature, rainfall, education, exposure to chemicals and underlying diseases.[121]

Communities in southwest coastal Bangladesh have adopted several interventions to provide low-salinity water such as rainwater harvesting, pond sand filter, bio-sand filter and desalination water. One hydrogeological intervention to lower aquifer salinity is Managed Aquifer Recharge (MAR), which pumps rainwater and pond water into the brackish aquifer to crease a lens of fresh water. [30]

Added value of this study

Despite MAR systems being considered a hydrogeological success in terms of lowering brackish aquifer salinity levels, the public health impacts of MAR systems have not been explored. This project was undertaken to determine whether MAR systems can provide low-salinity water that confers health benefit thereby justifying scale-up in southwest coastal Bangladesh. It also reports community acceptability of the MAR intervention and recommendations for the utility of MAR systems in this region.

Implication of all the available evidence

Evidence so far suggests that drinking water salinity can contribute to sodium intake and impact cardiovascular health. There is little evidence of whether lowering drinking water salinity can reduce blood pressure and other cardiovascular markers. An earlier non-randomized evaluation of MAR systems suggests access to MAR did not reduce blood pressure of the community.

Introduction

Salinity of the groundwater aquifers has affected fresh drinking water availability in many coastal communities across South and Southeast Asia [48,122,123], and other parts of the world.[124] In particular, salinity of the coastal aquifers has contributed to drinking water scarcity in southwest coastal Bangladesh.[125] Contributors to water salinity in southwest coastal Bangladesh includes low-lying topography,[47] decreased upstream fresh river water flow [48] frequent cyclones and shrimp cultivation.[49] More than 90% of the population in Bangladesh depends on groundwater as the primary source of drinking water and therefore many are exposed to brackish water from of the shallow and deep aquifers in southwest coastal Bangladesh.[51]

One potential solution for increased availability of low-salinity drinking water is to enhance the recharge of coastal aquifers.[25] The Department of Geology, University of Dhaka, the Department of Public Health Engineering of the Government of Bangladesh and the UNICEF, Bangladesh have installed managed aquifer recharge (MAR) systems to provide access to low-salinity water in some coastal communities. MAR systems increase the quantity of groundwater by artificially enhancing aquifer recharge and are used both to address water quality and water scarcity concerns.[29] In southwest coastal Bangladesh, MAR systems were installed to infiltrate rainwater and pond water into the brackish aquifer to create a lens of freshwater on top of the brackish aquifer for drinking and cooking purposes (Figure 1). [30, 31]

MAR systems are designed to reduce the intake of sodium through drinking water compared to untreated brackish aquifer water, but it remains unclear whether MAR systems can impact blood pressure or kidney function.[126] A non-randomized pilot assessment of four communities suggests drinking MAR water did not reduce blood pressure, however, small sample size and imbalanced socio-demographic confounders among the enrolled communities precludes the inference from the pilot study (unpublished data). Low sodium intake through low-salinity MAR water will likely reduce blood pressure and improve kidney function but intake of MAR water could reduce the intake of other beneficial minerals such as calcium and magnesium that may have adverse health consequences. [77] It is essential to assess the health benefits of MAR systems prior to advocating widespread scale-up in the coastal Bangladesh. We conducted a community-based randomized controlled trial to evaluate whether MAR systems can reduce communities' blood pressure and improve kidney function in southwest coastal Bangladesh.

Methods

Study design, enrollment and baseline

We conducted a stepped-wedge cluster randomized controlled trial in 16 communities in three districts (Khulna, Satkhira and Bagerhat) of southwest coastal Bangladesh (Figure S2). The communities for this study were part of MAR implementation by UNICEF, Bangladesh and University of Dhaka to provide access to MAR water during the dry season, when aquifer salinity is high. We conducted the study between December, 2016 and April, 2017. Before December 2016, none of these 16 communities had access to MAR water. Prior to the study commencing, research assistants visited these 16 communities to list the households and household members at least 20 years old. A few household members (N=45) reported they occasionally managed MAR water prior to the official access if their households faced drinking water scarcity. Households were eligible for enrollment if they reported they had never consumed MAR water and expressed willingness to consume MAR water during the study timeline. Research assistants returned to the eligible households to seek informed written consents from the household head, all household members over 20 years old, and pregnant women of any age. The study design, rationale, sample size calculation and site selection procedure have been reported in detail elsewhere.[39] The evaluation was led by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), and the study was approved by the human subject committees of icddr,b (PR-15096). The trial was registered at ClinicalTials.gov (NCT02746003).

Randomization and masking

Following enrollment, each of the communities was randomly assigned a month in which they would begin receiving access to MAR water. The randomization schedule was assigned by a co-investigator at Emory University who was not involved in the fieldwork. In the first (baseline) step, none of the communities had access to MAR water. The study was randomized to provide access to MAR water in a step-wedge manner for four communities at each step after the baseline visit.[39] Thus, communities gained access to MAR water in a randomized order throughout 2nd to 5th visit. Once access to MAR water was provided, communities had continued access. Deviating from the protocol, three communities (one each in steps 3, 4 and 5) did not gain access to MAR water at the randomly assigned times because of ongoing groundwater-quality problems that would have made it impractical to provide the water yet, such as presence of sand in MAR water. Neither the research assistants nor the participating households could be blinded to access to MAR water.

The Intervention

The intervention consisted of providing access to MAR water at the community level and promoting its use as an alternative to brackish water source. The design and implementation of the MAR systems was undertaken by UNICEF, Bangladesh and University of Dhaka and has been describe elsewhere. [30] We employed two separate group of research assistants for intervention promotion and data collection. The intervention was promoted by locally employed community health promoters (CHPs) trained by the behavioral change communication experts of icddr,b. CHPs met with householders in the intervention group to describe the potential health benefits of drinking low-salinity MAR water using promotional materials such as flipcharts and encouraged households to consume MAR water for drinking and cooking when access was provided. CHPs recommended that householders carry a bottle of MAR water when away from the household premises. Promoters attended multiple training sessions and refreshers and visited households at least once per week. Promoters were expected to work an average of 60 hours per month and received a remuneration equivalent to US$30 per month.

Exposure and confounder measurement

Research assistants used a pre-tested structured questionnaire programmed in open-source Open Data Kit (University of Washington, Department of Computer Science and Engineering) installed on Android smart phones to collect exposure and outcome data from participating household members in all communities during all visits irrespective of intervention status. Research assistants requested information about the source of drinking and cooking water for the visit day, and asked participants whether they had stored drinking or cooking water in the home. If stored water was available, research assistants collected the water in a pre-sterile 50 mL conical Falcon™ tube and measured the electrical conductivity using a Hanna Salinity™ meter (Romania, model: H198192, range: 0.000 µS/cm to 400 mS/cm electrical conductivity, accuracy: ± 1% of reading) at their home. Electrical conductivity is widely used as an indicator of salinity, which measures how easily electrons pass a certain distance in water and is correlated with the concentration of dissolved ions in water. [127] Research assistants collected demographic and socioeconomic information, presence of hypertension and use of hypertensive medications during the first step. Participant weight was measured in all visits but height was measured once.

Outcome measurement

Systolic blood pressure measured among participants at least 20 years old in all visits was the primary outcome. Trained research assistants measured blood pressure at community-level using a HEM-907 blood pressure device. [112] Eating, smoking and heavy exercise was proscribed for 30 minutes and participants rested for 5 minutes with arms and back supported by a chair prior to blood pressure measurement. [113,114] Blood pressure was measured three times during each visits and the arithmetic mean was used for analysis. Diastolic blood pressure, and 24-hour urine total protein were the secondary outcomes.

Research assistants collected 24-hour urine samples from each available household member ≥20 years old. Urine samples were transported at between 2 and 8°C to a local field laboratory for analysis of electrolytes, creatinine and total protein. [128] Four electrolytes (Na, K, Ca and Mg) were measured as intermediate outcomes to assess the MAR water-blood pressure relationship.

Urinary total protein was estimated using colorimetric method by a semi-auto biochemistry analyzer (Evolution 3000, BSI, Italy, coefficient of variation: < 1%). Urine creatinine was measured by a colorimetric method (Jaffe reaction). [129] Direct Ion Selective Electrode (ISE) methods, a common method in clinical biochemistry laboratories with high agreement to the conventional flame photometer, [130] were used to measure the urinary sodium & potassium with a semi-auto electrolyte analyzer (Biolyte2000, Bio-care Corporation, Taiwan, coefficient of variation: ± 5%). Urinary calcium and magnesium were measured by photometric titration method using Biolyte2000. [131]

Statistical analyses

We calculated summary statistics for variables including the median and inter-quartile ranges for continuous variables, and proportions for categorical variables.

The primary analyses were intention-to-treat: participants were analyzed based on the randomization schedule. Participants' systolic and diastolic blood pressures were both assumed to follow normal distributions, and urine protein was assumed to follow a gamma distribution. We used multi-level linear models to analyze the effect of access to MAR water on blood pressure, and multi-level parametric quantile regression models to assess the effect of access to MAR water on urine protein. In all models, we used three-level random intercepts to account for multi-level clustering of longitudinal visits within person, persons within households, and households within communities. [132] [133] The blood pressure models were estimated by maximum likelihood. [132] [133] Adaptive quadrature with 7 quadratic points was used to obtain an approximation to the maximum likelihood estimation for urine protein. [133] We used cluster-robust standard errors with all models.[134] We sequentially fit three models for each outcome considering different combinations of fixed effects of confounders. Model 1 adjusted for age, sex, body mass index (BMI) and visit; Model 2 further adjusted for smoking status (never smoker, current smoker and former smoker), work-related physical activity (vigorous physical activities, moderate physical activity and sedentary activity) determined by WHO Global Physical Activity Questionnaire,[135] and marital status (married or unmarried); and Model 3 additionally adjusted for educational attainment, religion, household income and wealth score. Urine total protein was additionally adjusted for urine creatinine in the final model.

Additionally we conducted as-treated and per-protocol analyses to take into consideration households' adherence to the intervention. The as-treated approach analyzed participants as they actually received the intervention: any households that did not use MAR water were included as "untreated".[136] Although field staff planned to visit households at least four weeks following access to MAR water, some households in the 3rd and 4th visits were visited prior to completion of four weeks of intervention, reducing the duration of receiving intervention when outcomes were assessed. Households that received less than pre-specified four weeks of intervention between visits were excluded from the as-treated and per protocol analyses. The per-protocol approach restricted the analysis to households that were fully or partially adherent to the protocol compared to households without access to MAR water.[136] For this purpose, we calculated the proportion of households that adhered to the intervention when they had access to MAR water. We defined fully adhering households as those which reported exclusive use of MAR water for both drinking and cooking purposes. Households were considered to have partial adherence if they reported exclusive use of MAR water either for drinking or cooking. Households were considered to be non-adherent if they reported that they did not use MAR water at all. In as-treated and per protocol analyses, multilevel linear models for blood pressure and multilevel parametric quantile regression models for urine protein were estimated with similar parameterization as described before.

We also assessed whether urinary sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) concentration were affected by households' MAR usage in as treated and per protocol analyses. Urinary Na and K were modeled with multilevel linear regressions, while skewed Ca and Mg were modeled by multilevel parametric quantile regression assuming gamma distributions. We plotted confounder adjusted participant mean blood pressure and urine electrolytes across visits to determine the trend across visits for different MAR users.

Missing data (n=90 urine protein and n=90 BMI) were imputed by multiple imputation by chained equations conditional on the variables included in the main analysis linear regression and parametric quantile regression models. Analyses was conducted using STATA (version 15) and R (version 3.3.1) statistical software.

Role of funding sources

The study funder reviewed and approved the study design but was not involved in enrollment, data collection, data analysis, interpretation, manuscript writing and decision to publish. The funder did not have access to study data.

Results

Study participants

Research assistants identified 1,307 participants from 16 communities during initial eligibility screening; 1,191 were enrolled from 542 households during the baseline visit following informed written consent between November 17, 2016 and December 22, 2016 (Figure 1). Over the period of five visits, research assistants completed 5,725 blood pressure measurements and collected 5,667 urine samples. The proportion of participants who were unavailable during the 2nd and subsequent visits were 2.0%, 3.6%, 5.3% and 2.2%, respectively. Few participants refused (n=17, 1.4%) or moved out (n=4) during the study period (Figure 1).

Baseline characteristics of the enrolled participants and households are reported in Table 1. Median age of the participants was 41 (IQR: 31 —54) years and median BMI was 21.8 (IQR: 19.4 —24.3) kg/m^2. Most participants were female (59 %), never smoker (51 %), married (96 %), and of Hindu religion (59 %).

Households' drinking and cooking water sources

The drinking and cooking water sources of households with or without MAR water access are illustrated in Figures 3 and 4. Prior to MAR water access, 60% (range: 51—79%) of households reported exclusive use of pond water for drinking and 90% (range: 89 — 95%) for cooking. (Figure 3 & 4). After communities had MAR water access, 47% (range: 38 — 67%) of households reported exclusive use of pond water for cooking and 15% (7 — 25%) for drinking (Figure 4). During the baseline (Visit 1) visit, 24% of households reported groundwater use for drinking (Figure 3), which decreased to $\leq$ 10% households in subsequent visits (Figure 3 & 4). Rainwater and groundwater were rarely used for cooking.

Adherence to MAR intervention

Households reported poor adherence to the MAR intervention. After MAR water access was provided, 42% (range: 26 — 50%) households fully adhered to the MAR intervention (exclusive use for drinking and cooking), and 28% (range: 24 — 34%) partially adhered (exclusive use of MAR either for drinking or cooking) and 30% (range: 20 — 40%) never used MAR water (Table 2).

Salinity of stored drinking water

The median electrical conductivity of rainwater was 71 (IQR: 28 —144) µS/cm, pond water was 974 (IQR: 666 — 1190) µS/cm, MAR water was 1624 (IQR: 1245 — 2013) µS/cm, and groundwater was 3225 (IQR: 2311— 4220) µS/cm after combining data from all visits (Figure S2).

Intention to treat (ITT) estimates

Participants among communities that were randomized to receive MAR water had 1.89 [95% CI: 0.51, 3.27; p-value: 0.007] mmHg increase in mean systolic blood pressure and 1.39 [95% CI: 0.23, 2.55; p-value: 0.018] mmHg increase in mean diastolic blood pressure compared to participants of communities randomized to receive no MAR water access after adjusting for community-specific average baseline blood pressure and all listed confounders in the final model (Table 3). The ratio of median urine protein for communities with MAR water access versus no access were 1.10 [95% CI: 0.96, 1.25; p-value: 0.159] after adjusting for community-specific average baseline blood pressure and confounders in the final model (**Table 3**).

As-treated and per protocol estimates

Compared to the participants of households that did not use MAR water, participants of the fully- and partially-adherent households did not have any statistically significant difference in mean systolic or diastolic blood pressure or urinary total protein in as-treated and per protocol analyses **(Table 4)**. In per protocol analyses, participants of the fully adherent households had 1.13 [95% CI: -0.43, 2.70; p-value: 0.157] mmHg change in mean systolic and 0.46 [95% CI: -0.58, 1.49; p-value: 0.387] mmHg change in mean diastolic blood pressure compared to the participants of households that did not have access to MAR water after adjusting for community-specific average baseline blood pressure and confounders in the final model **(Table 4)**. In as-treated analyses, participants of the fully adherent households had 0.49 [95% CI: -0.83, 1.80; p-value: 0.468] mmHg change in mean systolic and 0.09 [95% CI: -0.74, 0.92; p-value: 0.830] mmHg change in mean diastolic blood pressure compared to the participants of households that did not use or did not have access to MAR water after adjusting for community-specific average baseline blood pressure and confounders in the final model **(Table 4)**.

The ratio of median 24-hour urinary total protein for the participants of fully adherent households versus no MAR user households were 1.07 [95% CI: 0.86, 1.33; p-value: 0.541], and for the participants of the partial adherent versus no MAR user households were 1.08 [95% CI: 0.89, 1.32; p-value: 0.437] after adjusting for community-specific average baseline blood pressure and confounders in the final model in per protocol analyses **(Table 4).**

Trend in blood pressure, urine protein and urinary electrolytes across MAR users

There were no differences in the urinary concentrations of sodium, potassium, calcium and magnesium among different MAR user categories (Table S2). Compared to the no MAR users, the fully adherent participants had -3.7 [95% CI: -16.4, 9.1; p-value: 0.575] mmol/day change in urinary sodium in fully adjusted models. We observed an upward trend of 24-hour urinary sodium, potassium, calcium and magnesium, and a downward trend of systolic and diastolic blood pressure for all categories of MAR users (Figure S3 & S4).

Discussion

Our findings show no evidence that MAR systems provide health benefits in terms of blood pressure and urine protein reduction. Contrary to our hypothesis, the ITT analyses suggested that access to MAR water actually increased blood pressure. We believe communities' practice on relying pond water prior to access to intervention may describe such adverse effects of MAR systems. Our water quality data suggests that pond water had consistently lower median electrical conductivity than the MAR water, and MAR water had consistently lower median electrical conductivity than the brackish groundwater across visits. When communities had no MAR water access, the predominant counterfactual water source was pond water for both drinking and cooking purposes. Therefore, access to MAR water actually exposed communities to higher salinity drinking and cooking water, which may explain why access to MAR water is associated with high blood pressure. This findings is different from what we had expected as we hypothesized that communities would be shifting from high salinity brackish groundwater to lower-salinity MAR water.

We believe the poor adherence of the intervention is also explained by the presence of lower electrical conductivity pond water to the communities. The poor adherence was observed despite active promotional visits to the households. Non-adherent households predominantly used pond water across visits. It was likely difficult to convince communities to switch to higher salinity MAR water when they had access to low-salinity pond water as their usual source. . As treated and per protocol analyses suggest no difference in blood pressure and urine protein among MAR users than non-users. Urinary excretion of sodium and other chemicals was not different among the categories of MAR users, which explains the null effect of the intervention. We observed a similar upward trend in urinary sodium and other electrolytes over the progression of study period, during the dry season, among participants from different adherence categories , which suggests that increments in mineral intake was not due to MAR water usage but commonly occurred among all participants.

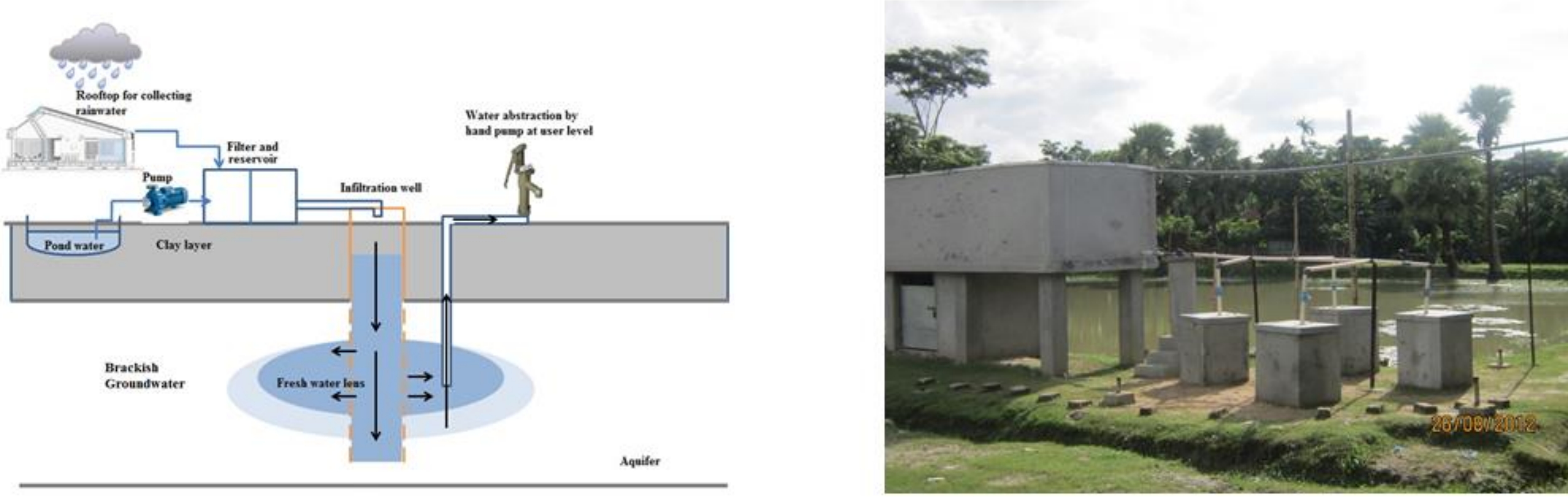
MAR systems were hydro-geologically successful at reducing the salinity of the brackish aquifers. The electrical conductivity of MAR water was consistently lower than the untreated brackish groundwater. The southwest coastal Bangladesh frequently faces inundation by seawater due to tidal surges associated with cyclones. The last two cyclones that rampaged the region was cyclone Sidr in 2007 causing a 5—6 meter tidal surges, and cyclone Aila in 2009 causing a 3 meter surge.[137] Ponds in the region were inundated by seawater and were not suitable for consumption for some years following these two cyclones. These cyclones highlighted the need of a more reliable and disaster resilient drinking water sources based on which MAR systems were first piloted in southwest coastal Bangladesh in 2009.[138] MAR system represented a strategy for increasing low-salinity water availability and sustaining a year-round drinking water supply that is expected to be resilient to tidal storms, cyclones and salinity of surface water bodies since freshwater infiltration and storage is done at groundwater level.[32] While technical advancements of MAR systems have been accomplished so far, several years of rainfall after 2009 also cleaned up the brackish ponds that was inundated by cyclones and communities have resumed consumption of pond water. Communities' practice of relying on a particular water source may changes over time and the evaluation of any drinking water salinity lowering intervention needs careful attention to communities' water consumption practices.

There are some limitations of our study and analyses. Three communities did not receive MAR access according to the randomization schedule due to technical issues including the presence of sand in water. These three communities were included as intervention communities in ITT analyses but as non-MAR users in per protocol and as treated analyses. This reduced the number of MAR users that may have widened confidence intervals of estimates in as-treated and per protocol analyses. Our definition of adherence relied on self-reported water source information from household members, which may have overestimated use. Household members may have consumed water from other sources particularly when they were outside the homestead or at work. Although we tested urinary electrolytes, we did not measure these in MAR water or participants' diet thereby limiting of our understanding of mineral exposure through the drinking water intervention.

The study was conducted as an interdisciplinary collaboration. The hydro-geologists provided a clear insight about how MAR systems work, saline water exposure measurement, and the hydro-geological context that supplemented the exploration of possible human health outcomes of MAR systems by the epidemiologists. Although MAR systems reduced the salinity of the brackish aquifer, we did not identify health benefits of MAR systems as communities were consuming lower salinity pond water rather than brackish groundwater. MAR systems may not be highly acceptable to the community in presence of alternative low-salinity water sources such as rainwater and pond water but may be a potentially acceptable source of drinking water when these sources are compromised.

Acknowledgement:

The study has been funded by Wellcome Trust, UK, Our Planet, Our Health Award. We acknowledge with gratitude the commitment of Wellcome Trust, UK to its research efforts. We are grateful to the study participants for their support and cooperation. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support. We are grateful to UNICEF and Dhaka University for their assistance to conduct the study. Dr. Gribble's effort was supported in part by funding from the National Institute of Environmental Health Sciences (P30 ES019776)



Schematic diagram of MAR systems

MAR systems in southwest coastal Bangladesh

Figure 1: MAR systems in southwest coastal Bangladesh

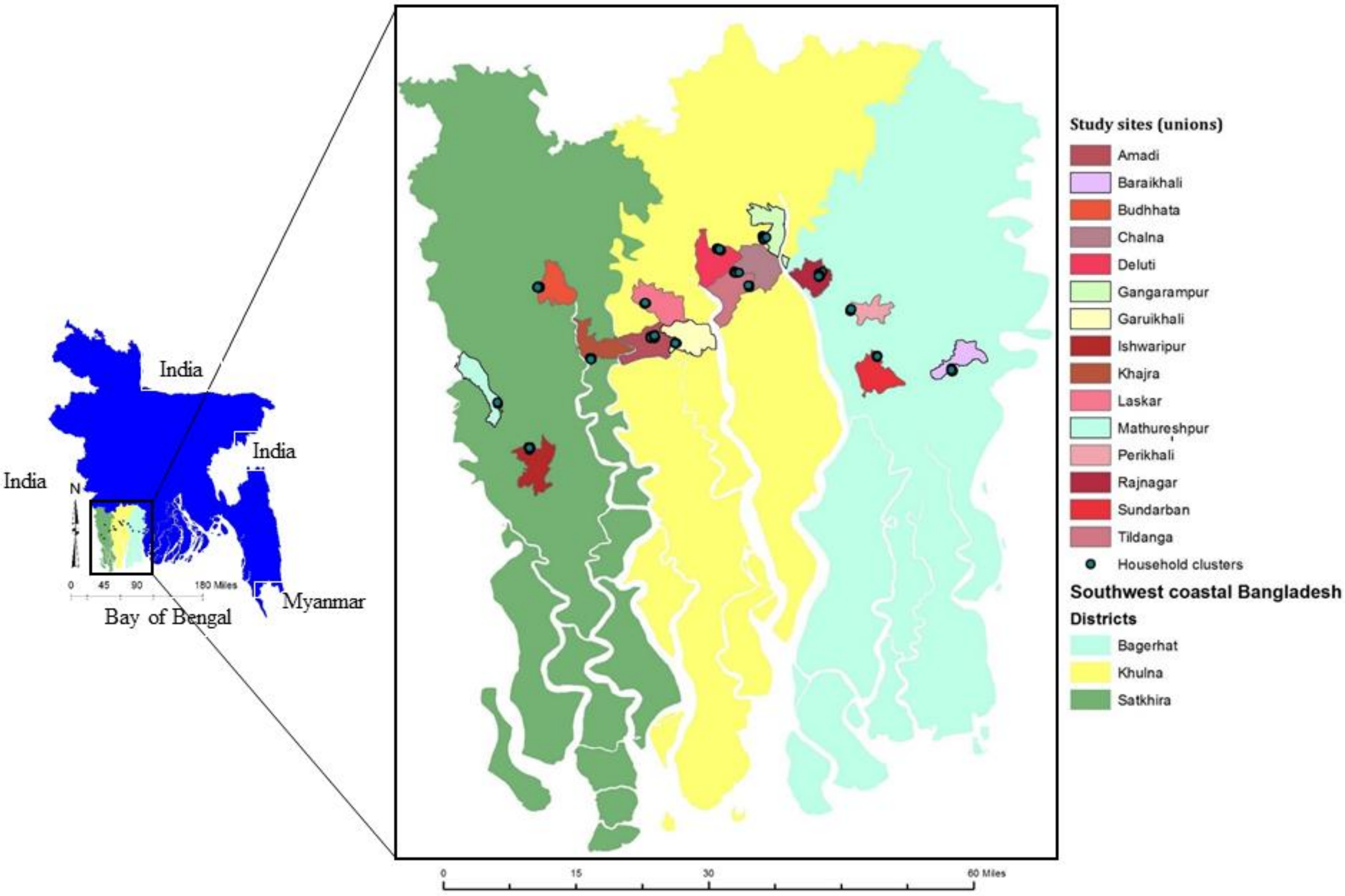
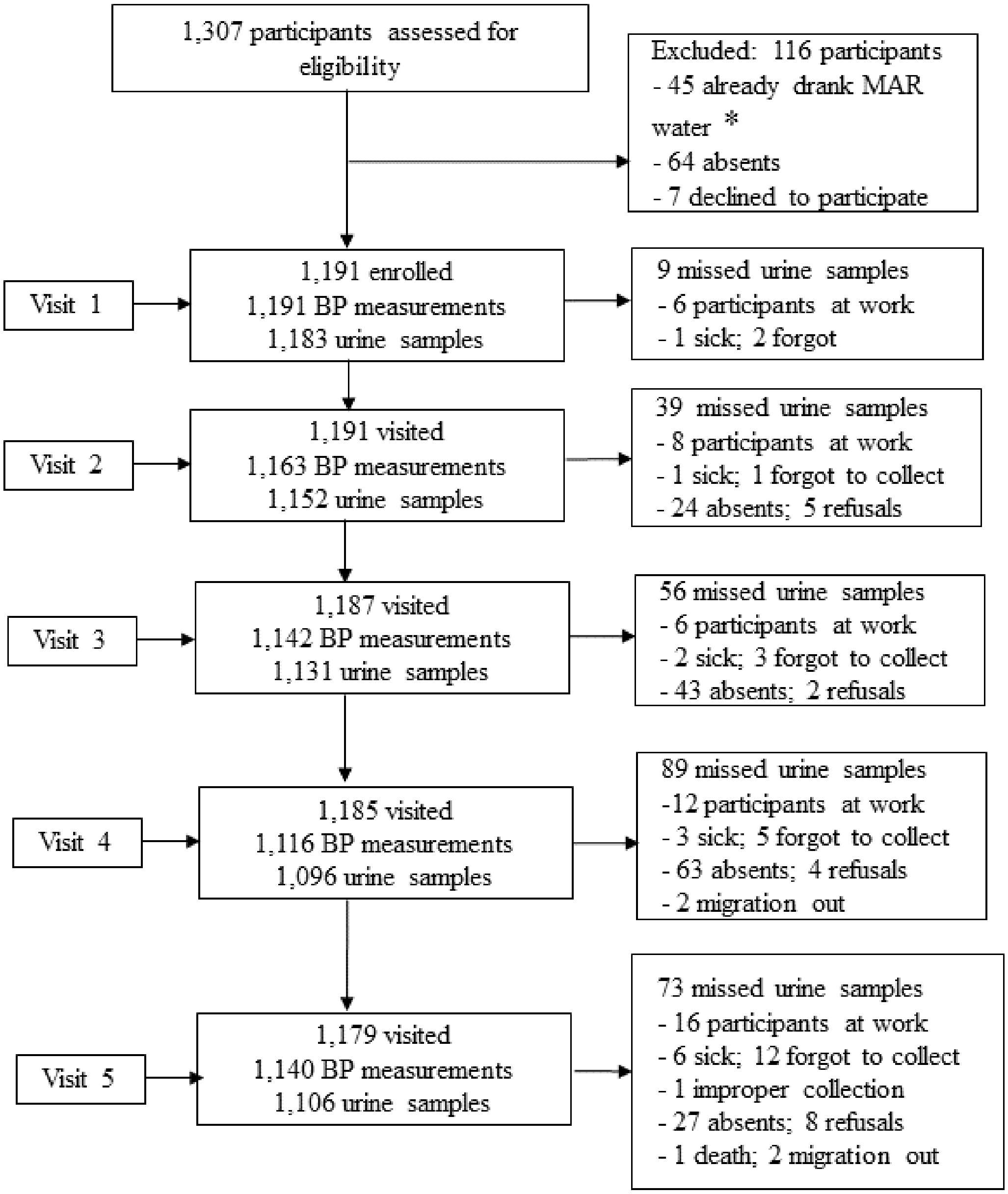


Figure S1: MAR study sites in southwest coastal Bangladesh



* 45 individuals consumed MAR water prior to their communities officially received access to MAR water. This happened as some households requested drinking water from the MAR systems during fresh water scarcity in irregular basis. These 45 individuals were excluded from the study.

Figure 3: Trial Profile

Table 1: Baseline characteristics of the trial participants

Variables	Median (IQR) or % (n)
Age (years)	41 (31 — 54)
20 ─ < 30 years	21.75% (259)
30 ─ < 40 years	25.94% (309)
40 ─ < 50 years	21.33% (254)
50 ─ <60 years	16.20% (193)
60 ─ <70 years	10.08% (120)
≥ 70 years	4.70% (56)
Male sex	40.72% (485)
BMI	21.83 (19.38 — 24.28)
Underweight (<18.5)	15.62% (186)
Normal weight (18.5 -<25)	64.23 (765)
Overweight (≥25-<30)	16.88 (201)
Obese (≥30)	3.27 (39)
Smoker	
Never	50.55% (602)
Former	9.15% (109)
Current	14.02% (167)
Exposed to passive smoking	26.28% (313)
Moderate to severe physical activities	39.95% (2298)
Education	
No institutional education	19.40% (231)
Up to 5th grade	28.97% (345)
Up to 10th grade	38.46% (458)
Up to 12th grade	8.23% (98)
>= 12th grade	4.95% (59)
Married	96.09% (5,527)
Religion	
Muslim	40.91% (2353)
Hindu	59.09% (3399)
Systolic BP (mmHg)	112.33 (104.33 — 124)
Diastolic BP (mmHg)	67.33 (61.67 — 74.67)
Hypertension	
Normal	68.35% (814)
Pre-hypertensive	22.08% (263)
Hypertensive	9.57% (114)

Household annual income (USD)	1,171 (780 — 1829)
Households annual income quintiles	
Lowest (<731.7)	24.54% (133)
2nd (731.7 — <975.6)	15.87% (86)
3rd (975.6 — <1463.4)	27.31% (148)
4th (1463.4 — <2195)	15.50% (84)
Highest (≥2195)	16.79% (91)
Drinking water EC (µS/cm)	970 (225 —1663)
Households drinking water quintiles	
Lowest (<113.6)	20.11% (109)
2nd (113.6 — <800)	19.93% (108)
3rd (800 — <1179)	20.11% (109)
4th (1179 — <1878)	19.93% (108)
Highest (≥ 1878)	19.93% (108)
Cooking water EC	1275 (1015 — 1867)
Households cooking water quintiles	
Lowest (<909)	20.30% (110)
2nd (909 — <1205)	18.27% (99)
3rd (1205 — <1542)	24.17% (131)
4th (1542 — <2000)	20.30% (110)
Highest (≥ 2000)	16.97% (92)

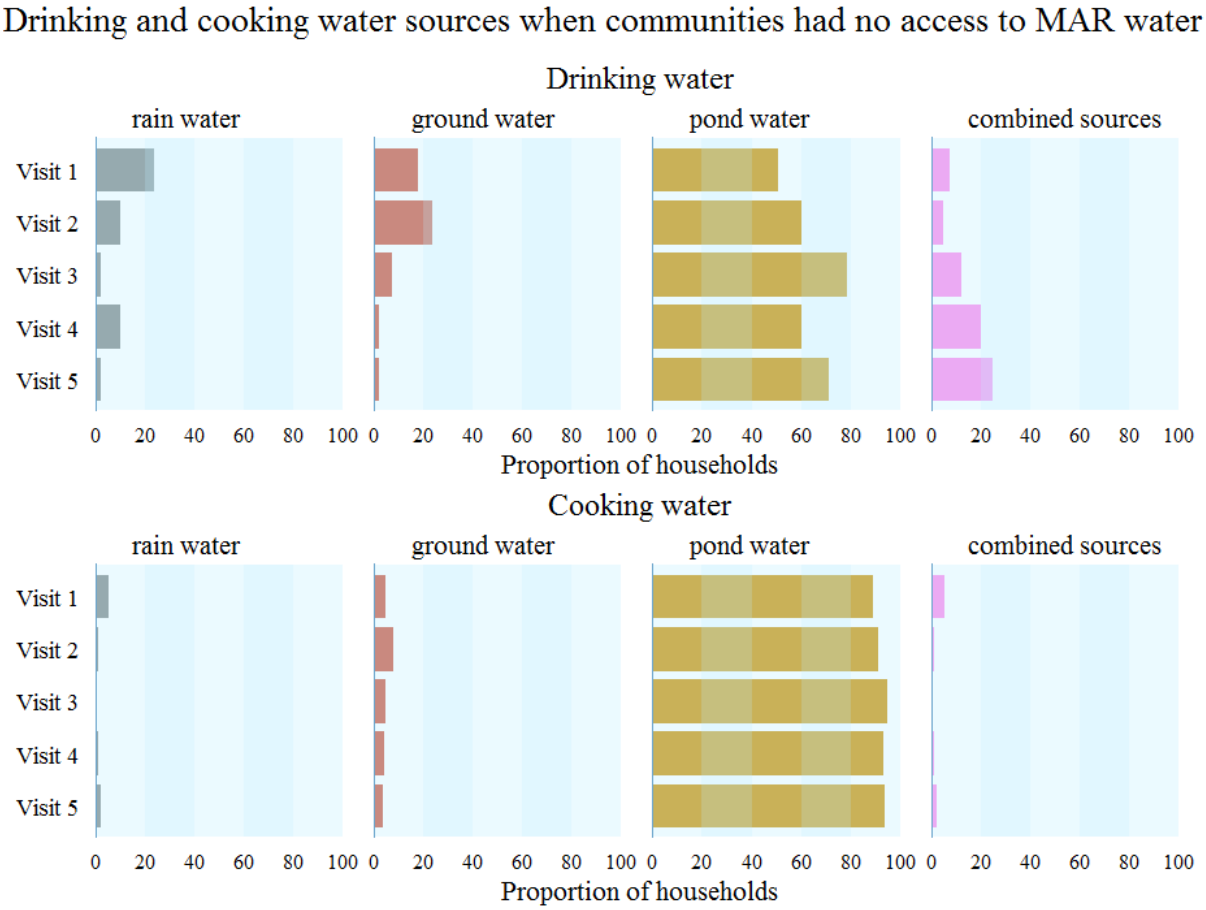


Figure 3: Drinking and cooking water sources for communities without access to MAR water

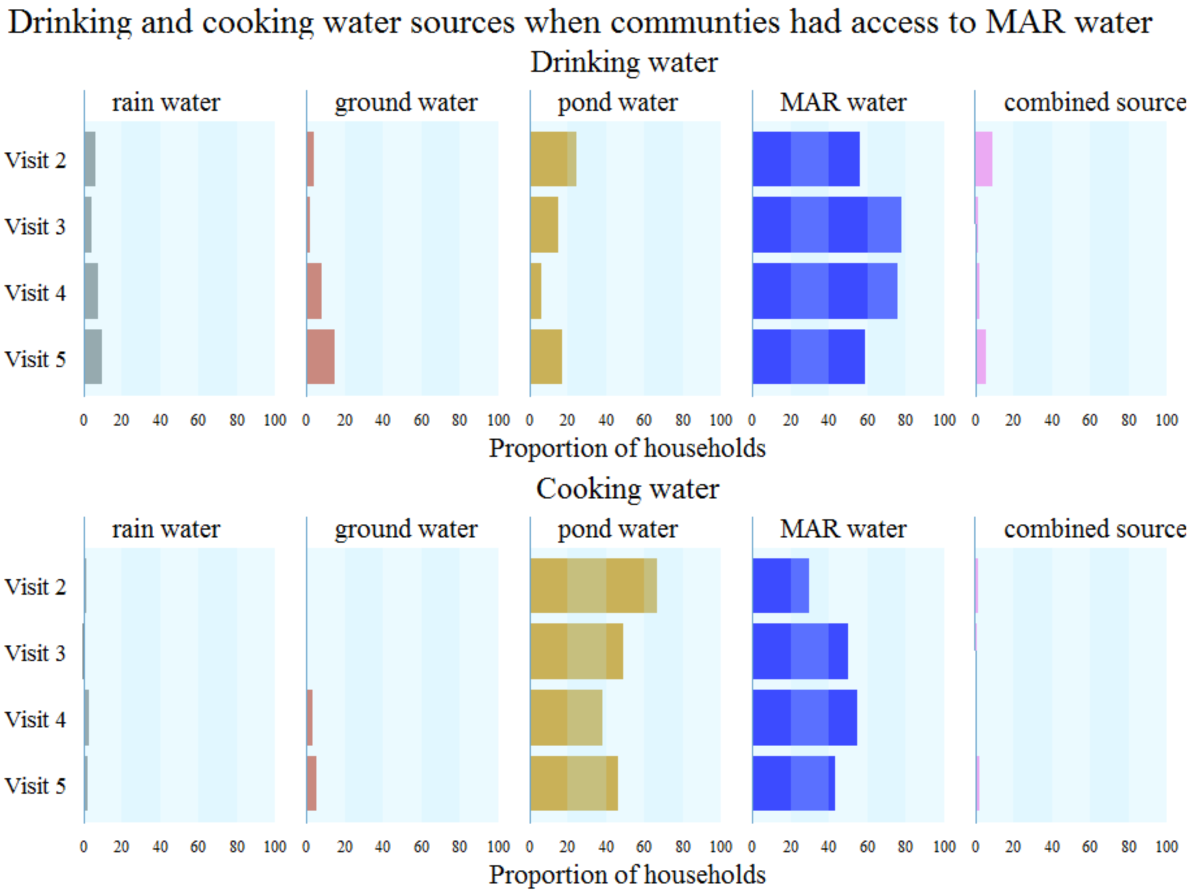


Figure 4: Drinking and cooking water sources for communities with access to MAR water

Table 2: Households' adherence to consume MAR water when communities were given access to MAR water

Households, % (n)	Visit 1 (Dec, 16) (N = 0 households)	Visit 2 (Jan, 17) (N = 150 households)	Visit 3 (Feb, 17) (N = 242 households)	Visit 4 (Mar, 17) (N = 339 households)	Visit 5 (Apr, 17) (N = 439 households)
Used MAR water for both drinking and cooking	----	26 (39)	49 (118)	50 (171)	36 (157)
Used MAR water for either drinking or cooking	----	34 (51)	30 (73)	30 (101)	24 (107)
Did not use MAR water	----	40 (60)	21 (51)	20 (67)	40 (175)

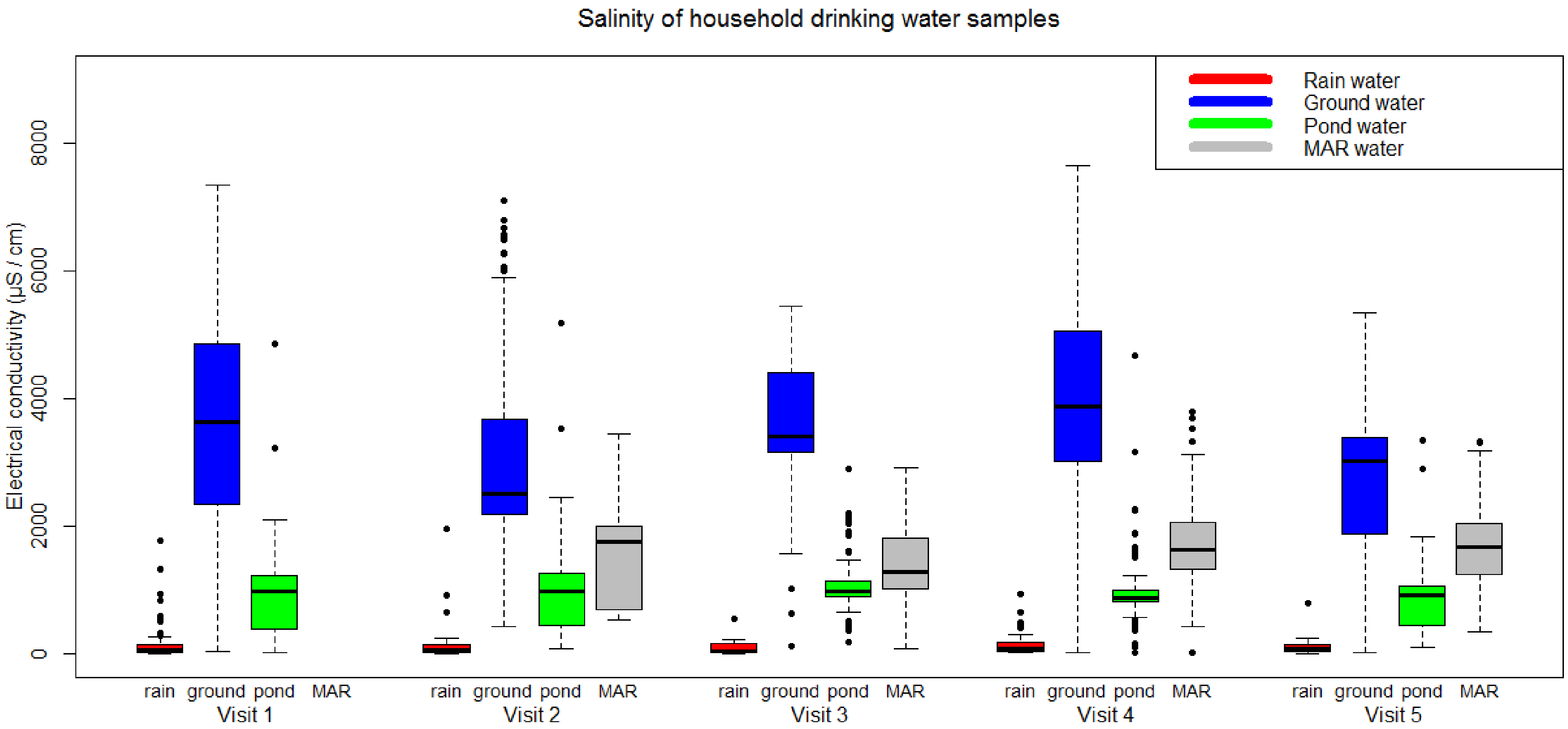


Figure S2: Salinity (electrical conductivity) of the households' stored drinking water samples across visits.

Table 3: Intention to treat effects of access to MAR water on blood pressure and urine protein of the study participants compared to no access

Outcomes	Model 1[a]		Model 2[b]		Model 3[c]	
	Regression coefficient (β)	**95% confidence Interval (p value)**	**Regression coefficient (β)**	**95% confidence Interval (p-value)**	**Regression coefficient (β)**	**95% confidence Interval (p-value)**
Systolic BP in mmHg (mean difference)	1.91	0.54, 3.27 (0.006)	1.89	0.52, 3.27 (0.007)	1.89	0.51, 3.27 (0.007)
Diastolic BP in mmHg (mean difference)	1.41	0.28, 2.54 (0.014)	1.40	0.24, 2.55 (0.018)	1.39	0.23, 2.55 (0.018)
Urinary total protein (ratio of median)	1.06	0.93, 1.20 (0.405)	1.06	0.93, 1.20 (0.401)	1.10	0.96, 1.25 (0.159)

[a] Adjusted for visit, age, sex and BMI

[b] Adjusted for visit, age, sex, BMI, physical activities, smoking and marital status

[c] Adjusted for visit, age, sex, BMI, physical activities, smoking, marital status, educational attainment, religion, household income and wealth score

Table 4: Effect of using MAR water on blood pressure and urine protein in as treated and per protocol analyses

		Outcomes	Model 1[a]		Model 2[b]		Model 3[c]	
			Regression coefficient (β)	**95% confidence Interval (p-value)**	**Regression coefficient (β)**	**95% confidence Interval (p-value)**	**Regression coefficient (β)**	**95% confidence Interval (p-value)**
Per protocol analysis	Full adherence	Systolic BP (mean difference)	1.16	-0.42, 2.75 (0.149)	1.14	-0.43, 2.71 (0.155)	1.13	-0.43, 2.70 (0.157)
		Diastolic BP (mean difference)	0.47	-0.56, 1.51 (0.370)	0.45	-0.57, 1.48 (0.384)	0.46	-0.58, 1.49 (0.387)
		Urinary protein (ratio of median)	1.01	0.80, 1.27 (0.950)	1.01	0.80, 1.27 (0.954)	1.07	0.86, 1.33 (0.541)
	Partial adherence	Systolic BP (mean difference)	0.79	-0.63, 2.2 (0.275)	0.75	-0.66, 2.17 (0.296)	0.73	-0.65, 2.11 (0.299)
		Diastolic BP (mean difference)	0.01	-0.96, 0.99 (0.983)	-0.002	-0.98, 0.97 (0.996)	-0.02	-0.99, 0.95 (0.968)
		Urinary protein (ratio of median)	1.01	0.82, 1.25 (0.893)	1.02	0.82, 1.25 (0.889)	1.08	0.89, 1.32 (0.437)
As treated analysis	Full adherence	Systolic BP (mean difference)	0.48	-0.83, 1.79 (0.470)	0.48	-0.84, 1.79 (0.476)	0.49	-0.83, 1.80 (0.468)
		Diastolic BP (mean difference)	0.07	-0.76, 0.91 (0.860)	0.08	-0.74, 0.90 (0.850)	0.09	-0.74, 0.92 (0.830)

		Urinary protein (ratio of median)	1.01	0.85, 1.20 (0.897)	1.01	0.85, 1.20 (0.896)	1.03	0.88, 1.20 (720)
	Partial adherence	Systolic BP (mean difference)	0.21	-0.89, 1.30 (0.710)	0.20	-0.89, 1.30 (0.719)	0.20	-0.87, 1.27 (0.718)
		Diastolic BP (mean difference)	-0.25	-0.92, 0.42 (0.472)	-0.22	-0.89, 0.44 (0.506)	-0.23	-0.88, 0.42 (0.490)
		Urinary protein (ratio of median)	1.01	0.90, 1.13 (0.843)	1.01	0.90, 1.13 (0.822)	1.03	0.92, 1.14 (0.623)

[a] Adjusted for visit, age, sex and BMI

[b] Adjusted for visit, age, sex, BMI, physical activities, smoking and marital status

[c] Adjusted for visit, age, sex, BMI, physical activities, smoking, marital status, educational attainment, religion, household income and wealth score. Urine protein additionally adjusted for urine creatinine.

Table S2: Urinary excretion of Na, K, Ca and Mg across different MAR users

Outcomes	As treated analyses			Per protocol analyses		
	No MAR user	Partial adherence	Full adherence	No MAR user	Partial adherence	Full adherence
Na (difference in mean)						
Model 1[a]	Reference	4.93 (-0.87, 10.73)	17.58 (12.4, 22.73)	Reference	-3.25 (-16.6, 10.1) [0.635]	-6.62 (-19.52, 6.29) [0.315]
Model 2[b]	Reference	5.38 (-0.29, 11.05)	17.88 (12.8, 22.97)	Reference	-3.09 (-16.4, 10.2) [0.649]	-6.62 (-19.18, 5.95) [0.302]
Model 3[c]	Reference	4.98 (-0.79, 10.75)	17.57 (12.40, 22.74)	Reference	-2.89 (-16.03, 10.27) [0.668]	-3.66 (-16.4, 9.13) [0.575]
K (difference in mean)						
Model 1	Reference	0.74 (-1.33, 2.81)	0.19 (-1.80, 2.17)	Reference	-1.39 (-3.92, 1.13) [0.278]	-1.44 (-3.87, 0.99) [0.245]
Model 2	Reference	0.81 (-1.27, 2.89)	0.24 (-1.70, 2.17)	Reference	-1.25 (-3.79, 1.29) [0.333]	-1.39 (-3.83, 1.04) [0.263]
Model 3	Reference	0.86 (-1.24, 2.96)	0.32 (-1.65, 2.28)	Reference	-1.27 (-3.79, 1.23) [0.318]	-1.41 (-3.84, 1.02) [0.255]
Ca (ratio of median)						
Model 1	Reference	1.30 (1.16, 1.47)	1.23 (1.09, 1.38)	Reference	1.02 (0.90, 1.15) [0.782]	1.06 (0.92, 1.21) [0.428]
Model 2	Reference	1.30 (1.16, 1.47)	1.23 (1.10, 1.38)	Reference	1.02 (0.91, 1.15) [0.757]	1.06 (0.92, 1.21) [0.414]
Model 3	Reference	1.32 (1.18, 1.49)	1.23 (1.09, 1.39)	Reference	1.02 (0.91, 1.15) [0.759]	1.06 (0.92, 1.22) [0.413]
Mg (ratio of median)						
Model 1	Reference	1.21 (1.07, 1.37)	1.28 (1.17, 1.47)	Reference	1.16 (0.96, 1.40) [0.123]	1.18 (0.98, 1.43) [0.087]
Model 2	Reference	1.22 (1.08, 1.37)	1.29 (1.12, 1.48)	Reference	1.16 (0.96, 1.41) [0.118]	1.18 (0.98, 1.43) [0.083]

Model 3	Reference	1.22 (1.08, 1.37)	1.28 (1.10, 1.48)	Reference	1.16 (0.96, 1.40) [0.114]	1.18 (0.98, 1.42) [0.088]

[a] Adjusted for visit, age, sex and BMI

[b] Adjusted for visit, age, sex, BMI, physical activities, smoking and marital status

[c] Adjusted for visit, age, sex, BMI, physical activities, smoking, marital status, educational attainment, religion, household income and wealth score

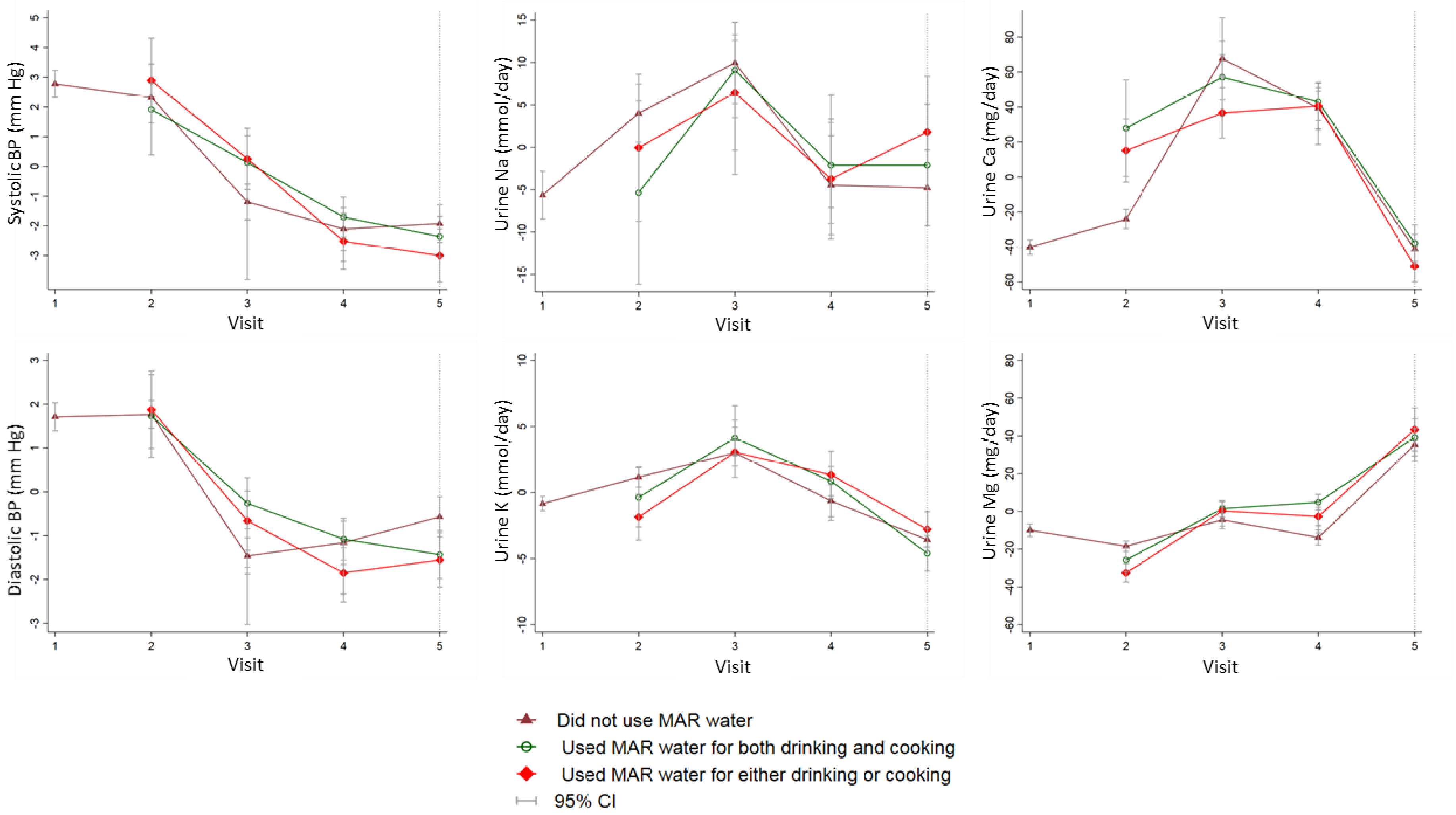


Figure S3: Trend of blood pressure and urine electrolytes across five visits among different MAR users (per protocol analyses)

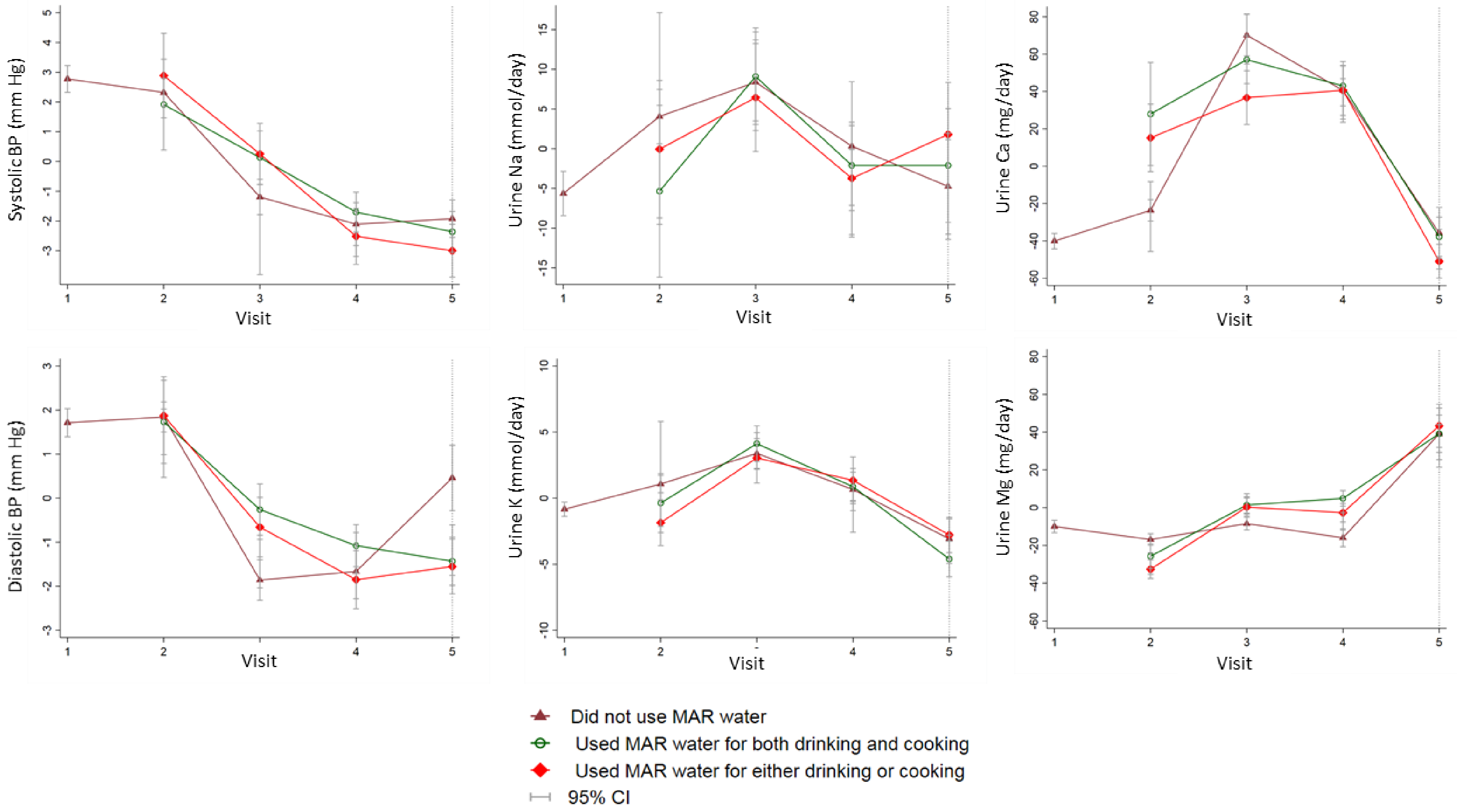


Figure S4: Trend of blood pressure and urine electrolytes across five visits among different MAR users (as treated analyses)

Chapter 4. Dose-response association of drinking water salinity with blood pressure and urinary protein excretion in southwest coastal Bangladesh[4] [5]

Abu Mohd Naser[1*], Mahbubur Rahman[2] , Leanne Unicomb[2], Solaiman Doza[2], Ruhel Gazi[2], Raisul A Robin[2], Rabiul Karim[2], Mohammad Nasir Uddin[2], Kazi Matin Ahmed[3], Mohammad Shamsudduha[4], William Burgess[5] , Shuchi Anand[6], Nargis Akther[7], Boluwaji Onabolu[7], K.M. Venkat Narayan[8], Howard H. Chang[9], Stephen P. Luby[10], Matthew O. Gribble[1], Thomas F. Clasen[1]

[1] Department of Environmental Health Sciences, Rollins School of Public Health, Emory University, Atlanta, USA
[2] International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), GPO Box 128, Dhaka 1000, Bangladesh
[3] Department of Geology, University of Dhaka, Dhaka, Bangladesh
[4] Institute for Risk and Disaster Reduction, University College London, UK
[5] Department of Earth Sciences, University College London, London, UK
[6] Division of Nephrology, School of Medicine, Stanford University, Stanford, California, USA
[7] Water Sanitation & Hygiene Section, UNICEF, Bangladesh
[8] Emory Global Diabetes Research Center, Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA
[9] Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, USA
[10] Woods Institute for the Environment, Stanford University, Stanford, California, USA

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[4] This chapter is a manuscript prepared for submission to Annals of Internal Medicine. The structure, length and format are in keeping with journal requirements.

[5] AMN and TFC developed the analyses concept. AMN, MR, LU, SD, KMU, MS, WB were involved in study implementation. RG, RAR, RK and MNU were involved in laboratory analyses of the environmental and biological samples. AMN and MOG developed the statistical analysis plan. AMN conducted the statistical analyses and MOG reviewed the analyses. AMN drafted and all author reviewed the manuscript.

Abstract

Background: Evaluation of the relationship between overall drinking water salinity (i.e., electrical conductivity) and blood pressure or urine protein outcomes has been limited.

Method: We pooled longitudinal data of a population-based cohort in Bangladesh comprised of 1,574 participants at least 20 years old who were followed-up for maximum five months. In total, 6,945 blood pressure and 6,404 urine protein data. We used multilevel linear regression models to estimate the association of drinking water electrical conductivity categories with blood pressure, and multilevel parametric quantile regression models were used to estimate the association of electrical conductivity with urine protein excretion. Models were adjusted for age, sex, BMI, physical activity, smoking status and household wealth. We also assessed the associations of drinking water salinity with urinary sodium, potassium, calcium and magnesium.

Results: Persons consuming moderate salinity drinking water (≥ 2 mS/cm and <10 mS/cm) had -1.60 [95% Confidence Interval (CI): -3.04, -0.15] mm Hg lower mean SBP, and -1.21 [95% CI: -1.99, -0.43] mm Hg lower mean DBP compared to consumers of fresh salinity water (< 0.7 mS/cm) in fully adjusted models. The average adjusted ratio of median 24-hour urine protein was 0.90 [95% CI: 0.76, 1.05] among consumers of moderate salinity water compared to consumers of fresh drinking water. Persons consuming moderate salinity water had on average +17.57 [95% CI: +12.40, +22.74] mmol/day higher Na, +0.32 [95% CI: -1.65, +2.28] mmol/day higher K, 1.23 [95% CI: 1.09, 1.39] times higher median Ca, and 1.28 [95% CI: 1.10, 1.48] times higher median Mg in urine compared to persons consuming fresh drinking water in fully adjusted models.

Conclusion: Higher drinking water salinity was associated on average with lower blood pressure and urine protein excretion, perhaps due to higher levels of calcium and magnesium in higher-salinity water.

Introduction

More than one billion people globally rely on coastal aquifers as the source of water (1). Of them, nearly 204 million currently reside in areas affected by seawater intrusion (2), a process of groundwater salinity due to movement of fresh-saline groundwater interface towards the inland along the shores (3). More coastal regions will be affected by seawater intrusion in future due to global climate change such as change in precipitation pattern affecting groundwater recharge, decrease flow of upstream rivers, frequent cyclones and sea-level rise (4, 5). Projections suggest high extraction of groundwater to meet the water demand of the population will contribute more to seawater intrusion than the climatic variables such as sea-level rise(4).

Many seawater intrusion affected communities depend on saline water for drinking purposes (2). Densely populated communities in seawater intrusion affected coastal regions of Bengal, Mekong, and Red River deltas in South and Southeast Asia are exposed to drinking saline water, which is a source water of chemicals and minerals affecting human health(6). There are limited data on human health impacts of drinking saline water in coastal communities. Few observational studies report high blood pressure and hypertension among coastal communities in South Asia, Southeast Asia and China (7-13). Evaluating the health impact of drinking saline water is imperative in context of progressive stress on coastal aquifers due to high groundwater demand and global climate change.

One particular example of seawater intrusion is southwest coastal Bangladesh, where communities drink groundwater from shallow and deep aquifers (14). Drinking saline water has been associated with high sodium intake and blood pressure among communities in southwest coastal Bangladesh. People consume an estimated 5 — 16 gm sodium during the dry season through drinking water (15) whereas the daily target sodium intake from all sources is < 2gm/day (16). A cross sectional study of 19-25 years old adults suggests high salinity water (> 600 mg/L sodium) consumption was associated with 3.5 (95% CI: 0.75 — 6.17) mm Hg higher systolic blood pressure and 2.8 (95% CI: 0.31 — 5.24) mm Hg higher diastolic blood pressure compared to those who consumed low salinity water (< 600 mg/L sodium) (12). Adjusted analysis for age, parity, socioeconomic status, and mid-upper arm circumference from a case-control study suggest pregnant women with >900 mg/L sodium in drinking water had 5.5 times (OR: 5.48, 95% CI: 3.30–9.11) more risks for pre-eclampsia and gestational hypertension compared to pregnant women whose drinking water had <300 mg/L sodium (17).

Accurate exposure of chemicals and minerals through drinking water is essential to evaluate the health impact of drinking saline water. Water salinity is typically expressed as Total Dissolved Solids (TDS) — i.e, milligrams dissolved solids per liter of water, or Electrical Conductivity (EC), which means the ability of water to conduct an electrical current where the dissolved ions are the conductors. The major cations contributing to the electrical conductivity are sodium, calcium, potassium and magnesium (18). The dissolved ions in saline water vary highly from one location to another, both in terms of specific ions and concentration level (2). Nevertheless, studies from southwest coastal Bangladesh have focused on only sodium intake through drinking saline water. These studies measured sodium in water as a measure of salinity exposure but the ignored other potential chemicals in saline water contributing to blood pressure regulation. Epidemiological studies suggest potassium, magnesium and calcium intake, which may be also derived from drinking saline water, have inverse association with blood pressure (19, 20) as opposed to sodium. Currently, no data exists on how all minerals together in saline water contribute to blood pressure.

We analyzed data from two studies that measured drinking water electrical conductivity compared to only sodium measurement in water, and urinary concentration of sodium, potassium, calcium and magnesium of the participants compared to measuring only urinary sodium. The objective of the analysis was to explore the impact of drinking water electrical conductivity or all minerals together in saline water on blood pressure and urinary total protein of the population.

Methods

Study Population

We pooled data from two studies led by International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) across southwest coastal Bangladesh. Study population of both studies are described in Figure 1. The studies were implemented as part of health impact evaluation of an drinking water salinity lowering intervention called Managed Aquifer Recharge (MAR), a technology of artificially recharging brackish groundwater aquifers with rainwater and pond water to lower salinity (25). The first was an observational pilot study that followed participants (n=383 from 166 households) in 4 communities during two time points. The pre-monsoon (10 May 2016 – 20 June 2016) visit corresponded when participants consumed predominantly groundwater and the monsoon (20 July 2016 – 20 August 2016) visit corresponded when they consumed predominantly rainwater.

The second study was a stepped-wedge cluster randomized trial to assess the health impacts of the MAR across 16 communities during the dry season of December, 2016 – April, 2017 (26) . The stepped-wedge trial (n=1,191 participants from 542 households, followed for 5 visits) was conducted in 16 communities in southwest coastal Bangladesh. The rationale, objectives and design of the study have been published elsewhere (26). No communities had access to MAR water systems during the first visit; 4 communities were randomized at each successive visit to receive access to MAR water.

In summary, we pooled 1,574 participants' longitudinal data from both studies. From all visits, 6,494 blood pressure measurements and 6,404 urine samples were compiled.

Saline Water Exposure or Electrical Conductivity Measurement

During each visit of both studies, we recorded household-reported primary drinking water sources in the previous 24 hours. We collected available water samples stored for drinking and measured the temperature-adjusted electrical conductivity at 25°C using a Hanna Salinity™ meter (model: H198192, accuracy: ±1%). Electrical conductivity is a widely used indicator of water salinity that measures how easily electrons pass a certain distance of water □ high electrical conductivity means more dissolved ions in water facilitating easy conductance of electron (24). The Salinity™ meters were calibrated every morning prior measuring electrical conductivity.

High Blood Pressure Risk Factor Data

Baseline data on demographics, participant-reported smoking status (never smoker, current smoker and former smoker), work-related physical activity (vigorous physical activities, moderate physical activity and sedentary activity), and information about household assets were collected in both studies using structured questionnaire by trained research assistants. We used WHO Global Physical Activity Questionnaire for determining status of physical activities among the participants. Participants' weight in kg was measured in all visits using Seca weight machine (Model: 874-1321009; accuracy: 0.05|0.1 kg, Hamburg, Germany) but height in one visit using Shorr board (accuracy: 1/8" or 0.1 cm; Olney, Maryland).

Outcome Data

Blood Pressure

Blood pressure (systolic and diastolic) was measured at each visit using an Omron® HEM–907 (accuracy: within ± 4 mmHg, Kyoto, Japan) digital blood pressure monitor, a device that meets the Association for the Advancement of Medical Instrumentation's (AAMI) criteria (27). Caffeine (tea, coffee, carbonated beverages), eating, heavy physical activities and smoking were proscribed for 30 minutes prior to measuring blood pressure. Participants rested for 5 minutes on a chair with both arms supported. An appropriately sized cuff and calibrated instrument were used. Blood pressure was measured three times; first left arm, then right arm, then again left arm. The arithmetic mean of three measurements was used for analyses.

24-Hour Urine Collection and Processing

Each participant received a 4 L plastic container for 24-hour urine collection and a plastic mug to transfer the voided urine to the 4-L plastic container. Research staff instructed the participants to discard the first morning urine and start collecting from the second void. They were instructed to transfer all other voids, and the next first morning. If containers became full, participants were instructed to use a container from their household. Volume of 24-hour urine samples were measured at household-level by trained research staff, and a 15 ml samples from the 4-L plastic container were taken after stirring. All urine samples were transported to a field laboratory at 2-8° C for processing, aliquoting and analysis on the same day.

24-Hour Urinary Total Protein

Urinary total protein was estimated using a light sensitive colored reagent (Randox.UK) in a semi-auto biochemistry analyzer (Evolution 3000, BSI, Italy, coefficient of variation: < 1%). The laboratory staff ensured quality control of the measurements by running blank samples and controlled solutions every day prior starting measuring the urine protein. Calibration of the instruments were done regularly using the solutions provided by the manufacturers. To assess the validity of the measurements, one replicate of the last sample from each batch was conducted (one batch consists of 25 samples). Four samples — the lowest two and the highest two concentrations—were also repeated for validity checking every day.

24-Hour Urinary Creatinine, sodium, potassium, calcium and magnesium

Urine creatinine was measured by a colorimetric method (Jaffe reaction) (28) . Direct Ion Selective Electrode (ISE) methods, a common method in clinical biochemistry laboratories with high agreement to the conventional flame photometer (29), were used to measure the urinary sodium & potassium with a semi-auto electrolyte analyzer (Biolyte2000, Bio-care Corporation, Taiwan, coefficient of variation: +5%). Urinary calcium and magnesium (magnesium only in the trial) were measured by photometric titration method using Biolyte2000. Laboratory staff followed manufacturer guidelines for conditioning and calibration.

Statistical analysis

Descriptive Statistics

We calculated the mean and standard deviation (SD) of approximately normally distributed variables, median and interquartile range (IQR) of skewed continuous variables, and proportions for categorical variables. We derived the household wealth score by principal component analysis using the household asset data for ownership of refrigerator, television, mobile phones, motorcycle, bicycle, sewing machine, chair, table, wrist watch, wardrobe wooden cot, motor pump, paddy husking machine, engine rickshaw, car and access to electricity (30). We calculated pair-wise Spearman correlations between drinking water electrical conductivity, systolic BP, diastolic BP and urine protein.

Dose-Response Modeling

The dose-response analyses were not pre-specified. Statistical models were finalized after examining the distribution of each outcome.

All regression models included three-level random intercepts to account for multilevel clustering of longitudinal visits within participant, participants within household, and households within communities. We estimated all models by maximum likelihood method and reported cluster robust standard errors.

The associations of concurrent electrical conductivity categories with mean systolic and diastolic blood pressure were modeled using multilevel linear models. Electrical conductivity categories were defined by the Food and Agricultural Organization (31): fresh water (electrical conductivity < 0.7 mS/cm), low salinity (electrical conductivity ≥ 0.7 and < 2 mS/cm), moderate salinity (electrical conductivity ≥ 2 and <10 mS/cm), or severe salinity (electrical conductivity ≥10 mS/cm). To determine the shape of the dose-response curve, electrical conductivity was modeled by restricted cubic splines.

The association of concurrent electrical conductivity with urinary total protein was assessed through multilevel parametric quantile models assuming a two-parameter gamma distribution of urinary protein. Adaptive quadrature with 7 quadratic points was used to approximate the likelihood for urine protein.

To understand the intermediate biological pathways, we examined whether water electrical conductivity is associated with daily urinary mineral excretion and whether urinary minerals are associated with blood pressure. 24-hour urinary sodium and potassium were modeled with multilevel linear models, while skewed calcium and magnesium were modeled by multilevel parametric quantile models assuming two-parameter gamma distributions. Model were; further adjusted for age, sex, body mass index (BMI); and additionally adjusted for smoking, physical activities and household wealth score. Age, sex, BMI and wealth score were used as continuous covariates whereas physical activities and smoking status were used as categorical variables. Urine total protein was also adjusted for urine creatinine in the fully-adjusted model.

In a sensitivity analysis, a propensity score-matched analysis matched person-visits from the highest 40% of the electrical conductivity distribution to person-visits from the lowest 20% of the electrical conductivity distribution, using nearest-neighbor matching by Mahalanobis distance. Variables used for matching were age, sex, BMI, smoking status, physical activities and wealth score. In matched analyses, 1299 person-visit were included in the low electrical conductivity group and 1299 in the high electrical conductivity group. Aside from a propensity-score matched subpopulation, and high vs. low electrical conductivity modeled as a binary variable, models were identical to the main analysis.

Missing data (n=90 urine protein, n=90 BMI, n=57 electrical conductivity) were imputed by multiple imputation by chained equations conditional on the variables included in the main analysis linear regression and parametric quantile regression models. Statistical analyses were performed in Stata, version 15.0 and R, version 3.3.1.

Ethical approval

Informed written consent was obtained from all the participating household members and the household heads, and study protocols were approved by the Ethical Review Committee of icddr,b (PR-15096). Data were provided to Emory University under a data transfer agreement with icddr,b.

Results

Study participants

The characteristics of the participants are given in Table 1. Median age and BMI of all participants were 40 (IQR: 31 —54) years and 21.8 (IQR: 19.4 —24.3) kg/m2. Most participants had normal weight (63%) as per WHO classification of BMI, were normotensive (70%), female (59 %) and never smoked (52%). Characteristics of the participants and households at enrollment for different drinking water electrical conductivity are reported in Table 1. In all participant-visit, 27% drank fresh water, 49% mildly saline water and 24% moderately saline water.

Drinking water electrical conductivity, blood pressure and urine protein excretion

The pair-wise univariate Spearmen's correlation between water electrical conductivity, urine minerals and systolic BP are illustrated in Figure S1. Participants with mild drinking water salinity water category had – 1.45 [95% CI: -3.05, +0.16] mm Hg change in mean systolic BP, -1.13 [95% CI: -2.02, -0.24] mm Hg change in mean diastolic BP compared to the fresh drinking water category in fully-adjusted model (Table 2). The ratio of median 24-hour urine protein for mild salinity to fresh drinking water category was 0.89 [95% CI: 0.76, 1.04] after adjusting for all confounders.

Participants with moderate salinity drinking water categories had -1.63 [95% CI: -3.10, -0.1 5] mm Hg change in mean systolic BP, -1.21 [95% CI: -1.99, -0.43] mm Hg change in mean diastolic BP in fully-adjusted model (Table 2). The ratio of median 24-hour urine protein for moderate salinity to fresh drinking water category was 0.90 [95% CI: 0.76, 1.05] after adjusting for all confounders (Table 2).

Sensitivity analyses suggest that compared to the lowest 20% drinking water electrical conductivity (electrical conductivity <394 µS/cm) person-visits, participants from the matched high electrical conductivity (electrical conductivity > 1440 µS/cm) person-visit had a -1.54 [95% CI: -2.65, -0.43] mm Hg change in mean systolic blood pressure, -1.16 [95% CI: -1.77, -0.55] mm Hg change in mean diastolic blood pressure in fully adjusted models (Table 3). The ratio of median 24-hour urine protein for high to low salinity drinking water group was 0.83 [95% CI: 0.73, 0.95] in fully adjusted models (Table 3).

The restricted cubic spline plots suggests a non-linear (Wald test, p value = <0.001) inverse association between drinking water electrical conductivity and blood pressure, and water electrical conductivity and urinary total protein (Figure 2).

Drinking water electrical conductivity and 24-hour urinary mineral concentrations

In fully adjusted models, participants from moderate drinking water salinity (EC: > 2 mS/cm) group had 17.57 [95% CI: 12.40, 22.74] mmol/day increase in mean urinary sodium, 0.32 [95% CI: -1.65, 2.28] mmol/day increase in mean urinary potassium, 1.23 [95% CI: 1.09, 1.39] ratio of median for urinary calcium, and 1.28 [95% CI: 1.10, 1.48] ratio of median for urinary magnesium compared to participants from fresh drinking water (EC: <0.7 mS/cm) group (Figure 3). Participants with mild drinking water salinity (EC: 0.7 □ < 2 mS/cm) group had 4.98 [95% CI: -0.79, 10.75] mmol/day increase in urinary sodium, 0.86 [95% CI: -1.24, 2.96] mmol/day increase in urinary potassium, 1.32 [95% CI: 1.18, 1.49] ratio of median for urinary calcium, and 1.28 [95% CI: 1.10, 1.48] ratio of median for urinary magnesium compared to participants from fresh drinking water group (Figure 3).

Discussion

Our analyses suggest that drinking water salinity has an inverse relationship with systolic and diastolic blood pressure and urinary total protein. We also identified high drinking water salinity is associated with increased urinary concentration of sodium, calcium and magnesium.

The increase urinary excretion of cardio-protective minerals such as calcium, and magnesium may explain the inverse association of drinking water salinity and blood pressure or urinary total protein despite increase in sodium concentration. Similar findings have been demonstrated by some epidemiological and experimental studies. A review of observational studies suggest that when individuals meet the recommended intake of potassium, calcium and magnesium, high sodium is not associated with high blood pressure but associated with low blood pressure (32). In the presence of optimum calcium intake, high sodium intake does not increase the blood pressure (33). Studies suggest high sodium intake increase blood pressure only when there are deficiency of calcium (34, 35).

We did not measure which ions contribute to the electrical conductivity of drinking water. Nevertheless, hydrogeological analyses suggest the major dissolved cations in groundwater are also those cations present in Earth’s crust at large scale such as calcium, magnesium, sodium and potassium (36). Hydrogeological survey of groundwater chemical constitutes in Bangladesh suggest that groundwater hardness — a measure of calcium and magnesium salts — is the highest in southwest Bangladesh compared to other regions of Bangladesh (37). Hydrogeological exploration suggests groundwater in coastal Bangladesh is Na-Ca-Mg-HCO3-Cl type (38). We hypothesize high salinity water in our study area may be the source of high intake of calcium and magnesium along with high intake of sodium, which is evident by high urinary concentrations of these minerals among our study participants.

Both blood pressure and urinary protein are markers of vascular health □ sodium, potassium, calcium and magnesium are involved in multiple pathways influencing vascular health. Extracellular potassium, calcium and magnesium release vasodilators such as nitric oxide (NO) and prostaglandin E1 (39, 40) whereas sodium decrease the production of vasodilator NO and releases vasoconstrictors (41). Ionic entry of sodium across cell membrane of vascular smooth muscle precedes the smooth muscle contraction that increase vascular tone and blood pressure (42, 43). Intake of optimum calcium and magnesium decrease the blood pressure by stabilizing the cell membrane of the vascular smooth muscle by binding to the plasma membrane of the vascular smooth muscle (44-47), which in turns interferes with the ionic conductance across cell membrane that subsequently diminish the vascular tone (48, 49). Calcium and magnesium concentration below the physiological concentration destabilizes the cell membrane, causes more sodium entry and smooth muscle contraction (50). Increase dietary intake of calcium, and magnesium also facilitates the urinary excretion of sodium in a variety of mechanisms including increase release of atrial natriuretic peptide, reduced sympathetic outflow, reduced angiotensin 2 expression, reduced circulating PTH, and interfering sodium reabsorption in the proximal convoluted tubules of kidneys (51, 52).

This study has several limitations. First, we did not measure concentrations of individual minerals in saline water. This precludes the understanding of exact mineral exposure through drinking saline water. Moreover, lack of dietary mineral intake data also limits our understanding of contribution of drinking water salinity to the total intake of these minerals over the progression of dry season. Measurement of 24-hour protein and electrolyte concentrations may be biased by over- or under-collection of 24-hour urine samples. Further studies are warranted to explore the concentrations of different ions in saline water and measuring the biomarkers associated with endothelial dysfunction of the communities who drink saline water.

Notwithstanding these limitations, this study has important implications that warrant further research. The study suggests households' shifting to low salinity water sources from high salinity sources may have high blood pressure and abnormal kidney function due to low intake of essential minerals of cardiovascular importance. WHO recommends in situations where a drinking water supply moves from a calcium and magnesium rich source to a low-mineral source, it would be appropriate considering remineralization of the water with calcium and magnesium (53). This findings are not in accordance with the promotion no or less mineralized water (e.g. rain water, desalination water) to mitigate the drinking water salinity problem in southwest coastal Bangladesh unless these water sources are re-mineralized with calcium and magnesium . Further research are warranted to measure the actual concentrations of important minerals in drinking water of varying salinity, and evaluate their health effects.

Acknowledgement:

The study has been funded by Wellcome Trust, UK, Our Planet, Our Health Award. We acknowledge with gratitude the commitment of Wellcome Trust, UK to its research efforts. We are grateful to the study participants for their support and cooperation. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support. We are grateful to UNICEF and Dhaka University for their assistance to conduct the study. Dr. Gribble's effort was supported in part by funding from the National Institute of Environmental Health Sciences (P30 ES019776)

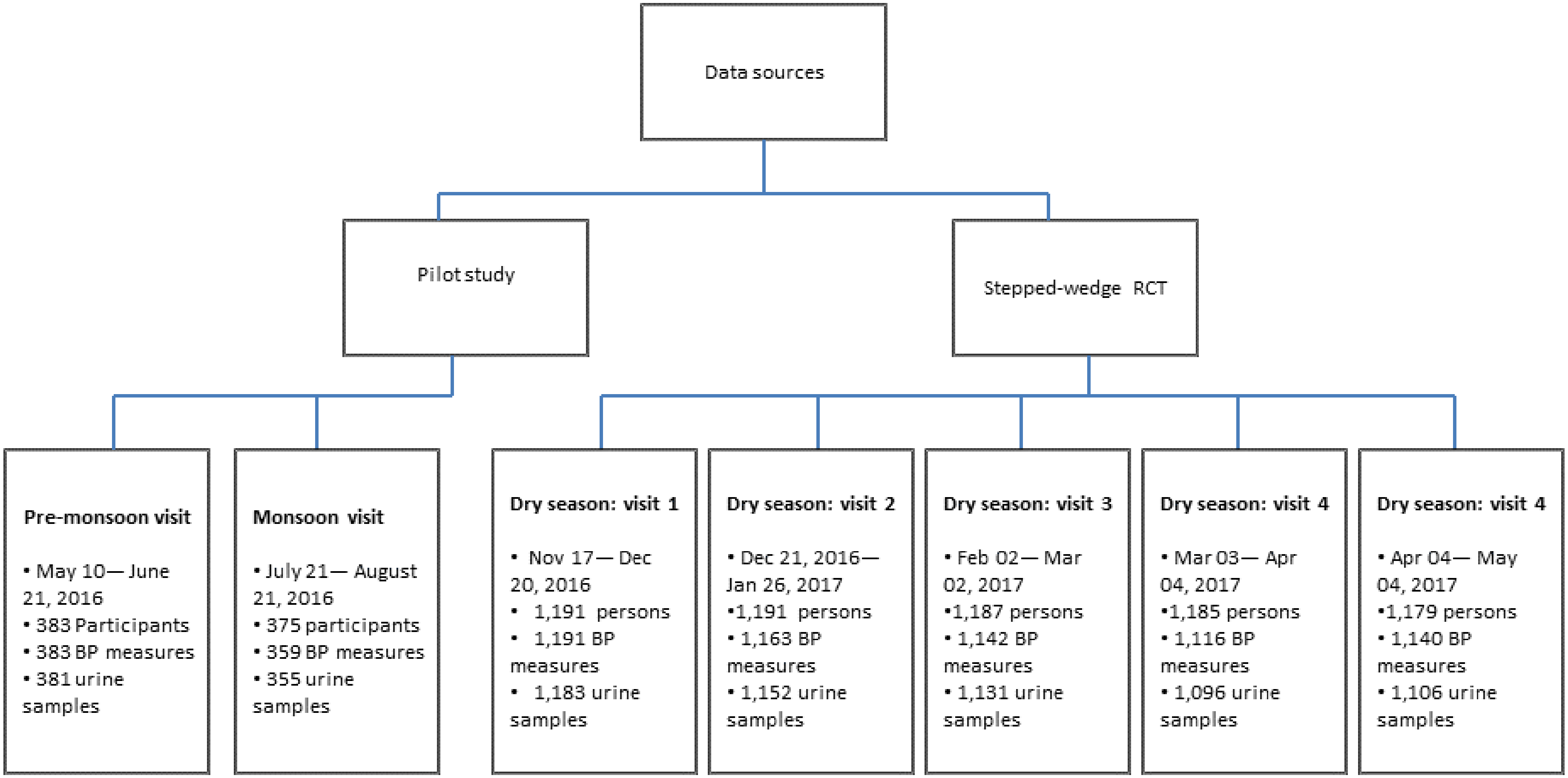

Figure 4: Data sources and study profiles

Table 2: Characteristics of the participants and households at enrolment

Characteristics	**Fresh drinking water (EC<0.7 mS/cm, N=554)**	**Slightly saline drinking water (EC: 0.7 — <2** mS/cm, N=538)	**Moderately saline drinking water (EC: 2 — 10 mS/cm, N=481)**
Age (years), median (interquartile range [IQR])	40 (31, 54)	41 (30, 54)	40 (30, 54)
Age categories, % (n) 20 — < 30 years	21.3 (118)	22.7 (121)	21.3 (102)
30 — < 40 years	27.3 (151)	25.1 (134)	27.4 (131)
40 — < 50 years	20.6 (114)	20.3 (108)	20.9 (100)
50 — <60 years	14.8 (82)	16.0 (85)	17.5 (84)
60 — <70 years	10.8 (60)	10.3 (55)	8.8 (42)
≥ 70 years	5.2 (29)	5.6 (30)	4.2 (20)
Male sex, % (n)	41.2 (228)	41.3 (222)	40.8 (196)
BMI, median (IQR)	22.3 (19.5, 25.3)	21.7 (19.4 23.9)	21.4 (18.9 23.9)
BMI categories, % (n) Underweight (<18.5)	14.8 (82)	16.4 (88)	18.3 (88)
Normal weight (18.5 -<25)	58.8 (326)	66.4 (357)	64.9 (312)
Overweight (≥25-<30)	22.0 (122)	14.5 (78)	14.1 (68)
Obese (≥30)	4.3 (24)	2.8 (15)	2.7 (13)
Smoking categories, % (n)			
Never	54.0 (299)	49.4 (266)	52.7 (253)
Former	8.7 (48)	11.7 (63)	7.5 (37)
Current	37.4 (207)	38.9 (209)	39.8 (191)
Work-related physical activity, % (n)			
Sedentary	37.4 (207)	41 (220)	30.7 (483)
Moderate	39.2 (217)	34.5 (185)	47.5 (746)
Vigorous	23.5 (130)	24.6 (132)	21.8 (342)
Systolic BP (mmHg), median (IQR)	113 (104, 124)	112 (104, 124)	109 (101, 119)
Diastolic BP (mmHg), median (IQR)	68 (61, 75)	68 (62, 75)	66 (60, 72)
Urinary Creatinine for male (mg/Kg body weight)	1544 (1162, 1951)	1470 (1136, 1776)	1401 (1069, 1787)
Urinary Creatinine for female (mg/Kg body weight)	1197 (944, 1501)	1112 (881, 1390)	1107 (934, 1310)
Hypertension status, % (n)			
Normal	66.6 (369)	69.3 (373)	74..8 (360)
Pre-hypertensive	23.5 (130)	216 (116)	19.5 (94)
Hypertensive	9.9 (55)	9.1 (49)	5.6 (27)
Sedentary activity, % (n)	35.4 (196)	29 (156)	72.7 (350)
Households wealth categories, % (n)			
lowest	14.2 (35)	18.3 (44)	29.1 (64)
2nd	14.2 (35)	22.8 (55)	23.2 (51)
3rd	18.3 (45)	22.8 (55)	18.6 (41)
4th	22.8 (56)	21.2 (51)	15.5 (34)
Highest	30.5 (75)	14.9 (36)	13.6 (30)

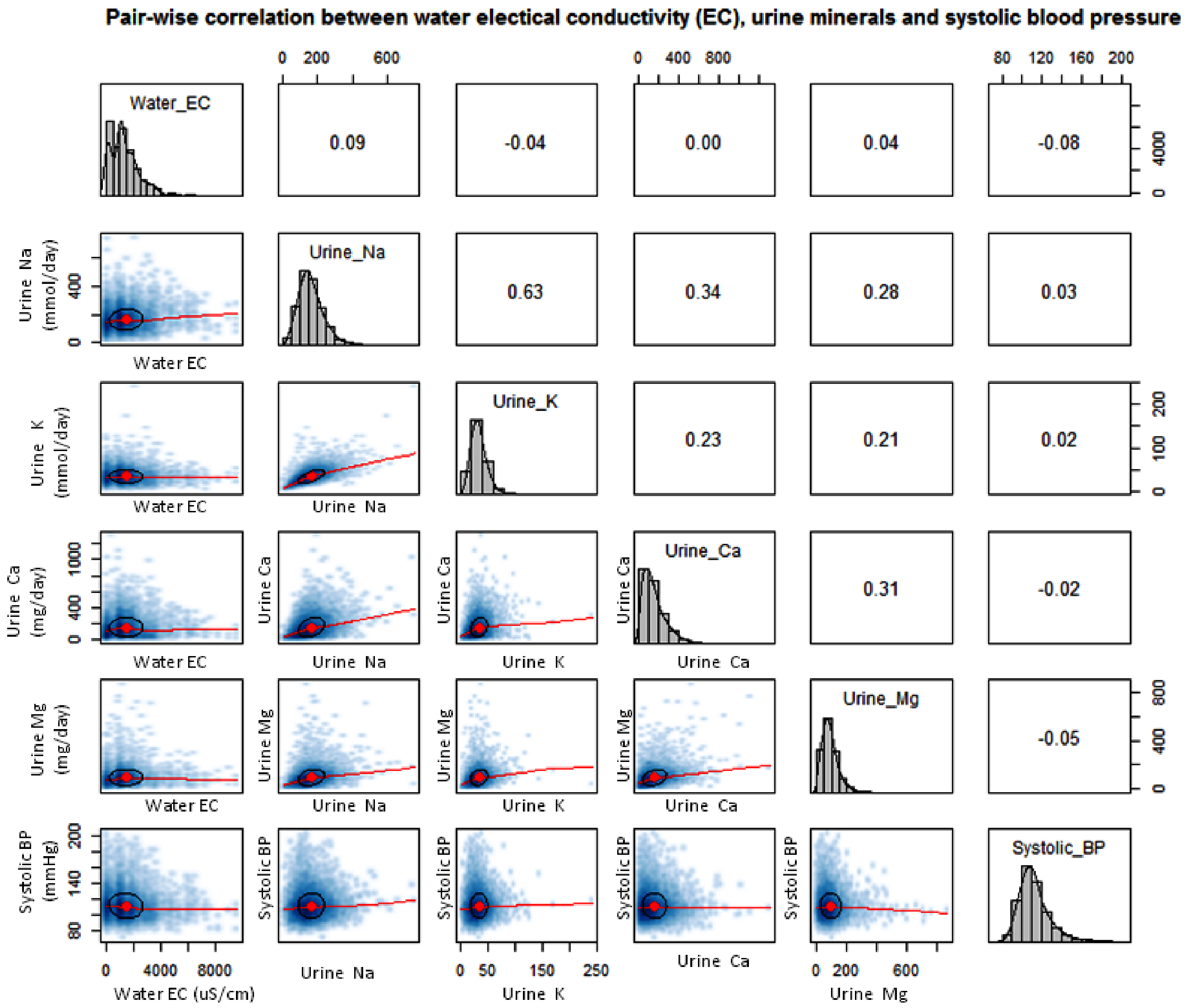

Figure S1: Pair-wise correlation between households’ drinking water electrical conductivity, urinary minerals and systolic blood pressure.

Table 2: Association between households' drinking water salinity categories and household members' blood pressure and 24-hour urinary total protein.

Outcomes	Drinking water electrical conductivity (EC) categories		
	Fresh water (EC: 0 — <0.7 mS/cm)	Mild saline water (EC: 0.7 — < 2 mS/cm)	Moderately saline water (EC: 2.0 — 10 mS/cm)
Systolic BP (mean difference from the reference group)			
Model 1[a]	Reference	-1.64 (-3.26, -0.02)	-1.84 (-3.33, -0.35)
Model 2[b]	Reference	-1.61 (-3.26, 0.04)	-1.76 (-3.25, -0.27)
Model 3[c]	Reference	-1.45 (-3.05, 0.16)	-1.60 (-3.04, -0.15)
Diastolic BP (mean difference from the reference group)			
Model 1	Reference	-1.29 (-2.19, -0.40)	-1.39 (-2.18, -0.60)
Model 2	Reference	-1.27 (-2.20, -0.34)	-1.36 (-2.17, -0.55)
Model 3	Reference	-1.13 (-2.02, -0.24)	-1.21 (-1.99, -0.43)
Urinary 24-hour total protein (ratio of medians with respect to reference)			
Model 1	Reference	0.85 (0.72, 0.99)	0.86 (0.72, 1.03)
Model 2	Reference	0.85 (0.72, 1.00)	0.86 (0.73, 1.03)
Model 3	Reference	0.89 (0.76, 1.04)	0.90 (0.76, 1.05)

[a] Unadjusted model

[b] Adjusted for age, sex, and BMI

[c] Adjusted for age, sex, BMI, physical activities, smoking, and wealth score. Urine protein was additionally adjusted for urinary creatinine level.

Table 3: Sensitivity analyses for association between households' drinking water salinity categories and household members' blood pressure and 24-hour urinary total protein in matched analyses.

Outcomes	Drinking water electrical conductivity (EC) categories	
	Low salinity (lowest 20% EC percentiles, EC: < 394 µS/cm)	Matched high salinity (> 60% EC percentile, EC: > 1440 µS/cm)
Systolic BP (mean difference from the reference group)		
Model 1[a]	Reference	-1.67 (-2.76, -0.58)
Model 2[b]	Reference	-1.61 (-2.66, -0.56)
Model 3[c]	Reference	-1.54 (-2.65, -0.43)
Diastolic BP (mean difference from the reference group)		
Model 1	Reference	-1.33 (-1.96, -0.71)
Model 2	Reference	-1.29 (-1.91, -0.68)
Model 3	Reference	-1.16 (-1.77, -0.55)
Urinary 24-hour total protein (ratio of medians with respect to reference)		
Model 1	Reference	0.79 (0.68, 0.90)
Model 2	Reference	0.79 (0.69, 0.91)
Model 3	Reference	0.83 (0.73, 0.95)

[a] Unadjusted model

[b] Adjusted for age, sex, and BMI

[c] Adjusted for age, sex, BMI, physical activities, smoking, and wealth score. Urine protein was additionally adjusted for urinary creatinine level.

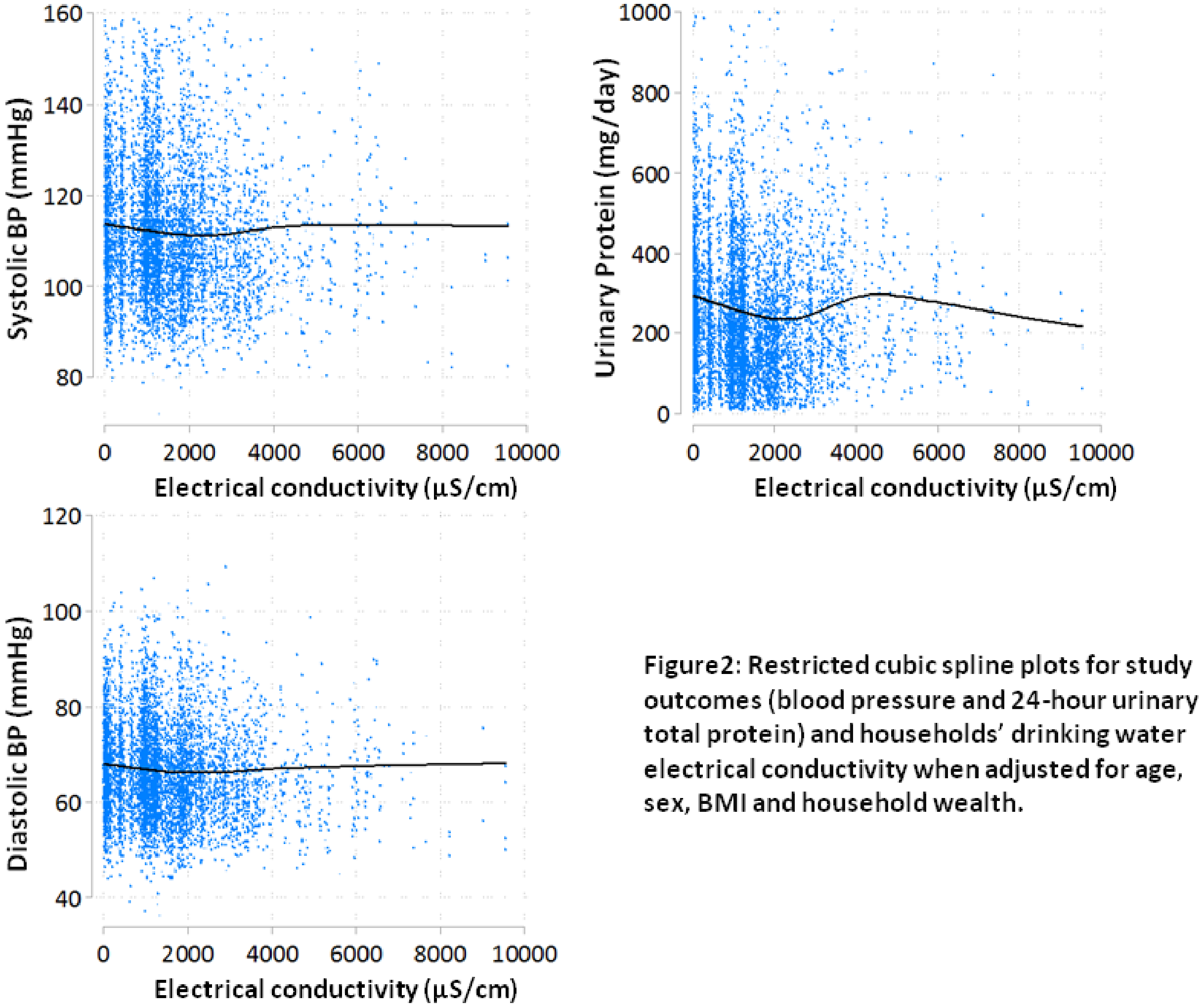

Figure2: Restricted cubic spline plots for study outcomes (blood pressure and 24-hour urinary total protein) and households' drinking water electrical conductivity when adjusted for age, sex, BMI and household wealth.

Figure 5: Restricted cubic spline plots for study outcomes (blood pressure and 24-hour urinary total protein) and households' drinking water electrical conductivity when adjusted for age, sex, BMI and household wealth.

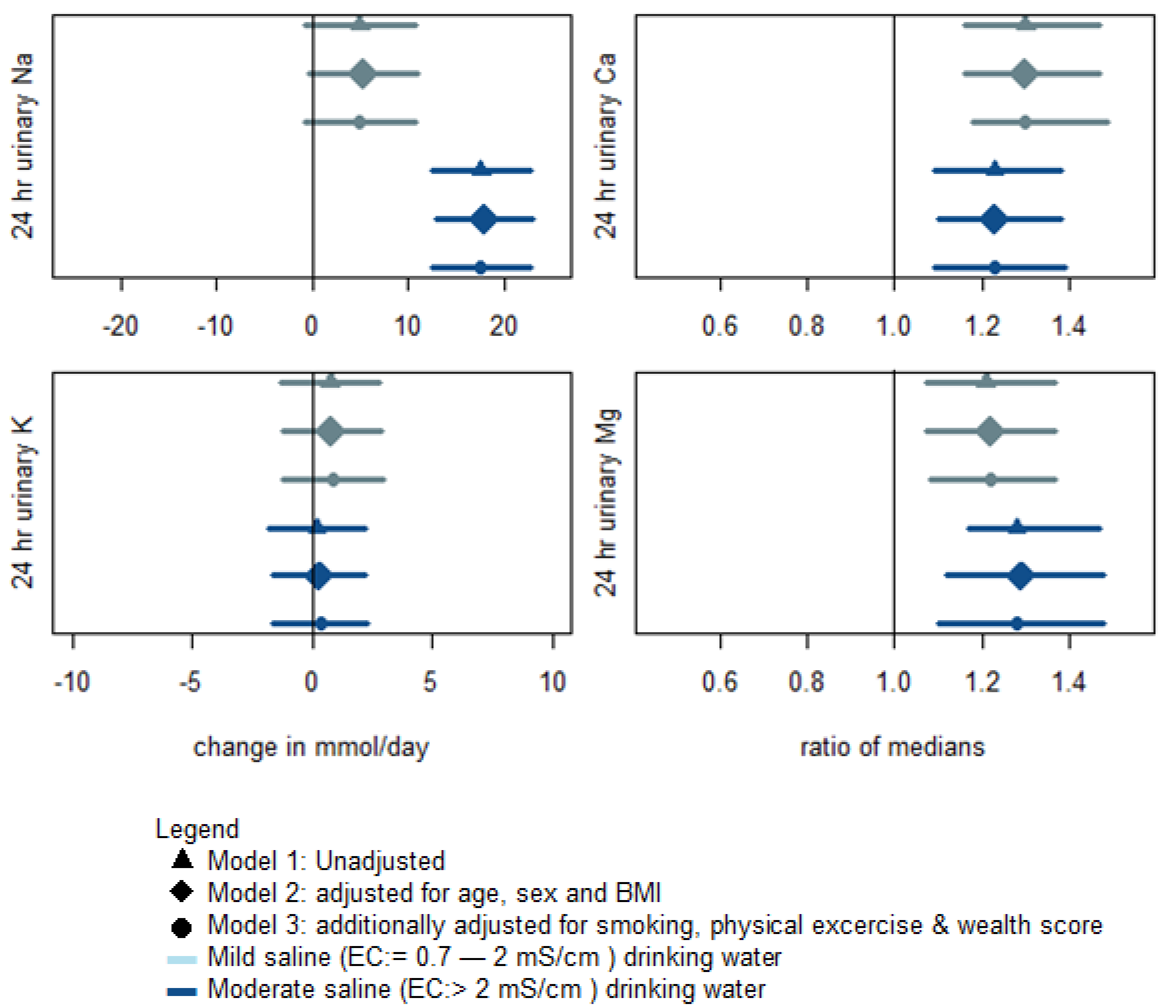

Figure 6: Change in mineral intakes (sodium, potassium, calcium and magnesium) in mild (EC: 0.7 — 2 mS/cm) and moderate (EC: >2 mS/cm) drinking water salinity households than the fresh drinking water households (EC: <0.7 mS/cm)

Chapter 5: Groundwater chemistry and systolic blood pressure: a cross-sectional study in Bangladesh[6][7]

Abu Mohd Naser[1*], Thomas F. Clasen[1], Mahbubur Rahman[2], Leanne Unicomb[2], Solaiman Doza[2], Ruhel Gazi[2], Raisul A Robin[2], Rabiul Karim[2], Mohammad Nasir Uddin[2], Kazi Matin Ahmed[3], Mohammad Shamsudduha[4], Jennifer Stowell[1], K.M. Venkat Narayan[5], Shivani Patel[5], Howard H. Chang[6], Stephen P. Luby[7], Bethany O'Shea[8], Matthew O. Gribble[1]

[1] Department of Environmental Health Sciences, Rollins School of Public Health, Emory University, Atlanta, USA
[2] International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), GPO Box 128, Dhaka 1000, Bangladesh
[3] Department of Geology, University of Dhaka, Dhaka, Bangladesh
[4] Institute for Risk and Disaster Reduction, University College London, UK
[5] Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA
[6] Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, USA
[7] Woods Institute for the Environment, Stanford University, Stanford, California, USA
[8] Bethany O'Shea, Department of Environmental and Ocean Sciences, University of San Diego, CA, USA

Target Journal: EHP

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[6] This chapter is a manuscript prepared for submission to Annals of Internal Medicine. The structure, length and format are in keeping with journal requirements.

[7] AMN, TFC and MOG developed the analyses concept. AMN received access to the datasets, combined and cleaned the data. AMN and MOG developed the statistical analysis plan. AMN conducted the statistical analyses and MOG reviewed the analyses. AMN drafted and all author reviewed the manuscript.

Abstract

Background: The relationship of drinking water chemical mixtures with blood pressure remains unclear.

Objectives: To assess the association of groundwater chemicals with systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Methods: Blood pressure, anthropometric and socio-demographic data for adults ≥35 year old was measured by the Bangladesh Demographic and Health Survey in 2011. Groundwater sodium, calcium, silicon, magnesium, potassium, iron, manganese, barium, zinc, sulfate and arsenic in 3,534 well water samples from Bangladesh were measured by the British Geological Survey (BGS) and Department of Public Health Engineering (DPHE) in a 1998-1999 survey. Participants who reported groundwater as their primary source of drinking water were assigned chemical measures from the nearest BGS-DPHE well as a surrogate of their contemporary drinking water chemical concentrations. Survey-estimation linear regression methods were used to model log-transformed blood pressure as a function of groundwater chemicals, controlling for age, sex, body mass index, smoking status, geographical region, household wealth, rural or urban residence, and educational attainment.

Results: The geometric mean ratio of SBP for 10 mg/L increase in groundwater magnesium concentration (versus no increase) was 0.992 (95% CI: 0.986, 0.999) after controlling for all chemicals and confounders. The magnesium-SBP association was consistent across different-level of confounder adjustments and was not modified by the presence of other chemicals. No significant associations of any other chemicals were consistently observed with SBP or DBP after adjustment for confounders and water chemicals.

Conclusions: Groundwater magnesium was negatively associated with SBP. Further research on drinking-water magnesium and blood pressure is warranted.

Introduction

The chemical properties of the sedimentary rock and soil in the Earth's upper crust are important for human health and nutrition as chemicals from the Earth's crust appear in food and drinking water (Selinus et al. 2013). Approximately 99% of the minerals in Earth's crust are made up of 8 elements: oxygen, silicon, aluminum, iron, calcium, sodium, potassium and magnesium (Hudson 2016). The most common dissolved cations in groundwater are those that predominate Earth's crust, such as calcium, magnesium, sodium and potassium (Marier 1980).

Nutritionally essential macronutrients such as calcium, phosphorus, magnesium, sodium, potassium and chloride are needed in bulk amounts to sustain human life (g/kg diet) whereas essential micronutrients such as iron, copper, zinc, iodine, selenium, manganese, molybdenum, chromium, fluoride and cobalt are needed in smaller concentration (µg to mg/ kg diet) (Combs Jr 2013; Lindh 2013). The optimum functioning of human body depends on the balanced intake of minerals and elements (Cook 2013). Essential macronutrients such as calcium, magnesium, sodium and potassium are related to the regulation of blood pressure (Kesteloot and Joossens 1988; McCarron et al. 1982).

Groundwater may also contain toxic elements such as arsenic (He et al. 2005; Shankar and Shanker 2014), uranium (Nolan and Weber 2015) and chromium (Batayneh 2012). These can occur in minerals of rock or soil usually at low concentrations, and be released into water via geological processes (Chowdhury et al. 2016; He et al. 2005). Groundwater concentrations of toxic chemicals can sometimes become elevated through activities such as mining, industry, and agriculture (Bradl 2005; He et al. 2005). Local geochemical contexts, such reducing conditions in sediments, can also influence the release of compounds such as arsenite into groundwater (Kinniburgh and Smedley 2001a).

Although geological macronutrients, micronutrients, and toxic chemicals often co-occur in drinking water, few epidemiological studies have investigated the relationships of real-world drinking water chemical mixtures with blood pressure. This is a major limitation as there may be countervailing physiological impacts from the different constituents in water. An 5-week experiment of normal-salt (0.3% NaCl), high-salt (8% NaCl) and high-salt plus high-potassium (8% KCl) diet on 27 Dahl salt-sensitive rats suggest protective effect of potassium against development of vascular damage and high blood pressure induced by high-salt diet (Kido et al. 2008). A meta-analysis of 33 randomized controlled clinical trial conducted between 1981 and 1995 comprising of 2,609 participants suggest oral supplementation of potassium was associated with -3.11 (95% CI: -1.91, -4.31) mmHg reduction in systolic blood pressure and -1.97 (-0.52, -3.42) reduction in diastolic blood pressure, and the associations were more pronounced in studies in which participants were concurrently exposed to high sodium intake (Whelton et al. 1997). An analysis of the first National Health and Nutrition Examination Survey (NHANES I) data suggest at low calcium intake (<400 mg/day), the ratio of sodium to potassium is significantly associated with SBP (β: 2.88, p value: <0.05) and DBP (β: 1.12, p value: <0.05) after adjusting for known confounders, but the association was non-significant for SBP (β: 0.003, p value: >0.05) and DBP (β: 0.30, p value: <0.05) among individuals with high calcium intake (>800 mg/day) (Gruchow et al. 1988).

Bangladesh, home to ~163 million people (World Bank 2017), is the largest delta in the world, formed by the deposition of sediments from the Himalayas by the Ganges, Brahmaputra and Meghna (GBM) rivers (France-Lanord et al. 2013). The GBM produces the largest total sediment load globally, bringing eroded rocks from the high Himalayas to the Bay of Bengal, passing through Nepal, India and Bangladesh (Kinniburgh and Smedley 2001a). Sediments of different rivers have different chemical compositions: for example, Ganges-derived sediments have a higher calcium-magnesium carbonate content than the sediments of Brahmaputra and Meghna rivers (Brammer 1996). Most of the groundwater in Bangladesh is of the CaHCO3 type, but Na-Ca-Mg-HCO3-Cl groundwater is abundant in salinity-affected coastal regions (Kinniburgh and Smedley 2001). The southern areas of the Delta were previously inundated by sea water, and chemicals such as sodium and magnesium have remained in the soil from the original sea water source (Kinniburgh and Smedley 2001).

In Bangladesh, 97% of the country's rural population depends on groundwater for drinking purposes (British Geological Survey 2001). In this population, groundwater is an important source of daily intake of minerals such as calcium, magnesium, iron and zinc (Hoque and Butler 2015). There has also been mass poisoning from high arsenic in groundwater in many areas of Bangladesh (Ahsan et al. 2006), associated with higher blood pressure among the higher-arsenic water consumers (Jiang et al. 2015). In coastal regions, high sodium intake through drinking groundwater has also been associated with high blood pressure of the adult population (Scheelbeek et al. 2016). The objective of this analysis was to characterize the cross-sectional, potentially interacting associations of local groundwater chemicals with blood pressure among groundwater-drinking adults age ≥35 years old in Bangladesh.

Methods

Study Population

The Bangladesh Demographic and Health Survey (BDHS) in 2011 recruited participants in 600 clusters representing rural and urban areas of the 7 administrative divisions (regions) of Bangladesh; each cluster was comprised of ~30 households [139]. Funded by the United States Agency for International Development (USAID) and Implemented by ICF, the Demographic and Health Surveys (DHS) are nationally-representative households surveys conducted in low- and middle-income countries approximately every 5 years to gather data on indicators of population health and nutrition [140]. In addition to the questionnaires, surveyors collect data on anthropometry, test blood for anemia and HIV, and in 2011 for the first time, blood pressure.

Blood Pressure Measures

One in three households of each cluster was randomly selected for blood pressure measurement [139]. All men and women ≥35 years old in the selected households were eligible. Of the 7,992 eligible adults contacted for blood pressure measurement, 105 refused to participate. Trained research staff used the LIFE SOURCE® UA-767 Plus Blood Pressure Automatic Monitor to measure blood pressure for 7,887 participants following the manufacturer's recommended protocol. Cuff sizes were selected to be appropriate for the participant's arm circumference. Participants did not eat and drink caffeinated or carbonated drinks, or smoke within the 30 minutes prior to blood pressure measurement. There were 3 measurements taken at approximately 10-minute intervals between measurements, for both systolic blood pressure (SBP) and diastolic blood pressure (DBP), and the arithmetic mean of the second and third measurements was used in the analyses [139].

Clinical and Demographic Determinants of Blood Pressure

Age, sex, body mass index (BMI), educational attainment in years, smoking categories of the participants (current smoker versus not smoker), rural or urban residence, geographical region and household wealth quintiles were compiled from the households and biomarker questionnaires of the 2011 BDHS. BMI was calculated from measured weight and height as kg/m^2. The wealth index of the households was created in three steps. First, a set of household-level indicators common to both urban and rural areas was input into a principal components analysis to produce a (first component) nation-wide wealth score for each household. Second, local wealth scores were generated separately for households in urban or rural areas, using principal components analysis of area-specific indicators. In the final step, local wealth scores were regressed on the nation-wide wealth scores to generate a combined wealth index for analysis [139]. Additional information regarding the BDHS 2011 survey is provided in **Table S1** [139]**.**

Drinking Water Source Data

The primary source of drinking water of the households was ascertained by BDHS in 2011. We considered groundwater to be the primary source of drinking water if households reported using tube well or borehole, and protected and unprotected well as their primary source of drinking water in the 2011 BDGS survey. In rural Bangladesh, households occasionally store water in a storage tank by connecting electric pump with the tube well in order to have piped water supply in their homesteads [141-143]. Therefore, households in the countryside reporting owning a water pump and having piped water supply in the households or yard were included as groundwater-drinkers for this analysis.

Hydro-geological Data on Groundwater Chemistry

Well water chemical concentration data were obtained from the British Geological Survey (BGS), which collects groundwater information within the United Kingdom and internationally (**Table S2**). BGS, in collaboration with the Department of Public Health Engineering (DPHE) of the government of Bangladesh, conducted a groundwater chemicals survey in 1998-1999 to develop maps showing the regional distribution of arsenic and other elements in the groundwater of Bangladesh, and to estimate of the percentage of wells exceeding various limits for arsenic and other elements. Funded by the UK Department for International Development, the survey was conducted in two phases: the first phase (1998) covered the most arsenic-impacted southern and eastern districts and the second phase (1999) covered the remaining districts except the three districts in Chittagong Hill Tracts [144]. The survey used stratified random sampling to ensure sampling sites representative of the entire country, and collected 3,534 well water samples across Bangladesh with coverage of one sample per 37 km^2 area [145]. Samples were collected from drinking water wells ranging in depth from 7 to 362 meters [146]. GPS coordinates of each well were collected. All samples were tested for arsenic, and all but 4 samples were tested for other chemicals in the BGS laboratories in UK. Arsenic was measured using hydride generation-atomic fluorescence spectrometry (HG-AFS); other chemicals were measured by inductively-coupled plasma-atomic emission spectrometry (ICP-AES) [145].

We excluded aluminum, boron, cobalt, chromium, copper, lithium, phosphorus and vanadium from analysis as >25% of the samples were below the limit of detection (LODs). The LODs and measurement techniques for each chemicals are given in **Table S3**. Although 28% samples had arsenic concentration below the limit of detection (<0.5 μg/L), we included arsenic in analysis due to the importance of arsenic to the government of Bangladesh and the many previous epidemiological studies reporting an association of well water arsenic and blood pressure in Bangladesh [147,148]. Therefore, in our analysis, we included 5 macronutrients (calcium, magnesium, silicon, sodium and potassium), 5 micronutrients (barium, zinc, manganese, sulfate, iron and silicon), and 1 toxic chemical (arsenic). Additional information regarding the BGS-DPHE surveys are provided in **Table S3**.

Exposure Assignment

Shapefiles of Bangladesh administrative units were obtained from DIVA-GIS, a free online resource for shapefiles of different countries worldwide [149]. We imported the GPS locations of the BDHS clusters and BGS-DPHE wells and projected them onto the Bangladesh shapefiles in ArcGIS 10.4.1 using the UTM 1984 45 N projection system. In order to protect the identity of the households, one randomly selected GPS location was taken per BDHS cluster. We determined the nearest BGS-DPHE wells for each BDHS 2011 clusters using spatial joining in ArcGIS **(Figure 1)** and calculated the nearest distance in kilometers. We assigned the chemical exposure measures from to the nearest BGS-DPHE well to each of the BDHS 2011 participants whose blood pressure was measured (i.e., the nearest well to their cluster, as BDHS geographic data were limited to cluster-level) as a surrogate of source water chemical concentrations contemporary to the blood pressure measures.

Statistical Analysis

The study sample for this analysis was comprised of participants who reported groundwater as the primary drinking water source and who had blood pressure measurements (N=7,000). Population means and standard deviations (SD) of the continuous variables, and population proportions of the categorical variables, were calculated using survey estimation methods. Concentrations of all water chemicals were right-skewed, and 25th, 50th and75th percentiles of the water chemicals were reported. Spearman correlations were calculated between chemical-pairs for all ground chemicals included in the analyses: sodium, calcium, silicon, magnesium, potassium, sulfate, iron, barium, zinc, manganese and arsenic.

Survey estimation linear regression methods were used to estimate associations of water chemicals with log-transformed SBP or log-transformed DBP in the subpopulation who reported groundwater as the primary source of drinking water. We sequentially fit three models to assess the independent association of each water chemical with SBP and DBP. Model 1 adjusted for age, sex and BMI as continuous variables. Model 2 further adjusted for current smoking status (current smoker versus never of former smoker), education attainment (no institutional, ≤ 5 years, 6 to ≤ 10 years and ≥ 11 years education) , rural or urban residence, wealth score quintiles, and regional location of the households . Model 3 further adjusted for all other chemicals in drinking water: sodium, calcium, silicon, magnesium, potassium, arsenic, sulfate, iron, manganese, barium, and zinc, using restricted cubic splines.

When a significant association was detected between a single chemical and a blood pressure outcome, pairwise interactions were modeled to test whether there were significant effect modification by other chemicals.

Missing data were imputed by multiple imputation by chained equations [150]. Statistical analyses were performed in Stata SE version 15.0 and graphs were prepared in R version 3.3.1 and ArcGIS version 10.4.

Ethical approval:

The study protocol was approved by Emory University IRB (IRB00088075). The DHS Program, which is the authority and compiles all DHS surveys for different countries and different year [151], provided the survey and GPS data Permission was obtained from the copyright section of the British Geological Survey Environmental Science Centre to use the publicly available BGS-DPHE dataset.

Results

Table 1 presents the characteristics of the participants, households and clusters included in our statistical analysis. Among the population ≥ 35 years old relying on groundwater as the primary source of drinking water, the mean age was 51.7 (95% CI: 51.4, 52.1) years old, and mean BMI was 20.6 (95% CI: 20.4, 20.7) kg/m^2; 31.8% were underweight, 57.3% were normal weight, 9.9% were overweight and 1.7% were obese. Nearly half of the population were male and 13.8% were active smokers. The mean educational attainment in this population was 3.1 (95% CI: 2.9, 3.2) years. More than half of the population had no formal institutional education, 26.0% had primary, 16.7% had secondary and 5.5% had college or higher-level education. Only 16.5% of the population resided in urban areas. The highest wealth quintile contained 15.1% of the households, and 20.8% of households were in the lowest wealth quintile. The most clusters (n=109) were selected from Dhaka division, and the fewest clusters (n=70) were selected from Sylhet division**.** The mean distance of the nearest BGS well to the BDHS clusters was 2.3 (95% CI: 1.3 — 3.6) kilometers. Mean SBP in the population was 118.7 (95% CI: 118.0, 119.4) mmHg, and DBP was 77.9 (95% CI: 77.5, 78.5) mmHg. SBP was higher among females, elderly, obese and among college- or higher-level educated participants (**Table S4).**

The percentiles of chemical concentrations in groundwater from areas inhabited by the study participants are presented in **Figure 2**. The order of chemicals in groundwater from high to low concentrations were sodium, calcium, silicon, magnesium, potassium, sulfate, iron, manganese, barium, zinc, and arsenic. Of the major chemicals, median concentrations of the groundwater samples of all clusters were 34.3 (IQR: 15.7 — 89.6) mg/L for sodium, 25.4 (IQR: 12—60.1) mg/L for calcium, 19.6 (15.2 —24.0) mg/L for silicon, 12.1 (IQR: 6.0—25.3) mg/L for magnesium, and 3.0 (1.8 —5.2) mg/L for potassium. Among the minor chemicals, SO4 and iron had a median concentration of 0.8 (IQR: 0.2— 4.0) mg/L and 0.7 (IQR: 0.1— 4.2) mg/L. The concentration of manganese, barium and zinc was very low. Arsenic had a median concentration of 3.3 (IQR: 0.4 —33.6) µg/L. WHO has not setup health-based guidelines for most of the chemicals we analyzed except barium (<700 µg/L) and arsenic (<10 µg/L) [152]. However, the median concentrations of all chemicals across Bangladesh were below the standard set by Bangladesh's Department of Environment (**Figure 2**).

Well water concentrations varied by region. Sodium concentrations were higher in three coastal regions (Barisal, Khulna and Chittagong) compared non-coastal regions, and the median concentration of sodium in Barisal region was above the Bangladesh drinking water standard (**Figure 2**). The median calcium concentration exceeded the Bangladesh standard in the Khulna region. The magnesium concentration was below the Bangladesh drinking water standard in all regions (**Figure 2**). Arsenic concentrations were relatively higher in southern and eastern regions, including Chittagong, Dhaka and Khulna, but median arsenic concentrations were within the Bangladesh standard in all regions. Potassium concentrations were relatively higher in coastal areas including Chittagong and Barisal regions, but were below the Bangladesh standard in all regions (**Figure 2**). The groundwater concentrations of iron, zinc, manganese and sulfate were high above the Bangladesh standard in all regions. The barium concentration was higher in Khulna division, and silicon concentration was uniform in all regions, however, there was no Bangladesh standard for barium and silicon (**Figure 2**).

The Spearman correlations among chemical-pairs are illustrated in **Figure 3**. The association of well water chemicals with SBP and DBP are illustrated in **Figure 4**. Magnesium concentration in the well water was negatively associated with SBP at 5% level of significance in all three models and with DBP in the first two models**.** A 10 mg/L increase (versus no increase) in well water magnesium concentration was associated with a 997 (95% CI: 0.994, 0.999) geometric mean ratio (GMR) of SBP when adjusted for age, sex, and BMI (Model 1); 0.996 (95% CI: 0.993, 0.999) GMR when further adjusted for smoking, education, geographic regions, rural or urban residence and wealth index (Model 2); and 0.992 (95% CI: 0.986, 0.998) GMR when further adjusted for other chemicals in water in Model 3 (**Figure 4**). A 10 mg/L increase in well water magnesium was associated with a 0.997 (95% CI: 0.994, 0.999) GMR of DBP in Model 1, 0.996 (95% CI: 0.993, 0.998) GMR in Model 2, and 0.995 (95% CI: 0.989, 1.002) GMR in Model 3.

A 5 mg/L increase in water potassium was associated with 0.994 (95% CI: 0.988, 1.000) GMR of SBP and 0.993 (95% CI: 0.988, 0.999) GMR of DBP in Model 1; however, the association disappeared in Model 2 & 3. In Model 1, a 50 μg/L increase in water arsenic concentration was associated with 0.995 (95% CI: 0.993, 0.998) GMR of SBP and 0.995 (95% CI: 0.993, 0.998) GMR of DBP. In Model 2, a 50 μg/L increase in water arsenic concentration was associated with 0.997 (95% CI: 0.994, 0.999) GMR of DBP.

We did not find any significant associations between well water micronutrients (iron, manganese, barium, zinc, silicon and sulfate) and blood pressure in any models. We also did not find significant pairwise interactions between well water magnesium and other chemicals for SBP or DBP **(Table 3)**.

Discussion

Magnesium in groundwater was negatively associated with SBP among adults at least 35 years old in Bangladesh. To our knowledge, this is the first finding of an association of drinking water magnesium and blood pressure in Bangladesh. There are several mechanisms by which magnesium may reduce blood pressure [153,154]. Magnesium increases the availability of several vasorelaxation factors such as prostacyclins, cyclic AMP, and nitric oxide that decrease vascular tone and reduce blood pressure [155,156]. Magnesium is an essential cofactor for the synthesis of gamma linoleic acid, which is the precursor for the vasodilator prostaglandin E1 [156,157]. Magnesium inhibits contraction of smooth vascular muscle by lowering the free intracellular calcium concentration and subsequently reduces vascular smooth muscle tone and blood pressure [158-160].

Several epidemiological studies have highlighted the negative associations between intake of magnesium-rich foods and blood pressure [37,161], but evidence is limited regarding the association between magnesium in drinking water and blood pressure. A meta-analysis of 9 case-control studies found an overall negative association of drinking water magnesium with cardiovascular mortality [pooled odds ratio: 0.75 (95% Confidence Interval 0.68, 0.82)] [162]. In Israel, after adjustment for socio-demographic and clinical parameters, patients in desalinated areas, compared to non-desalinated areas, had lower blood magnesium concentrations (2.08 ± 0.27 Vs 1.94 ± 0.24 mg/dL, P < 0.0001 from T-test) and an estimated hazard ratio for one year all-cause mortality following hospitalization of 1.87 (95% Confidence Interval: 1.32 — 2.63).

Magnesium in drinking water is highly bioavailable because it occurs in readily absorbable ionic forms [163]. Although drinking water is not the primary source of magnesium, it may make appreciable contribution when diets have low intakes of magnesium [164]. A secondary analysis of 5,256 well water from three Asian mega-deltas (i.e., Bengal, Mekong and Red River), where 70% population rely on groundwater for drinking water, suggests drinking water is the important source of daily intake of minerals; and individuals can obtain up to half of the daily recommended intake of magnesium from drinking two liters of groundwater in some areas of Bangladesh [38].

Our analyses have some important limitations. There may be substantial exposure measurement error resulting from a lack of temporal alignment between water chemistry data and the other variables included in our analysis that could introduce some information bias [165]. Water chemical concentrations in groundwater have temporal patterns, and chemicals may not all shift in the same direction or by the same magnitude over time. For example, in China and Bangladesh, greater annual temporal variation was observed for redox-sensitive chemicals such as Fe, Mn and As [166,167]. Temporal variation may also depend on the depth of the water: in Bangladesh, the major chemicals in groundwater (Na, K, Mg, Ca and Cl) from shallow wells (<30 m deep) varied around ± 90% of baseline concentration over a period of 2-3 years, however, these chemicals varied <10% in deeper wells (>30 m) [166]. The instability of chemical concentrations over time in shallow wells may be due to greater interaction with freshwater (e.g., rainwater) [166]. The median depth of the BGS-DPHE wells were 35 (IQR: 22, 56) meters, so there may be differential measurement error in assigned chemical exposures across participants according to well depth. There may be additional heterogeneity in the extent of measurement error if some wells were measured during wet vs. dry seasons.

A second source of exposure measurement error is misclassification of the nearest wells to individual participants. In rural Bangladesh, millions of hand pumps have been installed over the last three decades to provide microbiologically safe water to the communities and to prevent waterborne diseases [76]. The BDHS 2011 reports 71.6% rural households had hand pumps within their household premises [139]. Although smoothed map of each chemical concentrations in Bangladesh from BGS-DPHE survey suggests broad geographical patterns in the concentrations of chemicals in Bangladesh [168], there is also small-scale spatial variation [169,170]. Average distance of the nearest BGS-DPHE well was 2.3 kilometers, and it is possible that actual household wells had different chemical concentrations than the nearest selected BGS-DPHE wells.

Our assigned exposure metric is likely to include both classical error components and non-differential Berkson measurement error. In general, regression coefficients are biased towards the null under classical error models but unbiased under Berkson error [171]. However, the extent to which our findings are compromised by information bias is uncertain. The negative association of magnesium with blood pressure is biologically plausible and consistent with epidemiological reports on related outcomes. The loss of precision from Berkson error could help explain why some of the chemicals which have been previously associated with blood pressure, such as arsenic and sodium, were not statistically significantly associated in this study. An advantage of our exposure metric data coming from a different source than our demographic confounders and outcome data is that measurement errors are likely independent between water chemicals and the other variables. Moreover, there could be dependent measurement error between the water chemicals especially those measured in the same assay. This could potentially bias the results from models with two or more chemicals simultaneously included as predictors. However, the analytical chemistry measurement of these water chemicals is precise, so the dependent component of the overall measurement error will be low.

Although the association estimates are specific to the population ≥35 years old in Bangladesh, the general findings may have broader relevance to persons of similar age elsewhere. We did not see evidence of other water chemicals pairwise-modifying the association of magnesium with blood pressure, so the putative benefit of magnesium may apply to other settings irrespective of those other settings' geology. It is likely our findings may be relevant for other settings where populations have inadequate magnesium intake through food, but the linear, substantial association of magnesium with blood pressure may be also important even for nutritionally rich settings such as Israel, where there are large differences in water magnesium exposure such as with desalination programs [172]. Further studies with measurement of blood pressure, along with concurrent precise measurement of magnesium in drinking water, are needed to better understand the contribution of drinking water magnesium on blood pressure regulation.

Acknowledgement: AM Naser's effort on the analyses was supported by a grant from Unilever Ltd. Dr. Gribble's effort was supported in part by funding from the National Institute of Environmental Health Sciences (P30 ES019776). We thank the DHS, BGS, and study participants for sharing their data for this analysis.

Conflict of interest: The authors have no conflicts of interest to declare.

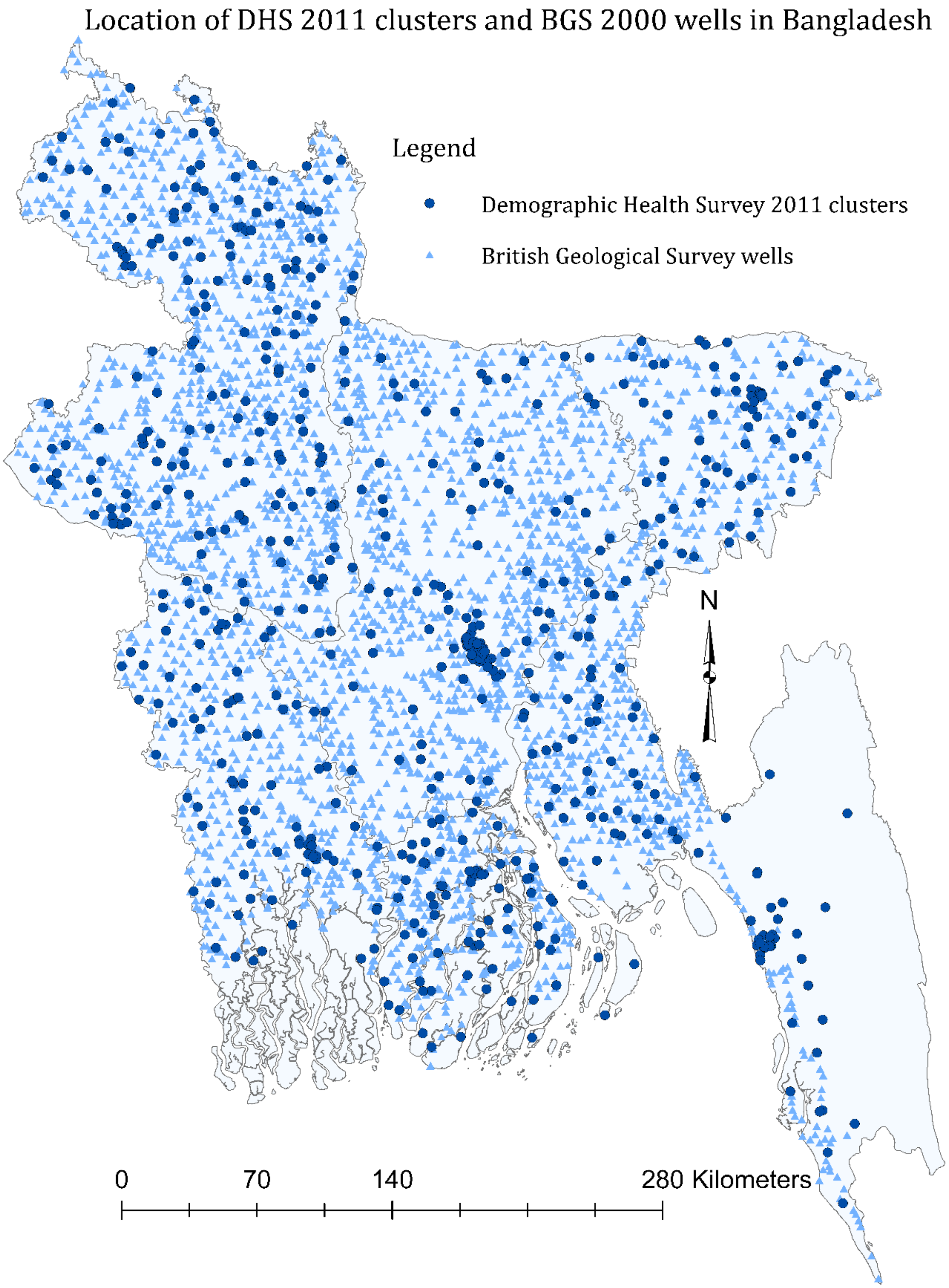


Figure 1: Location of DHS 2001 clusters and BGS 1998-1999 wells in Bangladesh.

Table 3: Characteristics of the ≥ 35 years old participants whose blood pressure were measured in BDHS 2011.

Participants' characteristics	All participants with blood pressure measurement (N=7,887)	Groundwater drinkers with blood pressure measurement (N=7,000)	Participants contacted for blood pressure measurement (N=7,992)
Age , mean (95% Confidence Interval)	51.4 (51.1, 51.7)	51.7 (51.4, 52.1)	51.5 (51.1, 51.8)
BMI (kg/m^2) , mean (95% CI)	20.9 (20.7, 21.0)	20.6 (20.4, 20.7)	20.9 (20.7, 21.0)
BMI categories, % Underweight (<18.5 kg/m^2) Normal weight (≥18.5 to <25 kg/m^2) Overweight (≥25 to <30 kg/m^2) Obese (≥ 30 kg/m^2)	29.1 (27.7, 30.6) 57.4 (56.0, 58.8) 11.3 (10.5, 12.3) 2.1 (1.8, 2.5)	31.1 (29.6, 32.6) 57.3 (55.9, 58.9) 9.9 (9.0, 10.9) 1.7 (13.7, 2.1)	29.1 (27.7, 30.6) 57.4 (56.0, 58.8) 11.4 (10.5, 12.3) 2.1 (1.8, 2.5)
Years of education, mean (95% CI)	3.3 (3.3, 3.5)	3.1 (2.9, 3.2)	3.4 (3.2, 3.6)
Education categories, % (95% CI)			
No institutional education	49.6 (47.7, 51.4)	52.0 (50.0, 53.9)	49.6 (47.7, 51.4)
Primary level (≤ 5 years)	25.8 (24.4, 27.1)	25.9 (24.5, 27.3)	25.8 (24.4, 27.1)
Secondary level (6 to ≤ 10 years)	17.5 (16.3, 18.7)	16.7 (15.5, 17.9)	17.5 (16.3, 18.7)
College level or higher (≥ 11 years)	6.9 (6.0, 7.9)	5.5 (4.7, 6.3)	7.2 (6.2, 8.3)
Male sex, % (95% CI)	49.4 (48.6, 50.2)	49.4 (48.5, 50.3)	49.5 (48.7, 50.2)
Current Smoker, % (95% CI)	13.6 (12.2, 15.1)	13.8 (12.3, 15.3)	13.6 (12.2, 15.1)
Household characteristics			
Urban residence, % (95% CI)	23.3 (22.2, 24.4)	16.5 (15.0, 18.1)	23.8 (22.8, 25.0)
Wealth index, (95% CI)			
Quintile 1	19.4 (17.7, 21.3)	20.8 (18.9, 22.8)	19.3 (17.5, 21.1)
Quintile 2	19.2 (17.8, 20.7)	21.1 (19.6, 22.8)	19.1 (17.7, 20.6)
Quintile 3	19.8 (18.3, 12.4)	21.5 (19.9, 23.2)	19.8 (18.2, 21.3)
Quintile 4	20.7 (19.1, 22.3)	21.4 (19.8, 23.2)	20.6 (19.0, 22.2)
Quintile 5	20.9 (19.2, 22.7)	15.1 (13.5, 16.9)	21.3 (19.6, 23.2)
Cluster characteristics	% (n/N)		
Distance of nearest well in kilometers , mean (95% CI)	3.2 (2.8, 3.7)	3.3 (2.9, 3.8)	3.2 (2.8, 3.7)
Divisional distribution, % (95% CI)			
Dhaka	32.1 (30.9, 33.3)	29.0 (27.3, 30.7)	32.3 (31.1, 33.6)
Barisal	5.9 (5.5, 6.4)	6.3 (5.8, 6.9)	5.9 (5.5, 6.4)
Chittagong	17.0 (16.1, 17.9)	17.6 (16.6, 18.6)	16.9 (16.0, 17.8)
Khulna	13.0 (12.3, 13.8)	12.9 (11.9, 14.0)	12.9 (12.2, 13.7)
Rajshahi	14.5 (13.6, 15.4)	15.5 (14.5, 16.6)	14.6 (13.7, 15.5)
Rangpur	11.7 (11.1, 12.4)	12.9 (12.2, 13.7)	11.7 (11.1, 12.3)
Sylhet	5.7 (5.3, 6.1)	5.8 (5.3, 6.3)	5.8 (5.4, 6.2)

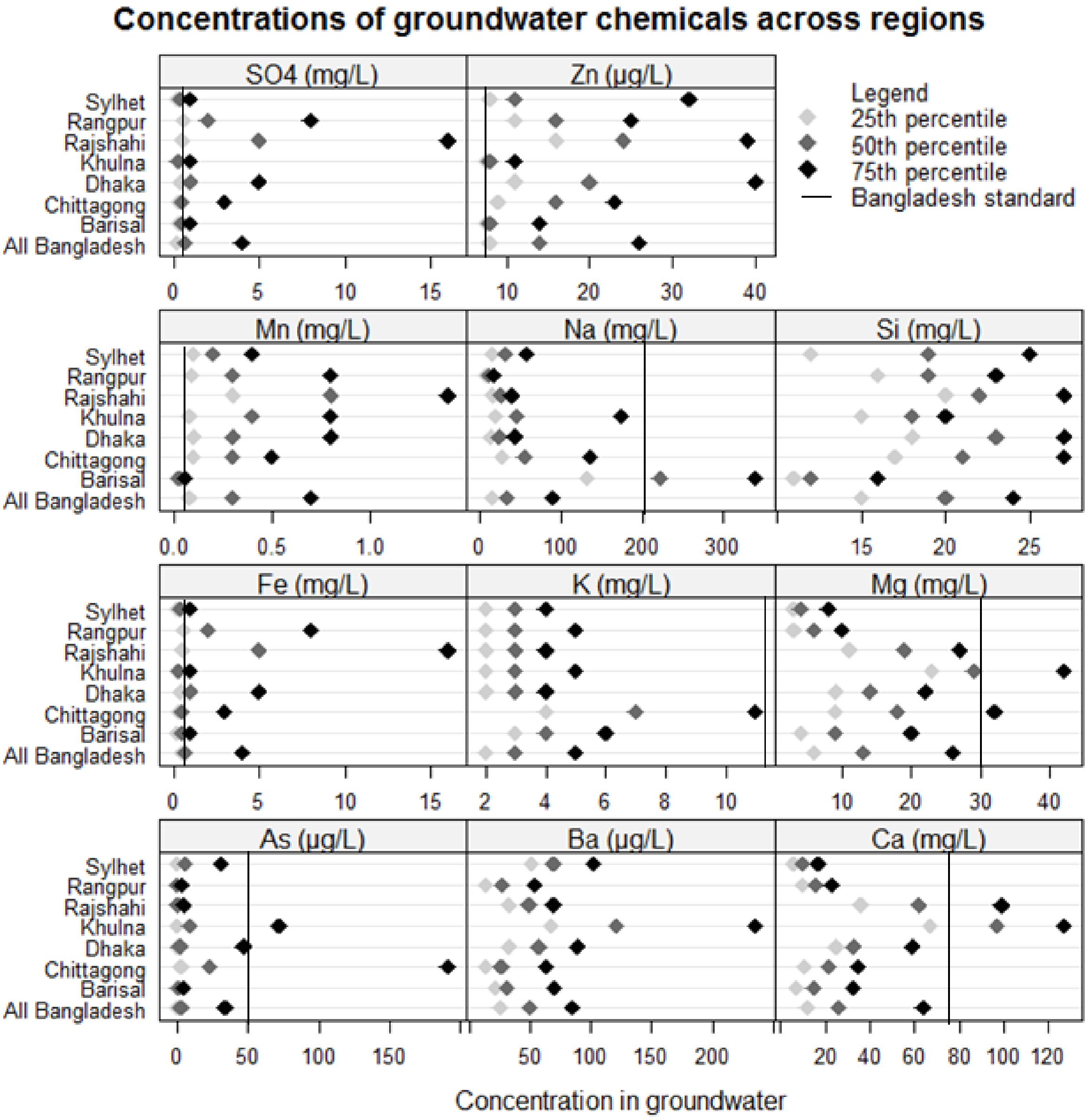
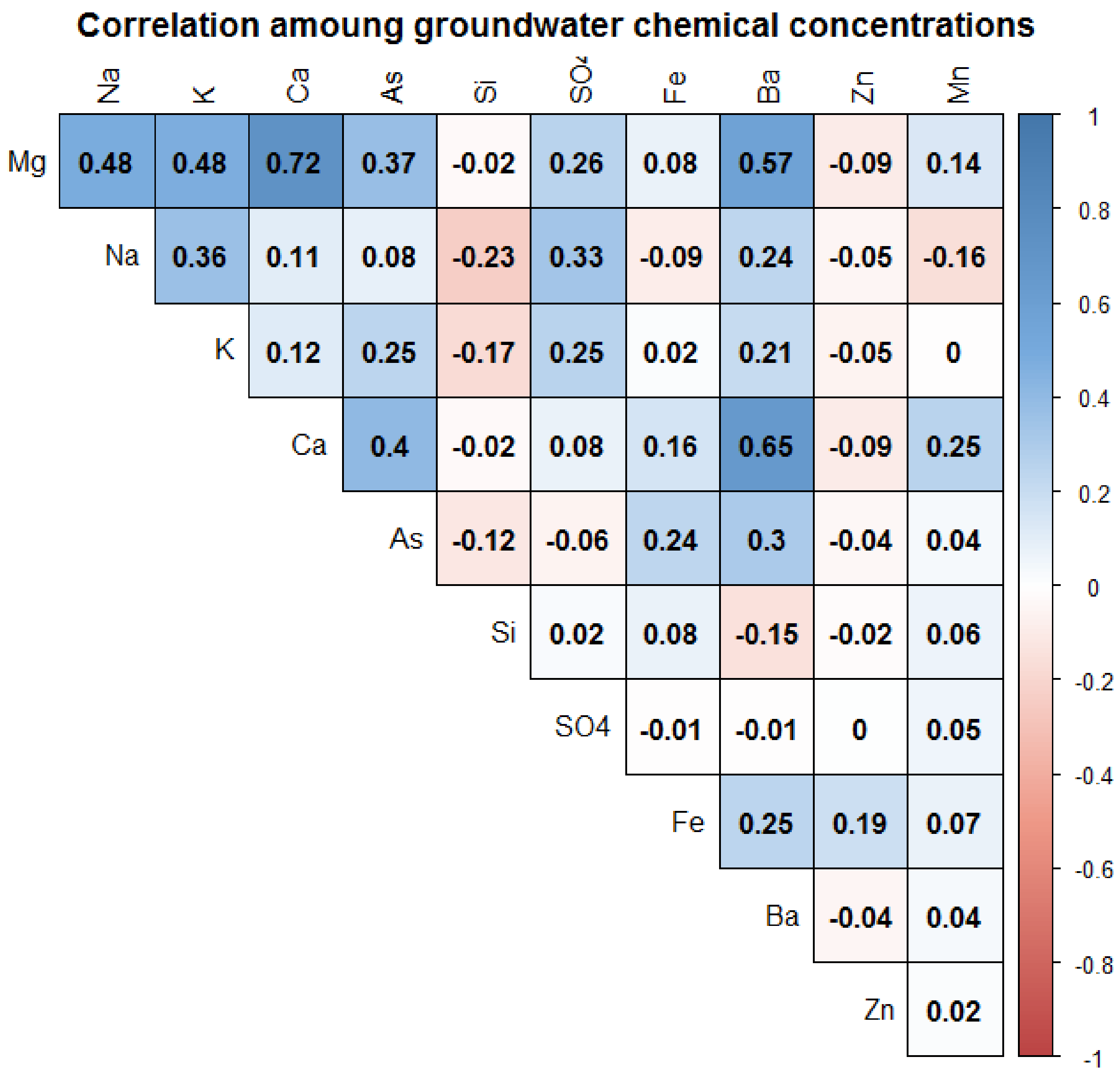


Figure 2: Concentration of groundwater chemicals across regions



	Na	K	Ca	As	Si	SO_4	Fe	Ba	Zn	Mn
Mg	0.48	0.48	0.72	0.37	-0.02	0.26	0.08	0.57	-0.09	0.14
Na		0.36	0.11	0.08	-0.23	0.33	-0.09	0.24	-0.05	-0.16
K			0.12	0.25	-0.17	0.25	0.02	0.21	-0.05	0
Ca				0.4	-0.02	0.08	0.16	0.65	-0.09	0.25
As					-0.12	-0.06	0.24	0.3	-0.04	0.04
Si						0.02	0.08	-0.15	-0.02	0.06
SO4							-0.01	-0.01	0	0.05
Fe								0.25	0.19	0.07
Ba									-0.04	0.04
Zn										0.02

Figure 3: Correlation between the groundwater chemical concentrations in water samples of selected BGS-DPHE wells.

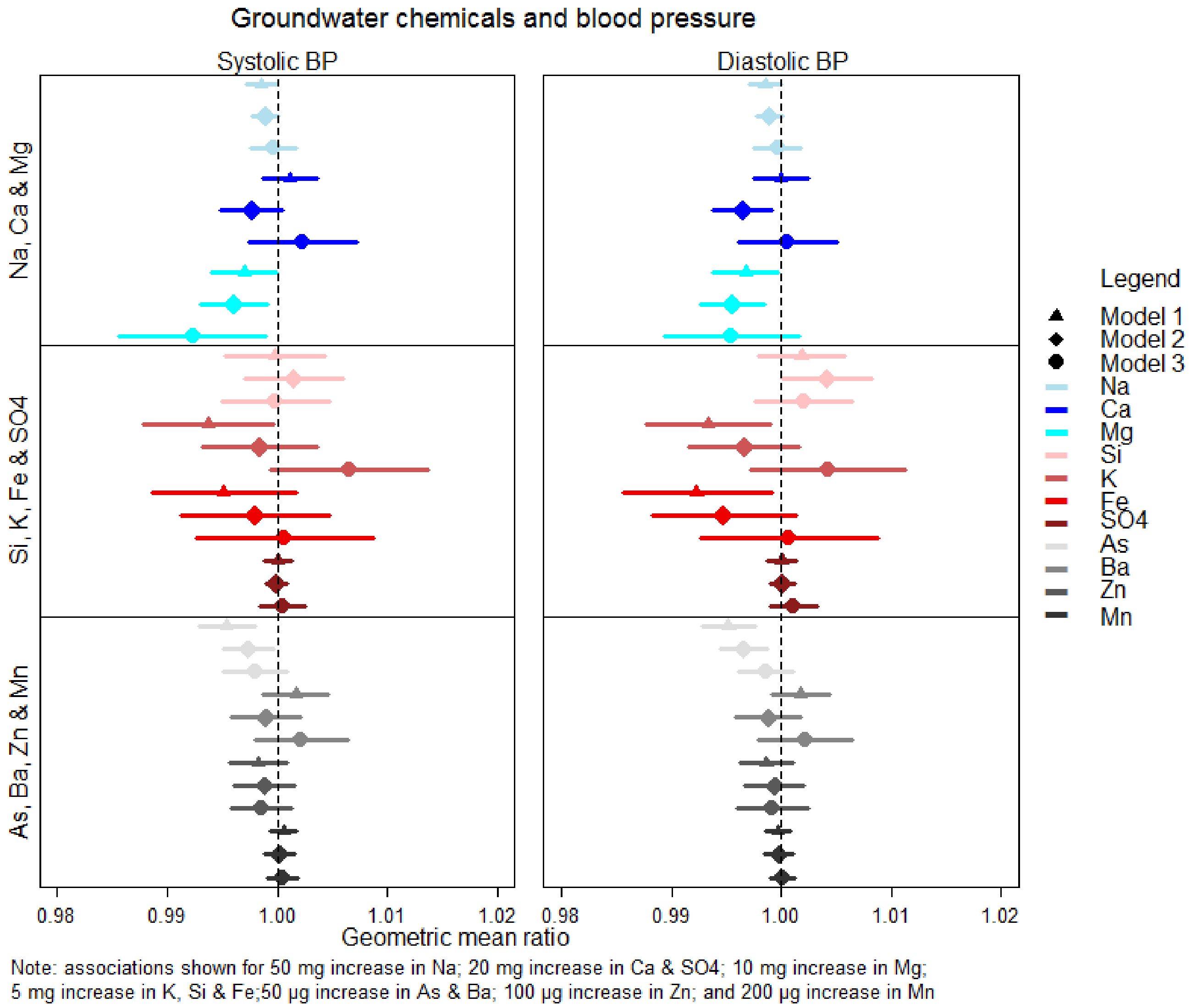


Figure 4: Association between changes in geometric mean ratios of systolic blood pressure for different groundwater chemicals.

Table 2: Pair-wise linear interaction between magnesium and other chemicals. No pairwise interactions with magnesium were Bonferroni-significant at $P < 0.005$.

Pair of Chemicals	P values	
	Systolic BP	Diastolic BP
Mg & Ca	0.78	0.45
Mg & Na	0.46	0.57
Mg & K	0.38	0.80
Mg & As	0.72	0.74
Mg & Si	0.40	0.76
Mg & Ba	0.76	0.79
Mg & Zn	0.82	0.94
Mg & Fe	0.78	0.69
Mg & SO_4	0.80	0.92
Mg & Mn	0.18	0.14

Table S1: Bangladesh Demographic and Health Survey 2011

Survey Name	Bangladesh Demographic and Health Survey
Survey year	2011
Sampling method	Nationally representative covering the entire population residing in non-institutional dwelling units.
Total clusters	600
Total households	Average 30 households per cluster.
Report	https://dhsprogram.com/pubs/pdf/FR265/FR265.pdf
Implementing organization	Conducted under the authority of the National Institute of Population Research and Training (NIPORT) of the Ministry of Health and Family Welfare and implemented by Mitra and Associates of Dhaka.
Funding source	ICF International provided financial and technical assistance for the survey through USAID/Bangladesh
Data source/website	https://dhsprogram.com/Data/ [permission required to access data]

Table S2: British Geological Survey and Department of Public Health Engineering well survey in Bangladesh

Survey name	British Geological Survey and Department of Public Health Engineering Survey
Survey year	2000
Sampling method	Across all districts and sub-districts of Bangladesh except for three districts of Chittagong.
Total wells	3,534
Chemicals tested	Arsenic, sodium, potassium, calcium, magnesium, boron, barium, cobalt, chromium, copper, iron, lithium, manganese, phosphorus, silicon, sulfate, zinc, strontium, vanadium
Method for analysis	Arsenic by hydride generation-atomic fluorescence spectrometry (HG-AFS) in the UK but some of the early samples collected in the Phase I survey were analysed by hydride generation-ICP-AES. Additional elements in the survey samples were measured by ICP-AES .
Laboratory used for analysis	British Geological Survey (BGS) laboratories
Report	http://www.bgs.ac.uk/research/groundwater/health/arsenic/Bangladesh/reports.html
Implementing organization	The Department of Public Health Engineering (DPHE) and BGS
Data source/website	http://www.bgs.ac.uk/research/groundwater/health/arsenic/Bangladesh/data.html

Table S3: Chemicals measured in BGS-DPHE survey.

Chemical name	Limit of detection (LOD)	% of samples below LOD	Method of testing
Arsenic	0.5 µg/L	28%	Hg-AFS[8]
Aluminum	0.04 mg/L	44%	ICP-AES[9]
Boron	0.1 mg/L	56%	ICP-AES
Barium	0.002 mg/L	0.2%	ICP-AES
Calcium	0.01 mg/L	0.03%	ICP-AES
Cobalt	0.008 mg/L	97%	ICP-AES
Chromium	0.008 mg/L	96%	ICP-AES
Copper	0.008 mg/L	95%	ICP-AES
Iron	0.006 mg/L	0.4%	ICP-AES
Potassium	0.5 mg/L	0.5%	ICP-AES
Lithium	0.008 mg/L	46%	ICP-AES
Magnesium	0.04 mg/L	0.03%	ICP-AES
Manganese	0.002 mg/L	0.7%	ICP-AES
Sodium	0.01 mg/L	0%	ICP-AES
Phosphorus	0.2 mg/L	28%	ICP-AES
Silicon	0.03 mg/L	0.03%	ICP-AES
Sulfate	0.2 mg/L	24%	ICP-AES
Vanadium	0.006 mg/L	87%	ICP-AES
Zinc	0.008 mg/L	16%	ICP-AES

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[8] Hydride generation-atomic fluorescence spectrometry

[9] Inductively coupled plasma atomic emission spectroscopy

Table S4: Blood pressure (mmHg) among different categories of participants

Characteristics	Systolic blood pressure mean (95% CI) mm Hg	Diastolic blood pressure mean (95% CI) mm Hg
Average	118.7 (118.0, 119.4)	77.9 (77.5, 78.5)
Age categories		
≥35 — 50 years	114.1 (113.5, 114.8)	77.7 (77.2, 78.2)
≥50 — 65 years	120.2 (119.1, 121.3)	78.2 (77.6, 78.8)
≥ 65 years	129.7 (127.9, 131.5)	78.1 (77.4, 78.9)
Sex		
Male	116.0 (115.2, 116.8)	76.3 (75.8, 76.8)
Female	121.4 (120.5, 122.3)	79.5 (79.0, 80.0)
BMI categories		
Underweight (<18.5 kg/m^2)	115.1 (113.8, 116.3)	74.2 (73.6, 74.8)
Normal weight (≥18.5 to <25 kg/m^2)	118.5 (117.8, 119.3)	78.3 (77.9, 78.9)
Overweight (≥25 to <30 kg/m^2)	123.8 (122.2, 125.3)	83.3 (82.4, 84.2)
Obese (≥ 30 kg/m^2)	127.4 (123.7, 131.1)	85.7 (83.8, 87.6)
Education categories		
No institutional education	119.9 (118.9, 120.8)	77.4 (76.9, 78.0)
Primary level	116.7 (115.7, 117.7)	77.5 (76.8, 78.1)
Secondary level	117.5 (116.4, 118.7)	78.9 (78.2, 79.6)
College level or higher	121.4 (119.7, 123.0)	81.0 (80.1, 81.9)

Chapter 6. Summary, Reflections and Future Research

Summary of findings

This dissertation describes the health effects of managed aquifer recharge (MAR) initiatives in southwest coastal Bangladesh that infiltrates rainwater and pond water into the brackish water to create a lens of freshwater into the brackish aquifer. It also describes the dose-response association between drinking water electrical conductivity (a measure of water salinity) with blood pressure and kidney function. It presents the association between drinking

The health effects of MAR initiatives were determined by a stepped-wedge trial that enrolled 1,191 participants in 16 communities of southwest coastal Bangladesh. Participants were visited for five times between December 2016 and April 2017. Following baseline, four communities were randomized to either receive access to MAR water or continue to serve as controls. We collected households' self-reported drinking and cooking water sources, measured electrical conductivity of the stored drinking and cooking water, participants' blood pressure and 24-hour urinary total protein, sodium, potassium, calcium, magnesium and creatinine concentration in all five visits.

The dose-response association between drinking water electrical conductivity and blood pressure and urine protein were determined by pooling the drinking water and health outcome data of stepped-wedge trial and the preceding pilot study. In total, we complied longitudinal data of 1,574 participants from 709 households, and analyzed 6,494 blood pressure and 6,404 urine data.

In the secondary analyses, blood pressure data among adults ≥35 years of age (N=7,887) were obtained from the BDHS 2011. Groundwater concentrations of sodium, calcium, silicon, magnesium potassium, iron, manganese, barium, zinc, sulfate and arsenic in 3,534 well water samples from Bangladesh were extracted from the BGS and Department of Public Health Engineering (DPHE) 1998-1999 survey. Participants who reported groundwater as the primary source of drinking water were assigned chemical measures from the nearest BGS-DPHE well as a surrogate of their contemporary drinking water chemical concentrations. Survey-estimation linear regression methods were used to model log-transformed SBP as a function of groundwater chemicals.

In Chapter 3, we described the health effects of MAR systems. We identified that using intention-to-treat analyses households that were randomized to receive MAR water statistical significant high systolic and diastolic blood pressure. Nevertheless, we identified poor adherence of the intervention among households when randomized to receive access to MAR water. One possible explanation of poor adherence was that households had access to relatively lower-salinity pond water (compared to MAR water) prior to the access to MAR water, and were predominantly consuming pond water for drinking and cooking purposes. Therefore, households did not shift from lower salinity pond water to higher salinity MAR water that resulted in poor adherence. Per-protocol and as-treated analyses considering the adherence identified that no statistical significant difference in systolic and diastolic blood pressure among the MAR water users and non-users. Therefore, in this study MAR systems did not provide any health benefits in terms of reduction in blood pressure and urinary total protein.

In Chapters 4, we described the dose-response association between drinking water electrical conductivity and participants' blood pressure and urinary total protein. We identified an inverse association between drinking water electrical conductivity and blood pressure. Compared to the low electrical conductivity category water consumers, moderate electrical conductivity category water consumers had statistical significant lower blood pressure. We also found that drinking high electrical conductivity category water was associated with high 24-hour urinary sodium, calcium and magnesium. These findings were consistent with scientific viewpoint that we published in Environmental Science and Technology [77] **(Appendix 4)**.

In Chapter 5, we presented data from the secondary data analyses. We identified that ground water magnesium concentration was associated with lower systolic blood pressure of the participants. We did not find any pair-wise interaction of other chemicals with magnesium to influence systolic blood pressure. Other chemicals were not associated with blood pressure. Measurement error of the exposure limits the findings from this study.

Reflections on study limitations and potential for improvements

There are several limitations to the research included in this dissertation. Some of these limitations have been discussed previously within earlier chapters, but are discussed in more detail below. Most of these limitations were occasioned by funding or logistical factors. Others, however, were due to a lack of understanding at the time that has now with the benefit of hindsight.

Drinking water mineral concentrations

Our water salinity exposure metric was electrical conductivity, which tells us about the presence of all ions in water but does not specify and quantify any chemicals. In the MAR trial, we only measured the electrical conductivity of the drinking and cooking water. This precludes a full understanding of proportion of different minerals/chemicals of cardiovascular importance in saline water. If we could have measured the concentration of sodium, calcium, potassium and magnesium in all or a subset of water samples, we would have a more complete understanding of exposure. Even if we had chemical concentrations measured for a subsample, we could have conducted a regression analyses to determine the association between each chemical in water and electrical conductivity. We could have also developed a conversion factor for water electrical conductivity and each chemical concentration in water. The BGS-DHS analyses had the mineral concentration data in groundwater, however, have measurement error issue. If we had the chemicals measured in drinking water from MAR study, we could have done the same analyses from MAR data.

Mineral intake through food

We did not measure minerals in food among the population residing in southwest coastal Bangladesh. Neither did we use an instrument such as the food frequency questionnaire (FFQ) to gain insight into dietary practices. Lack of any information on dietary sources of sodium and other minerals is an important limitation of the MAR study. Application of a FFQ for each households along with food sample collection from a subsample for mineral measurement could have strengthen the study.

Follow-up of the cohort in wet season

The MAR study was implemented during the dry season. The drinking water exposure increased over the progression of dry season. However, we don't have any wet season data of this cohort. Exposure and outcome data collection in wet season could allow for a better understanding of how participants' blood pressure responds to the lowest water salinity. Currently we have wet season blood pressure data from another cohort from the pilot study. Following up the same cohort during both wet season and dry season would be very helpful to understand the health effect of drinking saline water.

Recommendation for future research

1. This dissertation suggests that saline water is a source of harmful sodium, but also cardio-protective minerals such as calcium, magnesium and potassium. Therefore, interventions that reduce salinity carry the risk of adverse health impacts in populations that do not have other sources of these essential minerals in their diets. We described this in a paper published by Environmental Science & Technology [77] (Appendix 4). In these populations, there is a need to critically consider remineralization of cardio-protective minerals. In particular, effectiveness of rainwater harvesting plus remineralization should be evaluated in future studies. A possible household level intervention to address salinity may be blending of rainwater with saline groundwater. However, this needs to be tested prior scale-up.

2. Although this dissertation focuses on mineral intake through drinking water, data on mineral intake through diet is important. Currently, there is a scarcity of published research on dietary patterns and mineral intake through food among communities in southwest coastal Bangladesh. Given the significant positive association between drinking water electrical conductivity (salinity) and urinary mineral excretion, we hypothesis that bioavailability of minerals from drinking water may be higher compared to dietary sources. Further research needs to be conducted to compare the bioavailability of minerals from food versus drinking water. If the bioavailability from drinking water seems higher than the diet, supplementation of minerals

in drinking water may open a new approach to reducing blood pressure and other cardiovascular diseases in these populations.

3. People in rural Bangladesh typically prepare food by boiling. The staple food is rice that requires a substantial volume of water to boil. Traditionally, communities use excess volume of water for cooking rice and then discard additional water from the cooked rice. Communities get much minerals from rice, which can be compromised by the chemical property of water used to boil rice. If the cooking water have low salinity or mineral contents, minerals from the rice can be lost. Salinity of the cooking water may also influence the mineral contents of cooked legume and vegetables in Bangladesh. Future research needs to be conducted to explore this.

4. Our research and previous studies were focused on adult populations. Children may also be vulnerable. Mineral deficiency and high sodium may have detrimental impact on their cardiovascular system [173]. Future research needs to be conducted to explore this.

5. High sodium intake through saline water may have adverse health consequences other than blood pressure. One potential health hazard of high sodium intake is that it causes calciuria. Therefore, urinary calcium excretion may be observed as part of kidney role. Women in post-menopausal age are particularly vulnerable for this high urinary calcium excretion and may suffer from osteoporosis. Further research needs to be conducted to explore this. Similarly, high sodium intake may be associated with high blood pressure independent from a change in cardiovascular system such as arterial stiffness and hypertrophy of heart muscle. Further research needs to be conducted to explore this.

Selected planned future analyses during post-doctoral fellowship

1. Determining the dose response association between urinary sodium, potassium, magnesium and calcium excretion on blood pressure and urinary protein.

> **Significance:** Literature suggests both linear association and "U-shaped" or "J-shaped" association between urinary sodium excretion and cardiovascular diseases. If there is "U-shaped" or "J-shaped" association, low sodium intake under the current guidelines may increase the blood pressure. A limitation of the many studies is that they relied on spot urine to measure the sodium exposure, which may be biased as affected by diurnal variation in urinary sodium excretion. In MAR studies, we measure the 24-hour urinary concentrations of all these minerals. We believe that the dose-response relationship from our data will have important public health significance.

2. Combined effect of sodium, potassium, magnesium and calcium on blood pressure and urine protein.

Significance: Although sodium, potassium, calcium and magnesium have influence on blood pressure, most published articles have only explored the association between sodium on blood pressure, or at best, the influence of the combination of sodium and potassium on blood pressure. There is a scarcity of evidence on the combined association of sodium, potassium, calcium and magnesium on blood pressure.

3. Confounding precludes inference about health benefits from a community water treatment program in coastal Bangladesh.

Significance: In the pilot study, we found that MAR communities have higher blood pressure than non-MAR communities. However, we also found that these two communities have substantial socio-economic differences. We consulted the Dhaka University team to select the communities ─ MAR communities had a MAR systems functional from where people were consuming water, non-MAR communities had a MAR system that was not rolled out then. We believe that the communities were matched hydro-geologically, but inference of health impact of MAR is confounded by several socio-demographic factors. The objective of this analysis is to highlight that socio-demographic confounders

4. Utility of spot urine to determine the association of urinary sodium excretion and blood pressure compared to 24-h collected urine— evidence from southwest coastal Bangladesh.

Significance: In the pilot study, we collected two spot urine (fasting and second morning urine) and also 24 hour urine from the same participants. The objective was to understand which type of urine samples we should collect in main study. Analysis suggests that spot urine may be good for estimating 24 hour sodium intake base on which formula is used, but do not provide a good exposure-outcome association. We propose to write a paper comparing the exposure-outcome association between 24-hour urinary sodium and spot urine sodium, and highlight the pros and cons of both approaches.

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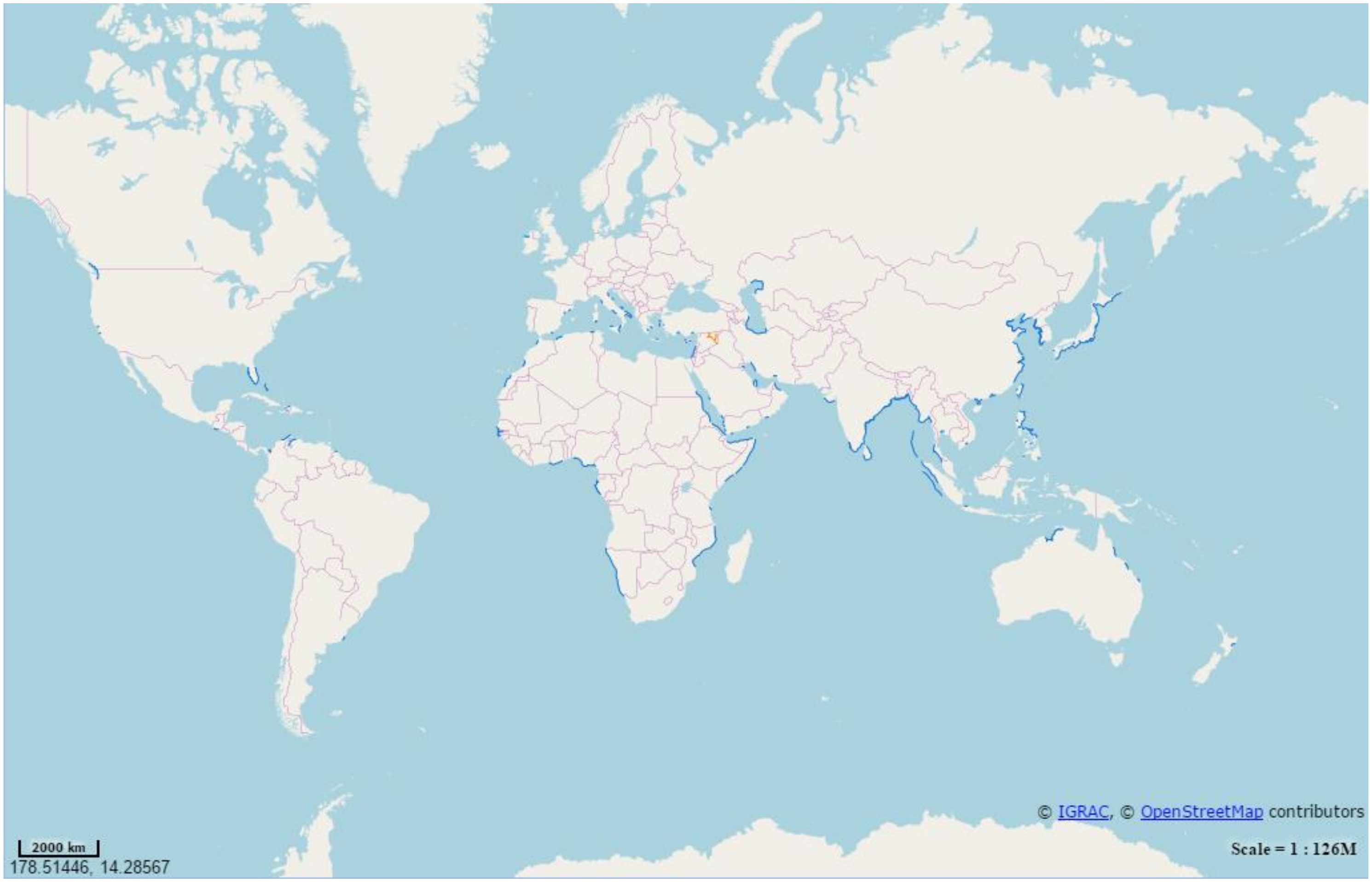
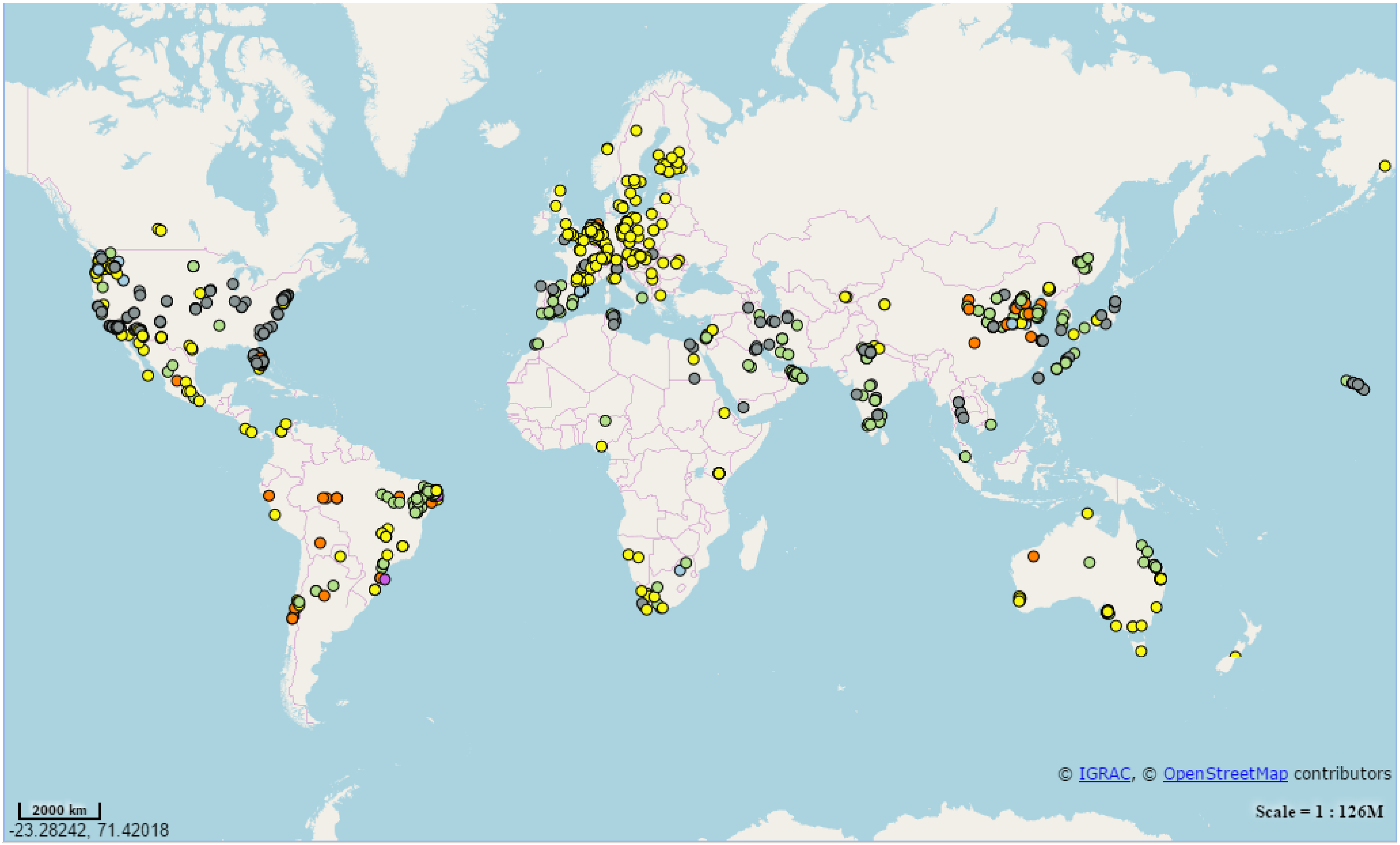
Appendix 1: Salt water intrusion into coastal aquifers due to marine reason across the world. Map downloaded from IGRAC (https://www.un-igrac.org/global-groundwater-information-system-ggis)



Appendix 2: Use of manage aquifer recharge (MAR) across the globe for different purposes. Map downloaded from IGRAC (https://www.un-igrac.org/global-groundwater-information-system-ggis



Appendix 3: A pilot non-randomized longitudinal study of managed aquifer recharge initiative in southwest coastal Bangladesh: rationale, objectives and study design

The health impact evaluation of managed aquifer recharge (MAR) required environmental assessment of drinking water salinity prior and after implementing the intervention as well as measurement of biomarkers of exposure, and biomarker of outcomes. We designed a stepped-wedge trial (described in subsequent chapters) for the health impact evaluation of MAR systems. Nevertheless, prior implementing the trial we conducted a pilot study as a preparatory step with the following objectives:

1. To pre-test the data collection instruments, environmental and biological sample collection procedures, and laboratory processing methods.

2. To identify the appropriate urine samples to be used in the upcoming trial for accurate measurement of urinary sodium exposure. In the pilot study we compared three urine samples from each participants ─ 24-hour sample, a 15-ml first morning and a 15-ml second morning samples. We focused on first and second morning urine samples rather than the convenient random spot urine to ensure adequate time for transportation of samples to the central field laboratories from remote rural communities, laboratory processing and analysis of samples in the same day. One objective of the pilot study was—

    (a) To understand whether the mean estimated 24-h urinary sodium excretion from first morning or second morning urine by different published formulae were comparable with the measured 24-hour sodium,

    (b) To understand the association of estimated 24-h urinary sodium on blood pressure from first morning or second morning urine were comparable with the association of directly measured 24-h sodium on blood pressure.

3. To gather preliminary information on mean and standard deviation of blood pressure and availability of the participants during study visits that will help us to re-calculate the sample size for the main stepped wedge study. We initially calculate the sample size from published study in other settings of Bangladesh that captured blood pressure of the population. Nevertheless, MAR study was designed for adult population ≥ 20 years. We realized revisiting the sample size of stepped-wedge trial with mean and standard deviation of blood pressure of ≥ 20 population from southwest coastal Bangladesh would be advantageous.

Study population: We conducted a survey in four communities of Dacope and Batiaghata sub-district of Khulna district that were neighbors to the communities targeted for the main MAR trial. Two communities were exposed and two communities were unexposed to MAR. We consulted the implementers of MAR program (Dhaka University team) to identify MAR communities where people had access to MAR water, and nearby non-MAR communities that have similar hydrological characteristics (confined aquifers where connection between the land surface and target aquifer is impeded by the presence of intervening impermeable layer) of the MAR communities, a MAR had been installed but was not operational in that time.

We conducted two visits in each community during pre-monsoon (May 10 — June 21, 2016) and monsoon (July 21 — August 21, 2016). A total 166 households from four communities were selected (78 households from MAR communities, 88 households from non-MAR communities). In the pre-monsoon visit, research staff collected household members' socioeconomic and demographic information, cardiovascular disease risk factors (e.g. smoking, physical exercise, stress), and inquired whether household members or their parents had any cardiovascular disease. They also measured height and weight, and collected information on household assets during first visit. All study participants were at least 21 years old. Informed written consent was taken from all the participating household members and the household heads.

Urine sample collection and analysis: Each participant received a 4-L plastic container for 24 hour urine collection, two 15-ml tubes for first and second morning urine collection, and a plastic mug to collect the voided urine and transfer to the 4-L plastic container and the 15-ml tubes. Research staff instructed the participants to discard their first morning urine and start collecting urine from the second morning void. Participants were instructed to transfer portion of second morning urine to the 15-ml tube, and the remaining second morning urine to the 4-L plastic container. They were instructed to transfer the entire voids of the day and night to the 4-L plastic container, and transfer the next first morning urine in 15-ml tube and the 4-L plastic container. If the 4-L containers were became full, participants were instructed to use any container from their household. Participants also received written printed instructions in native language for collecting urine samples, and documenting time of start and end of collection and the amount of urine loss if any. Volume of 24 hour collected urine were measured at household-level, and a sample of 15 ml samples from the 4-L plastic container was taken after making a homogenous mixture. All urine samples were transported to a field laboratory in 2-8° C for processing and analysis in the same day. Direct Ion Selective Electrode (ISE) method was used for urinary sodium and potassium measurements using a semi-auto electrolyte analyzer (Biolyte2000, Bio-care Corporation, Taiwan) [174].

Outcome measurement: Blood pressure of $\geq$20 year available household members of the selected households was measured during both visits. Trained research staff used Omron® HEM–907 digital blood pressure monitors to measure blood pressure, which are comparable to the gold standard mercury sphygmomanometers in terms of measurement and meets the Association for the Advancement of Medical Instrumentation's (AAMI) criteria [112]. Blood pressure was measured following the guidelines described by Pickering et. al. 2005 and Giorgini et. al 2014 [113,114]. Caffeine (tea, coffee, carbonated beverages), eating, heavy physical activities and smoking were proscribed for 30 minutes prior to measuring blood pressure. Participants rested for 5 minutes on a chair with both arms supported. An appropriate sized cuff and calibrated instrument was used. Blood pressure was measured three times; first left arm, then right arm, then again left arm. Both systolic and diastolic blood pressure was recorded from all measurements. The arithmetic mean of three systolic blood pressure measurements was used as the primary outcome.

Ethical approval: Informed written consent was taken from all the participating household members and the household heads. The study was approved by the Ethical Review Committee of icddr,b (PR-15096).

Appendix 4: First do no harm: the need to explore potential adverse health implications of drinking rain water[10]

Abu Mohd Naser[1], Reynaldo Martorell[2], K.M. Venkat Narayan[2], Thomas F. Clasen[1]

[1]Department of Environmental Health Sciences, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

[2] Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

Corresponding author:

Abu Mohd Naser Titu, MBBS, MPH
Department of Environmental Health Sciences
Rollins School of Public Health, Emory University
2nd floor, CNR building, Room # 2030E
Atlanta, GA 30322, USA
email: atitu@emory.edu

Seawater intrusion of coastal aquifers in has caused potable water scarcity in coastal communities of Bangladesh— the problem is severe in southwest coastal Bangladesh [175]. Global warming is expected to aggravate the condition due to rising sea levels and more frequent and stronger cyclones. High drinking water salinity has contributed to high salt intake and hypertensive disorders among population in southwest coastal Bangladesh. Pregnant women drinking groundwater have higher prevalence of preeclampsia and gestational hypertension compared to those drinking rainwater or pond water [120]. The estimated intake of sodium is 5-16 g/day in the dry season when the population relies on groundwater while the World Health Organization recommended daily intake is less than 2g.

The potable water scarcity problem is largely seasonal, with the main challenge occurring during the dry season between November and early May. Due to less rainfall and upstream decrease river flow, salinity of the surface water bodies such as rivers and canals increases during dry season. Moreover, the ponds often dry up during dry season leaving people with few or no alternatives but to abstract saline groundwater using hand pumps for drinking and cooking purpose. During the wet season (May— October), communities usually collect rainwater through household- or community-level rainwater harvesting systems and use it for drinking and cooking purposes. Rainwater has a very low content of sodium and other minerals.

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[10] This chapter is a manuscript has been published in Environmental Science & Technology. Reprinted with permission from American Chemical Society. Copyright 2017 American Chemical Society. 77. Naser AM, Martorell R, Narayan KV, Clasen TF. First Do No Harm: The Need to Explore Potential Adverse Health Implications of Drinking Rainwater. ACS Publications; 2017.

To address salinity-induced drinking water scarcity, interventions such as rainwater harvesting systems, pond sand filters near rainwater-supplied surface ponds, and rainwater-supplied managed aquifer recharge are currently being promoted. These systems capture rainwater during wet season when sufficient rainfall is available and store for the future use in dry season. While these interventions may be effective in reducing sodium exposure in drinking water, they may also have unintended adverse effects on human health. We outline these below.

The major cations of groundwater and surface water are calcium, magnesium, sodium and potassium — all four are the essential minerals for human. Although drinking water is not the primary source of calcium and magnesium, it may serve an appreciable contribution of these minerals when normal diets, especially in low resource environments, have low or borderline intakes in these elements [164]. Analyses from Bengal, Mekong and Red River deltas suggest individuals can obtain up to half of the dietary reference intake (DRI) of calcium, magnesium and iron is from drinking two liters of groundwater [38]. Readily-absorbable ionic forms in drinking water facilitates the higher bioavailability of minerals from drinking water relative to food[164] .

Several epidemiological studies suggest that drinking water low in calcium and magnesium salts is associated with higher cardiovascular mortality and morbidity [176]. In most large-scale studies, an inverse relationship between drinking water hardness and cardiovascular diseases has been reported [176]. Studies also suggest protective effects of drinking water with greater calcium and magnesium levels from death due to cerebrovascular diseases , as well as rectal, colon, gastric, breast, and prostate cancers.

Water chemical analyses suggest groundwater in southwest coastal Bangladesh has high concentrations of calcium and magnesium alongside with high sodium [145]. While high sodium is responsible for increased groundwater salinity, the groundwater hardness (a measure of calcium and magnesium salts in water) of southwest coastal Bangladesh is also greater than other parts of Bangladesh (Figure 1)[145]. While promoting rainwater to address the water salinity problem may lower sodium intake and benefit cardiovascular health, it may also reduce intake of essential cardio-protective minerals such as magnesium and calcium. The World Health Organization recommends remineralization of the desalinated water with calcium and magnesium to ensure the cardiovascular safety of desalinated water [177]. Promotion of rainwater without remineralization may expose communities at cardiovascular risks. A recent study from Israel highlights the potential adverse cardiovascular health effects if desalinated water is not remineralized. Israel has established many desalination plants, accounting for 75% of the tap water consumption to improve fresh water supply [172]. A nationwide acute coronary disease survey of Israeli patients identified a significant fall in blood magnesium levels and all-cause mortality rise in areas served by desalinated water compared to non-desalinated water areas [172].

Up to 60% of the magnesium and calcium content in food, and a higher proportion of manganese, copper and cobalt can be lost from vegetables, meat and cereal if demineralized water is used for washing and cooking [164]. Thus, cooking food with rainwater may reduce the mineral content of food and cause substantial loses of cations. Calcium concentrations of vegetables and potatoes increase when hard water is used for cooking but decrease when soft water is used [164]. Magnesium concentration decrease in cooked vegetables for both hard and soft water used, but the loss is more pronounced for soft water. Therefore, promoting rainwater for cooking may cause unwanted loss of essential minerals from Bangladeshi food.

In the 1970s there was a massive campaign in Bangladesh to reduce exposure to microbiologically contaminated surface or shallow well water by sinking millions of tubewells to tap into aquifers in the Ganges river delta. While the initiative succeeded in reducing diarrhea and enteric infection, the tubewells had a significant unintended consequence – they exposed tens of millions to naturally occurring arsenic, a major risk for heart disease, cancer, stroke, chronic lower respiratory diseases and diabetes. Considering this past example, we urge caution in rainwater promotion in Bangladesh given potential unintended health consequences related to rainwater low mineral contents. Research should be undertaken to understand 1) the proportion of DRI of calcium, magnesium and potassium that comes from different sources of drinking water in this setting, 2) the scope of remineralization of rainwater or food fortification with calcium and magnesium, and 3) whether a mixture of rainwater and groundwater can ensure intake of calcium and magnesium beside mitigating the salinity problem.

Acknowledgement: The authors are grateful to British Geological Survey for permitting use of the groundwater map.

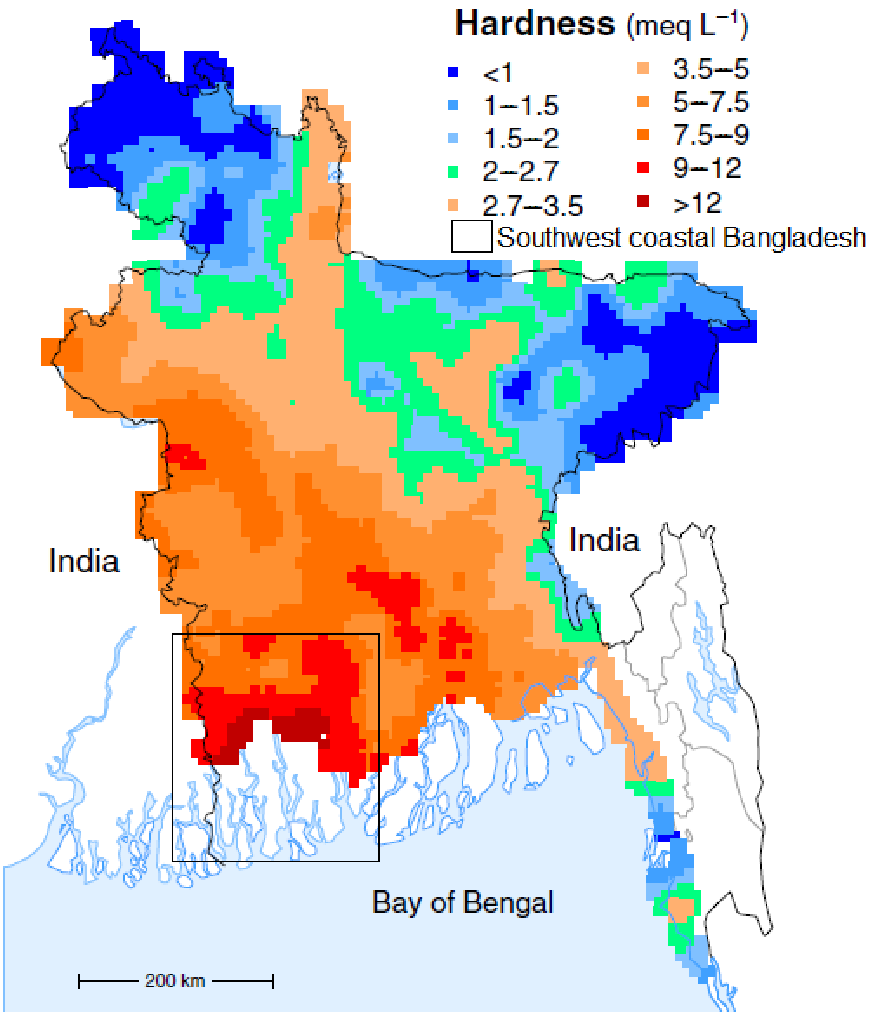
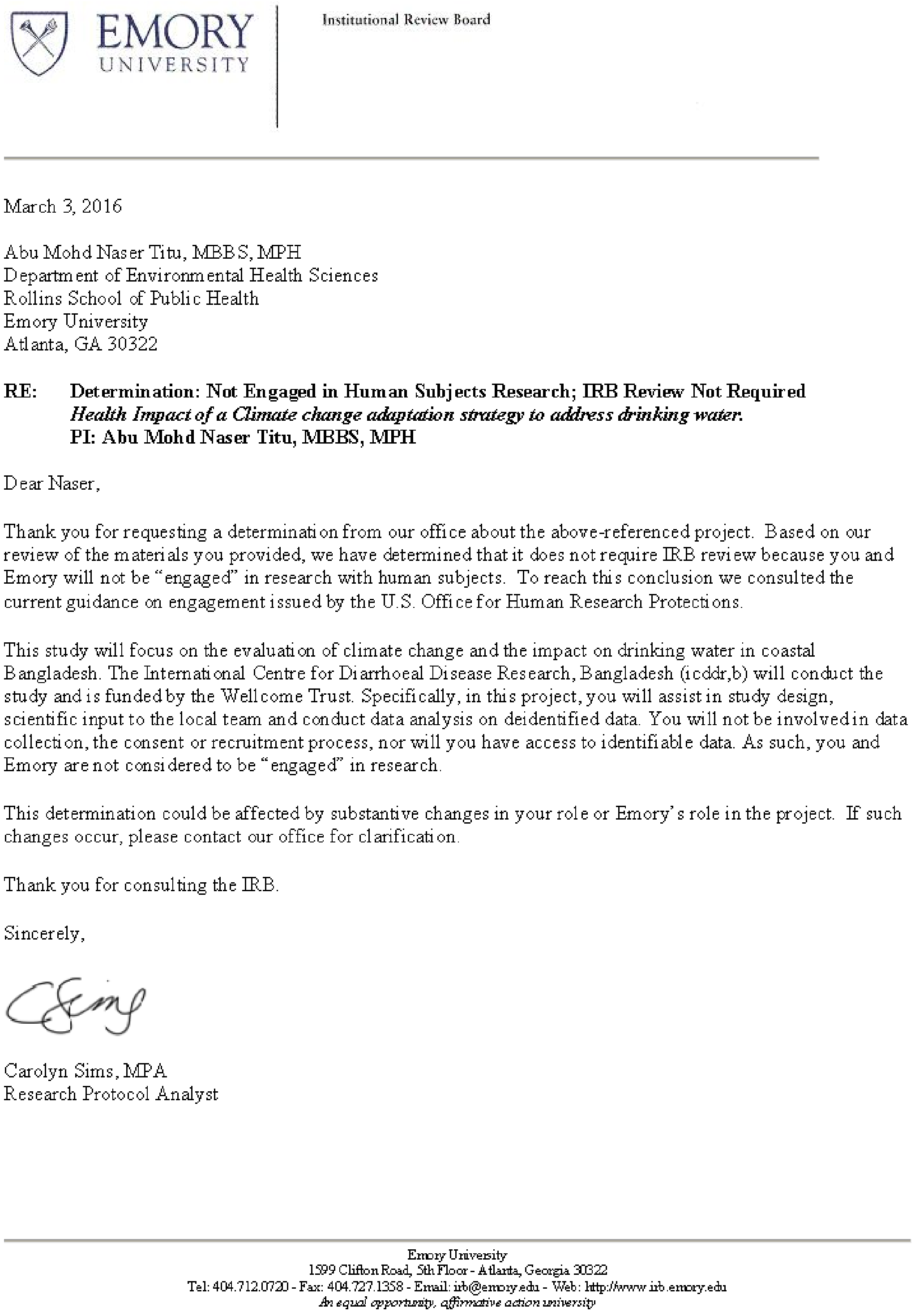


Figure 7: Groundwater hardness in different parts of Bangladesh (Contains British Geological Survey Materials © *NERC 2017*).

Appendix 5: IRB approvals for conducting the stepped-wedge trial and DHS data analysis



EMORY
UNIVERSITY

Institutional Review Board

March 3, 2016

Abu Mohd Naser Titu, MBBS, MPH
Department of Environmental Health Sciences
Rollins School of Public Health
Emory University
Atlanta, GA 30322

RE: Determination: Not Engaged in Human Subjects Research; IRB Review Not Required
Health Impact of a Climate change adaptation strategy to address drinking water.
PI: Abu Mohd Naser Titu, MBBS, MPH

Dear Naser,

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because you and Emory will not be "engaged" in research with human subjects. To reach this conclusion we consulted the current guidance on engagement issued by the U.S. Office for Human Research Protections.

This study will focus on the evaluation of climate change and the impact on drinking water in coastal Bangladesh. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) will conduct the study and is funded by the Wellcome Trust. Specifically, in this project, you will assist in study design, scientific input to the local team and conduct data analysis on deidentified data. You will not be involved in data collection, the consent or recruitment process, nor will you have access to identifiable data. As such, you and Emory are not considered to be "engaged" in research.

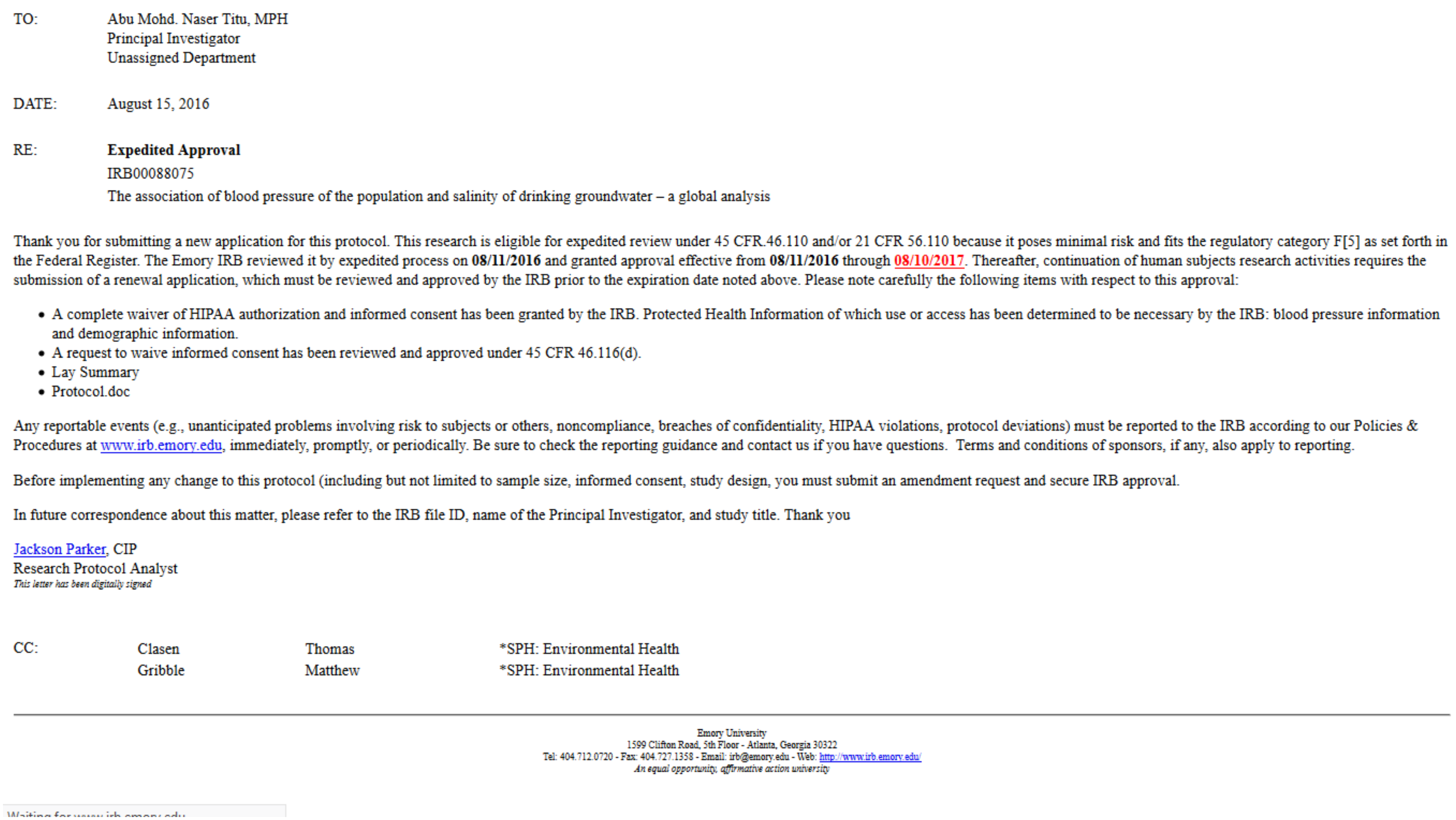
This determination could be affected by substantive changes in your role or Emory's role in the project. If such changes occur, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Carolyn Sims, MPA
Research Protocol Analyst

Emory University
1599 Clifton Road, 5th Floor - Atlanta, Georgia 30322
Tel: 404.712.0720 - Fax: 404.727.1358 - Email: irb@emory.edu - Web: http://www.irb.emory.edu
An equal opportunity, affirmative action university



TO: Abu Mohd. Naser Titu, MPH
Principal Investigator
Unassigned Department

DATE: August 15, 2016

RE: **Expedited Approval**
IRB00088075
The association of blood pressure of the population and salinity of drinking groundwater – a global analysis

Thank you for submitting a new application for this protocol. This research is eligible for expedited review under 45 CFR.46.110 and/or 21 CFR 56.110 because it poses minimal risk and fits the regulatory category F[5] as set forth in the Federal Register. The Emory IRB reviewed it by expedited process on **08/11/2016** and granted approval effective from **08/11/2016** through **08/10/2017**. Thereafter, continuation of human subjects research activities requires the submission of a renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. Please note carefully the following items with respect to this approval:

- A complete waiver of HIPAA authorization and informed consent has been granted by the IRB. Protected Health Information of which use or access has been determined to be necessary by the IRB: blood pressure information and demographic information.
- A request to waive informed consent has been reviewed and approved under 45 CFR 46.116(d).
- Lay Summary
- Protocol.doc

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design, you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you

Jackson Parker, CIP
Research Protocol Analyst
This letter has been digitally signed

CC:	Clasen	Thomas	*SPH: Environmental Health
	Gribble	Matthew	*SPH: Environmental Health

Emory University
1599 Clifton Road, 5th Floor - Atlanta, Georgia 30322
Tel: 404.712.0720 - Fax: 404.727.1358 - Email: irb@emory.edu - Web: http://www.irb.emory.edu/
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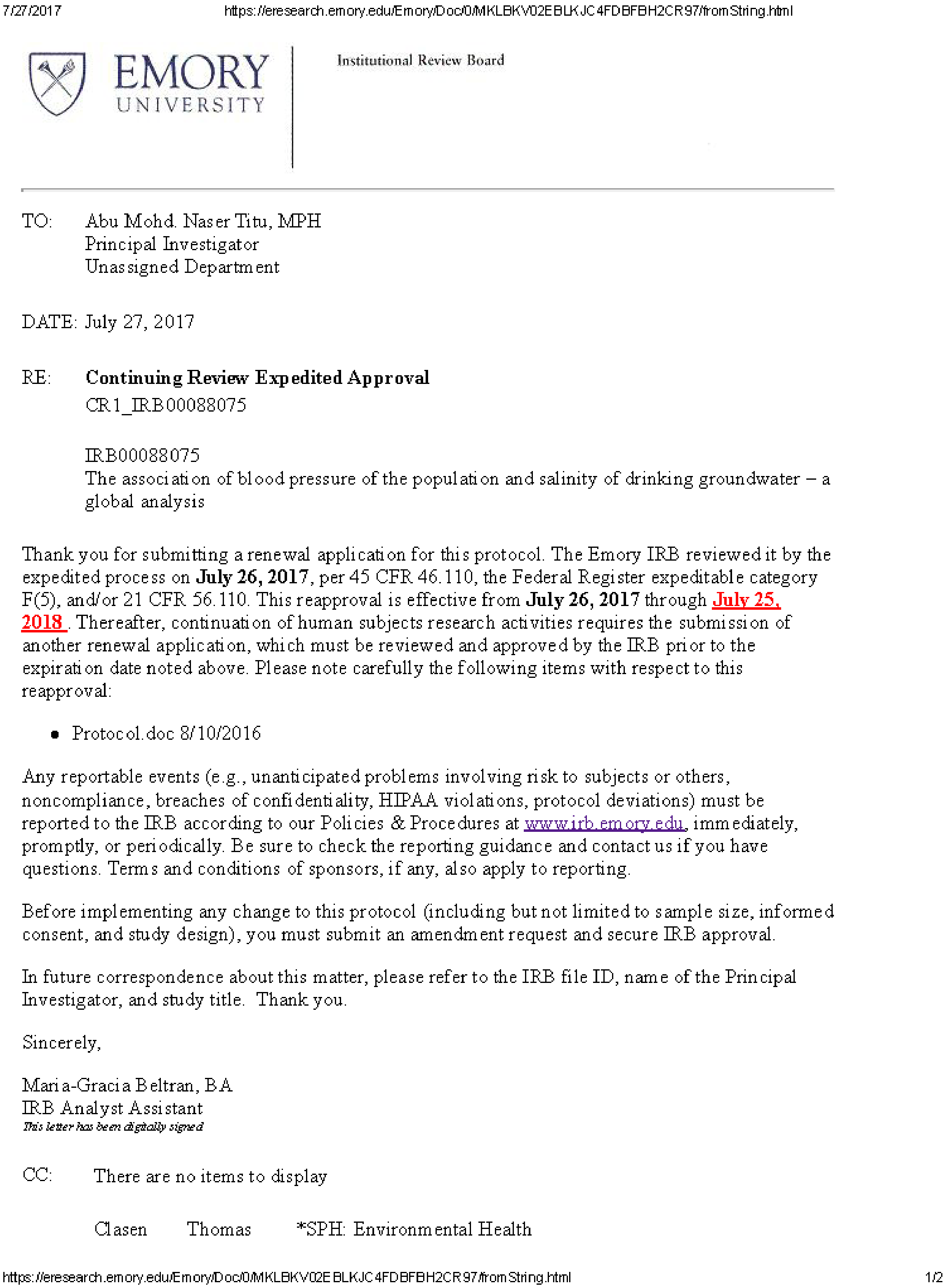

EMORY
UNIVERSITY

Institutional Review Board

TO: Abu Mohd. Naser Titu, MPH
Principal Investigator
Unassigned Department

DATE: July 27, 2017

RE: **Continuing Review Expedited Approval**
CR1_IRB00088075

IRB00088075
The association of blood pressure of the population and salinity of drinking groundwater – a global analysis

Thank you for submitting a renewal application for this protocol. The Emory IRB reviewed it by the expedited process on **July 26, 2017**, per 45 CFR 46.110, the Federal Register expeditable category F(5), and/or 21 CFR 56.110. This reapproval is effective from **July 26, 2017** through **July 25, 2018** . Thereafter, continuation of human subjects research activities requires the submission of another renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. Please note carefully the following items with respect to this reapproval:

- Protocol.doc 8/10/2016

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, and study design), you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you.

Sincerely,

Maria-Gracia Beltran, BA
IRB Analyst Assistant
This letter has been digitally signed

CC: There are no items to display

Clasen Thomas *SPH: Environmental Health

Gribble	Matthew	*SPH: Environmental Health

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icddr,b

Approval for continuation of research activity

This is to certify that icddr,b research protocol # PR-15096 titled "Health impacts of a climate change adaptation strategy to address drinking-water salinity in coastal Bangladesh": PI – Dr Mahbubur Rahman of the Infectious Diseases Division (IDD) had been approved by Ethical Review Committee (ERC) on 10 March 2016.

The ERC undertakes annual/periodic review of all ERC-approved protocols for reappraisal. The ERC approval for implementation of any research protocol is not, however, affected unless any unanticipated problems involving risks to the study participants or any serious or continuing noncompliance of the ERC Guidelines are detected in the implementation of the study, during the review period.

The review undertaken as of 12 March 2017 to oversee the implementation of the above protocol reveals no Adverse Event (AE) or Serious Adverse Event (SAE) or unanticipated problems involving risks to the study participants or any serious or continuing noncompliance of the ERC Guidelines. Therefore, the ERC is pleased to **approve** the protocol for continuation of its activity for next one year starting from **10 March 2017** to **09 March 2018.**

The continuing review application must be submitted to the IRB Secretariat for this study to continue beyond 09 March 2018. All necessary materials for continuing review must be reviewed with sufficient time for review and issuing continued approval before the expiration date. Failure to initiate a continuing review application in a timely fashion may result in discontinuation of study activities until approval can be renewed. Performing study activities, including data analysis, beyond the expiration date results in noncompliance of federal regulations.

Other terms and conditions for implementation of your research protocol, as contained in our memo dated 10 March 2016 according initial approval of the research protocol shall, however, remain unchanged.

Professor Kazi Zulfiquer Mamun
MBBS, M.Trop. Med, PhD.
Chairperson
Ethical Review Committee of icddr,b
14 March 2017

Cc: Coordination Manager, Grants, RA (G-01336)