

An Inexpensive Water Filtration System for Removing Arsenic from Tube Well Water and Improving
Health in Rural Populations

Ishraq A. Haque

South Carolina

I. Abstract

Approximately 200 million people worldwide are affected by arsenic-contaminated water for reasons such as drinking tube well groundwater. Last year, the author created a novel and inexpensive One Step Red Soil Filtration (OSRSF) system for villages in Bangladesh. The goal of this current study was to investigate the long-term biological effects of utilizing the OSRSF with a slight modification of the container for a more efficient system. After one-year usage, hair and nail arsenic levels of villagers significantly decreased. The percentage of immune cell survival was also improved by using the OSRSF. This result was verified in the laboratory by using the cell viability assay to check how arsenic trioxide and sodium arsenite affect survival for two B-cell lines. Then, an immunofluorescence staining assay revealed how mitochondrial stress was attenuated due to a decrease in the cell death marker Bax caused by the filtration system. Western blot analysis showed caspase-3 protein expression decreased, causing a reduction in immune cell death. Overall, this study presents how an economical filtration system can improve human health and provide clean water for low-income populations.

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III. Key Words: Arsenic, tube well water, arsenicosis, laterite soil, arsenic filtration, Bangladesh, cell viability, Bax, caspase-3

IV. Abbreviations and Acronyms: World Health Organization (WHO), Composite Iron Matrix (CIM), Parts Per Billion (ppb), One Step Red Soil Filtration (OSRSF), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Fluorescein Isothiocyanate (FITC)

V. Acknowledgements

First, I would like to recognize my mother and father for their support throughout the project. I would also like to thank Mr. Redwan Mehidi and Mr. Nazmul Haque, who helped me talk to local villagers and collect samples. Also, I would like to acknowledge Mr. Rafiqul Haque for helping me gather information about arsenic affected populations in Bangladesh, assisting me with handling the filtration system, and advising me on how to handle samples. Additionally, I would like to thank the Medical University of South Carolina for allowing me to use its facilities. Finally, I would like to thank all the participants who helped me with the sample collection for my study. Without these contributions, this project would not have been possible.

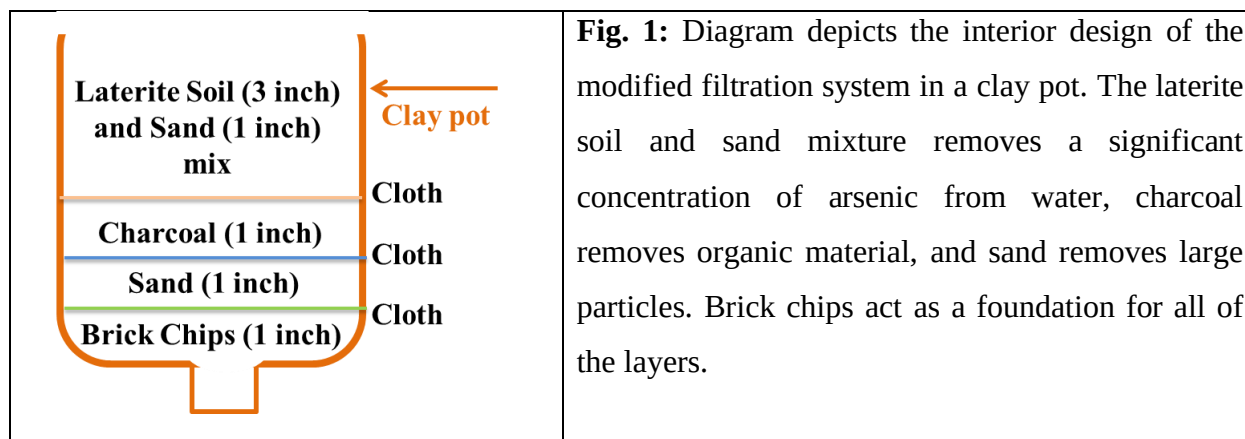
VI. Biography

Ishraq Haque is a senior from Academic Magnet High School in North Charleston, South Carolina. Ishraq has had a passion for science throughout his life with his parents being scientists at a medical university. He has developed projects in multiple fields such as osteology, environmental engineering, and cardiology. Additionally, he has been able to go to many national and international competitions by presenting his work. He got third place in Environmental Science at the National Junior Science and Humanities Symposium, went to the International Science and Engineering Fair after winning in the Lowcountry fair, and he was the South Carolina representative for the U.S. Stockholm Junior Water Prize in 2019 and 2020 at the national competition where he also got the Bjorn von Euler Innovation in Water Scholarship. Outside of school, Ishraq is an avid pianist, martial artist, community volunteer, writer, and participant in his school's science club, getting an opportunity to represent his school in a team for the National Science Olympiad. Ishraq enjoys assisting those around him and hopes to one day become a prominent scientist serving both the environment and medicine in order to provide people a higher quality of life.

1. Introduction

Arsenic is a toxic element in its inorganic form found within the Earth's crust and natural surroundings, including water and air [1]. Arsenic-contaminated water can be characterized as the pervasive presence of arsenic in groundwater. In many rural regions across the developing world, poverty-stricken families use tube wells to pump out groundwater often contaminated with arsenic [2,3]. Due to the arsenic contamination of groundwater, over 200 million people globally have been reported drinking high levels of arsenic-contaminated water. Consuming high concentrations of inorganic arsenic can often lead to arsenicosis, and this is a significant occurrence found within thousands of people in countries such as Peru, India, and Bangladesh. As a result, complications such as diarrhea, affected hair and nails, darkened skin tone, and types of cancer like skin and bladder cancer appear [3-6].

This study was initially held in Bangladesh in order to find a new approach to reduce arsenic for rural low-income populations. In Bangladesh, it has been found that over 20 million people drink arsenic-contaminated water, and as a result, over 43,000 people die from arsenicosis every year [5,7]. As the author demonstrated in his research paper last year, one of the



most efficient solutions to remove arsenic from potable water has been to use affordable filters [8]. The SONO Filter has gained international recognition for its ability to remove a significant concentration of arsenic from potable water. It easily met the standards of nations affected by arsenic contamination and met the threshold of the World Health Organization: 10 ppb. Although the SONO Arsenic Filter specifically had unprecedented success due to the presence of the composite iron matrix (CIM) along with other layers in the system including sand and charcoal [9-11], it has important drawbacks that can be found such as easy breakage, maintenance, weak sludge-disposal guidance, and a slow flow of the water, and high cost of up to US\$50 [10]. This is significant because poverty-stricken families in Bangladesh and around the world often generate incomes as low as US\$3 daily. Due to these problems, alternate household tube wells, such as the “3-Kolshi” have been created with a layer of cast iron fillings replacing the expensive and specifically designed CIM [11], but these alternatives also have problems like buffering time in the release of water, a complicated set-up and maintenance, high cost, and not being able to remove enough arsenic often, a significant concern. As a result, many filters have not been used for several years as plentiful time has been lost cleaning, replacing, and taking care of the necessary materials and supplies of arsenic filters [10]. Thus, the creation of a much simpler and affordable filter is necessary.

In order to create an affordable filter, it was determined that a cheap alternative to the expensive CIM or cast iron turnings would be required. Based on past literature, using natural substances that strongly bind to arsenic due to the latter’s attraction to Fe^{+3} would be recommended. Zeolites and soils have been found to have a high presence of Fe^{+3} and thus could act as inexpensive sorbents to acquire from the ground to remove arsenic from potable water. Specifically, laterite soil was used as it is widely available in the Indian subcontinent. After

thorough literature search, the author hypothesized that applying laterite soil in lieu of an iron matrix will remove arsenic. To materialize this novel concept, the author developed a one-step arsenic filtration system that would be economic and efficient in removing a significant amount of arsenic from drinking water (Fig. 1). With the inclusion of laterite soil commonly known as red soil and the replacement of iron, a cheaper alternative to iron came along with an extra benefit comes into the filter: the pH of the water does not change at all after arsenic removal and iron is not drained away that easily. Although drawbacks can be found with the replacement of CIM with laterite soil such as less arsenic filtration, it is important to consider that poverty-stricken families often have daily incomes as low as US\$3-5 [10], and they cannot even afford maintaining expensive filters like the SONO filter with the CIM. Extreme low-income families will have a cheaper option to drink water, and the poverty-stricken groups will still have a way to drink improved water with a filter that may be in usage for a long time. With the information provided, the author decided to help introduce a novel approach to the removal of arsenic for low-income rural populations, a One Step Red Soil Filtration (OSRSF). After the OSRSF was developed, specific modifications were made. The OSRSF's exterior was changed from utilizing a tin container to a clay pot, or kolshi. The unique properties of clay enable the substance to adsorb arsenic after exposure to the inorganic element [12]. In addition, using a clay kolshi in lieu of a tin container is also cheaper as many villagers have the former due to the fact that it can be homemade.

This study presents a filter consisting of a mixture of a 3:1 ratio of laterite soil and sand, cloth, charcoal, more sand and cloth, and brick chips. Filtered water can be collected in a kolshi. An additional sheet of cloth may be inserted on top of the container holding the filtered water as a final safeguard to filter out large molecules. The 3:1 mixture of laterite soil and sand will remove a significant amount of arsenic from the contaminated tube well water and it will also filter large molecules from the water. The charcoal and the brick chips remove toxins along with organics, and the cloth holds the layers within the filtration system together. Finally, the second container serves to store the filtered water so that people may pour the filtered water out for drinking. It is important to consider that this study aims to reduce arsenic to a tolerable level for poor people, unlike previously created filters which significantly reduced arsenic to specific domestic standards and even the standard of the WHO, 10 ppb. This study aims to at least meet the standard of water quality for Bangladesh in regards to arsenic presence: 50 ppb [6,11].

Through this novel filtration system, a more economic option will be available for poverty-stricken populations, filter maintenance will be easier compared to past filtration systems, and improved water will be available to the low-income rural populations. Thus, the quality of life may be enhanced for thousands of people through the creation of this filtration system that improves the water quality of arsenic-contaminated tube well water.

Arsenic easily enters human food and may accumulate in significant amounts in both meat and plant-based food. It often enters and contaminates groundwater, and groundwater from tube wells is heavily used for cooking in low-income populations. Arsenic also easily enters rice (13), as groundwater may be used for cooking and it may even enter the crops through watering them. Unfortunately, rural and poverty-stricken families do not understand this concept and suffer significant health complications due to arsenicosis. This arsenic contamination is often detectable in hair and nail samples from people using tube well water. Use of my low-cost novel filtration system may help overcome this problem in rural areas for reasons besides just drinking water.

Chronic arsenic exposure can cause cardiovascular disease, diabetes, central nervous system disorders, skin lesions, immunosuppression, and inflammation as arsenicosis [14,15]. While arsenic influences many cellular pathways, this study looked at the effects of arsenic-contaminated tube well water on cellular survival of B-cells which have multiple roles in controlling immunity and inflammation. Several laboratory experiments were also conducted next using major arsenic compounds arsenic trioxide (As_2O_3) and sodium arsenite (NaAsO_2) to see how the OSRSF could help improve cellular survival because these compounds are commonly seen in groundwater. These studies were also expanded to investigate possible causes of cellular death through various experiments such as the cell viability assay, immunofluorescence staining, and Western blotting. Overall, these studies suggested that higher amounts of arsenic in tube well water in rural populations carry significant risk in health and human life, and use of the affordable OSRSF may revolutionize their life in maintaining good health.

2. Materials and Methods

2.1. Measurement of arsenic levels by EZ Arsenic High Range Test Kit

The author travelled to a small village in Satkhira, Khulna Division, Bangladesh where water samples from 30 tube wells were collected and tested for arsenic concentrations using the Hach

“EZ Arsenic High Range Test Kit” [16]. A range (100 ppb, 250 ppb, and 500 ppb) of the lowest and highest amount of arsenic concentrations was found in my previous study when 30 tube well water samples were analyzed. The novel filtration system, the OSRSF, was used to filter samples from 100 ppb, 250 ppb, and 500 ppb tube wells, and these water samples were also used in the cell viability assays.

2.2. Measurement of arsenic levels by mass spectroscopic analysis

Arsenic levels in the hair and nail samples from people used OSRSF for 12 months were analyzed as described previously [17]. Briefly, samples were submerged in 1% Triton X-100, sonicated for 20 minutes, washed with milli-Q water, and dried at 60°C for overnight. Samples were then digested with nitric acid (70°C for 15 mins, and 115°C for 15 minutes), cooled, and diluted with 1.0% nitric acid containing yttrium (10 ppb), and arsenic concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS).

2.3. Cell lines and culture conditions

Human B-cell lines Priess and Frev were grown in T-25cm or T-75cm cell culture flasks (Corning, Corning, NY, USA) and cultured (2×10^5 /ml) in Iscov's Modified Dulbecco's Medium (IMDM) containing 10% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 µg/ml) (Mediatech Inc, Herndon, VA, USA) [18]. The cells were split (1:5) twice a week and maintained in tissue culture flasks until cell survival assays were performed.

2.4. MTS cell viability assay

Priess and Frev cells were cultured for 48 hours at 37°C, counted, and cell concentrations were adjusted to 5×10^5 cells/mL were used. One hundred µL of cells were added to each well of a 96 well tissue culture plate, centrifuged, and supernatant was aspirated. Tube well water samples containing various concentrations of arsenic as well as OSRSF filtered water samples were added to the wells. Each water sample tested in triplicate for both Priess and Frev cells. One hundred µL of media was then added to three wells as a background control. For inorganic arsenic compounds, arsenic trioxide (As_2O_3) and sodium arsenite (NaAsO_2), 0-100 ppb concentrations in double distilled water were tested on B-cells. The plate was placed in a 37° C humidified incubator with 5% CO_2 and incubated for 24 hours. After this incubation, 20 µL of 3-

(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium [19], inner salt (MTS) solution was added to each well and the plate was incubated for three hours. Absorbance readings at 490 nm were taken using a plate reader (Biotek). This data was converted to percent cell survival after subtracting the background value.

2.5. Immunofluorescence staining

Priess B-cells were treated with purified water (0 ppb), 500 ppb arsenic water sample (before filtration) and 50 ppb arsenic water sample (after filtration) for 24 hours at 37°C. Cells were placed on a cover slip, fixed with absolute methanol and placed onto glass microscope slides [20]. A prepared solution of phosphate-buffered saline (PBS)/0.01M Glycine/ 0.1% Triton-X was placed over the cover slips, enough to cover the whole slip for 10 minutes. Then 5% bovine serum albumin (BSA)/PBS solution was added to the cells for 10 minutes. Excess solution was removed and 5% normal serum was added for 10 minutes. Primary antibody against Bax protein (Santa Cruz) diluted in 1% BSA/PBS was added and incubated at room temperature for 1 hour, and then rinsed twice with 1% BSA/PBS for 10 minutes. Matched secondary antibody (Santa Cruz) labeled with fluorescein isothiocyanate (FITC) diluted in 1% BSA/PBS was added and incubated at room temperature for 1 hour. The cover slips were rinsed twice with 1% BSA/PBS for 10 minutes and then rinsed twice with PBS for 5 minutes. DAPI was added to the cover slips for 10 minutes, rinsed twice with PBS for 5 minutes. Slides were then analyzed under microscope, and overlay images were assembled.

2.6. Preparation of cell lysates and protein estimation

Priess and Frev cells were washed with phosphate buffered saline (PBS) and cell pellets (5×10^6) were made and lysed using 100 μ L of complete lysis buffer of pH 7.4 (10 mM Trizma Base, 150 mM NaCl, 1% Triton-X 100) plus protease inhibitor (10 mM PMSF, 10 mM TLCK) on ice. The sample was spun at 7,000 RPM for 8 minutes and the supernatant was aliquoted and stored in -80°C until use. Aliquots were also added to a stock solution of 1.36 mg/mL BSA and double distilled water. 25 μ L of Reagent A and 200 μ L of Reagent B (Bio-Rad Laboratories, Hercules, CA) were added to the sample which was then placed in a plate reader (Biotek Inc., Winooski, UT, USA) to detect absorbance at 630 nm. The experimental value was compared to a standard protein value graph to determine protein concentration and the volume of protein per

sample to be used in western blot analysis.

2.7. Western blot analysis

Samples (30 µg of protein) were diluted with the same volume of sample buffer, heated at 70°C on a heat block with dithiothreitol, and were used in the SDS-PAGE (NuPage 4-12% Bis-Tris gels) according to a standardized laboratory procedure at the Medical University of South Carolina. After electrophoresis, proteins were transferred onto nitrocellulose membranes for immunoblot analysis. The nitrocellulose membranes were blocked using TBS-Tween and 5% nonfat dry milk (Nestle USA, Inc., Solon, OH) on a rotating balance. After blocking, the membranes were washed 5 times with TBS-T, and the blots were probed at 4 degrees C overnight with the primary antibodies against caspase-3, and beta-actin. Then horseradish peroxidase conjugated goat anti-mouse or anti-rabbit secondary IgG antibody was applied to the blots at 37 degrees C for 2 hours. Blots were immediately processed to digitalize image using FluorChem. Protein bands were quantified by using Image J software program. Compared with the beta-actin loading control, the percent change in amount of caspase-3 protein was calculated.

2.8. Statistical Analysis

Statistical analyses were performed by Student's t test. It is important to consider that any p-value ≤ 0.05 is considered to be statistically significant.

3. Results

Arsenic levels in tube well water from a rural village in Bangladesh and their effects on immune cells

My previous study collected and tested water samples from 30 tube wells to find the range of arsenic concentrations in the Satkhira village region in Bangladesh. As described in the methods, the author went to the village and talked to the people to have water samples from their tube wells. With the villagers' consent, a total of 30 tube wells were selected and marked for this project. Water samples were then collected and tested using Hach's "EZ Arsenic High Range Test Kit" as described in the methods. It was found that the OSRSF filtered the arsenic more efficiently than the locally used 3-Kolshi system. According to the data previously obtained, 500 ppb

arsenic-contaminated water samples filtration went from 500 ppb to 100 ppb

Table I. Arsenic amounts in select tube well water samples before and after filtration with OSRSF and their effects on cell survival.

Tube Well	Owner	Arsenic Amount (ppb)		Cell Survival (%)	
		Before Filtration	After Filtration	Before Filtration	After Filtration
04	Ebadul Sardar	500	50	62.526 ± 1.07	95.815 ± 1.16*
06	Shahidul Islam	500	50	64.218 ± 1.59	96.051 ± 1.21*
09	Alamgir Hossain	500	50	59.495 ± 3.46	94.632 ± 0.92*
12	Mizanur Dhali	250	50	85.114 ± 0.85	97.258 ± 0.68**
19	Sayed Mali	250	25	82.163 ± 1.17	97.045 ± 1.01**
22	Zahiruddin Dhali	250	50	86.962 ± 1.53	96.354 ± 1.22**
23	Abdul Quddus	100	25	91.414 ± 0.62	99.106 ± 0.08**
26	Mukter Hossain	100	25	88.695 ± 1.09	99.862 ± 0.73**
28	Abdul Hai	100	50	90.057 ± 1.36	99.911 ± 0.54**

Water samples from selected tube wells were collected and tested before and after filtration with OSRSF using Hach's "EZ Arsenic High Range Test Kit" as described. Priess B-cells were cultured in water samples and percent cell viability was determined. *p<0.01, **p<0.05.

and then 50 ppb with the OSRSF. Also, 250 ppb water samples dropped to 50 ppb and 100 ppb samples were reduced to having only 25 ppb of arsenic present. The current study selected nine tube wells out of 30 tube wells based on higher arsenic contents (Table 1) and tested their effects on immune cells before and after filtration with the OSRSF. Specifically, water samples from tube wells 04, 06, 09, 12, 19, 22, 23, 26, were analyzed for arsenic concentrations before and after filtration, and tested their effects on the survival of B-cells. This study was performed in the laboratory at the Medical University of South Carolina where Priess B-cells were treated with different water samples and tested for their survival by using the MTS viability assay (Table I). Results suggest that filtration of arsenic (200-500 ppb) containing water with OSRSF significantly removed arsenic contamination and improved survival of B-cells. These data may provide more insights into the actual significant effects that the OSRSF can provide as it can restore immune function as shown by the increase in B-cell viability.

This idea of restoring the body's immune function prior to drinking groundwater can be

related to other functional outcomes such as analyses of arsenic contents in hair and nail samples. After 12 months of OSRSF usage, hair and nail samples were taken from individuals in the Satkhira village with written consents. Based on this longitudinal part of the project, a significant decrease in arsenic contents in hair and nail samples was observed. An inductively coupled plasma mass spectrometry was performed on the acquired hair and nail samples.

Table II: Hair and nail status of arsenic levels before and after use of OSRSF.

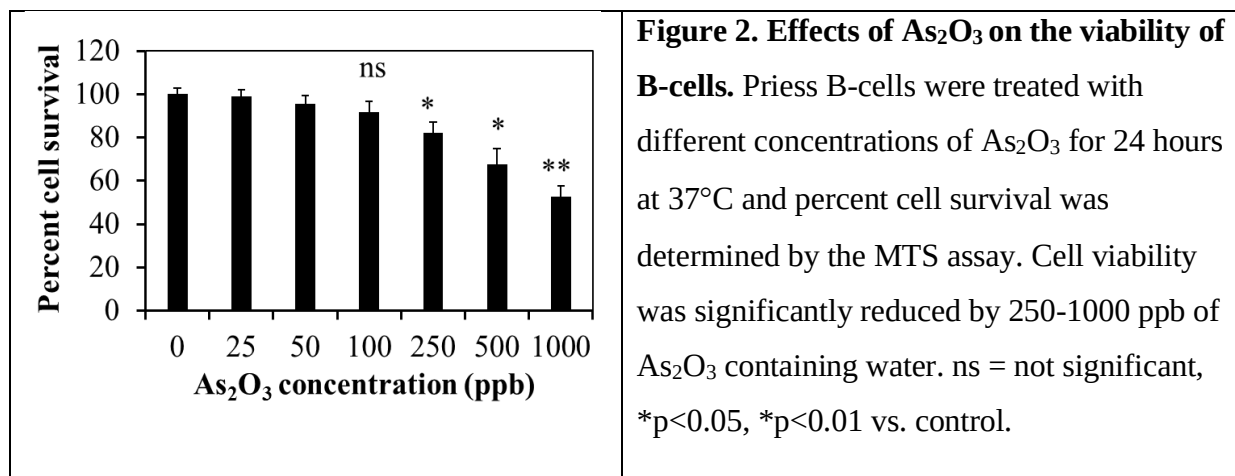
Tube well	Hair ($\mu\text{g/gm}$)		Nail ($\mu\text{g/gm}$)	
ID#	Before	After	Before	After
#04	2.119	0.538*	6.474	2.462*
#06	2.014	0.515*	6.813	2.109*
#09	2.461	0.652*	5.704	1.963*

Mass spectroscopic analyses of hair and nail samples from individuals from the 500 ppb tube well owners' household members. Water samples obtained before and after use of OSRSF for 12 months were used in this analysis. Hair and nail samples were collected, washed, and digested in Teflon vessels with 1 mL of concentrated nitric acid. After the decomposition at 180°C for 10 min, the volume of samples was made up to 10 mL with Milli-Q water. The concentrations of arsenic were determined by ICP-MS. * $p < 0.01$, before vs. after filtration.

The hair samples dropped in arsenic concentration levels from ~ 2.014 - $2.461 \mu\text{g/gm}$ to ~ 0.515 - $0.652 \mu\text{g/gm}$: a significant drop. Similarly, the nail samples lost a significant concentration of arsenic from ~ 5.704 - $6.813 \mu\text{g/gm}$ to ~ 1.963 - $2.462 \mu\text{g/gm}$ (Table II). Since arsenic accumulates in the integumentary system, analyzing its status before and after OSRSF usage was necessary.

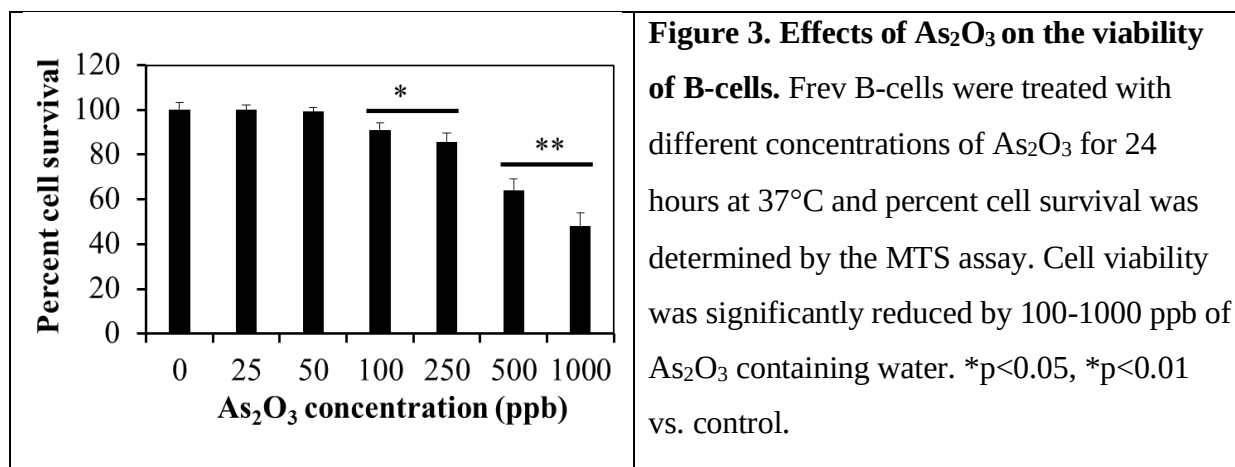
Effects of two major inorganic arsenic compounds on the survival B-cells

In general, humans are most likely to be exposed to inorganic arsenic through drinking groundwater. Since the OSRSF filtration system significantly decreased the concentrations of arsenic in groundwater and improved B-cell viability, the author decided to test how various concentrations of inorganic arsenic compounds affect the viability of two B-cell lines (Priess and Frev cell lines) in the laboratory. Specifically, two inorganic arsenic compounds, arsenic trioxide (As_2O_3) and sodium arsenite (NaAsO_2) were tested for their effects on B-cell survival.



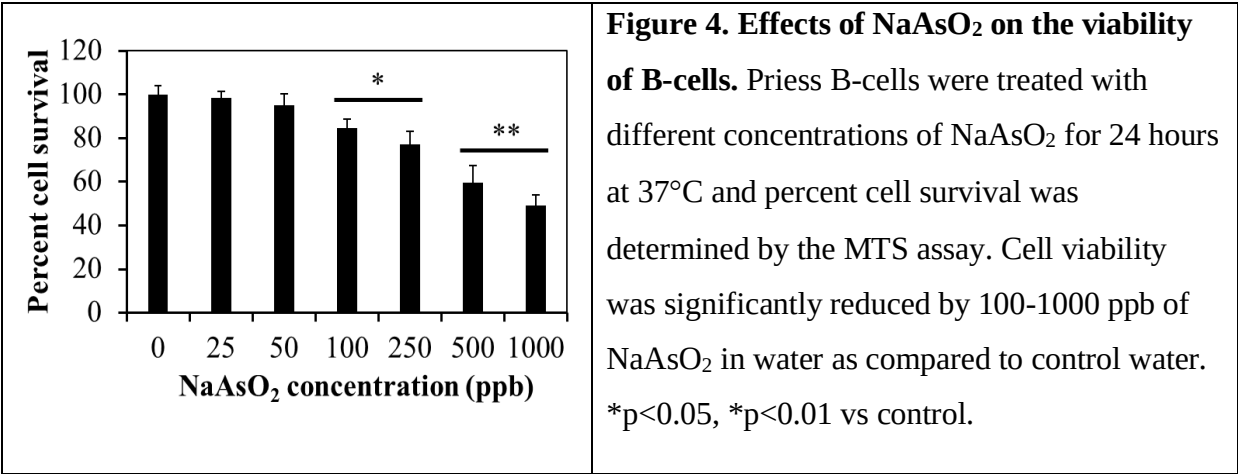
Various As₂O₃ concentrations (0, 25, 50, 100, 250, 500, 1000 ppb) were used to treat Priess B-cells for 24 hours at 37°C. It was revealed that the percent B-cell viability decreased with an increase in concentration of the arsenic compound (Figure 2). A significant decrease in cell viability was observed in the range of 250-100 ppb As₂O₃ in water. No significant drop in B-cell viability was observed in the 100 ppb of As₂O₃ in water.

The same range of As₂O₃ concentrations was used to treat Frev B-cells. The percent of cell survival also decreased with an increase in arsenic levels, and 100-1000 ppb As₂O₃ significantly inhibited Frev B-cell viability (Figure 3). These data suggest that different B-cell populations may differentially respond to arsenic compounds in water.

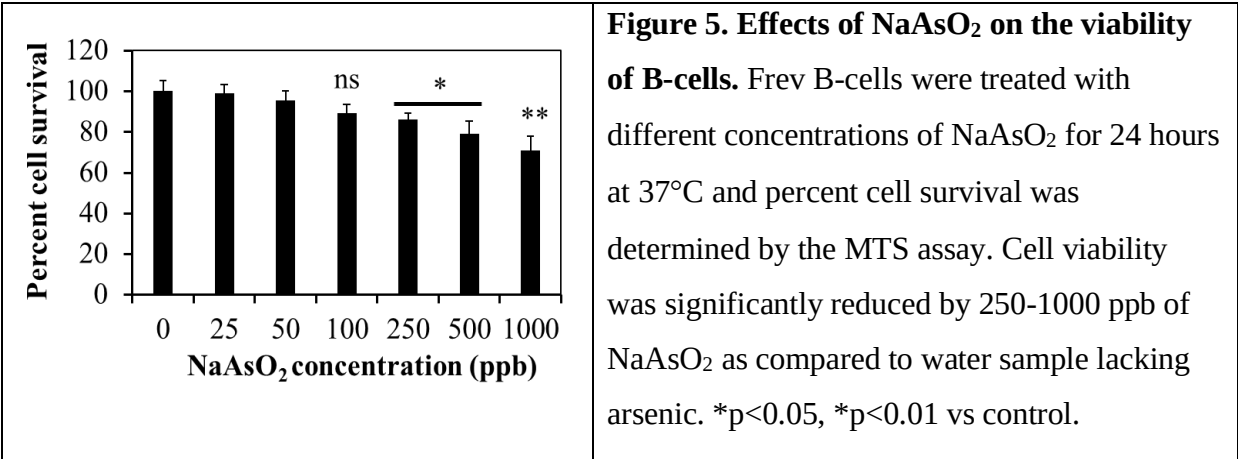


Then, various NaAsO₂ concentrations (0, 25, 50, 100, 250, 500, 1000 ppb) were tested on Priess and Frev B-cells. First, Priess B-cells were treated with varying concentrations of NaAsO₂ and consequently examined for cell viability. The data acquired shows that the percent cell survival decreased with an increase in concentration of the arsenic compound (Figure 4). As seen

in the figure, 100-1000 ppb NaAsO₂ significantly inhibited Priess B-cell viability. Similarly, Frev



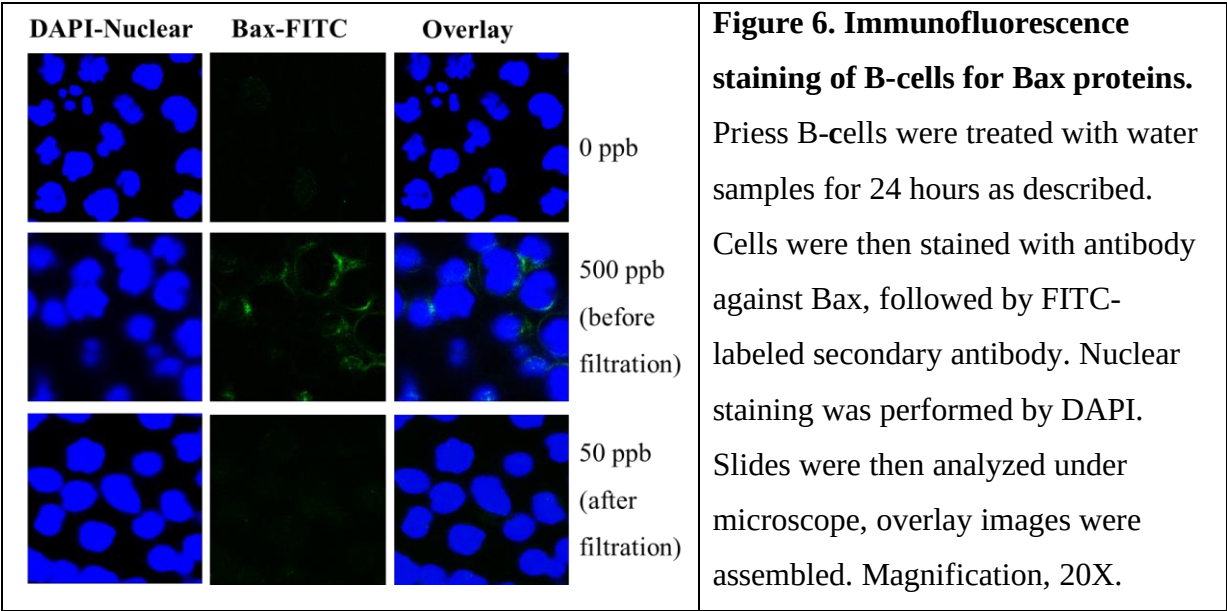
B-cells were also treated with the same concentrations of NaAsO₂, and it was revealed that the percent cell survival decreased with an increase of concentration as well (Figure 5). Overall, figures 2-5 indicate that the arsenic compounds actually have significant roles on B-cell survival.



Effects of arsenic contaminated tube well water on cell death

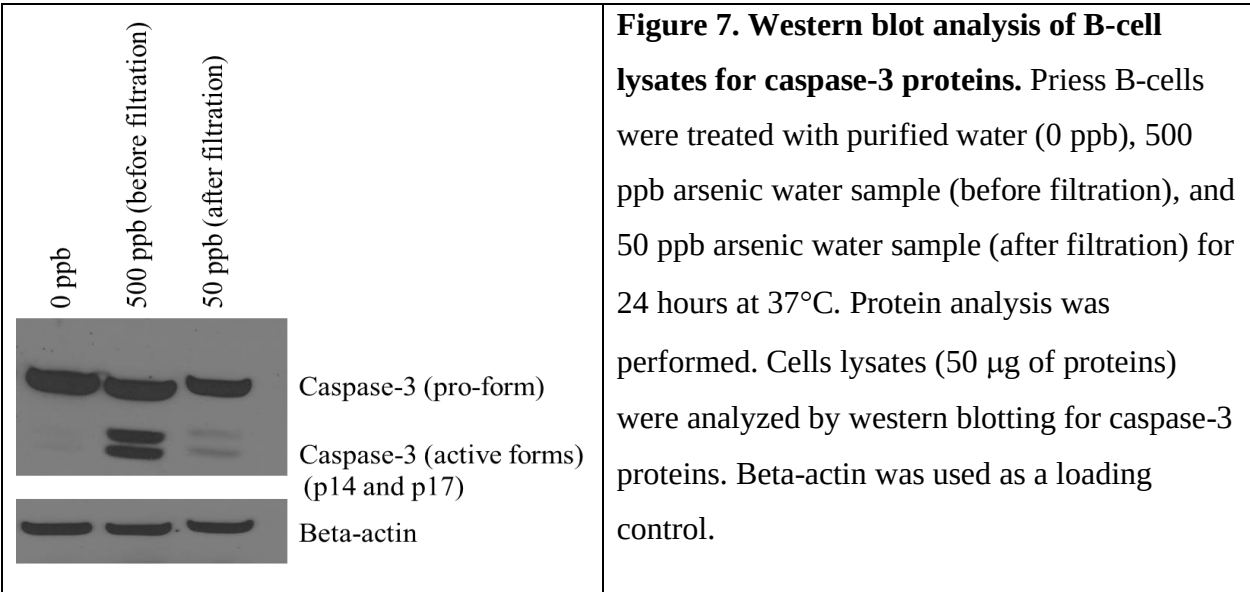
After finding out that the viability of Priess and Frev B-cell lines was affected by increasing concentrations of arsenic in water, the author decided to examine the effect of treating just the Priess B-cell line and see what happens to the viability of the cell, specifically with the mitochondrial Bax protein, an inducer of mitochondrial stress and cell death. An immunofluorescence assay was performed on the Priess B-cell line in order to see the effect of 500 ppb and 50 ppb arsenic on Bax protein expression. The Bax protein can actually cause mitochondrial stress and cell death if it is present in a high concentration [21]. After preparing the B-cells and treating them with unfiltered water (500 ppb) and filtered water with the OSRSF

(50 ppb), an immunofluorescence assay was performed (Figure 6). It was revealed



that treatment with 500 ppb of arsenic containing water induced Bax protein in the B-cells as compared to double distilled water which contains no traceable arsenic by the Test Kit. Interestingly, it was found that there was a profound reduction of Bax protein in the B-cells that were treated with OSRSF filtered water containing 50 ppb of arsenic.

Next, the author decided to further investigate how the inner mechanisms of B-cells are affected by arsenic concentrations. The expression of the caspase-3 protein was studied to see how it changed due to arsenic interaction (Figure 7). Caspase-3 is a protein that is responsible for



the execution of cell death. An excess of this protein can induce apoptosis which is also called a programmed cell death. Priess B-cells were treated with 500 ppb of arsenic groundwater and 50

ppb OSRSF filtered water.

Cell lysates were obtained and then 50 µg of protein from each sample was analyzed by Western blotting to see what happens with caspase-3 expression before and after OSRSF filtration. It was showcased by the data that the 500 ppb of arsenic caused a high expression of active caspase-3 in Priess B-cells (Figure 7). However, this expression was significantly reduced after use of filtered water as indicated by the Western blotting and subsequent densitometry analysis (data not shown). This data reveals that the OSRSF helped reduce caspase-3 expression in B-cells, suggesting that the cell viability was improved.

4. Discussion

The OSRSF successfully reduced the arsenic level from arsenic-contaminated ground water to a significant degree as seen in the author's previous project. By implementing a cheaper strategy, the OSRSF was able to reduce the arsenic level of water in Satkhira district to at most the Bangladeshi standard of 50 ppb. Through the usage of this novel filtration system, poverty-stricken people may be able to afford arsenic filtration that may not have been affordable to them, and they may be able to drink safer water than their former status quo. Thus, the aim is to significantly reduce the amount of arsenic in contaminated water for poor people was reached. The factors that made the OSRSF novel on reducing arsenic levels in water include the combination of the usage of laterite soil, a one-step filtration process, a cheaper filter available to the poor, and an easier maintenance process.

Numerous biological implications can be recognized from this project. After participating in this one-year longitudinal study, people experienced drinking safer water (Table I), having reduced arsenic levels in hair and nail samples (Table II), and less arsenic spots in their body (data not shown). This is relevant as villagers used the OSRSF often which decreased arsenic presence in their bodies and consequently attenuated the signs of arsenicosis. This highlights how a diminished existence of arsenic in the body was caused by the utilization of the OSRSF. Thus, the use of OSRSF will likely weaken the complications of arsenicosis and ultimately improve the health of poverty-stricken families.

In this study, water samples from nine tube wells which showed higher levels of arsenic were tested for their effects on immune cells before and after filtration with the OSRSF. The significant reduction of arsenic levels in water samples also improved survival of immune cells

(Table I). These data may provide more insights into the actual significant effects that the OSRSF can provide safer drinking water for rural people and maintain health and immune function as shown by the increase in B-cell viability. Further experiments were done in the laboratory to examine the interaction between arsenic based molecules and cell viability. These data (Figures 2-5) were examined to see what exactly occurs in the cellular and biochemical levels based on results the author procured. The influence of As_2O_3 and NaAsO_2 on the Priess and Frev B-cell lines was investigated because these compounds are most prevalent in arsenic-contaminated groundwater. It was revealed that the viability of both Priess and Frev B-cells decreased with their increasing amounts of As_2O_3 and NaAsO_2 . This is significant because this showcases that immune cell death is more prevalent with increasing levels of arsenic in drinking water. Additionally, the aforementioned data (Figures 2-5) suggest that usage of OSRSF could decrease cell death. As an illustration, Priess B-cells experienced a ~60% cell survival when they were subjected to 500 ppb of NaAsO_2 treatment but approximately 100% cell survival when treated with only 50 ppb of the same compound. Since the OSRSF can significantly decrease the presence of arsenic in potable groundwater (from 500 ppb to 50 ppb for instance), B-cell viability may increase which can allow for enhanced immune responses against many diseases.

Results in Figures 6 and 7 were obtained using filtered and unfiltered water obtained from Bangladesh. The immunofluorescence assay and Western blot were specifically performed to see how arsenic affects the inner mechanisms of human biology, namely in Priess B-cells. Data in Figure 6 indicated how the Bax protein significantly decreased in expression for the 50 ppb sample contrasted with the 500 ppb water sample. This diminished presence of Bax protein actually attenuates mitochondrial stress, allowing for better cellular function, interactions, and responses against various infections and diseases. Western blot analysis in Figure 7 revealed how the expression of caspase-3 was significantly weakened in the 50 ppb sample from the unfiltered 500 ppb water, and this was verified by the subsequent densitometry analysis. This data suggests how the execution of cell death by caspase-3 will happen less frequently; apoptotic conditions triggered by arsenic will decrease and allow for better cellular function as well. Overall, the OSRSF can significantly decrease arsenic presence in water and allow for enhanced cell viability and utility, allowing for the OSRSF owners to have a better quality of life.

The OSRSF is much cheaper than the 3-Kolshi or SONO filter and most of the villagers reported the OSRSF to be easier to use and maintain as compared to locally used 3-kolshi after

reviewing a questionnaire that was released (data not shown). Groundwater also has a high presence of lead due to its location in the ground which is surrounded by natural elements like lead and arsenic. The properties of laterite soil namely the rich iron content of the substance may enable it to attract lead molecules and thus remove it from ground water [22]. Thus, the possibility of removing lead by OSRSF can be tested in future.

Throughout the study, the inexpensiveness of the OSRSF strengthened the project. The OSRSF is more beneficial for low-income rural populations in comparison to the 3-Kolshi and the SONO filter systems economically. The total construction of the OSRSF took approximately US\$3, and maintaining the OSRSF has been found to be almost free of costs by simply replacing the laterite soil and using a clay pot. However, the locally made 3-Kolshi has been reported by Satkhira villagers to cost US\$10 to make and produce; the SONO filter has been seen to cost up to US\$50. The laterite soil is locally available in Satkhira for free and can be acquired for multiple uses. Additionally, the usage of the clay kolshi is free as it can be made at home, the sand is locally available for free, the cloth is also available for free, and the purchase of charcoal and brick chips may be bought for less than US\$1. By extension, the owner of the OSRSF may use his or her own private collection of laterite soil, sand, cloth, brick chips, and charcoal. If necessary, the owner of the OSRSF may clean the filter frequently or replace it with another version of OSRSF from time to time to have access to safer drinking water in rural villages.

Regarding limitations, the author only examined the effect of arsenic on B-cells and not other types of cells in the immune system. This is significant because other cells in this system, such as T-cells, macrophages, and dendritic cells, play crucial roles in protecting the body as well. Investigating the influence of arsenic on other immune cells is essential to determining how the inorganic element harms humans. Besides this limitation, creating a more diverse filtration system is essential as this OSRSF was localized for Bangladesh only.

Future goals of this study may include broadening the utility of the filtration system and the scope of the study. A larger group of participants in the project could strengthen reliability as there would be more data. Also, the examination of all 30 villagers who used the OSRSF for one-year should be considered as they may also enhance the work. Additionally, the implementation of the OSRSF should happen for cooking as well because water is used for multiple purposes besides quenching thirst and maintaining a hydrated body.

As far as future biological work is concerned, the author plans to see how the OSRSF affected cell viability, hair, and nail statuses of all 30 users of the system. An inspection of the users' skin condition and other symptoms should be checked too as victims of arsenicosis often have dark spots all over their body along with severe health complications. Additionally, Bax and caspase-3 expression for all 30 OSRSF owners should be observed as well to see if this information supports the project's data. Overall, this project has made considerable investigations into the beneficial consequences of using the OSRSF, but more work is required to further enhance the filtration system.

5. Conclusions:

Based on the results obtained from this novel study, a number of conclusions can be drawn on the effectiveness and benefits of OSRSF usage:

- (1) Using a clay kolshi in lieu of a tin container allowed for enhanced adsorption of arsenic and provided the filter's users a more inexpensive system.
- (2) Arsenic concentrations present in the body, namely in hair and nails, significantly decreased.
- (3) The viability of immune cells such as B-cells improved significantly with the utilization of the OSRSF.
- (4) Arsenic compounds such as As_2O_3 and NaAsO_2 reduced the viability of B-cells, indicating how the use of OSRSF could benefit arsenic affected rural populations.
- (5) The use OSRSF diminished cell death markers such as Bax, and caspase-3 when tested in the laboratory using B-cells.

In conclusion, this study investigated a novel arsenic filtration system for the poverty-stricken people by analyzing its benefits and influence on the body. By utilizing the OSRSF, poverty-stricken people worldwide may be able to drink water of a higher quality, creating a potential equalizer for all.

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